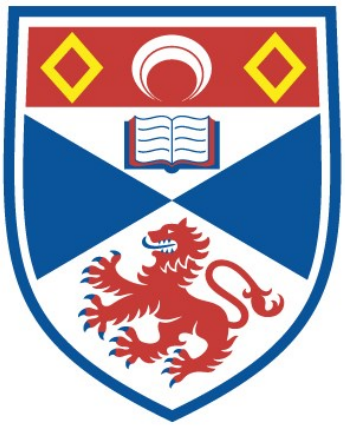
THE ROLE OF ANGIOTENSIN II IN OSMOREGULATION IN TELEOST FISH

Christal Elizabeth Grierson

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



1991

The Role of Angiotensin II in Osmoregulation in Teleost Fish

by

Christal Elizabeth Grierson

Thesis submitted for the degree of

Doctor of Philosophy

in the University of St.Andrews

August, 1990.

DECLARATION

a) I Christal Elizabeth Grierson, hereby certify that this thesis has been composed by myself, that it is a record of my own work, and that it has not been accepted in partial or complete fulfilment of any other degree or professional qualification.

Signed Date 14/8/90

b) I was admitted to the Faculty of Science of the University of St. Andrews under Ordinance General No. 12 in October 1986, and as a candidate for the degree of Ph. D. in October 1986.

Signed Date 14/8/90

c) I hereby certify that the candidate has fulfilled the conditions of the Resolution and Regulations appropriate to the degree of Ph.D.

Signature of Supervisor Date 14/8/90

COPYRIGHT

UNRESTRICTED

In submitting this thesis to the University of St. Andrews I understand that I am giving permission for it to be made available for use in accordance with the regulations of the University Library for the time being in force, subject to any copyright vested in the work not being affected thereby. I also understand that the title and abstract will be published, and that a copy of the work may be made and supplied to any bona fide library or research worker.

ACKNOWLEDGEMENTS

Firstly I would like to thank my supervisor Dr. Neil Hazon for giving me the opportunity of working at the Gatty Marine Lab. and for his advice and approachability during the project especially when time was critical.

I would also like to thank Professor I. Henderson and his group at Sheffield University for advice and inspiration and to Dr. C. Deacon for supply of iodinated peptides. Dr. J. McQueen (M.R.C., Blood Pressure Unit, Glasgow) advised on membrane preparation and radioreceptor assays.

I am indebted to Alberta Mandich and Catriona Paterson for lab assistance and to Karen Johnston for production of figures.

I also owe thanks to my colleagues at the Gatty, Dr. Liam O'Toole and Kenny Armour for invaluable computer tuition and especially to Marie Woolston and Jenny Boyle for coffee and lunch breaks.

Finally I would like to thank my family especially my father for support and encouragement and my children who have been my driving force.

This work was carried out during the tenure of a Science and Engineering Research Council Grant.

ABSTRACT

An osmoregulatory role for angiotensin II was investigated in the euryhaline European eel Anguilla anguilla L., plaice (Pleuronectes platessa) and dab (Limanda limanda).

1. Ile^5- and val^5-AII increased blood pressure in all species. Ile^5-AII produced a greater effect than val^5-AII, at equal concentrations, in eels, however in plaice and dab, this trend was reversed. Papaverine reduced blood pressure, followed by recovery to control in all cases.

2. Both AII sequences increased drinking rates except ile^5-AII in dab. Ile^5-AII again proved more potent than val^5-AII in eels and val^5- was greater than ile^5-AII in flatfish. Papaverine increased drinking in all species. Captopril had no effect on eel or dab drinking rates, but reduced those of plaice.

3. 1.0nM and 10.0nM AII increased ANP release from isolated eel myocytes, except from FW atria. At 1.0nM, ventricular release was greater than atrial, however at 10.0nM, AII ANP secretion was similar in both types of myocyte. In all cases ANP release was greater from SW than FW myocytes.

4. Tissue/plasma ratios revealed greatest binding in SW eel liver. Tissue receptor specific binding was also greatest in FW eel liver membranes, however in SW tissues gill was slightly greater than liver.

5. A FW eel liver membrane radioreceptor assay was developed. Binding was optimal at 22^{o}C, 25mM calcium, protein concentration of 700ug, ^{125}I-AII concentration of 25pM and an incubation period of 60 minutes.

6. Initial FW eel liver binding studies indicated two receptor classes with K_d=1.5x10^{-11}M and 2.46x10^{-10}M. However subsequent studies reveal K_d=3.31x10^{-8}M in FW liver and K_d=1.09x10^{-7}M in SW liver preparations.

7. ^{125}I-ile^{5}-AII produced greater binding than ^{125}I-val^{5}-AII in eel liver preparations. ^{125}I-val^{5}-AII produced greater binding in flatfish membranes.

8. Investigations of ^{125}I-ile^{5}-AII displacement from FW eel liver membranes revealed peptide potencies in the following order.

sar-AII>ile^{5}-AII$\geq$5-8AII>ile^{5}-AI>val^{5}-AI$\geq$
ile^{4}-AIII$\geq$val^{4}-AIII>bradykinin>1-4AII

ABBREVIATIONS

AI	angiotensin I
AII	angiotensin II
AIII	angiotensin III
A.C.	adenylate cyclase
ACE	angiotensin converting enzyme
ACTH	adrenocorticotrophic hormone
ANP	atrial natriuretic peptide
asn	asparagine
asp	aspartic acid
AVT	arginine vasotocin
B	bound
BSA	bovine serum albumin
cAMP	adenosine 3',5'-cyclic monophosphate
Capt	Captopril
cGMP	guanosine 3',5'-cyclic monophosphate
cpm	counts per minute
CRF	corticotropin releasing factor
C.S.	Corpuscles of Stannius
DG	diacyl glycerol
DTT	dithiothreitol
EDTA	ethylenediamine tetra acetic acid
EGF	epidermal growth factor
EGTA	ethyleneglycol-bis-(β-aminoethyl ether) tetra acetic acid
EMEM	Eagles minimum essential medium
F	free
FSH	follicle stimulating hormone
FW	fresh water

GARGG	goat anti-rabbit IgG serum
GFR	glomerular filtration rate
GH	growth hormone
gly	glycine
GPP(NH)p	5'-guanylylimidophosphate
H	hormone
his	histidine
HT	hypothalamus
^{125}I-AII	iodinated angiotensin II
IC50	concentration required to produce half maximal inhibition
ile	isoleucine
IP_3	inositol tri-phosphate
IT	isotocin
i.v.	intravenous
K_d	dissociation constant
LH	luteinising hormone
LHRH	luteinising hormone releasing hormone
MSH	melanocyte stimulating hormone
M.Wt	molecular weight
NHP	neurohypophysial peptides
NRS	normal rabbit serum
NSB	non-specific binding
Pap	papaverine
PD	pars distalis
phe	phenylalanine
PRL	prolactin
pro	proline
PtdIns(4,5)P	phosphatidyl inositol 4,5 bisphosphate

PTH	parathyroid hormone
R	receptor
RAS	renin angiotensin system
RIA	radioimmunoassay
S.A.	specific activity
sar AII	saralasin angiotensin II
SB	specific binding
S.E.M.	standard error of the mean
STI	soybean trypsin inhibitor
SW	sea water
T_3	triiodothyronine
T_4	thyroxine
TLC	thin layer chromatography
TSH	thyroid stimulating hormone
tyr	tyrosine
UI	urotensin I
UII	urotensin II
val	valine
VIP	vasoactive intestinal peptide.

LIST OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

1. GENERAL INTRODUCTION

1.1 OSMOREGULATION

Osmoregulation is a homeostatic mechanism involved in maintenance of body fluid and electrolyte balance. Organs involved in this regulation include the kidney, gills, gut, urinary bladder, gall bladder, integument of amphibia, rectal gland of elasmobranchs and cephalic salt glands of reptiles and birds. The way in which each vertebrate group osmoregulates depends to a large extent on habitat.

1.1.1 Terrestrial environment

Terrestrial vertebrates may lose water by evaporation across respiratory surfaces and skin, in urine and faeces and through sweating and panting. Water loss can be minimised by production of hypertonic urine or in reptiles and birds a virtually water free urine, reduction of skin permeability and reduction of respiratory evaporation by nasal exhalation and habitation of environments with high humidity.

1.1.2 Fresh Water

Fresh water (FW) vertebrates are at risk from hyperhydration due to the osmotic difference between the dilute external media and body fluids. This situation is remedied by copious production of dilute urine and active reabsorption of sodium and chloride ions across the gills of fish (Maetz, 1971) and by the skin of amphibians (Middler, et al, 1968; Bentley, 1973).

1.1.3 Sea Water

Marine teleosts maintain plasma osmolarity 25-30% that of seawater, (1000mOsm/l) creating a concentration gradient leading to water loss and solute accumulation. This is compensated by drinking large volumes of seawater (SW) along with excretion of sodium and chloride across the gills (Keys and Wilmer, 1932), as teleosts are unable to produce hypertonic urine, and magnesium and sulphate by the kidneys (Hickman and Trump, 1969). Marine reptiles are unable to produce urine hyperosmotic to plasma and bird kidneys cannot produce urine more concentrated than twice the blood osmolarity therefore these species secrete excess sodium and chloride via cephalic salt glands (Schmidt-Neilson, 1960). Marine mammals produce a hyperosmotic urine. Other vertebrate classes which maintain body fluids hyperosmotic to seawater do so by retention of organic compounds such as urea and trimethylamine oxide. This reduces water loss, but substantial ionic regulation for example in elasmobranchs at the gills, kidneys and rectal gland is still required to maintain all aspects of homeostasis. Hagfish, SW stenohaline members of the primitive cylostome family, are unusual in that they are the only true vertebrates to maintain body sodium and chloride concentrations similar to that of SW. Although they do not have to cope with the problem of osmotic water movement, ionic regulation may occur through the relatively impermeable gills, with elimination of divalent ions at the kidney.

1.2 HORMONAL CONTROL OF OSMOREGULATION

Many hormones affect osmoregulation, playing specific roles in different organs. A hormone is defined as a chemical substance synthesised by an endocrine gland, secreted into the blood and transported to its site of action in the body. Endocrine glands are, the adrenal/interrenal, thyroid, parathyroid, hypothalamus, pituitary, pancreas, kidney, ovary and testis. Some of these glands are distinct organs, such as the pituitary, whereas others are found within larger organs performing non-endocrine functions e.g. the pancreas. In contrast, a paracrine agent is a chemical which when released affects tissues located near the site of secretion and includes hypothalamic hormones, somatostatin, prostaglandins and renin.

1.2.1 Pituitary Gland

This gland is present in all vertebrates and is morphologically similar, being composed of the neurohypophysis and the adenohypophysis.

1.2.1a Adenohypophysis

The adenohypophysis is formed from epithelial cells differentiated to secrete peptide hormones. These hormones are:- growth hormone (GH), prolactin (PRL), the glycoprotein hormones - thyrotropin (TSH), luteinising hormone (LH) and follicle stimulating hormone (FSH) and the corticomelanolipotropins - which include and melanotropin (MSH) and adrenocorticotropin (ACTH). GH and

PRL are secreted by distinct cells of the pars distalis (PD) and ACTH is produced by cells from both the PD and pars intermedia.

Prolactins actions can be grouped into 5 categories 1) reproductive 2) osmoregulatory 3) growth and developmental 4) effects on ectodermal structures 5) synergism with steroid hormones. However no unifying principle is apparent in vertebrates (Hirano, 1986). GH stimulates body growth by actions on carbohydrate and protein metabolism and in some vertebrates may also have an osmoregulatory role. ACTH influences corticosteroid release from the adrenal cortex and is itself under control of corticotropin releasing factor (CRF) produced in the hypothalamus. TSH acts to stimulate the thyroid gland and LH and FSH stimulate the gonads. MSH affects colour change in certain vertebrates.

1.2.1b Neurohypophysis

All the neurohypophysial (NHP) peptides are nine residue peptides (nonapeptides), but distinct structural variations occur between different vertebrate groups. The fifth and sixth residues are cysteines, linked by a disulphide bond to form a cystine residue. In all peptides so far characterised the amino acids at positions 1,5,6,7, and 9 are conserved, whereas those at positions 3,4,8 are variable (Acher, 1974, 1988; Acher and Chauvet, 1988) (see table 1.1). In most vertebrates a vasopressor and a neutral peptide exist. Chemically, neutral hormone examples are oxytocin, mesotocin, isotocin, glumitocin, valitocin and

Table 1.1 Structure and Phylogeny of Vertebrate Neurohypophysial Hormes, (from Batten and Ingleton, 1987).

	Neutral Hormone	Basic Hormone
MAMMALS *Placentals* All except pigs	1 2 3 4 5 6 7 8 9 Cys-Tyr-*Ile*-Gln-Asn-Cys-Pro-*Leu*-Gly-NH_2 Oxytocin	1 2 3 4 5 6 7 8 9 Cys-Tyr-*Phe*-Gln-Asn-Cys-Pro-*Arg*-Gly-NH_2 Arginine vasopressin
Domestic pigs	Oxytocin	1 2 3 - 4 - 5 6 - 7 Lys 9 Lysine vasopressin
Marsupials Macropodidae	Oxytocin	Lysine vasopressin + 1 Phe 3 4 - 5 - 6 - 7 8 - 9 Phenypressin
Phalangeridae	1 - 2 - 3 - 4 - 5 - 6 - 7 Ile 9 Mesotocin	Arginine vasopressin
BIRDS, REPTILES, AMPHIBIANS AND LUNGFISHES	Mesotocin	1 - 2 - Ile - 4 - 5 - 6 - 7 - 8 - 9 Arginine vasotocin
TELEOSTEAN AND GANOID FISHES	1 - 2 - 3 - Ser - 5 - 6 - 7 Ile - 9 Isotocin	Arginine vasotocin
ELASMOBRANCHS *Selachii* Skates and Rays	1 - 2 - 3 - Ser - 5 - 6 - 7 - Gln - 9 Glumitocin	Arginine vasotocin
Sharks and Dogfishes	1 - 2 - 3 - 4 - 5 - 6 - 7 Val - 9 Valitocin + 1 - 2 - 3 - Asn - 5 - 6 - 7 - 8 - 9 Aspar(g)tocin	Arginine vasotocin
Holocephali	Oxytocin	Arginine vasotocin
CYCLOSTOMES	------	Arginine vasotocin

aspartocin and the basic group include the vasopressins and arginine vasotocin (AVT).

1.2.2 Adrenal and Interrenal Glands

The adrenal cortex is effectively unique to mammals comprising the outer zona glomerulosa, the zona fasciculata and an inner zona reticularis. Amphibians and fish, however have interrenal glands which tend to be less discrete due to mixing of cells with kidney tissue. These glands secrete a range of 21-carbon steroids, termed corticosteroids, which vary in different vertebrate groups (Table 1.2). Mammals produce aldosterone and cortisol, although rodents generally secrete corticosterone; amphibians reptiles and birds - aldosterone and corticosterone; teleosts predominantly cortisol although corticosterone and aldosterone have been found in some species (Sandor et al, 1976); and in elasmobranchs the novel steroid 1- hydroxycorticosterone. Adrenocorticosteroids have not been definitely identified in cyclostomes (Weisbart et al, 1978). On the basis of mammalian bioassays cortisol and corticosterone are termed glucocorticoids, affecting intermediary metabolism and are in general gluconeogenic and aldosterone is a mineralocorticoid affecting salt and water balance. There may be some overlap of function of the steroid hormones due to homology between the steroid binding and DNA binding domains of the respective receptors, (King, 1987).

Table 1.2. Major corticosteroids produced in vertebrate groups (From Balment and Henderson, 1987).

Group	Corticosteroids in blood
Elasmobranchs	1-*Hydrocorticosterone*, corticosterone, 11-deoxycorticosterone, 11-deoxycortisol
Bony fish	*Cortisol*, cortisone, 11-deoxycortisol, corticosterone
Lungfish	Cortisol, aldosterone, 11-deoxycortisol, 11-deoxycorticosterone, corticosterone
Amphibians	*Corticosterone*, 18-hydroxycorticosterone, aldosterone, 11-deoxycorticosterone, cortisol
Reptiles	*Corticosterone, 18-hydroxycorticosterone, aldosterone*
Birds	Corticosterone, aldosterone, 11-deoxycorticosterone
Mammals	*Cortisol/corticosterone*, aldosterone, cortisone, 18-hydroxycorticosterone, 11-deoxycorticosterone

* Italicised steriods are the major secretory product.

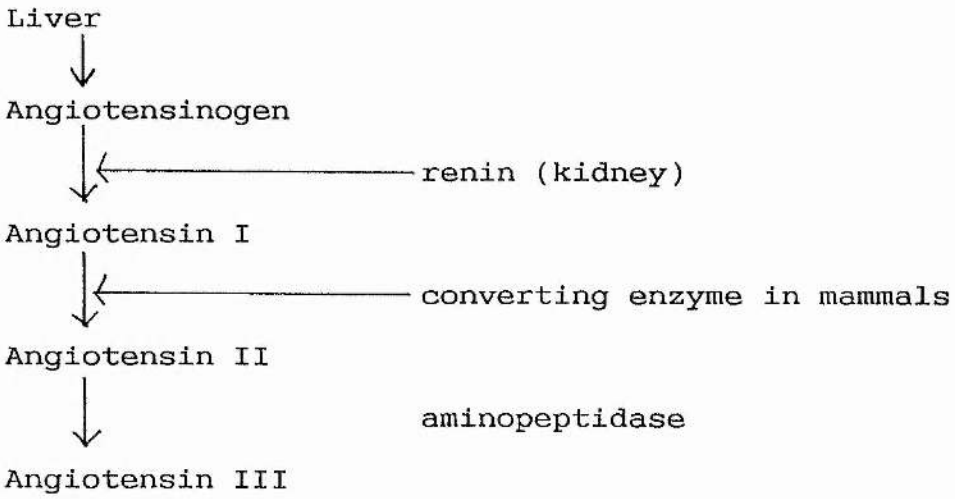
The adrenal medulla of mammalian vertebrates and the equivalent chromaffin tissue of non-mammalian vertebrates produce catecholamines of which epinephrine and norepinephrine are the principal examples.

1.2.3 Renin - angiotensin system

The renin angiotensin system (RAS) (see fig 1.1) (Peart, 1969) consists of a high molecular weight blood borne substrate, angiotensinogen (50,000-100,000 daltons), which is a glycoprotein synthesised in the liver. Renin, a proteolytic enzyme stored in the juxtaglomerular granules of mammalian kidneys, cleaves off 10 amino acids from the substrate, forming angiotensin I (AI). This first step is rate-limiting. Angiotensin converting enzyme (ACE) cleaves off amino acids 9 and 10 from AI leaving the biologically active octapeptide angiotensin II (AII). Angiotensinases degrade AII, however angiotensin II (AIII) (formed by aminopeptidase cleavage). the heptapeptide is thought to have some biological activity at least in mammals (Freeman et al, 1977). The basic structure of AII is conserved throughout vertebrate groups (see Table 1.3), with interchange of asparagine (asn) and aspartic acid (asp) occurring in position 1 and valine (val) and isoleucine (ile) at position 5. AI however is more diverse with several amino acids occupying position 9. Elements of this system have been identified in all vertebrate groups although controversy surrounds the cyclostomes and elasmobranchs (Nishimura et al, 1970; Henderson et al, 1981; Hazon and Henderson, 1985).

Fig 1.1

The mammalian renin-angiotensin system (from Balment and Henderson, 1987).



POSITIONS OF PEPTIDE CLEAVAGE

(Renin substrate)

	1	2	3	4	5	6	7	8	9	10	11	12
Angiotensinogen	Asp-	Arg-	Val-	Tyr-	Ile-	His-	Pro-	Phe-	His-	Leu-	Leu-	Val-

↑ renin

Angiotensin I	Asp-	Arg-	Val-	Tyr-	Ile-	His-	Pro-	Phe-	His-	Leu

↑ converting enzyme

Angiotensin II	Asp-	Arg-	Val-	Tyr-	Ile-	His-	Pro-	Phe

↑ aminopeptidase

Angiotensin II	Arh-	Val-	Tyr-	Ile-	His-	Pro-	Phe

Table 1.3. Angiotensin I Amino Acid Sequences from Various Vertebrates

	Amino acid sequence									
Common structure	1 -	2 Arg	3 Val	4 Tyr	5 -	6 His	7 Pro	8 Phe	9 -	10 Leu
Species Variation										
Eel	Asp/Asn				Val				Gly	
Salmon	Asn/Asp				Val				Asn	
Goosefish	Asn				Val				His	
Bullfrog	Asp				Val				Asn	
Snake	Asp/Asn				Val				Tyr	
Fowl	Asp				Val				Ser	
Bovine, turtle	Asp				Val				His	
Human, pig, rabbit, rat, dog, horse	Asp				Ile				His	

The RAS in mammals functions to regulate blood pressure and salt and water balance, via cardiovascular control and influences upon renal and adrenocortical functions. In non-mammalian vertebrates exogenous AII administration increases blood pressure both directly and also by the release of catecholamines. Intrarenal actions of AII include control of glomerular filtration rate (GFR), sodium excretion by constriction of efferent arterioles and stimulation of sodium reabsorption in the proximal and distal convoluted tubules (Hall, 1986). AII is also known to stimulate calcium mobilisation and sodium influx in vascular smooth muscle cells.

It has been well established that AII is a potent dispsogen in several mammalian, avian and reptilian species (Fitzsimons, 1975;Fitzsimons and Kauffman 1977; Kobayashi et al, 1979). The site of action AII appears to be located in the brain of mammals (Severs and Summy-Long, 1975; Johnson and Schwob, 1975) and birds (Wada et al, 1975; Takei 1977a, b; Schwob and Johnson, 1977). Dipsogenic receptors in the brains of amphibians have not been identified. It has been suggested that these receptors appeared at a later evolutionary stage than amphibia, however euryhaline teleosts and elasmobranchs have been shown to respond to AII by drinking (Carrick and Balment, 1983; Hazon et al, 1989) whereas frogs have not (Hirano et al, 1978). The RAS may, however play a role in maintenance of water balance in amphibians (Bolton and Henderson, 1987).

1.2.4 Atrial Natriuretic Peptide

Atrial natriuretic peptide (ANP) is a peptide hormone involved in extracellular fluid volume regulation. A high molecular weight precursor is stored in granules of mammalian atria (De Bold et al, 1981) and consists of 151/152 amino acids (Kangawa and Matsuo, 1984). This inactive prohormone is cleaved to a smaller biologically active peptide of around 24-28 amino acids which is released into the circulation (Yamanaka et al, 1984; Maki et al, 1984; Oikawa et al, 1984). ANP is a diuretic and natriuretic and affects blood pressure and volume, kidney function and smooth muscle tone (De Bold et al, 1981), although its physiological role is still unclear. ANP often acts in conjunction with other hormones and has been shown to inhibit basal production of aldosterone, although this is controversial, and aldosterone stimulated by AII, ACTH, serotonin and potassium (Atarashi et al, 1984; Chartier et al, 1984; Campbell et al, 1985). In contrast, ANP studies on lower vertebrates are less extensive. However immunoreactive ANP has been detected in members of agnatha, chondrichthyes and osteichthyes and is thought to be involved in salt rather than osmotic balance in fishes (Evans et al, 1989). Binding sites for mammalian ANP have been demonstrated in the heart, renal system and aorta of the hagfish Myxine glutinosa (Kloas et al, 1988).

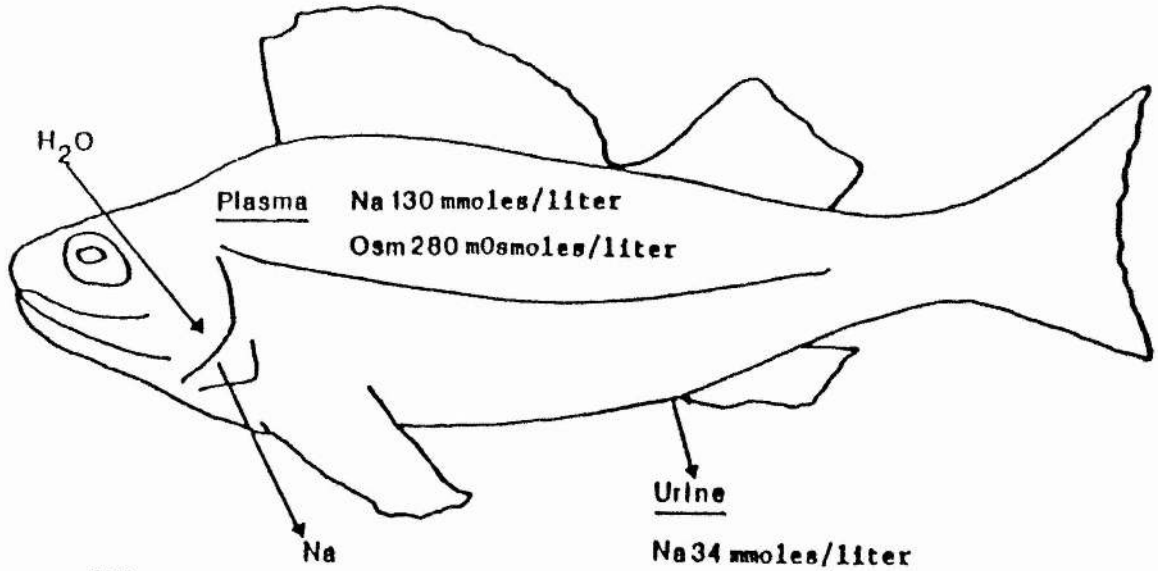
1.3 TELEOST OSMOREGULATION

Teleost fish comprising 20,000 species, successfully achieve osmotic balance although habitats range from freshwater with an osmolarity of <10 mOsmols/l to seawater with an osmolarity of 1,000 mOsmols/l. Fish in freshwater maintain blood plasma hypertonic and those in seawater hypotonic by 250-500 mOsmol/l in each case with respect to appropriate aquatic environments (McFarland, 1965; Parry, 1966; Maetz, 1970). See fig. 1.2.

Some euryhaline fish tolerate a wide range of salinities, as opposed to stenohaline fish which inhabit purely FW or SW environments. These euryhaline teleosts reverse the direction of ion flow between body fluids and the external environment and therefore maintain homeostasis. For example plasma sodium concentrations were measured in the eel (Anguilla anguilla L.) with mean values of 140mmols/l in FW and 160mmols/l in SW (Scheer and Langford, 1976), which is a small variation in relation to the change in external salinity. Therefore teleosts must be able to regulate electrolyte turnover rates. Measurements in FW fish revealed that branchial influx is 20-50% higher than outflux, compensating for renal loss of salts. Teleosts in SW, however, rapidly exchange sodium and chloride across gills at a rate 10-30 times per hour greater than can be accounted for by drinking, gut absorption and branchial extrusion (Motais, 1961, 1967; Oide and Utida, 1968; Potts, 1968). Organs involved in teleost osmoregulation include the skin, gut, kidney, urinary bladder and gills.

Fig 1.2 Teleost osmoregulation

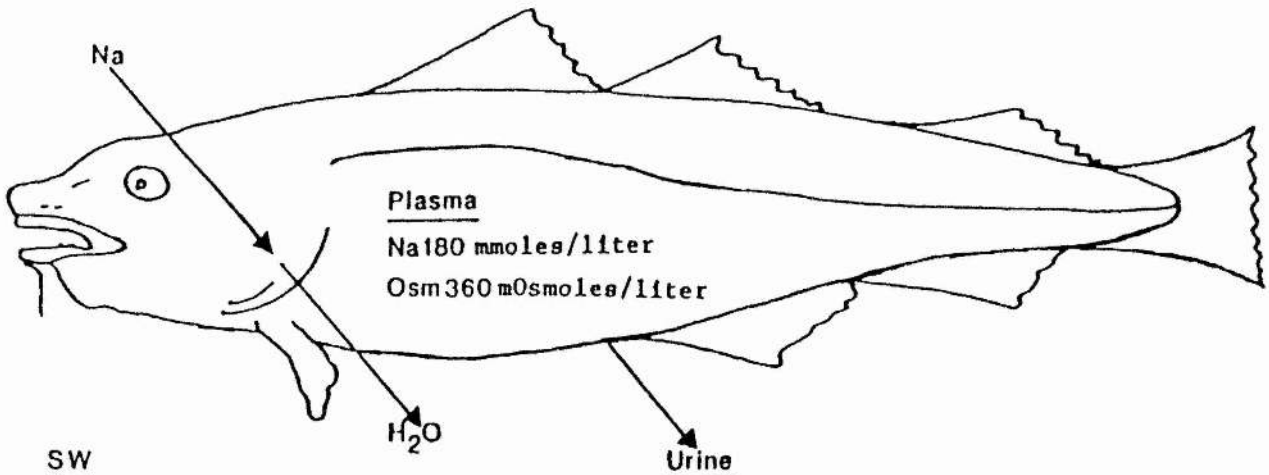
(from Balment and Henderson, 1987).



FW

Na 1 mmoles/liter

Osm 10 mOsmoles/liter



SW

Na 470 mmoles/liter

Osm 1000 mOsmoles/liter

1.3.1 Skin

The skin of fish is quite impermeable to water and solutes, thereby limiting exchanges with their FW and SW environments (Fromm, 1968; Kirsch, 1972). Skin from teleost head regions has been shown in vitro to secrete chloride by mitochondrion rich epithelial cells similar to the gill chloride cells (Karnaky, 1980; Marshall and Nishioka, 1980), which may contribute to excretion of salts obtained by ingestion of SW. Using Ussing chambers and the opercular skin from Fundulus heteroclitus, the backflux of chloride and unidirectional sodium fluxes have been shown to be passive and to follow existing electrochemical gradients (Degnan and Zadunaisky, 1980).

1.3.2 Oesophagus/Intestine

SW adapted teleosts drink water and excrete salts while those in FW do not drink. Therefore the gut of marine teleosts plays an essential part in compensating for the osmotic water loss through the gill. For water to be absorbed, the ingested fluid must be desalinated so that osmolarity is reduced below that of the body fluids. Perfusion experiments of the SW eel oesophagus in vivo demonstrated it was highly permeable to NaCl, but effectively impermeable to water. This allows desalination of the ingested water before it reaches the intestine. The oesophagus of the FW eel had a similar low water permeability, but in contrast to the SW eel also has low NaCl permeability. (Kirsch et al, 1975; Hirano and Mayer-

Gostan, 1976; Kirsch, 1978). The oesophageal mucous plays an important role in this process being fibrous close to the epithelial membrane which slows the flow of water and allows active uptake of ions. As the mucous becomes less fibrous further away from the epithelium water flow is faster, therefore ions move down the concentration gradient towards the epithelium and are absorbed with water following the local osmotic gradient created (Kirsch and Laurent, 1975; Kirsch et al, 1975; Hirano and Mayer-Gostan, 1976). Nearly 92% of the chloride concentration gradient is supported by the mucous layer (Kirsch et al, 1975; Parmalee and Renfro, 1983). The contents of the stomach are further diluted by the gastric secretion and water is reabsorbed in the posterior stomach and anterior intestine. In the posterior intestine sodium and chloride are actively reabsorbed and $CaCO_3$ precipitates out reducing the osmolality.

Generally, water and salt absorption rates, osmotic water permeability and Na^+,K^+-ATPase activity are higher in intestines from SW fish compared with FW, and all increase following SW transfer of euryhaline fish (Hirano et al, 1976).

Investigations into the structure of the epithelial lining of the SW goby intestine revealed a highly convoluted lining consisting of 3 cell types (Loretz, 1983a). These are the columnar absorptive cells, mucous and basal cells. The mitochondrion rich columnar cells appear to have greater conductance of chloride than sodium ions across the goby intestinal apical membrane. The weight of intestinal mucous

increased 32% during SW transfer as a result of hyperplasia of mucosal cells (Mackay and Janicki, 1979).

Isolated intestines from SW adapted Japanese cultured eels (Anguilla japonica) exhibited active sodium and water transport from mucosa to serosa, with the molar ratio of water to sodium in the fluid crossing the intestinal wall being greater than in FW adapted eels (Oide and Utida, 1967). Skadhauge (1969) also showed an increase in the epithelial osmotic permeability to water in the SW adapted yellow eel. Measurements of ionic composition of fluid in the different segments of the intestinal tract of the rainbow trout indicate that between 60-80% of SW ingested was absorbed and 95% of salt absorbed was in the form of NaCl, although other monovalent ions readily pass through the intestinal wall and enter the extracellular fluid compartment. Residues remaining in the lumen consist of magnesium, sulphate and insoluble carbonate salts (Conte, 1969). Shehadeh and Gordon (1969) have shown that insoluble carbonates of magnesium and calcium become trapped in mucous tubes. This could alter the bicarbonate-carbonate ratio which would affect intestinal pH and the reduced osmolarity of the ingested fluid could also change sodium and chloride absorption.

It has been demonstrated that net chloride and water fluxes depend on potassium ion transport (Ando, 1983), suggesting the existence of a coupled KCl transport system to which water may be linked. Ouabain treatment of Japanese cultured eel intestines inhibited this coupled transport,

proposing the linkage of H_2O absorption to the coupled Na^+-K^+-Cl^- transport system (Ando, 1981; Ando, 1985). The flounder intestine also appears to have a Na^+-K^+-Cl^- cotransport system which seems to be characteristic of salt absorbing epithelia (Musch et al, 1982).

1.3.3 Kidney and Urinary Bladder

The teleost kidney is usually divided into two portions, the head kidney and the trunk kidney. Generally the head kidney consists of lymphoid, haematopoetic, interrenal and chromaffin tissue with variable amounts of haematopoetic and pigment cells distributed among the tubules and vascular space of the trunk kidney. The Corpuscles of Stannius are usually located on the dorsal side of the middle to posterior part of the kidney. The teleostean kidney can be classified into 5 configurational classes, although exceptions may exist, depending on distinction between trunk and head kidney and the extent of fusion between the two kidneys (Ogawa, 1961).

FW teleost kidneys produce the most monovalent ion free urine of any vertebrates and the kidneys work on a filtration reabsorption system, with urine flow determined by the volume of glomerular filtrate. Marine teleosts have modified the versatile filtration - reabsorption principle into a tubular divalent ion secretory device and produce a concentrated urine with flow largely determined by tubular activities.

The FW teleost nephron structure is of a renal corpuscle linked by a ciliated neck region to an initial proximal segment followed by a second proximal segment, intermediate and distal segments and a collecting duct system. Measurements of glomerular size and total filtration surface (Marshall and Smith, 1930; Nash, 1931; Ogawa, 1962) indicate that the filtration surface and GFR is greater in FW than SW teleosts. GFR and urine flow respond secondarily to change in H_2O influx and are usually linearly related because a constant proportion of water filtered is reabsorbed by the tubule. It is because marine teleosts do not form a dilute urine, that nearly all have lost the distal and intermediate segments of the nephron present in the FW kidney. The dominant activity of the SW teleostean kidney is magnesium and sulphate excretion and glomeruli have become superfluous structures at least in those species which never venture into reduced salinities.

Ten times as many SW species enter FW than vice versa, suggesting the marine kidney is more adaptable. There is a tendency for the euryhaline fish kidney to resemble that of stenohaline fish of the primary environment. A definite correlation between glomerular development and habitat exists, with euryhaline forms living in reduced salinities possessing on average larger and more numerous glomeruli than those in marine environments. Generally SW adapted teleosts have reduced GFR (Hickman and Trump, 1969; Lahlou, 1970) compared to those in FW although there may be exceptions (Oide and Utida, 1968; Schmidt-Nielsen and Renfro, 1975). SW adaptation of trout leads to reduced

urine flow, reduction in the number of filtering nephrons, an increase in the number of nephrons not perfused, increased fractional sodium excretion, decreased generation of osmotically free water and increased tubular secretion of divalent ions (Henderson and Brown, 1978; Brown et al, 1980a).

Some teleost kidneys are aglomerular and work on a secretory principle. In SW the nephron appears to consist of only two regions, one which represents the second proximal segment in FW teleosts and a collecting duct system. (Grafflin, 1937; Smith, 1932). The marine aglomerular kidney has been studied in the midshipman (Porichthys notatus), and the toadfish (Opsanus tau) and nephrons consist of an initial segment with brush border and a terminal collecting duct system (Grafflin, 1931, 1937; Bulger, 1965; Bulger and Trump, 1965).

The teleost urinary bladder unlike those of tetrapods is of the same embryological origin as the kidney and represents an expansion of the fused mesonephric ducts. The urinary bladder modifies urine and itself plays an important role in teleost osmoregulation. Generally bladders from SW-adapted fish have higher water absorption and osmotic permeability and lower or equal NaCl absorption rates, compared with those in FW (Hirano et al, 1973). This is because for SW fish to reabsorb water to replace that lost osmotically, primary active ion reabsorption must occur followed by passive water reabsorption through the water permeable bladder epithelium. The mitochondrion rich

columnar cells are present in the bladder lining of most species and some also contain areas of cuboidal cells e.g. Gillichthys mirabilis. The columnar cell region is histologically similar to the collecting duct and is responsible for bladder ion transport (Loretz and Bern, 1980, 1983). FW and SW bladders have the same high rates of neutral NaCl cotransport, but SW bladders have an additional electrogenic sodium absorption mechanism (Loretz and Bern, 1983). Cuboidal cells from SW and 5% SW acclimated goby urinary bladders produced little mucosal to serosal NaCl transport (Loretz and Bern, 1990).

Bladders of stenohaline FW fish are almost impermeable to water. FW Gillichthys mirabilis have lower water transport and osmotic permeability than SW adapted fish (Doneen, 1976) and the urinary bladders of 3 species of flounder exhibited decreased osmotic permeability to water and increased permeability to sodium and chloride ions on acclimation to hypotonic media (Hirano et al, 1973). Bladders of euryhaline fish of FW origin appear osmotically impermeable irrespective of environmental salinity, while those of SW origin may change from permeable to relatively impermeable in FW (Hirano et al, 1973).

In the urinary bladder of the aglomerular marine teleost toadfish (Opsanus tau) adapted to different salinities, changes in urinary sodium and chloride concentrations and osmolality have been reported (Lahlou et al, 1969). Urine flow was slower in SW than FW acclimated fish. 60% of the urine secreted by the kidney was

reabsorbed (Howe and Gutknecht, 1978). The rates of reabsorption of sodium and chloride were approximately equal, however sodium was actively absorbed with chloride absorption appearing passive. Small amounts of magnesium and sulphate were also reabsorbed, but concentrations were doubled due to fluid reabsorption.

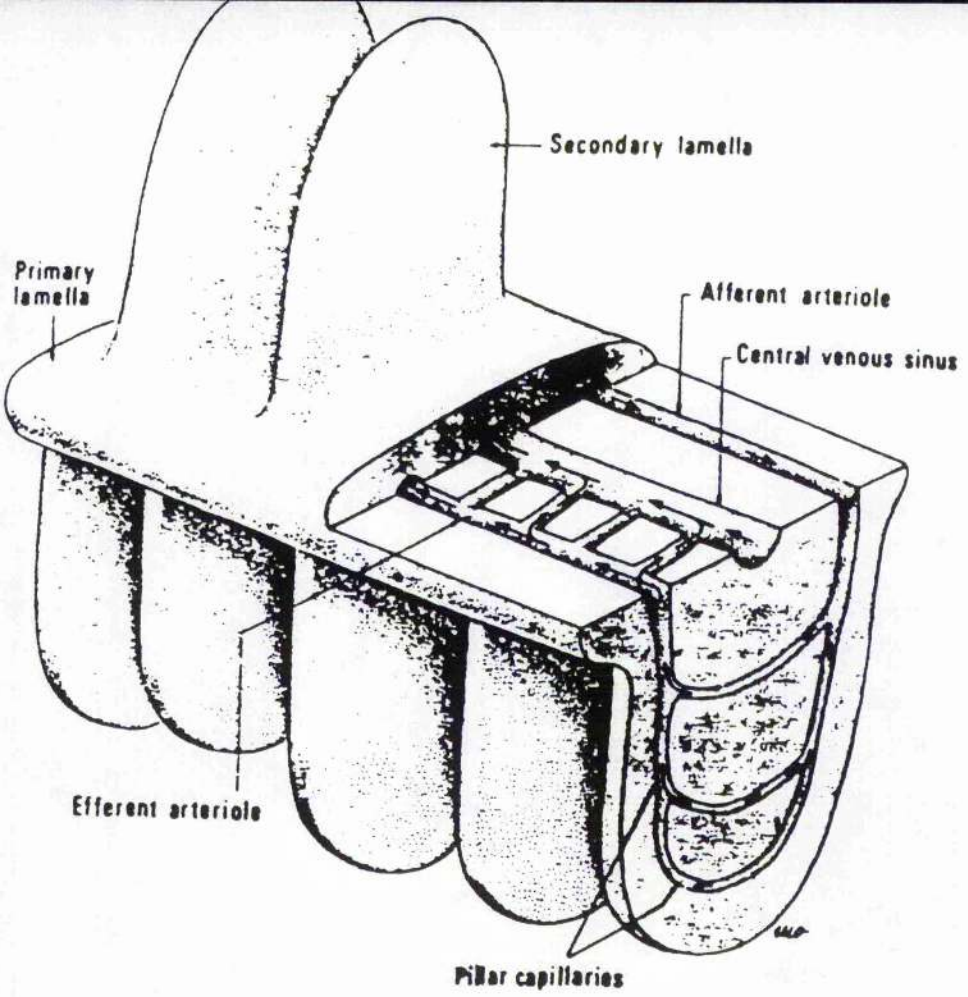
1.3.4 Gills

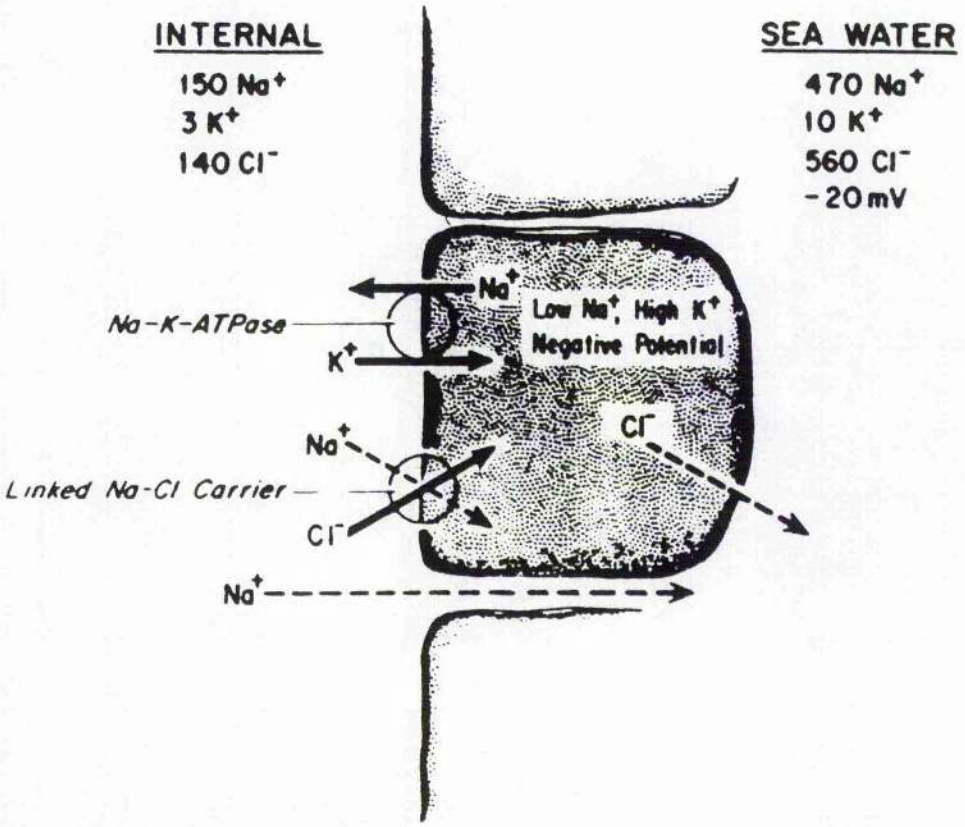
Teleost gills are composed of paired gill arches enclosed in an opercular cavity. Each arch carries two rows of filaments or primary lamellae which project at right angles from the outer convex edge of the gill arch. From these primary lamellae project the secondary lamellae (fig 1.3). The primary lamellar epithelium containing mostly salt secreting cells is functionally connected to the venous circulation. The secondary lamellar epithelium consisting of respiratory cells is irrigated by the arterioarterial circulation (Girard and Payan, 1980) and is responsible for respiration and the maintenance of acid-base balance in both FW and SW adapted fish.

Blood flow and pressure are thought to be important in the regulation of local bronchial electrolyte concentration. The combined activity of pillar cells and sphincters at the entrance and exit of the central venous system allows the secondary lamella and the interlamellar region to be perfused independently. Constriction of the pillar cells at the base of the lamellae may divert blood to the central compartments, as proposed in the European eel (Gilloteaux,

Fig 1.3 Gill structure (from Pisam et al, 1987).

Fig 1.4 Model of a chloride cell in seawater (from Epstein et al, 1980).


Secondary lamella
Primary lamella
Afferent arteriole
Central venous sinus
Efferent arteriole
Pillar capillaries


INTERNAL
150 Na+
3 K+
140 Cl-
SEA WATER
470 Na+
10 K+
560 Cl-
-20 mV
Na+
Low Na+, High K+
Negative Potential
Na-K-ATPase
K+
Cl-
Na+
Linked Na-Cl Carrier
Cl-
Na+

1969), although other studies in rainbow trout did not confirm this hypothesis (Richards and Fromm, 1969). In trout, blood reaching the central venous vessels is oxygenated while, in the eel it is both oxygenated and deoxygenated (Mayer-Gostan et al, 1987).

The respiratory epithelium covers both sides of each secondary lamella and consists of squamous pavement cells (Hughes and Grimstone, 1965), while the primary lamella contains squamous type epithelial cells, non differentiated cells, mucous goblet cells and mitochondrion rich chloride cells (Conte, 1969). The epithelial cells, also known as pavement cells, form a continuous covering except where the mucous and chloride cells are exposed to the surface.

Since Smith (1930) postulated an osmoregulatory role for the teleost gill, numerous studies have supported the concept that sodium and/or chloride ions are actively transported across the gill surface. Marine teleosts transport sodium from the plasma which has 140mmol/l into SW which contains approx 450mmol/l. Transport by FW fish is from water which varies in concentration from 0.1-20mmol/l into plasma which averages 150mmol/l. Thus the concentration gradient against which sodium transport takes place ranges from 3:1 for marine teleosts to 1500:1 in FW fish (Richards and Fromm, 1970).

Ion fluxes occur via the Na^+,K^+-ATPase enzyme, Na^+/Na^+, Cl^-/Cl^-, Na^+/NH_4^+, Na^+/H^+ and Cl^-/HCO_3^- exchanges. To

investigate these fluxes, much research has concentrated on the role of specialised cell types in gill epithelium, particularly the chloride cell.

1.3.5 Chloride Cells

Chloride cells (fig 1.4) are large, granular acidophilic cells and are found predominantly between the secondary lamellae in the interlamellar region close to the afferent branchial artery, although they have been reported to be on both filaments and lamella of gills (Keys and Wilmer, 1932). The site of ion transport across the gill epithelium is generally accepted to be the chloride cell (Motais and Garcia-Romeu, 1972 ; Maetz and Bornancin, 1975) and was first suggested to be salt secretory after studies using the European eel (Keys and Wilmer, 1932). The use of Ussing chambers, the vibrating probe technique, which can be used to measure extracellular current density immediately above the epithelial surface and the chloride cell containing opercular epithelium from Sarotherodon mossambicus have now provided direct evidence that chloride cells are salt secretory in teleosts and have high ionic permeability (Foskett and Scheffey, 1982).

The surface of the chloride cell is greatly extended by a tubular system continuous with the plasma membrane and contains many mitochondria (Doyle and Gorecki, 1961; Kessel and Beams, 1960, 1962; Philpott and Copeland, 1963; Threadgold and Houston, 1964). High levels of Na^+,K^+-ATPase are also indicative of high level electrolyte transport.

The apical crypt, the opening through which the chloride cell makes contact with the external medium, has been demonstrated using the vibrating probe studies to be the site of secretion by the chloride cell. Na^+, K^+-ATPase maintains a low level of intracellular sodium so that chloride is driven into the cell (Scheffey et al, 1983). Chloride leaves the cell conductively through the apical crypt, while sodium transport is driven by the voltage across the cell wall and occurs through the "leaky" tight junctions between chloride cells and accessory cells.

Adaptation of eels to SW increased both chloride cell number, size and Na^+,K^+-ATPase activity (Shirai and Utida, 1970; Utida et al, 1971). An increase in the number of mitochondria and the volume of the membranous tubular system and changes in the morphology of the apical pit of chloride cells in teleosts was demonstrated with increased environmental salinity (Maetz and Bornancin, 1975).

Respiratory cells in both FW and SW fish and are linked by tight junctions made of several interconnecting strands (Girard and Payan, 1980). No structural changes have been noted in higher salinities.

1.3.6 Na^+K^+-ATPase

Na^+,K^+-ATPase has been shown to be a common factor in transporting epithelia and therefore demonstrates high specific activity in osmoregulatory organs. In gills the enzyme activity has been located on the chloride cell (Mizuhira et al, 1970; Shirai, 1972; Utida and Hirano, 1972)

and has been shown to be proportional to salinity (Kamiya and Utida, 1969; Jampol and Epstein, 1970; Evans and Mallery, 1975) with sodium turnover being directly proportional to Na^+,K^+-ATPase activity (Epstein et al, 1980; Maetz, 1970; Utida et al, 1971; Butler and Carmichael, 1972). Autoradiographical studies revealed that the enzyme is situated on the serosal rather than mucosal side of the chloride cell in basolateral infoldings (Karnaky et al, 1976). Studies with ouabain also revealed Na^+,K^+-ATPase was inhibited from the blood rather than mucosal surface (Epstein et al, 1980). Inhibition of gill ATPase by intraperitoneal injection of ouabain drastically reduced sodium and chloride efflux (Schultz and Curran, 1970; Silva et al, 1977) although in SW, chloride is actively transported out of chloride cells and sodium extracted passively (Degnan et al, 1977; Epstein et al, 1973; House and Maetz, 1974; Kirschner et al, 1975). Therefore it appears that Na^+,K^+-ATPase provides the motive force for chloride efflux from the gill in seawater.

In kidneys of FW teleosts Na^+,K^+-ATPase acts to conserve sodium and the activity was found to treble on transfer of Platichthys stellatus from SW to FW (Utida et al, 1974). Enzyme activity also increased in killifish, rainbow trout and Japanese eels transferred from FW to SW (Epstein et al, 1969; Kamiya and Utida, 1969).

Urinary bladders of FW adapted Platichthys showed significantly greater sodium transport from mucosa to serosa

than did the SW adapted fish (Johnson et al, 1972; Hirano et al, 1973), suggesting Na^+, K^+-ATPase plays an important role in sodium reabsorption by the urinary bladder of the FW species (Utida et al, 1974).

In the gut, the enzyme activity of the intestinal mucosa of FW eels (Anguilla rostrata) doubled on SW transfer (Jampol and Epstein, 1970).

The integration of the various osmoregulatory sites in teleosts is controlled by hormones and both peptide and steroid hormones may play roles in adaptation of fish to environments of different salinities.

1.4 HORMONAL CONTROL OF TELEOST OSMOREGULATION

1.4.1 Putative Osmoregulatory Hormones

Many hormones are involved in teleost body fluid regulation, however in some cases specific roles have yet to be defined. The major osmoregulatory hormones will be dealt with in individual sections, however many others such as GH, VIP and those involved in calcium regulation may play a role.

Evidence for an osmoregulatory role for GH has been indicated by increased survival on adaptation of several salmonid species to SW (Miwa and Inui, 1985). Plasma sodium levels have been reduced in chum and sockeye salmon and trout after GH treatment of SW acclimated fish (Miwa and Inui, 1985; Clarke et al, 1977; Collie et al, 1985) and

bovine GH increased gill Na^+,K^+-ATPase activity of Oncorhynchus kisutch (Richman and Zaugg, 1987).

Vasoactive intestinal peptide (VIP) may also be involved in fluid and electrolyte balance, inhibiting fluid and ion absorption in Tilapia intestine (Mainoya and Bern, 1984) and by stimulation of chloride secretion by the opercular membrane (Foskett et al, 1982).

Maintenance of plasma calcium concentrations may be important in osmoregulation with hypocalcaemic regulation required in SW and hypercalcaemic control in FW. Hormones involved in hypocalcaemic regulation include calcitonin (Fouchereau-peron et al, 1986), hypocalcin (Fontaine, 1964), parathyrin (Milet et al, 1980) and teleocalcin (Ma and Copp, 1978). PRL (Hirano, 1986), Vit D_3 metabolites (Urist, 1976; Hayes et al, 1986) and a hypercalcaemic pituitary factor (Parsons et al, 1978) are thought to be involved in calcium control.

1.4.2 Prolactin

Prolactin is a peptide hormone released from the anterior pituitary. Its role in regulation of water and electrolyte homeostasis was originally identified by Pickford and Phillips (1959) who discovered that ovine PRL kept hypophysectomised Fundulus heteroclitus alive in FW. Salmon PRL has 31% sequence homology to mammalian (ovine) PRL although it lacks one of the 3 disulphide bridges (Kawauchi et al, 1986; Yasuda et al, 1986) and Specker et al (1985) found that the Tilapia pituitary secretes two

distinct PRL's in culture. Circulation concentrations of prolactin are higher in FW than SW adapted fish (Hirano et al, 1985), and its physiological actions promote efficient homeostasis of teleosts in FW.

The principal role of PRL in ion and water transport in the gills seems to be a reduction in permeability. PRL reduced the permeability of the branchial epithelium to water and ions by reducing Na^+,K^+-ATPase activity in FW adapted teleosts (Bern, 1975; Hirano, 1977) and decreased net excretion of sodium by 75% in Tilapia mossambicus (Dharmamba and Maetz, 1976). PRL treatment of fish in SW impairs their ability to excrete sodium and therefore increases plasma sodium levels (Hirano, 1986). PRL prevents passive loss of sodium from the gills of hypophysectomised fish in FW without affecting active uptake of the ion. Cortisol, secreted from the teleost interrenal gland acts synergistically with PRL by activating or maintaining ion pumps (Hirano and Mayer-Gostan, 1978). PRL is thought to increase chloride cell number during FW adaptation (Lam, 1972) and it has been shown to increase gill mucous secretion (Hirano, 1986). PRL receptors have been located on Tilapia gills (Edery et al, 1984).

Teleosts in SW drink and absorb water from the intestine together with monovalent ions. On transfer of eels from FW to SW intestinal absorption of water increases in close association with an increase in active transfer of sodium and chloride, and cortisol is known to be involved in the adaptation (Hirano et al, 1976). However, PRL treatment

of the SW adapted eel and trout decreases NaCl and water absorption by the isolated intestine (Hirano et al, 1976; Morley et al, 1981) but had no effect on fluid absorption of FW adapted eels (Hirano and Utida, 1968). In contrast, administration of PRL to SW Tilapia augmented sodium and water reabsorption. Na^+,K^+-ATPase activity of intestinal mucosa of FW adapted Fundulus heteroclitus was unaffected by PRL (Pickford et al, 1970b). Therefore the mechanism producing these effects may involve decreased osmotic and ion permeability (Mainoya, 1982).

The production of a copius dilute urine is a characteristic of FW fish and PRL has been shown to restore urine flow reduced by hypophysectomy in some FW fish (Clarke and Bern, 1980). This diuretic action may be due to increased GFR, increased number or functionality of glomeruli (Lam, 1972), and/or reduction in tubular reabsorption of water (Hickman, 1965). PRL is also known to decrease the sodium content of urine in some species suggesting stimulation of tubular reabsorption of the ion and Na^+,K^+-ATPase was stimulated in the kidney of hypophysectomised Fundulus in FW (Lahlou and Giordan, 1970; Pickford et al, 1970a). PRL may also reduce water reabsorption of the kidney (Foster, 1975).

Bladders isolated from FW adapted flounder absorb mainly sodium and chloride ions with greatly reduced water transport, whereas those in SW absorb both the ions and water. PRL stimulates sodium uptake (Johnson et al, 1972) and Na^+,K^+-ATPase activity in the FW Platichthys stellatus

bladder (Utida et al, 1974). The osmotic permeability to water was also reduced in the SW goby bladder although there was no effect on sodium or chloride absorption. Injection of PRL into SW flounder produced a reduction in water permeability while stimulating the sodium pump (Johnson et al, 1972). Na^+,K^+-ATPase activity of the urinary bladder of Platichthys stellatus increased after PRL injection of SW acclimated fish, simulating the results of FW transfer. In Kareius bicoloratus which does not survive FW transfer, PRL did not increase the enzyme activity (Hirano et al, 1973). Increased ion absorption by the goby bladder, observed after SW adaptation, results at least in part from stimulation by cortisol (Loretz, 1983b). PRL and cortisol appear to act in conjunction since cortisol is required to demonstrate the effects of PRL (Clarke and Bern, 1980) otherwise osmotic permeability decreased in vitro. Stimulation of bladder sodium reabsorption by teleost rostral PD transplantation can also be simulated by ACTH or cortisol treatment (Doneen, 1976).

Ovine PRL has been shown to have a hypercalcaemic effect on several teleost species, accompanied by stimulation of high affinity Ca^{2+}-ATPase activity in the gills (Flik et al, 1984). It was suggested that PRL activity was inversely related to osmolarity and calcium concentration of the media. However plasma PRL levels did not increase on adaptation of Tilapia to calcium deficient SW whereas they did on transfer to FW (Nicoll et al, 1981).

1.4.3 Cortisol

Cortisol is the major corticosteroid released by the interrenal gland of both FW and SW teleosts and secretion is influenced by ACTH (Henderson et al, 1976; Decourt and Lahlou, 1987). Generally it is accepted that this hormone is involved in water and electrolyte balance, particularly during migration from FW to SW in euryhaline species. The gills are an important target organ for cortisol which stimulates active ion transport of sodium and chloride ions in both FW and SW fish. Cortisol restores sodium balance in interrenalectomised eels (Chester-Jones et al, 1972).

Cortisol plasma levels of 3 FW teleosts:- the stenohaline carp, the euryhaline Tilapia and trout were measured during SW adaptation. Results suggest that the hormone is only necessary for acclimation, with the period of increase being shortest in Tilapia and longest in carp (Hegab and Hanke, 1984).

Cortisol administration has been shown to increase gill Na^+,K^+-ATPase activity in several FW species including eel, mullet and Tilapia (Epstein et al, 1971; Kamiya, 1972; Forrest et al, 1973; Gallis et al, 1979; Balm, 1986), and to increase Na^+,H^+-ATPase like activity in FW Tilapia gills (Balm, 1986). In contrast to PRL, cortisol does not reduced passive sodium flux across FW teleost gills (Dharmamba and Maetz, 1972; Forrest et al, 1973). Pharmacological doses of cortisol stimulated branchial water influx in the FW Japanese eel (Ogawa, 1975), however the hormone was without effect on water permeability of the

gills in FW mullet but did increase water influx in the SW adapted fish (Gallis et al, 1979).

Cortisol injections in SW adapted Tilapia stimulate chloride secretion across the opercular membrane (Foskett et al, 1983), increase the rate of sodium turnover (Mayer and Maetz, 1967), increase Na^+,K^+-ATPase activity and the number of branchial chloride cells (Kamiya, 1972; Doyle and Epstein, 1972; Thomson, and Sargent, 1977).

Na^+,K^+-ATPase activity of 3 osmoregulatory organs - gill, kidney (male only) and intestinal mucosa increased in cortisol treated Fundulus heteroclitus in SW (Pickford et al, 1970b). Branchial and renal ATPase were unaffected in the long term FW or SW adapted mullet (Chelon labrosus) but activity was inhibited in short term FW adapted fish (Gallis et al, 1979).

Cortisol restored high rates of water and ion intestinal absorption in hypophysectomised eels in SW (Hirano, 1967). ACTH and cortisol administration also produced a dose-dependent stimulation of sodium and water transport in FW adapted eels (Hirano and Utida, 1968). The restoration of sodium flux in the gut of hypophysectomised FW adapted goldfish (Ellory et al, 1972) and of fluid transport in adrenalectomised FW eels (Gaitskell and Chester-Jones, 1970) also indicate the necessity of cortisol in maintaining intestinal sodium and water transport in FW adapted eels, although this may involve synergism with prolactin (see above).

Increased sodium turnover, perhaps reflecting increased GFR and renal sodium retention was observed in the kidney of SW adapted Fundulus heteroclitus treated with cortisol (Pickford et al, 1970b).

Cortisol and ACTH increased water permeability and stimulated sodium and chloride reabsorption in the SW adapted Gillichtys mirabilis urinary bladder (Doneen 1976), but had no effect on the flounder bladder (Johnson et al, 1972).

1.4.4 Neurohypophysial hormones

There are two categories of neurohypophysial hormones, the antidiuretic vasopressor peptides and the neutral oxytocin-like peptides. There are also two distinct types of vasopressin receptor at least in mammalian tissues; V_2 receptors linked to the adenylate cyclase system and found in renal tubules, and the vascular and hepatic V_1 receptors involved in the breakdown of phosphatidylinositol (Michell et al, 1979). Arginine vasotocin (AVT) is an antidiuretic vasopressor peptide found in non-mammalian vertebrates including teleosts in which isotocin (IT) is the neutral peptide.

AVT and IT concentrations in pituitary glands of SW eels were higher than those in FW, although levels in the preoptic nuclei were similar (Holder , 1969). Bentley (1971) found plasma AVT levels did not alter in eels transferred from FW to SW, however Henderson et al (1986)

report values of 28.2±3.8pg/ml in distilled water and 778±95.8pg/ml in SW. Very low doses of AVT produced a glomerular antidiuresis in FW eels (Henderson and Wales, 1974). Higher doses of AVT which induced a pressor response and concomitant diuresis were thought to be pharmacological. Several authors also documented diureses following AVT or IT injection in goldfish, eels, bull frog and lung fish (Maetz et al, 1964; Lahlou and Giordan, 1970; Chester-Jones et al, 1969; Chester-Jones et al, 1971). Lahlou et al, (1969) proposed that the diuretic response to AVT was accompanied by increased GFR since the hormone was without effect on the aglomerular toadfish. It was also suggested that AVT constricts the efferent glomerular arteriole in fish (Sawyer, 1972). It is possible that the antidiuretic/diuretic effects may be due to the relative vasoconstrictor effects of AVT on different vascular beds. Studies using perfused kidney trunk preparations from lung fish and rainbow trout (Pang et al, 1981) suggest that the antidiuretic response seen in eels may be due to glomerular vasoconstriction without a change in systemic blood pressure. The diuretic response to higher doses of AVT may be attributed to peripheral vasoconstriction and elevated perfusion pressure overriding glomerular vasoconstriction and therefore increasing GFR. AVT is not thought to affect urinary composition or tubular water permeability, but may play a role in control of glomerular intermittency (Babiker and Rankin, 1978).

These studies on the effects of AVT on renal function of FW fish are pharmacological and as yet an endocrine role

for AVT, in particular pertaining to osmoregulation, has not been identified.

Another possible site of action of AVT is the gills where vasoconstriction may induce diversion of blood flow (Rankin and Maetz, 1971). A direct effect may be through inhibition of adenylate cyclase (A.C.) seen in rainbow trout gill plasma membranes (Guibbolini and Lahlou, 1990).

IT produced a glomerular diuresis without a sustained blood pressure increase in FW eels and Babiker and Rankin (1978) found low doses were antidiuretic and higher doses were diuretic. IT also inhibited A.C. of gill plasma membranes of rainbow trout adapted to FW and SW (Guibbolini and Lahlou, 1990).

1.4.5 Catecholamines

Catecholamines, epinephrine (adrenaline) and norepinephrine (noradrenaline), exert effects on blood flow through the gills, on active ion transport mechanisms and on branchial permeability to water, ions and organic substances. Studies on intact fish, perfused isolated heads or perfused gill arches have shown an initial transient vasoconstriction followed by a prolonged vasodilation (Wood, 1975). This response is in both FW (Girard and Payan, 1976) and SW fish (Claiborne and Evans, 1980). A transient increase of catecholamines was observed on SW acclimation of 3 types of teleost: the carp, Tilapia and trout (Hegab and Hanke, 1984). It is postulated that the main effect of catecholamines is a greater blood flow through the lamellae

and enlargement of the functional respiratory surface (Nilsson, 1984).

In the perfused head of the FW trout sodium influx was stimulated while efflux was unaffected (Payan et al, 1975). However in free swimming trout the opposite effect was found (Wood and Randall, 1973) and Perry et al, (1984) observed the same situation for chloride uptake. Epinephrine increases the branchial permeability to small molecules such as water and urea and these effects are more pronounced in FW than SW (Isaia, 1979; Haywood et al, 1977).

In the SW eel epinephrine increased the chloride concentration of the perfusion fluid (Keys and Bateman, 1932) and increased water loss (Pic et al, 1974). Sodium influx through the gill is stimulated via -receptors and is thought to be due to Na^+/NH_4^+ and Na^+/H^+ exchanges in SW adapted fish (Payan and Girard, 1978) while chloride entry is inhibited by receptor blockade (Mayer-Gostan and Maetz, 1980).

Catecholamines are involved in mammalian kidney function, since on denervation, renal tubular sodium and water reabsorption are decreased (Di Bona, 1983). - adrenergic nerves are thought to play a role in reduction of GFR on SW adaptation of trout, since adrenergic blockade partially blocked the normal decrease in GFR that occurs on transfer to a more hypertonic environment (Elger and Hentschel, 1983).

1.4.6 Thyroid Hormones

The thyroid hormones, thyroxine (T4) and triidothyronine (T3), are not thought to be directly involved in fluid and electrolyte balance of teleosts, but may have some effect on development of osmoregulatory mechanisms in marine fish. The thyroid does not appear to be involved in the immediate adaptive responses necessary for salmonids to survive in environments of higher salinity, however, SW survival of yearling coho solmon is correlated with a peak in plasma T4 (Folmar and Dickhoff, 1981; Young, 1986) and T3 during smoltification. This is substantiated by studies indicating greater adaptability of salmonids to SW by prior treatment with thyroid hormones (Refstie, 1982; Grau et al, 1985). Plasma T4 remained higher than in FW at all times, but T3 levels returned to those of FW fish within 24hrs of transfer (Redding et al, 1984). SW acclimation of Fundulus heteroclitus increased plasma T4 which appears to be associated with hydromineral homeostasis, since thiourea treatment resulted in increased plasma osmolality and sodium preceeding death. Thiourea had no effect in FW (Knoeppel et al, 1982). Similar findings were noted by Mohsen and Tourette (1962) with guppies.

T4 has been implicated in the control of branchial Na^+,K^+-ATPase activity of euryhaline fish, on the basis of an almost parallel increase in plasma T4 and enzyme activity during SW acclimation of salmon (Folmar and Dickhoff, 1979), however no dose dependent effect was observed (Leatherland, 1982). Studies by Miwa and Inui (1983) did not support this

finding, but it was suggested that T4 may work in conjunction with cortisol to affect enzyme activity. While cortisol alone stimulated branchial ATPase of SW adapted Tilapia, thyroxine did not affect activity in fish in FW or SW. However, a significant rise in enzyme activity was observed on administration of both hormones simultaneously under hypo- and hyperosmotic conditions (Dange, 1986). Thyroxine is thought to regulate branchial mitochondrial function during adaptation of fishes to hyperosmotic water, possibly by promoting mitochondrial protein synthesis (Shivakumar and Jayaraman, 1984).

1.4.7 Urotensins

Urotensins I and II (UI and UII) are produced in the urophysis, a neurohaemal organ located in the caudal spinal cord, and function to regulate water and ion transport. UI is a peptide hormone consisting of 41 amino acids and has structural homology with mammalian corticotropin releasing factor. UII contains 12 amino acids and resembles somatostatin. Each urotensin is derived from a separate prohormone (Bern, 1985) and belong to a diverse group of peptides.

Both peptides are vasopressor, although UI only weakly, in teleosts and it has been postulated that they may modulate osmoregulation by vascular effects or by influencing secretion of cortisol and/or prolactin (Bern, 1985). The urophysis appears depleted of neurosecretory material in Mollienesia sphenops (Kriebel, 1980) and in Tilapia (Takasugi and Bern, 1962) transferred from FW to SW.

However, the urophysis of FW acclimated Gillichthys mirabilis, was depleted of peptides (Berlind et al, 1972). Immunocytochemical studies of UII indicate higher levels in marine than FW species (Owada et al, 1985).

In general urophysial extracts increase urine flow and excretion of at least sodium (Maetz et al, 1964; Bern et al, 1967; Chester-Jones et al, 1969; Woo and Tong, 1981). These effects may be explained by the vasopressor activities of UII (and higher doses of UI) (Chan, 1975). Thus UII in particular could affect ion and water transport rates in various osmoregulatory organs such as the gill, kidney, intestine and skin by modulation of blood flow.

Injections of urophysial homogenates increased plasma sodium, chloride and magnesium levels of the SW adapted Gillichthys mirabilis (Bern and Nishioka, 1979). No difference was observed in plasma potassium, calcium or haematocrit. UII stimulated sodium and chloride absorption by the goby posterior intestine adapted to 5% SW and is thought to be due to excess apical ion influx rather than basolateral Na^+,K^+-ATPase activity (Loretz and Bern, 1981). UI inhibits anterior intestinal NaCl and water absorption. In SW adapted fish UII stimulates anterior intestinal water and NaCl absorption (Mainoya and Bern, 1984) and UI stimulates and UII inhibits active chloride efflux from goby skin (Marshall and Bern, 1981). Both peptides stimulate active sodium absorption in the urinary bladder (Loretz and Bern, 1981), although UII was by far the more potent hormone.

It has been suggested that the activities of UII would be consistent with FW adaptation and those of UI with SW adaptation (Loretz and Bern, 1981).

Effects seen in different species indicate inconsistent actions by these peptides which could be due to compensation by other osmoregulatory systems.

1.4.8 Atrial Natriuretic Peptide

Recent immunological studies have confirmed the presence of immunoreactive ANP in plasma, atria and ventricles of teleost, chondrichthyan and agnathan fishes (Evans et al, 1989). An eel natriuretic peptide (1-27) was isolated from atria and found to have 59% homology to mammalian (human or rat) ANP, 52% to fowl and 46% to frog ANP (Takei et al, 1989). Electron micrographs of atrial and ventricular cardiocytes from Gila atraria have demonstrated many perinuclear granules resembling the ANP containing secretory granules of mammalian atriocytes (Westenfelder et al, 1988). Further studies have shown that myocytes from atria and ventricles of Gila atraria secrete similar amounts of ANP, 3.9 and 2.8ng/culture respectively. These concentrations were comparable to those produced by rat and mouse atriocytes (5.2 and 4.3ng/culture), but were higher than those secreted by the mammalian ventricular myocytes (0.8 and 0.3ng in rat and mouse cultures) (Baranowski and Westenfelder, 1989). The fish peptide was found to have a significant degree of homology with mammalian ANP.

Teleost plasma ANP concentrations are lower in FW than SW (Galli et al, 1988; Evans et al, 1989; Westenfelder et al, 1988), suggesting ANF may mediate salt tolerance in teleosts. Intraarterial injections of trout heart extracts or synthetic ANP in FW trout increased arterial blood pressure. The pressor response was slower in onset and of longer duration than equipotent pressor injections of catecholamines and is thought to be the result of a direct effect of the hormone on the vasculature (Duff and Olson 1986). Olson and Meisheri (1989) in contrast demonstrated ANF injection relaxed FW trout blood vessels. Eel ANP also decreased aortic pressure of conscious eels in a dose dependent manner (Takei et al, 1989) and ANP has been shown to vasodilate preconstricted perfused gills of trout and the marine teleost Opsanus beta (Olson and Meisheri, 1989; Evans et al, 1989). Extracts from Opsanus beta atria and ventricles also produced dilation of aortic rings with the ventricular extracts proving more potent (Evans et al, 1989).

Peptides isolated from the eel and Gila atraria were found to be natriuretic (Takei et al, 1989; Westenfelder et al, 1988) and also diuretic in the latter case. Synthetic ANP and trout ventricular extracts produced increased sodium and chloride secretion, but only a mild diuresis in the rainbow trout (Duff and Olson, 1986). In the intestine atriopeptin III has been found to inhibit the Na-K-2Cl transport system in SW flounder (O'Grady et al, 1985), however the opercular epithelium did not respond to ANP.

Killifish operculum however did respond to ANP and chloride transport was stimulated in both FW and SW adapted fish (O'Grady et al, 1985). ANP was found to have no effect on gill permeability to water in trout in vitro (Olson and Meisheri 1989), however receptor binding to chloride cells has been demonstrated in both FW and SW adapted European eels (Broadhead et al, 1989). ANP has been shown to have a steriodogenic effect, producing increased plasma levels of cortisol in flounder (Reid-Arnold and Balment, personal communication).

1.4.9 Renin-Angiotensin System in Teleosts

Most, but not all teleosts possess the renin secreting granular epithelioid cells and renin-like pressor activity has been demonstrated in several marine teleosts, including those with aglomerular (Mizogami et al, 1968) as well as glomerular kidneys (Taylor, 1977). Renal renin concentrations are generally higher in FW than SW teleosts (Capelli et al, 1970; Mizogami et al, 1968) and Christensen et al (1982) have reported that FW salmon have more granular epitheloid cells than those in SW. Plasma renin concentrations were double in SW European eels, than in FW and increased when transferred from FW to SW (Henderson et al, 1976). Angiotensin II levels were also greater in SW adapted European eels (3598.8±780.8pg.ml) compared with those in FW (192.0±63.2pg/ml) (Henderson et al, 1986). Plasma renin activity decreased in Anguilla rostrata transferred from SW to FW, but also decreased in Japanese eels transferred from FW to SW (Sokabe et al,

1966). Sokabe (1968) also noted reduced renal renin content in Tilapia mossambica on FW adaptation, however Malvin and Vander (1967) were unable to detect any change. Nishimura et al (1976) found no change in plasma renin of the aglomerular toadfish Opsanus tau when adapted from 50 to 5% SW, however Capelli et al (1970) found increased renin activity in decreased salinity. From studies on the rainbow trout, Bailey and Randall (1981) have postulated that AII reacts directly on renin producing cells to inhibit renin release by a negative feedback mechanism, and that a baroreceptor response is responsible for renin secretion in fish.

Conversion of AI to AII has not been demonstrated directly in fish. Indirect evidence includes the fact that ACE inhibitors abolished the pressor response to AI and not AII in the American eel (Nishimura et al, 1978). The inhibitors also produced sustained hypotension in the toadfish (Nishimura et al, 1979) and decreased the drinking rate of killifish (Malvin et al, 1980). Increased drinking in response to AI by the euryhaline flounder was abolished by Captopril (an ACE inhibitor) (Carrick and Balment, 1983). This inhibitor also reduced raised drinking rates of FW carp induced by AI, however AII administration restored the increased drinking rate (Perrott and Balment, 1985). Gills could play an important role in fish AI activation since their function is similar to mammalian lungs, which convert 80% AI to AII, in that they receive the entire cardiac output and have an extensive vascular surface area. Studies

by Galardy et al (1984) support this concept as gills and Corpuscles of Stannius (C.S) exhibited the greatest ACE activity of tissues tested. Levels were barely detectable in skeletal muscle, liver and kidney.

Corpuscles of Stannius of some fish produce a renin like presser substance and C.S. extracts have been shown to produce elevated blood pressure in the rat and eel (Sokabe et al, 1970). An AII - like substance was formed following incubation of salmon C.S. extracts with homologous plasma (Sokabe et al, 1970) and incubation of salmon C.S. or kidney produced two angiotensin sequences - asp^{1},val^{5},asn^{9} and asn^{1},val^{5},asn^{9} in a ratio of 2:1 (Takemoto et al, 1983). Angiotensin substances generated by incubation of carp or goosefish C.S. with homologous plasma produced a dose related hypocalcaemia in a killifish bioassay. However synthetic human AII and angiotensins generated by incubation of carp or eel kidney with homologous plasma produced significant hypercalcaemia in the bioassay (Pang et al, 1981). Chester-Jones et al (1966) also reported significant angiotensinase activity in C.S. extracts, but were unable to demonstrate an angiotensin like pressor substance following incubation of C.S. extracts with ox renin substrate.

It is unknown whether the RAS evolved primarily as a dipsogenic hormone (Malvin et al, 1980), as a regulator of blood pressure (Nishimura and Sawyer, 1976) or to produce renal effects (Henderson et al, 1981).

1.4.9a Renal Effects of the RAS

It has been suggested that the decreased renal renin content of SW adapted fish may be involved in the reduction of GFR and urine volume which occurs in euryhaline teleost transferred from FW to SW. Plasma renin activity of the Japanese eel however, has been shown to increase for the first 24 hours following SW transfer in spite of decreased GFR and urine volume (Sokabe, 1968). Therefore it appears that changes in environmental salinity may only temporarily affect plasma renin levels in some euryhaline species.

In both FW and SW adapted trout, Captopril infusion doubled GFR, urine production rates and tubular transport maxima for glucose (TMG), without altering plasma composition (Kenyon et al, 1985).

Changes in nephrons similar to those seen on SW adaptation were observed in AII infused trout. These were a reduction in the number of filtering nephrons and an increase in the number not perfused in FW trout. In SW trout AII increased the number of nephrons not perfused and decreased the number not filtering (Brown et al, 1980a). Synthetic asn^{1},val^{5}-AII and semipurified eel angiotensins increased urine flow, inulin clearance, sodium and potassium excretion to the same extent and dose-dependently, when infused intraarterially into FW adapted American eels, (Nishimura and Sawyer, 1976). It was also noted that the increased GFR seen on infusion of FW adapted eels with AII-amide and semipurified eel angiotensins produced the diuresis, but only after clearly vasopressor doses of the

hormone. The marked kaliuresis and natriuresis were also found to follow the diuresis (Nishimura and Sawyer, 1976).

1.4.9b Pressor Effects of the RAS

Partially purified eel and rat renins produced a prolonged pressor response in intact and hypophysectomised European eels and in nephrectomised rats (Henderson et al, 1976) and synthetic asn^{1},val^{5}-AII and semipurified eel angiotensin increased blood pressure in FW adapted American eels (Nishimura and Sawyer, 1976). Angiotensin also produced a pressor response in intact, hypophysectomised and C.S. lacking eels (Anguilla anguilla L) (Henderson et al, 1976) and Gray and Brown (1985) observed a pressor response in AII infused rainbow trout. Sar-AII produced neither an agonistic nor antagonistic response on blood pressure and an ACE inhibitor inhibited vasopressor responses to eel angiotensin I in American eels (Nishimura et al, 1978).

1.4.9c Dipsogenic Activity of the RAS

Transfer of Anguilla japonica to SW induced drinking after 15 minutes and increased plasma angiotensin after 2 hours (Okawara et al, 1987). Intraarterial injections of synthetic AII and semipurified eel angiotensins accelerated drinking in water replete FW eels and dehydrated SW eels (Takei et al, 1979). Exogenous AII stimulated drinking in two marine teleosts the long horned sculpin and the flounder, but had no effect on 3 FW species studied - the common goldfish, mottled sculpin and the common shiner (Beasley et al, 1986). The high rate of drinking seen in SW

adapted flounder was associated with increased plasma chloride and osmotic concentrations. This dipsogenic effect appeared to rely on the RAS since it was abolished by simultaneous Captopril administration (Balment and Carrick, 1985). Captopril also blunted the response of increased drinking by FW carp on AI administration, with exogenous AII producing recovery (Perrott and Balment, 1985). In the killifish sar^1,ala^8-AII competitively inhibits AII induced and enhanced drinking during acute SW transfer (Malvin et al, 1980).

It has been suggested that baro- or volume receptors involved in the control of drinking may be located proximal to the branchial arteries (Hirano and Hasegawa, 1984).

Although some exceptions exist it is generally concluded that a drinking response to AII is a characteristic of fish which encounter water more hypertonic than their typical residence (Kobayashi et al, 1983).

1.4.9d Interaction of the RAS with other hormones involved in osmoregulation

The RAS can also influence secretion or synthesis of other hormones which are important for hydromineral homeostasis. Intravenous injection of a large pressor dose of asn^1,val^5-AII or an eel renin preparation into chronically catheterised hypophysectomised or intact FW eels increased plasma cortisol levels (Henderson et al, 1976).

Nishimura et al, (1976) also found a concomitant increase in plasma cortisol on eel and rat renin administration of intact and hypophysectomised eels. These authors found no clear correlation between plasma renin and plasma cortisol. Captopril did not effect electrolyte concentrations in SW adapted eels, however it doubled plasma cortisol levels in SW and decreased them in FW eels (Kenyon et al, 1985). AII administration produced a transitory increase in plasma cortisol of free swimming flounder (Balment et al, 1987).

Exogenous administration of AII in FW eels increased plasma AVT concentrations which in turn reduced renin levels. This interplay was not observed in SW adapted eels (Henderson et al, 1986).

A considerable portion of the vasopressor effect of angiotensin is indirect in nonmammalian species, being mediated by catecholamines, possible released from adrenergic nerve endings and/or the adrenal medulla (Carroll and Opdyke, 1982).

For any hormone to exert its effect on a particular tissue or cell its presence must be transformed into an intracellular signal. This is achieved by interaction with specific hormone receptors.

1.5 Hormone Receptors

Hormone receptors are protein macromolecules located on the plasma membrane or within the cell, which bind hormones with great specificity and high affinity and transduce agonist activity through a sequence of reactions into an

altered function of the target cell. Hormone binding can usually be described by the following equations

$$R + H \quad [RH]$$

$$K_d = \frac{[R]\ [H]}{[RH]}$$

where R and H are the respective concentrations of free receptor and hormone and [RH] is the concentration of the receptor hormone complex. K_d is the dissociation constant and is a measure of the affinity of the receptor for the hormone.

Indications that a hormone action is receptor mediated include organ and structural specificity, saturation, evidence of competitive inhibition and reduced responses in some tissues on repeated hormone administration. The response of a cell or tissue to specific hormones is dictated by the particular receptors it possesses and the intracellular reactions initiated by the binding of any one hormone to its receptor.

Steroids interact with receptors in the nucleus or cytosol to form complexes that accumulate in the nucleus. These complexes can bind regulatory DNA sequences and alter transcription rates of adjacent genes.

The binding of ligands to many cell surfaces receptors activates an enzyme which generates an increase in the concentration of an intracellular signalling compound, termed a second messenger, often initiating a cascade system. Second messenger molecules include cAMP, cGMP and

inositol phosphates, with changes in intracellular calcium concentration also acting as a mediator. The systems initiated by these molecules and ions can be found in figs 1.5-1.8.

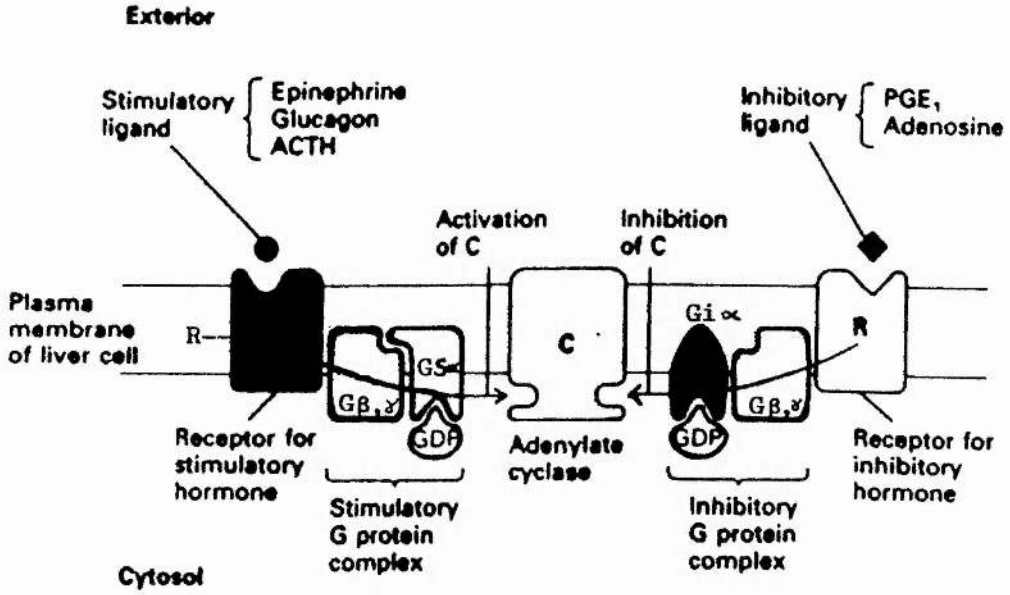
1.5.1 Techniques

Receptor binding has to be specific both in terms that it can be prevented by addition of unlabelled hormone and that it cannot be prevented by addition of any hormones not known to interact with this receptor.

One of the purposes of carrying out binding studies is to determine both affinity and abundance of specific sites in a membrane, that is the steady state kinetics of the binding process. Radioreceptor assays are radioligand assays which measure displacement of the specific binding of tracer amounts of labelled hormone from receptor sites by unlabelled hormone, during an incubation period of time long enough to establish equilibrium binding. The amount of labelled hormone bound at each hormone concentration is determined by centrifugal or filtration methods of separation. This type of assay was first introduced by Lefkowitz et al (1970) using ^{125}I-ACTH as a tracer to measure levels of ACTH in serum. The criteria used for developing radioreceptor assays are the same for hormone receptor binding. However disadvantages are that artifacts may arise suggesting specific binding sites, but which may not be true receptors. The major advantage of radioligand

Fig 1.5 G-protein mediated activation and inactivation of adenylate cyclase (from Gilman, 1984).

Fig 1.6 Guanylate cyclase stimulation, releasing the second messenger cGMP. (adapted from Maimer et al, 1988).


Exterior
Stimulatory ligand
Epinephrine
Glucagon
ACTH
Inhibitory ligand
PGE1
Adenosine
Activation of C
Inhibition of C
Plasma membrane of liver cell
R
GS
Gβ,γ
GDP
C
Gi∝
R
Gβ,γ
GDP
Receptor for stimulatory hormone
Adenylate cyclase
Receptor for inhibitory hormone
Stimulatory G protein complex
Inhibitory G protein complex
Cytosol

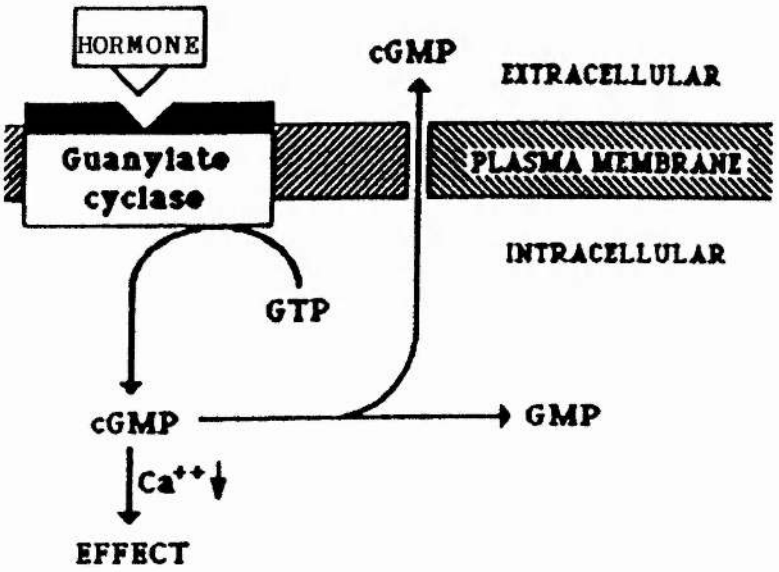
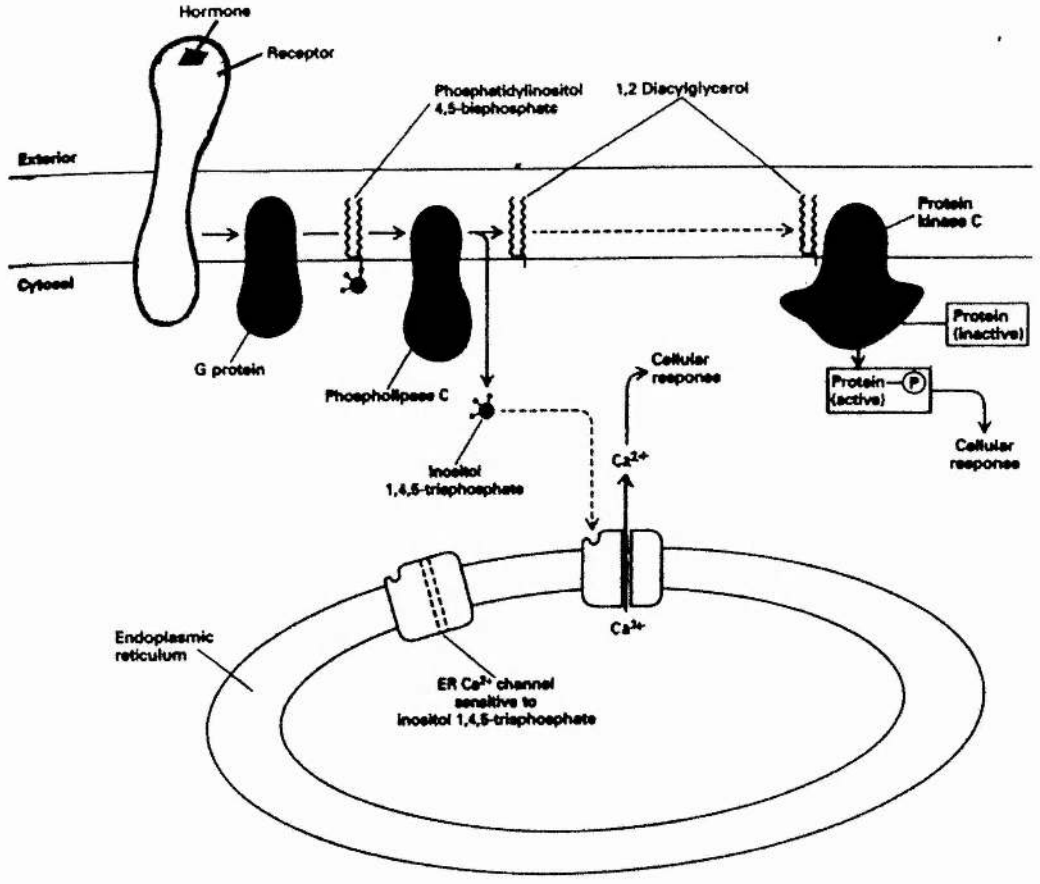
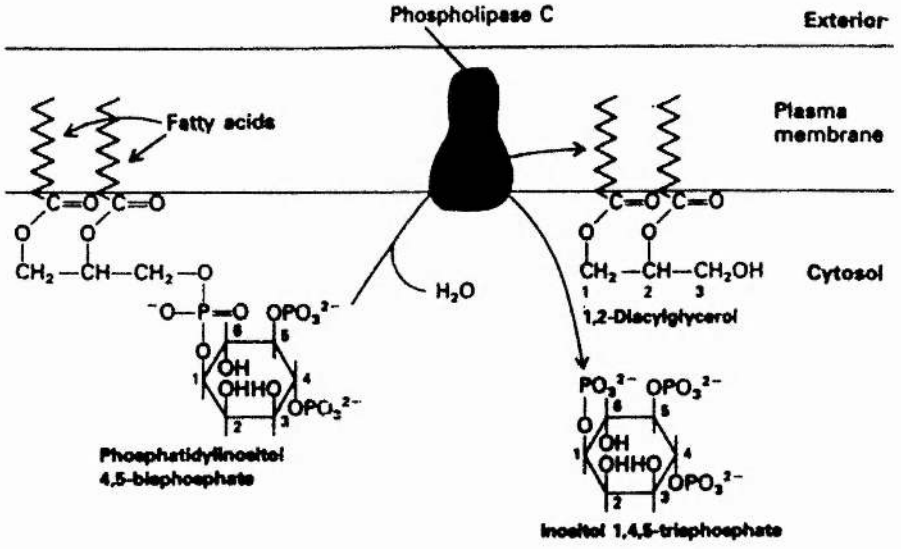

HORMONE
cGMP
EXTRACELLULAR
Guanylate cyclase
PLASMA MEMBRANE
INTRACELLULAR
GTP
cGMP
GMP
Ca++
EFFECT

Fig 1.7 Second messengers in the inositol-lipid signalling pathway (from Darnell et al, 1990).

Fig 1.8 Generation of the second messenger inositol 1,4,5 triphosphate (IP_3) and 1,2 diacylglycerol (DG) from phosphatidylinositol 4,5-bisphosphate (from Darnell et al, 1990).


Hormone
Receptor
Phosphatidylinositol 4,5-biphosphate
1,2 Diacylglycerol
Exterior
Cytosol
Protein kinase C
Protein (inactive)
G protein
Phospholipase C
Cellular response
Protein (active)
P
Cellular response
Inositol 1,4,5-triphosphate
Ca^{2+}
Ca^{2+}
Endoplasmic reticulum
ER Ca^{2+} channel sensitive to inositol 1,4,5-triphosphate


Phospholipase C
Exterior
Fatty acids
Plasma membrane
H_2O
Cytosol
1,2-Diacylglycerol
Phosphatidylinositol 4,5-biphosphate
Inositol 1,4,5-triphosphate

receptor assays is the ability of receptors to recognise the molecular peptide hormones, rather than antibodies recognising conformations other than those responsible for biological activity in radioimmunoassays. (Catt and Dufau, 1977).

Scatchard plots (Scatchard, 1949) which can often indicate secondary site presence and cooperativity may be used to analyse results. This plot is based on the equation.

$$\frac{B}{F} = \frac{-1}{K_d} (B-n)$$

where
- B = concentration of hormone bound
- F = concentration of free hormone
- K_d = equilibrium dissociation constant
- n = number of binding sites

A number of assumptions are made when Scatchard analysis is performed (Bolander, 1989):-

1. the labelled hormone is biologically identical to the native hormone.
2. the labelled hormone is homogenous
3. receptors are homogenous
4. receptors act independently - some can exhibit negative cooperativity
5. the receptor is unoccupied. Occupied receptors are said to be "masked" and an inability to bind the labelled hormone would result in an under estimation of n (Kelly et al, 1979).
6. the number of receptor sites remains constant during incubation

7. unbound hormone is equal to free hormone
8. the reaction is fully reversible throughout duration of the incubation.
9. the reaction is at equilibrium. This assumption also entails other assumptions.

a. both the hormone and receptor are stable
b. the reaction is reversible
c. equilibrium is not perturbed by separation of bound and free hormone

Basically, the Scatchard transformation represents ligand interaction with a single population of binding sites, via a simple reversible bimolecular reaction. A plot of B/F against B yields a straight line of slope = $^{-1}/K_d$ and an x-intercept equal to n. Curvilinear Scatchard plots indicate complex binding phenomena or technical artifacts, and upwardly concave plots suggest negative cooperativity, multiple binding sites or multiple affinities of the same receptor. Scatchard plots indicating the existence of two receptors may be separated into two individual plots, enabling estimation of the affinities and concentrations of each population (Barnett et al, 1978). This evaluation is based on the premise that the ligand interacts independently with the two sites and that binding to the high affinity site is saturated before binding to the low affinity site (Nahorski, 1981).

1.5.2 Fish Hormone Receptors

Receptor populations for hormones have not been extensively studied in fish tissue preparations. However some receptor types and locations are listed in Table 1.4. These include receptors for peptide hormones, the steroid hormone cortisol, glucocorticoids, and the thyroid hormone T3. The majority of studies are limited to brain, gills and liver. Insulin receptors have been demonstrated in brains of humpback salmon (Oncorhynchus gorbuscha) and lamprey (Lampetra fluviatilis) (Leibush, 1984), and glucocorticoid receptors in the gills of trout (Salmo gairdneri) and American eels (Anguilla rostrata) (Sandor et al, 1984). Liver studies centre on GH receptors, with binding sites located in Salmo gairdneri, Tilapia, goby (Gillichthys mirabilis) and salmon (Oncorhynchus tschaytscha) (Fryer, 1979). Receptors for adenosine have been identified in the brain of Sebastolobus altevelis and alascanus (Murray and Siebenaller, 1987).

Second messenger systems, in fish have also been rarely investigated, although the presence of adenylate cyclase activity in the gills of rainbow trout is well documented (Guibbolini and Lahlou, 1987 a, b, c). cAMP has also been shown to enhance steroid production from the interrenal of Coho salmon (Patino et al, 1986) and both cAMP and cGMP produce dose dependent increases in 1 -hydroxycorticosterone production from the perifused dogfish (Scyliorhinus canicula) interrenal gland (Armour, 1990 personal communication). cAMP has also been shown to stimulate

Table 1.4. **Location of Hormone Receptors in Fish**

Hormone Receptor	Fish Species	Tissue	Notes	Reference
Triiodothyronine	Salmo gairdneri	R.B.C. nuclei	Binding is specific, high affinity saturable, temperature and pH dependent	Sullivan et al, 1987
Insulin	Lampetra fluviatilis	Brain	Receptors were able to distinguish porcine, salmon and lamprey insulin and both bound porcine insulin suggesting the receptor has not altered functionally	Leibush et al, 1984
Insulin	Oncorhynchus gorbuscha	Brain		Leibush et al, 1984
Angiotensin	Carassius auratus	Corticotrope	Receptor recognises AI and AII	Weld and Fryer, 1987
Prolactin	Tilapia	Kidney	Receptor no. greater in FW than SW	Fryer, 1979a
Glucocorticoid	Anguilla rostrata	Gill	^{3}H triamcinolone acetonide	Sandor et al, 1984
Glucocorticoid	Salmo gairdneri	Gill	^{3}H triamcinolone acetonide used as ligand	Sandor et al, 1984
Cortisol	Anguilla rostrata	Gill nuclei	FW adapted fish used	Sandor et al, 1984
Neurohypophysial Peptides	Salmo gairdneri	Gill	Hormones (AVT and IT) inhibited basal and stimulated A.C. in Ca^{2+} absence	Guibbolini et al, 1988 Guibbolini and Lahlou, 1990
Growth hormone Growth hormone Growth hormone Growth hormone	Tilapia Gillichthys mirabilis Salmo gairdneri Oncorhynchus tschaytscha	Liver Liver Liver Liver	Single Class of binding sites which bind time and membrane concentration dependently. Slight binding was also detected in Tilapia kidney and gill	Fryer, 1979b Fryer, 1979b Fryer, 1979b Fryer, 1979b
Cortisol	Salmo gairdneri	Embryo	Association constant $2x10^{-11}$M, M.Wt of binding component – 50,000	Pillai and Terner, 1974

oxygen consumption and secretion of hypertonic saline by the dogfish rectal gland (Simpson and Sargent, 1985) although forskolin and cAMP inhibited incorporation of [^{32}P]orthophosphate into phospholipids. Forskolin also produced a dose dependent dispersion of pigment within leucophores of the teleost Oryzias latipes, suggesting cAMP acts as a second messenger in this response (Namoto and Yamada, 1987).

The subject and aims of this thesis were

1. to carry out initial whole animal studies on the physiological actions of angiotensin II, in selected euryhaline teleost species.
2. to examine the binding of angiotensin II in various tissues of the European eel (Anguilla anguilla L.).
3. to develop a specific radioreceptor assay for angiotensin II.
4. to utilise the radioreceptor assay to determine the number, affinity and specificity of angiotensin II receptors.

2. MATERIALS AND METHODS

2.1 Materials - see table 2.1.

2.1.1 Animals

Eels, Anguilla anguilla L. were supplied by both Mr. Hamish Wilson, Kirkbank, Kelso, and Dr. D. Trudgill, Newmill, Blairgowrie and were housed in the Gatty aquarium either in aerated running fresh water (FW eels) or 100% running seawater (SW eels). Plaice (Pleuronectes platessa) and dab (Limanda limanda) were provided by the Gatty Marine Lab specimen supply service and kept in seawater in the aquarium. All fish except those eels in seawater were stored short term and all were starved. The lighting regime was 10 light : 14 dark.

2.2 Cannulation of Blood Vessels

Eels were anaesthetised by immersion in fresh water or seawater containing 0.5% MS222 until cessation of breathing and loss of muscular tone. Maintenance of anaesthesia during surgery was achieved by irrigation of the gills with MS222 anaesthetic. A mid-ventral incision from the left of the heart to the posterior end of the liver exposed the pneumogastric artery and a branch of the splenic vein into which PE50 cannulae were inserted and secured to the body wall. The incision was closed by a running silk suture and fish were allowed 24hrs. recovery prior to study. The splenic cannula allowed intravenous (i.v.) injections and the pneumogastric artery the monitoring of blood pressure.

Table 2.1 - Materials

amastatin	Sigma Chemical Company, Poole, Dorset
ammonia	B.D.H., Poole, Dorset
angiotensins (see table 2.2)	Peninsula Laboratories Europe Ltd., St.Helens, Merseyside
ANP assay kit	Peninsula Labs
bacitracin	Sigma
bovine serum albumin (BSA)	Sigma
bradykinin	Peninsula Labs
butan-2-ol (sec butylalcohol)	Fisons Laboratory, Reagents, Loughborough
$CaCl_2$	B.D.H.
calcium free EMEM	Flow Labs., Irvine
Captopril	Peninsula Labs
collagenase (Worthington type II)	Worthington Biochemical Corporation, Freehold, New Jersey, U.S.A.
Coomassie brilliant blue G250	Sigma
^{51}Cr-EDTA	Amersham International PLC., Aylesbury, Buckinghamshire
DNAase	Sigma
Eagles minimal essential medium (EMEM)	Sigma
ethanol	James Burrough Ltd., Witham, Essex
heparin	Sigma
^{125}I-ile^5-AII	Amersham International and Dr. C. Deacon, Sheffield University
^{125}I-val^5-AII	Dr. C. Deacon, Dept. of Animal and Plant Science, Sheffield University
M_gSO_4	B.D.H.
microcentrifuge tubes	Scotlab, Bellshill, Glasgow
NaCl	B.D.H.
$NaHCO_3$	B.D.H.
orthophosphoric acid (85% v/v)	B.D.H.
papaverine	Sigma
PE50 cannulae	Portland Plastics, Hythe, Kent
pony vials	Canberra Packard Ltd., Pangbourne
soybean trypsin inhibitor (STI)	Sigma
sucrose	Sigma
TLC plates (LKSD Lineark silica gel preabsorbent)	Whatman Chemical Separation Inc,. Clifton
tricaine methanosulphate (MS222)	Sandoz Ltd., Switzerland
Tris Sigma 7-9 chemical buffer	Sigma
trypsin	Gibco, Paisley

Table 2.2 Sequence of Peptides Used to Displace Iodinated AII in Radioreceptor Assays

	AMINIO ACID SEQUENCE									
Position	1	2	3	4	5	6	7	8	9	10
Common Structure		Arg	Val	Tyr		His	Pro	Phe		
Peptide Notation Angiotensin I (human or ile^5-AI)	Asp				Ile				His	Leu
Angiotensin I (eel or val^5-AI)	Asn				Val					
Angiotensin II (human or ile^5-AII)	Asp				Ile				–	–
Angiotensin II (eel or val^5-AII)	Asp				Val				–	–
Saralasin Angiotensin II (sar^1,ile^5-AII)	Sar				Ile				–	–
Angiotensin III (human or ile^4-AIII)	–				Ile				–	–
Angiotensin III (val^4-AIII)	–				Val				–	–
AII(1-4) tetrapeptide	Asp				–	–	–	–	–	–
AII(5-8) tetrapeptide	–	–	–	–	Ile				–	–
Bradykinin	Arg	Pro	Pro	Gly	Phe	Ser				

Plaice and dab were cannulated via the caudal artery and vein.

2.3 Blood Pressure Studies

Injections (i.v.) of 0-100ng/kg ile^5-AII and 0.5mg/kg papaverine were administered to SW eels. 0-100ng/kg body weight each of ile^5- and val^5-AII and 0.5mg/kg papaverine were injected intramuscularly (i.m.) into plaice. 0-100ng/kg ile^5-AII, 0-50ng/kg val^5-AII were injected i.m. into dab and 0.5mg/100g papaverine as a bolus dose. Blood pressure was monitored via the pneumogastric artery in SW eels and the caudal artery in plaice and dab, using an Elcomatic EM750 pressure transducer and a George Washington 400 MD/4 pen recorder.

2.4 Drinking Rate Studies

Fish drinking rate determinations, a modification of the method of Carrick and Balment (1983), involved placing SW eels in 35 litres of seawater and plaice and dab in 25 litres containing 10uCi/litre ^{51}Cr-EDTA for 6hr periods. 50ng/kg each of ile^5- and val^5-AII, 100ug/100g Captopril or 0.5mg/kg papaverine were injected i.v. in 200ul 0.6% saline (which also acted as a control) to SW eels and 0.3mg/kg ile^5- and val^5-AII, 50mg/kg Captopril or 10mg/kg papaverine also in 200ul 0.6% saline i.m. into plaice and dab. Blood samples were removed from the caudal blood sinus, fish killed and guts removed after ligature at both ends. Gut contents were counted for ^{51}Cr activity on a Canberra-Packard Minaxi Auto Gamma R5000 series counter. Samples of

seawater containing ^{51}Cr were also counted. Drinking rates were estimated using the following calculation -

$$\text{Drinking Rate} = \frac{C}{MxT} \quad \text{ml/hr}$$

C = total counts incorporated into gut
M = concentration of bathing medium
T = time in tracer containing medium

Plasma chloride concentrations were evaluated using a Corning chloride analyser model 925.

2.5 ^{125}I-AII Tissue/Plasma Ratios

Tissue/plasma ratios were estimated by i.v. injection of 10uCi/kg ^{125}I-AII into seawater eels. Blood and tissue samples were removed after 20 mins, tissue washed, weighed and counted on a Gamma counter.

2.6 Isolation of Eel Myocytes

Atria and ventricles were removed and washed 3 times in heparinised (0.53mU/ml), calcium free, EMEM pH 7.6 (NaHC03 used to achieve pH if necessary). Tissue was then minced into c 2mm pieces in fresh EMEM on ice and divided into 10ml siliconised centrifuge tubes containing approx. 0.25g tissue each. 2ml EMEM/0.1% collagenase was added and tubes shaken for 5 mins at room temperature, followed by centrifugation at 50g for 5 mins. Supernatants were separated and retained. This was repeated until muscle blocks had dispersed (4-5 times) and supernatants were reserved at each stage. Pooled supernatants were centrifuged at 500g using an MSE Mistral 3000, for 5 mins at 4^{o}C, with resuspension of pellets in EMEM containing 66ug/ml

deoxyribonuclease 1 and 100ul/ml STI. A large bore pipette was used to mechanically disperse tissue by gentle pipetting approx. 50 times followed by agitation for 20 mins and centrifugation for 5 mins. This last centrifugation/resuspension step was repeated and all supernatants kept. Centrifugation was for 5 mins at 1200g and 4^{o}C, with resuspension of pellets in EMEM containing DNAase and STI, followed by filtration through a three stage Swinnex filter (250, 125 and 74uM nylon mesh). Cell numbers were assessed using a haemocytometer and 80% were found to be myocytes and cell viability checked using trypan blue exclusion. Cells were resuspended in EMEM plus calcium to a concentration of 10 million cells/tube, for assay. Myocytes were incubated in the absence and presence of 1.0nM and 10.0nM AII for 30 minutes at room temperature in a final incubation volume of 400ul.

2.7 Radioimmunoassay for Atrial Natriuretic Peptide

ANP standard (alpha-human-ANP) was reconstituted in 1.0ml radioimmunoassay (RIA) buffer containing triton X - 100 and rabbit anti ANP serum was reconsitituted in 13.0ml of RIA buffer and both solutions stored on ice. Standards were prepared by serial dilution and ranged from 1-128pg ANP/100ul with unknown samples also of a volume of 100ul. Addition of 100ul primary antibody (rabbit anti ANP) was made to the standard and incubated overnight. ^{125}I-ANP was reconstituted in 13.0ml RIA buffer (approx. 20,000cpm/100ul) and 100ul added to each RIA tube followed by overnight incubation at 4^{o}C. Goat anti-rabbit IgG serum (GARGG) and

normal rabbit serum (NRS) were both reconstituted with 13.0ml RIA buffer and 100ul of each added to every RIA tube, followed by incubation at room temperature for 2 hrs. Separation was with 0.5ml RIA buffer and centrifugation using an MSE Mistral 3000, at 1700g for 20 mins. Supernatants were aspirated off and samples counted on a gamma counter.

2.8 Preparation of Tissue Membrane Fraction

Eel tissue membranes were prepared as described by a modification of the method of Cuatrecasas (1972). Eels (200-500g) were decapitated, livers removed, weighed and placed on ice and homogenised at 0-4^{o}C in a ratio of 40-50g tissue per 250ml 0.25M sucrose (ice-cold) using a Polytron (model PT-1035, Kinematica, Switzerland) at setting 7 for 30 sec. The following centrifugation steps employed the use of either a Beckman, model J2-21M/E (Beckman Instruments Inc. Palo Alto, California) with the JA20 rotor or a MSE 25 High Speed centrifuge (MSE, Crawley, Sussex) using the 6x100ml (59582) head, with 50ml or 100ml polycarbonate tubes respectively. All steps were carried out at 4^{o}C. Homogenate was centrifuged at 100g for 10 mins, pellet discarded, resulting supernatant recentrifuged for 1 hr at 12000g and pellet discarded. The decanted supernatant was adjusted to 0.1M NaCl/0.2mM $MgSO_4$ (per 100ml add 5.3ml of 2M NaCl/4mM $MgSO_4$ in 0.25M sucrose) and centrifuged at 40,000g for 1hr followed by removal of the supernatant. The membrane pellet formed was washed by resuspension in 100ml of 50mM Tris buffer (pH 7.0) using a teflon glass

homogeniser, and centrifuged at 40,000g for 1hr. This last step was repeated. The final pellet was suspended in 50mM Tris buffer to a tissue concentration of 5g/ml giving an average protein concentration of 3.6mg/ml and $CaCl_2$ added to a final concentration of 25mM. The preparation was then frozen and stored overnight at -20°C for assay the next day.

2.9. Angiotensin II receptor Assay

This technique is based on the method by McQueen et al (1984). Membrane preparation was thawed at room temperature, and kept on ice with gentle shaking to suspend membrane material evenly, 0.2ml aliquots were transferred to microcentrifuge tubes - all assay tubes and reagents were handled on ice prior to incubation. Labelled and unlabelled AII were stored in acid ethanol (375ml ethanol, 125ml H_2O, 7.5ml conc HCL) and prepared for assay in either 50mM Tris/1%BSA/1% bacitracin pH 7.4 or 50mM Tris containing 10^{-6}M amastatin. The former buffer was stored 10-fold concentrated at -20°C and diluted daily for peptide preparation and the latter was made up fresh daily. Serial 2-fold dilutions of AII ranged from 1.56uM to 800uM and 0.1ml volumes transferred for incubation. 2.4mM AII was required to estimate non specific binding (NSB). Other peptides e.g. AI and bradykinin used in displacement curves, instead of unlabelled AII (see table 2.2) were added in similar concentrations, with the membrane preparation and labelled AII remaining unchanged. ^{125}I-AII supplied by Amersham had a specific activity (S.A.) of between 1223 and 2200Ci/mMol, and that provided by Sheffield University

approx. 653Ci/mMol. 0.1ml aliquots containing approx. 100pM ^{125}I-AII (around 250pM in the Sheffield case) were added to the incubation mixture.

The reaction mixture therefore comprised:

0.4ml Tris/NaCl + 0.5% BSA + 0.5% bacitracin

0.72mg of membrane protein

approx. 25pM ^{125}I-AII

0,0.39, 0.78, ...100,200uM unlabelled AII

All AII peptides utilised were of the ile^{5} sequence unless stated. Incubation was at 22^{o}C for 1hr with termination by removal of samples onto ice followed by the immediate addition of 1ml ice-cold 50mM Tris (pH 7.0) and centrifugation at 1300g at 4^{o}C for 10 mins to separate bound and free radioactivity. Supernatants were removed by aspiration and pellets counted for ^{125}I activity for 1 min in a Canberra Packard Gamma counter (microcentrifuge tubes were placed inside pony vials for counting).

2.10 Protein Determinations

Protein concentrations of the membrane preparation followed Bradford (1976). 100mg Coomassie blue G250 was dissolved in 50ml 95% ethanol and 100ml 85% w/v orthophosphoric acid and made up to 1 litre final volume with distilled water. 3ml of a 1:2 dilution of the assay buffer was added per 100ul standard (0-100ug BSA) or sample. The blue colour produced was measured using a Cecil CE599 Universal automatic scanning spectrophotometer (Cecil

Instruments, Cambridge) at 595nm (the colour is stable for 10-60mins).

2.11 Thin Layer Chromatography (TLC)

Solvent system: 3%v/v ammonium hydroxide: butan-2-ol (35:105, v/v). 50ul portions of unbound labelled peptide (supernatant from the separation spin during receptor assay) were spotted on individual lanes of the TLC plates. Plates were then placed in a tank containing the solvent system. Solvent was allowed to migrate for around 4hrs or until it had reached 12cm. 1cm bands were removed from the origin to the solvent front for measurement of radioactivity.

3.0 RESULTS

3.1 PHYSIOLOGICAL STUDIES INVOLVING ANGIOTENSIN II

3.1.1 Blood Pressure Studies

In SW eels ile^5-AII raised blood pressure by 5.3mmHg on injection of 10 and 50ng/kg doses and a further increase of 8mmHg was induced by 100ng/kg ile^5-AII (fig 3.1.1a). Val^5-AII did not have equivalent potency, only producing maximum increases of 4mm Hg over the range 0.5-100ng/kg tested. Papaverine injection (0.5mg/kg) produced a reduction in blood pressure of 10.3mmHg, 10 minutes post injection and a further decrease of 2mmHg at 20min following injection into SW eels. Recovery began at 30 minutes and was almost complete at 90 minutes, at 1.3mmHg below resting blood pressure levels.

Both sequences of AII peptide increased plaice blood pressure (fig 3.1.1b) with maximum increments of 8.0 (60, 100ng/kg) and 9.0mmHg (50ng/kg) for the ile^5 and val^5 structures respectively. However, val^5-AII does appear to have a greater effect in that blood pressure was consistently higher over the doses 5-50ng/kg than in equivalent ile^5-AII injected fish. Papaverine treatment again lowered blood pressure by 6.2 and 8.2mmHg, 15 and 30 minutes post injection, respectively. Recovery began at 45 mins and was almost complete at 90 minutes.

Ile^5 and val^5-AII again evoke similar maximum responses on blood pressure of dab (fig 3.1.1c), with increases of 9.3 and 9.5mmHg respectively. However, as in plaice, val^5-AII

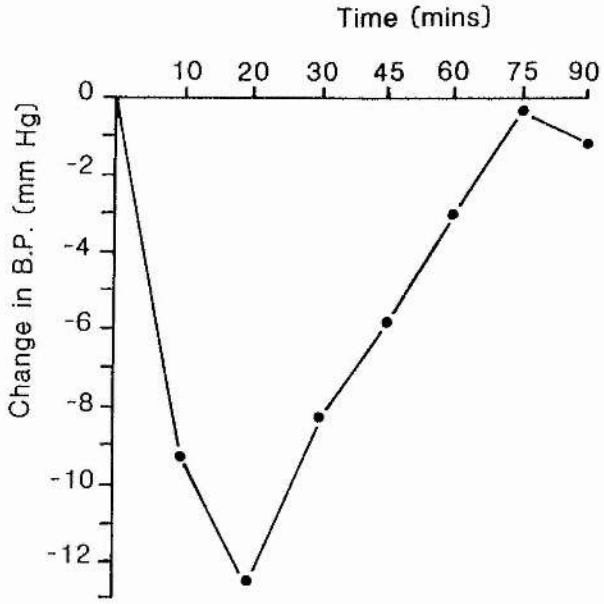
3.1.1 BLOOD PRESSURE STUDIES

Fig 3.1.1a **Blood Pressure Studies in SW Eels**

i.v. injections of 0.5mg/100g papaverine (pap) and 0-100ng/kg bodyweight ile^5-angiotensin II (ile^5-AII) were administered to SW eels. Blood pressure was monitored for 90mins, at 10-15min intervals post papaverine injection. results are mean values from 3 fish. Control injections consisted of equivalent volumes of 0.6% saline.

SW Eel

Pap



ile^5-A II

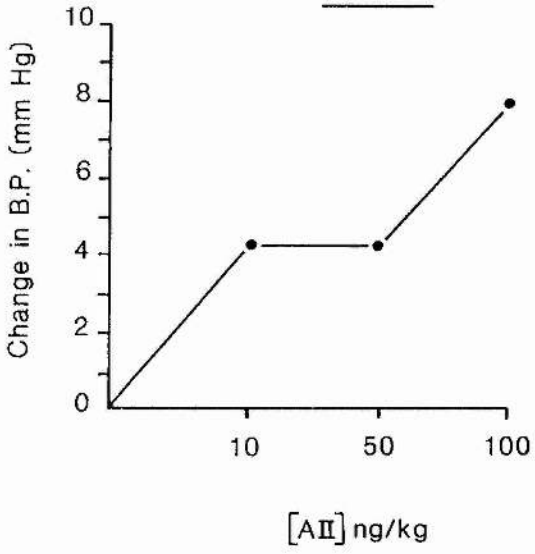
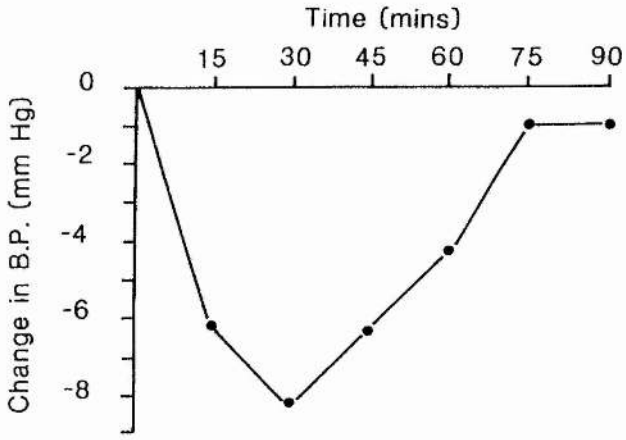


Fig 3.1.1b **Blood Pressure Studies in Plaice**

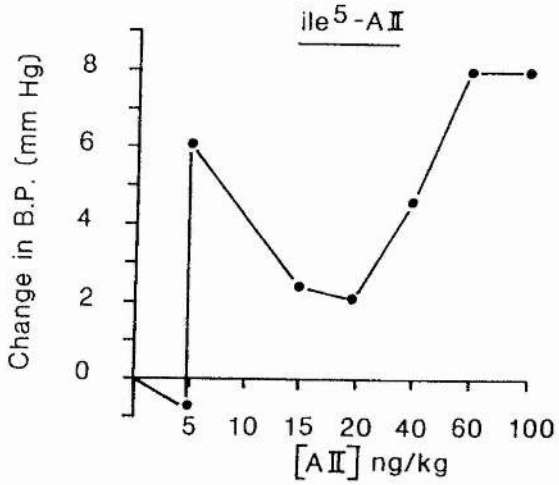
i.v. injections of 0.5mg/Kg papaverine (pap), 0-100ng/kg ile^5-AII, 0-100ng/kg val^5-angiotension II were administered to plaice. Blood pressure was monitored, and for 90 minutes post injection of papaverine. Results are mean values from 3 fish.

Plaice

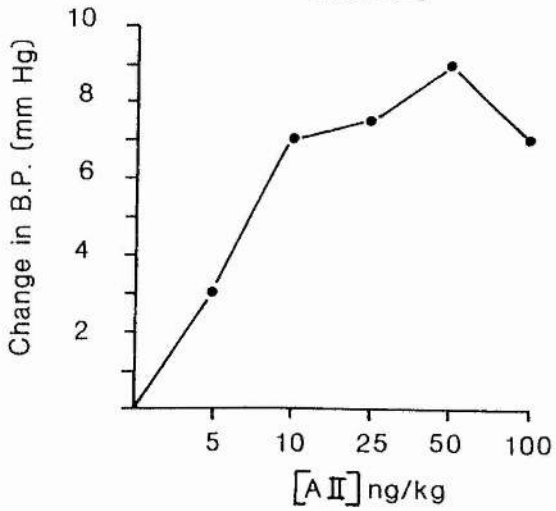
Pap



ile^5-AII



val^5-AII

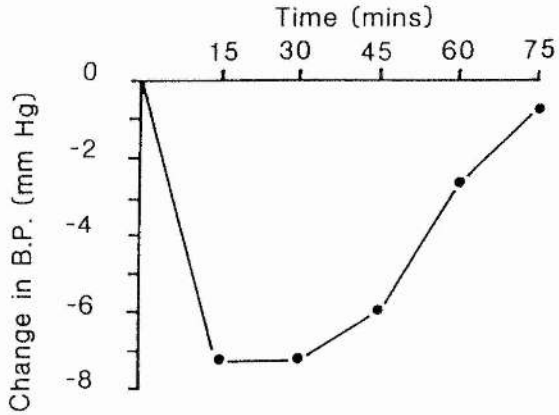


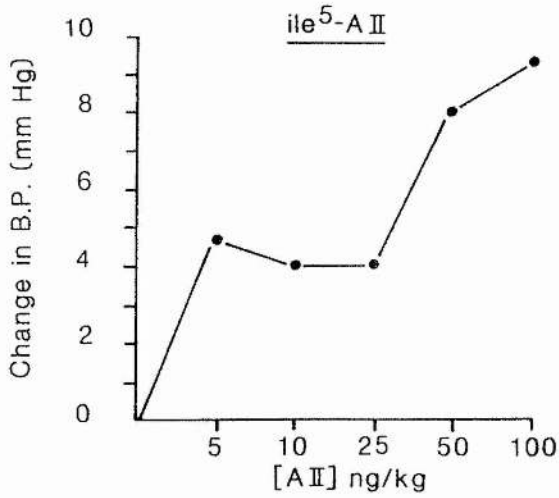
3.1.1c Blood Pressure Studies in Dab

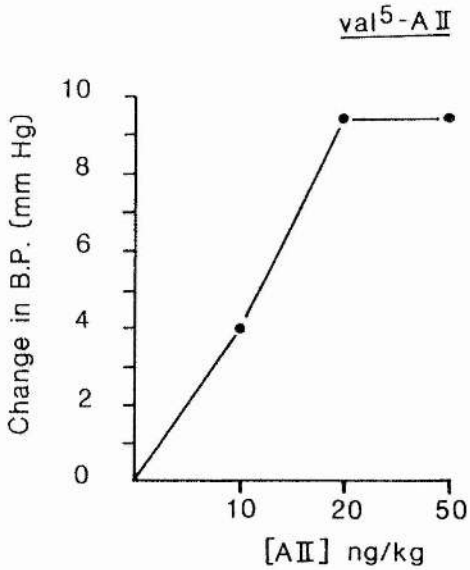
Papaverine (pap) (0.5mg/100g) as a bolus does and ile^5-AII (0-100ng.kg) i.v. were injected into dab, val^5-AII (o-50ng/kg) was injected i.v. Results are means of 3 (pap and ile^5-AII) and 2 fish (val^5-AII).

Dab

Pap


Time (mins)
15
30
45
60
75
0
-2
-4
-6
-8
Change in B.P. (mm Hg)


ile5-AII
10
8
6
4
2
0
Change in B.P. (mm Hg)
5
10
25
50
100
[AII] ng/kg


val5-AII
10
8
6
4
2
0
Change in B.P. (mm Hg)
10
20
50
[AII] ng/kg

appears to achieve this equivalent increment in blood pressure at a lower dose of 20ng/kg compared with 100ng/kg ile^5-AII. Papaverine produced a similar blood pressure profile to those of eels and plaice, with blood pressure reduced by 7.3mmHg at 15 and 30 minutes respectively, following injection, proceeded by recovery to control values at 90 minutes.

3.1.2 DRINKING RATE STUDIES

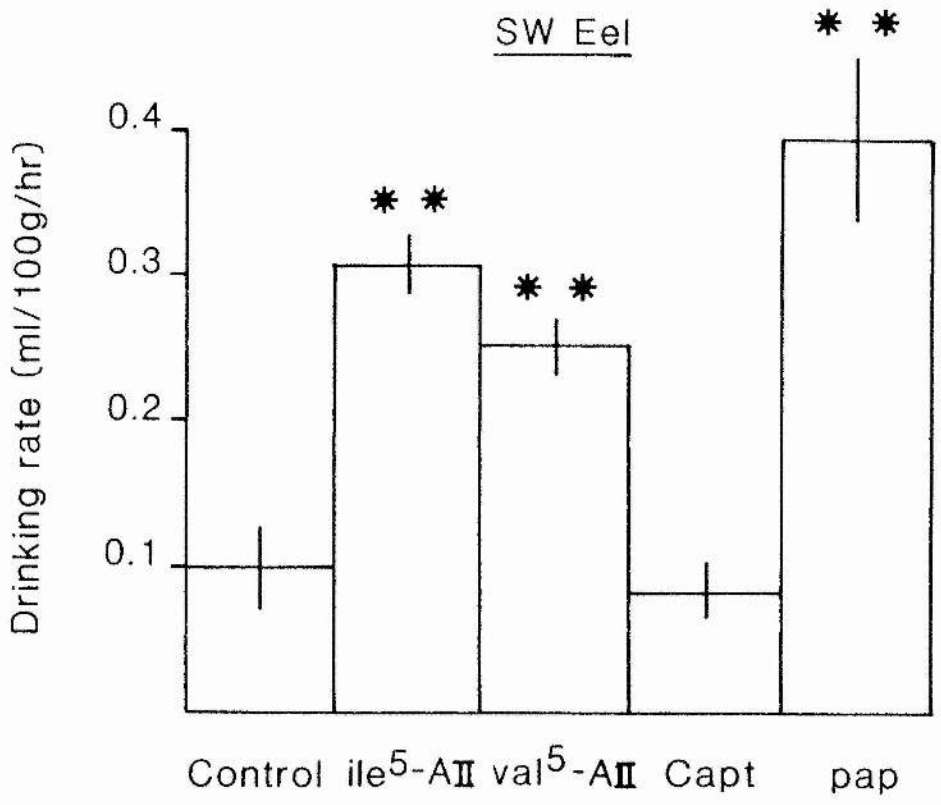
Basal drinking rates of SW eels (3.1.2a) were 0.10±0.03ml/100g/hr and basal plasma chloride was 130± 3mmol/l. Neither drinking rate nor plasma chloride concentrations were markedly affected by Captopril injection (100ug/100g). However, both sequences of AII increased drinking rates, ile^5-AII 3-fold and val^5-AII by greater than 2-fold to 0.31±0.02 and 0.25±0.0ml/100g/hr respectively ($p < 0.01$). Plasma chloride concentration was unaffected by val^5-AII, but ile^5-AII increased levels to 142mmol/l ($p < 0.01$). Papaverine injection resulted in the greatest enhancement of drinking rate creating a 4-fold increase to 0.39±0.06 ml/100g/hr over basal ($p < 0.01$) and also generated a significant increase in plasma chloride to 139±4mmol/l, $p < 0.01$.

Basal drinking rates of plaice (fig 3.1.2b) were 0.24±.03 ml/100g/hr and resting plasma chloride concentrations were 152±2mmol/l. Ile^5-AII caused slight increases over basal to 0.28±0.03ml/100g/hr in drinking and to 155±5mmol/l for chloride concentrations (this was not significant). However, val^5-AII appeared to have a more

3.1.2 DRINKING RATE STUDIES

Fig 3.1.2a **Drinking Rate Studies and Associated Plasma Chloride Concentrations in SW Eels**

Drinking rates and plasma chloride concentrations were determined after i.v. injection of 200ul 0.6% saline (vehicle and control), 50ng/kg ile^5-angiotensin II (ile^5-AII) and val^5-angiotensin II (val^5-AII), 100ug/100g Captopril (Capt) and 0.5mg/kg papaverine (pap) 7, 5, 4, and 8 SW eels, respectively. Significant difference from controls are indicated by **p < 0.01 (Mann-Whitney U test).


SW Eel
Drinking rate (ml/100g/hr)
0.4
0.3
0.2
0.1
Control
ile5-AII
val5-AII
Capt
pap

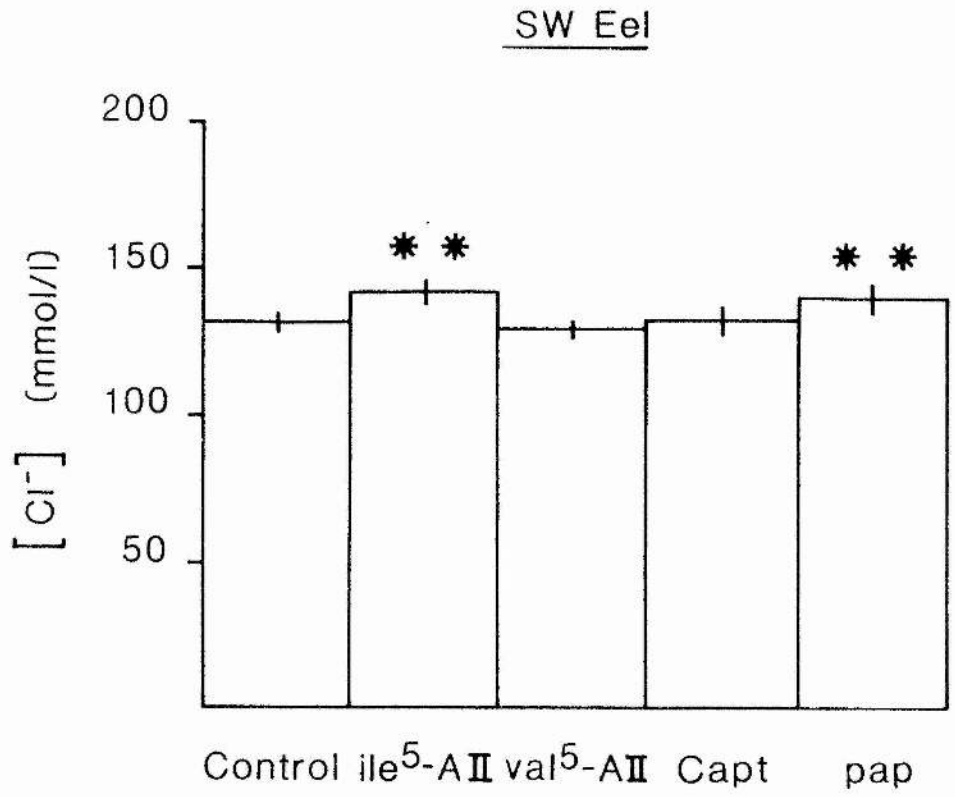
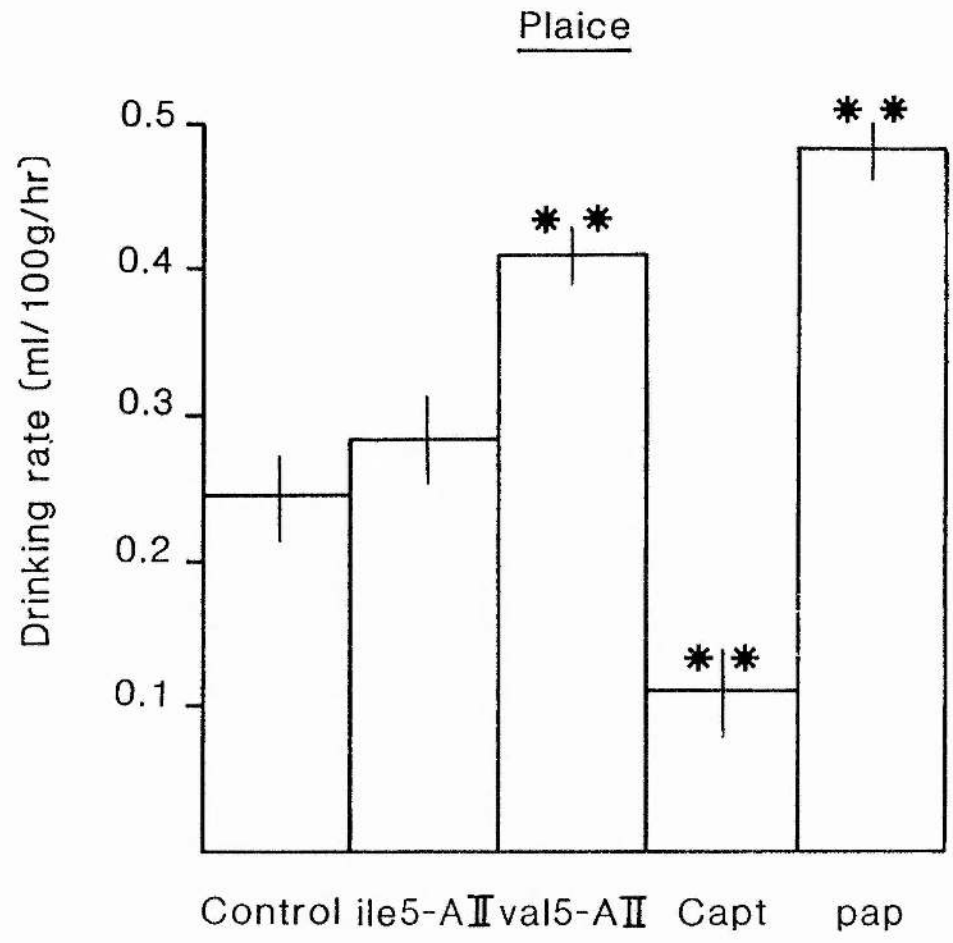

SW Eel
[Cl-] (mmol/l)
200
150
100
50
Control
ile5-AII
val5-AII
Capt
pap

Fig 3.1.2b **Drinking Rate Studies and Associated Plasma Chloride concentrations in Plaice**

Drinking rates and plasma chloride concentrations were determined after i.m. injections of 200ul 0.6% saline (vehicle and control) 0.3mg/kg ile^5 and val^5 angiotensinII (ile^5- and val^5-AII), 50mg/kg Captopril (Capt) and 10mg/kg papaverine (pap) to 8, 5, 10, 9 and 6 plaice, respectively. Significant difference from controls are indicated by **p < 0.01 (Mann-Whitney U test).


Plaice
Drinking rate (ml/100g/hr)
0.5
0.4
0.3
0.2
0.1
Control
ile5-AⅡ
val5-AⅡ
Capt
pap

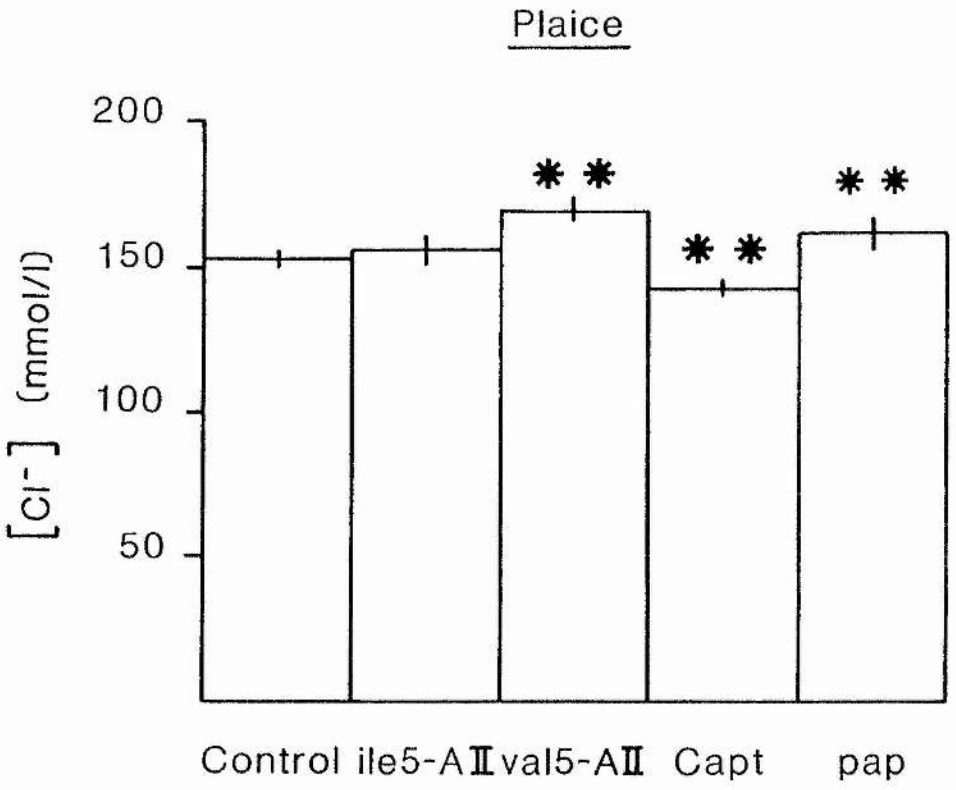
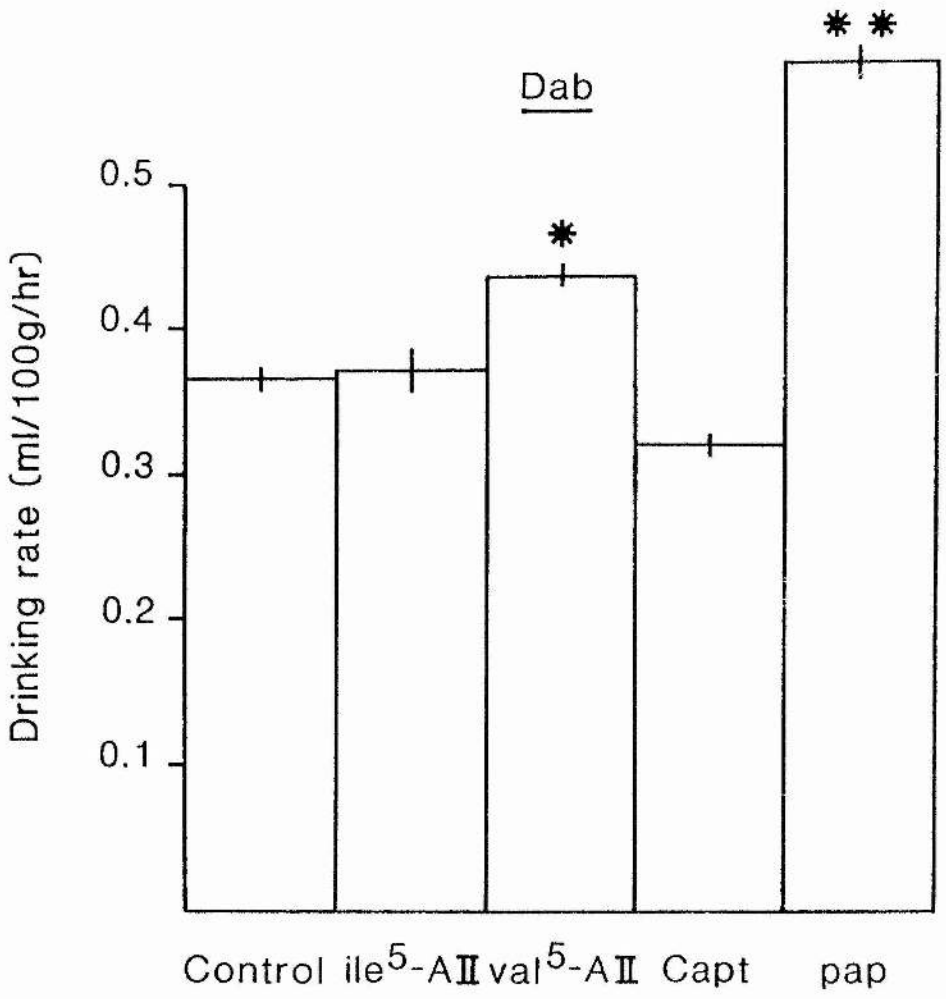
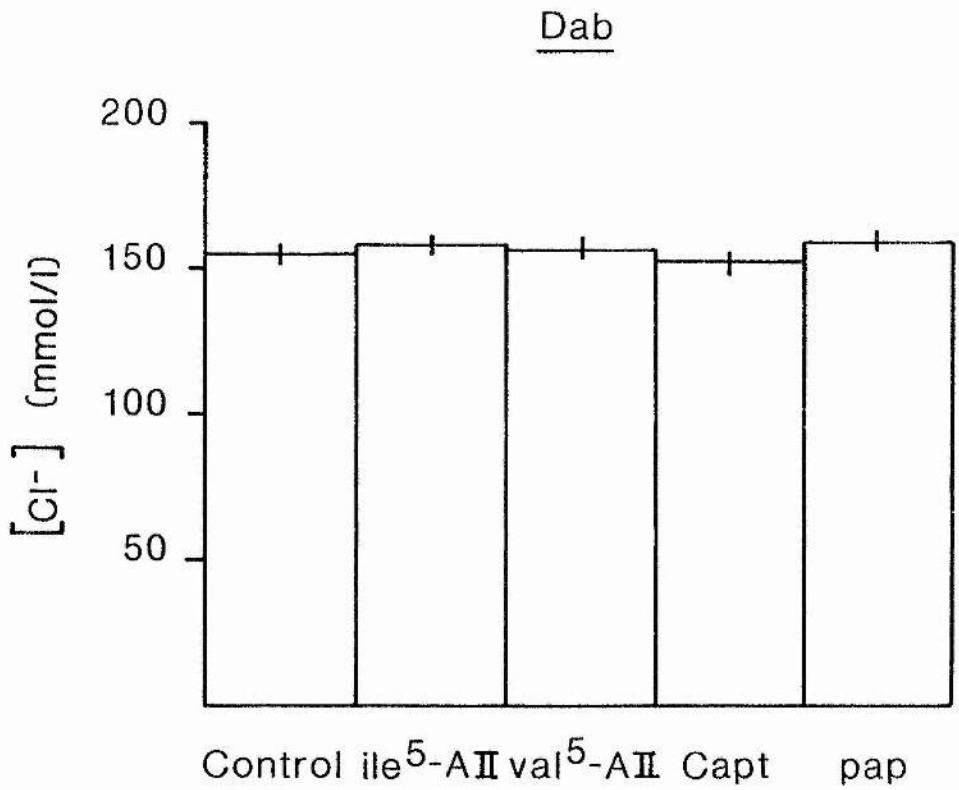

Plaice
[Cl-] (mmol/l)
200
150
100
50
Control
ile5-AⅡ
val5-AⅡ
Capt
pap

Fig 3.1.2c **Drinking Rate Studies and Associated Plasma Chloride concentrations in Dab**

Drinking rates and plasma chloride concentrations were determined after i.m. injections of 200ul 0.6% saline (vehicle and control) 0.3mg/kg ile^5- and val^5-angiotensin II (ile^5- and val^5-AII), 50mg/kg Captopril (Capt) and 10mg/kg papaverine (pap) to 7, 4, 7, 4 and 6 dab respectively. Significant difference from controls are indicated by *$p < 0.05$ and **$p < 0.01$ (Mann-Whitney U test).


Dab
Drinking rate (ml/100g/hr)
0.5
0.4
0.3
0.2
0.1
* *
*
Control
ile5-AII
val5-AII
Capt
pap


Dab
[Cl-] (mmol/l)
200
150
100
50
Control
ile5-AII
val5-AII
Capt
pap

potent action enhancing drinking to 0.41±0.02ml/100g1hr and plasma chloride concentration to 170±4mmol/l ($p < 0.01$). Papaverine also augmented drinking rate and plasma chloride concentration to 0.48+0.02ml/100g/hr and 163±5mmol/l respectively ($p < 0.01$), and Captopril reduced drinking rates and plasma chloride levels to 0.11±0.03ml/100g/hr and 144±1mmol/l respectively,($p < 0.01$).

Neither ile^5-AII nor Captopril had any significant effect on dab drinking (fig 3.1.2c), rates being 0.37±0.09 and 0.32±0.03ml/100g/hr respectively, in relation to the basal rate of 0.36±0.07ml/100g/1hr. Val^5-AII and papaverine both elevated drinking rates to 0.43±0.03 ($p < 0.05$) and 0.58±0.07ml/100g/hr ($p < 0.01$), respectively. None of the test substances resulted in any major deviance from basal plasma chloride concentrations all ranging between 152±3 to 158±2mmol/l compared to a basal level of 154±4mmol/l.

Tracer degradation was estimated by counting blood samples and distal gut sections. Low values (less than 5% in total) indicate neglible breakdown and therefore minimal loss of chromium anally.

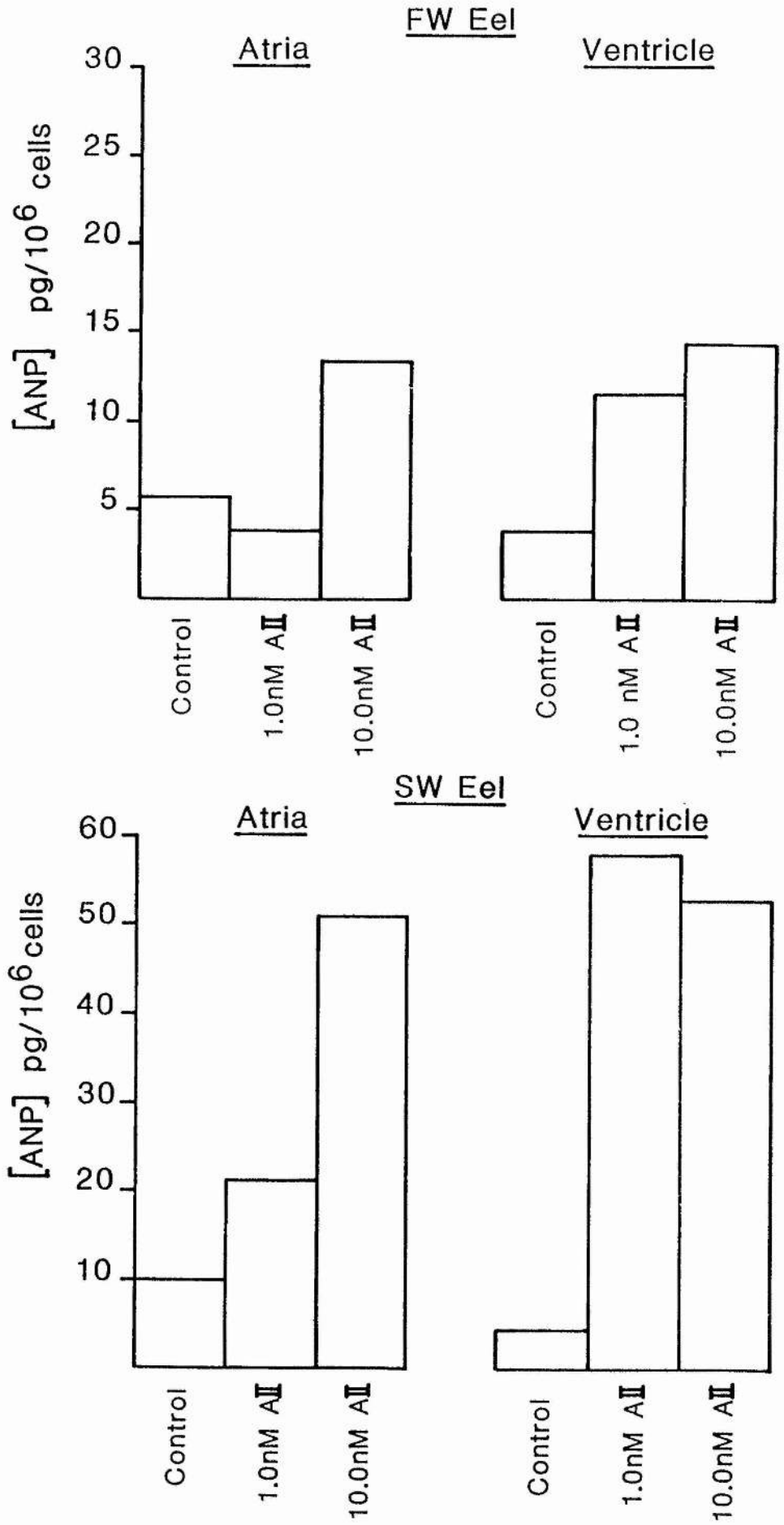
Fig 3.1.3 **Effect of Angiotensin II on Eel Myocyte ANP Release**

Effect of AII on ANP release during 30 minute incubation of isolated eel myocytes was investigated (fig 3.1.3). ANP secretion was similar in FW atrial, FW ventricular and SW ventricular myocytes, however control levels were higher in SW atrial myocytes. 1.0nM AII

Fig 3.1.3 **EFFECT OF ANGIOTENSIN II ON EEL MYOCYTE ANP RELEASE**

Fig 3.1.3. Effect of Angiotensin II on Eel Myocyte ANP Release

Incubations of eel ventricular and atrial myocytes (10^6 cells/tube) in the absence (control) and presence of 1.0nM and 10.0nM val^5-angiotensin II (val^5-AII). Results indicate ANP release during 30min incubations. Incubations and ANP determinations were performed in duplicate.


FW Eel
Atria
Ventricle
[ANP] pg/10^6 cells
30
25
20
15
10
5
Control
1.0nM AII
10.0nM AII
Control
1.0 nM AII
10.0nM AII
SW Eel
Atria
Ventricle
[ANP] pg/10^6 cells
60
50
40
30
20
10
Control
1.0nM AII
10.0nM AII
Control
1.0nM AII
10.0nM AII

increased ANP production over control in all cases except FW eel atrial myocytes. Ventricular ANP release was greater than atrial for both FW and SW eels following 1.0nM AII administration. 10.0nM AII also increased ANP secretion, over control, in all cases, with production being relatively constant between the two preparations from each fish. With the exception of SW eel ventricular myocytes, 10.0nM AII produced a greater stimulation of ANP release than 1.0nM. In all cases, ANP secretion was greater from SW eel that FW eel myocytes.

From the physiological effect induced by AII it was then decided to investigate receptor binding of AII in eels, also employing the use of the iodinated version of the peptide.

3.2 STUDIES TO ASSESS ^{125}I-ANGIOTENSIN II BINDING

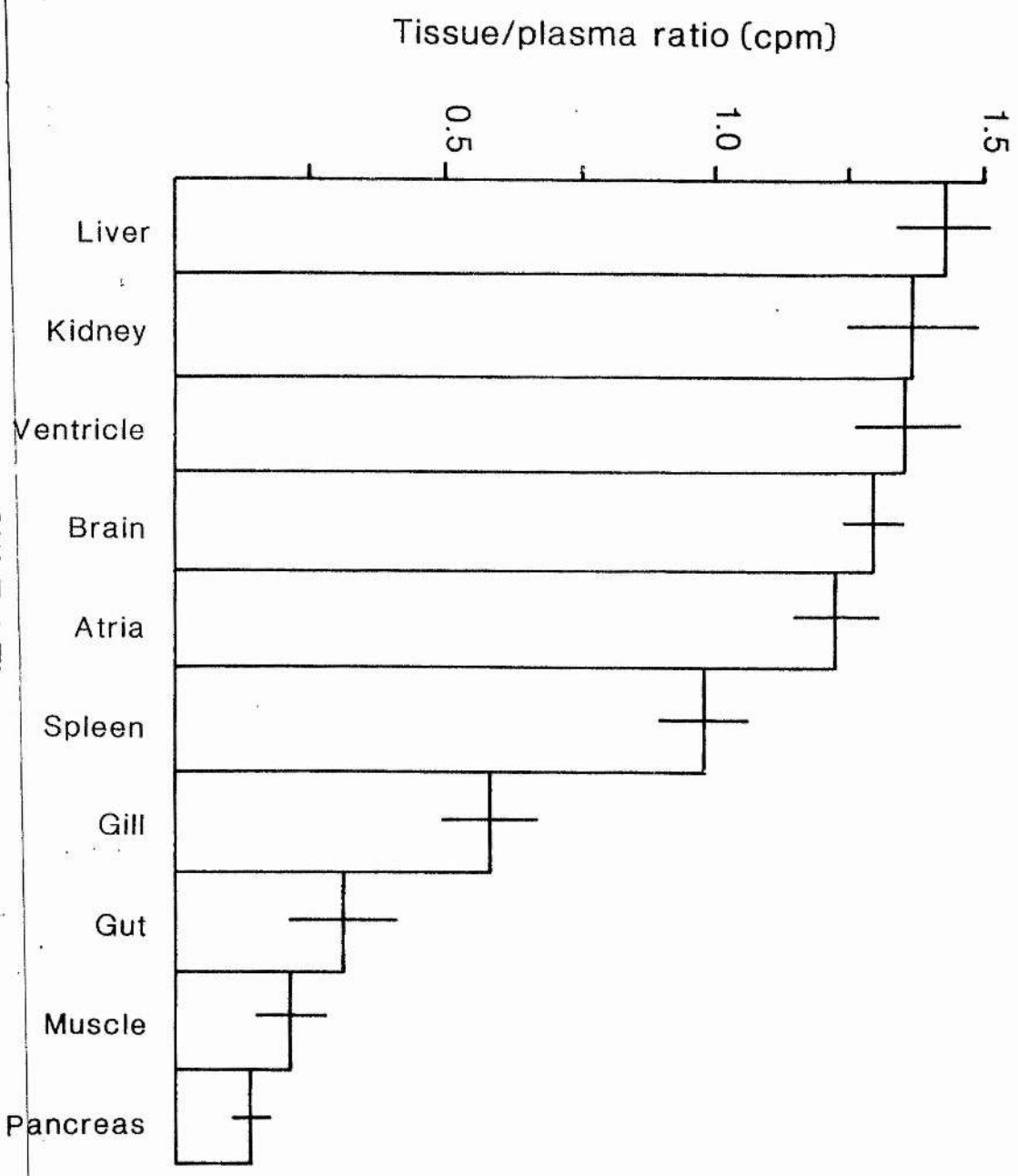
3.2.1 Distribution of ^{125}I-AII in Eels

Tissue/plasma ratios in SW eels (fig 3.2.1) indicated liver had the greatest binding of ^{125}I-AII at 1.43±0.09/ml, although kidney, ventricle and brain produced similar binding of 1.37+0.12, 1.37+0.14 and 1.3+0.06g/ml, respectively. ^{125}I-AII binding was 1.23±0.09 in atria, 0.97±0.09 in spleen and 0.57±0.09g/ml in the gill, but the remaining 3 tissues, gut, muscle and pancreas had much lower levels of binding at 0.3+0.1, 0.2+0.06 and 0.13+0.06g/ml, respectively.

Fig 3.2.1 DISTRIBUTION OF ^{125}I-AII IN SW EEL TISSUES

Fig 3.2.1 **Distribution of ^{125}I-AII in SW Eel Tissues**

Tissue/plasma ratios of 10 tissues from 3 fish were estimated in triplicate, 20 minutes after i.v. injection of 10uCi/kg ^{125}I-AII and expressed as mean $\pm$ S.E.M.


Tissue/plasma ratio (cpm)
0.5
1.0
1.5
Liver
Kidney
Ventricle
Brain
Atria
Spleen
Gill
Gut
Muscle
Pancreas
SW Eel Tissue

3.2.2 Tissue Receptor Binding Studies

Tissue receptor binding studies (Table 3.2.1) showed liver again produced greatest specific binding of ^{125}I-AII of the FW preparations, with head kidney also resulting in substantial specific binding. FW gill and spleen also produced specific binding, but binding in the heart preparation was very low and no ^{125}I-AII was bound specifically in the FW muscle membrane preparation. SW tissue specific receptor binding was greatest in the gill although liver was similar. SW spleen produced specific binding as did SW head kidney, however no specific binding was found in either the SW heart or muscle preparations.

With liver confirmed as a good model tissue in which to investigate ^{125}I-AII receptor binding in eels, various aspects of the technique were studied in this tissue to validate the receptor assay and enhance specific binding.

3.3 DEVELOPMENT OF AN AII RECEPTOR ASSAY

RECEPTOR BINDING AT EACH CENTRIFUGATION STEP DURING FW EEL LIVER MEMBRANE PREPARATION

The first step involved determination of specific binding at each centrifugation step during membrane preparation (Table 3.3.1). The membrane component of incubations consisted of 200ul of the pellet formed in the case of both the 1000g and 12,000g spins and 200ul of the pellet resulting from the remaining centrifugation procedures resuspended in appropriate volumes of Tris buffer (50mM, pH7.0).

Table 3.2. ^{125}I-AII BINDING IN MEMBRANE PREPARATIONS OF FW AND SW EEL TISSUES

Table 3.2. ^{125}I-AII Binding in Membrane Preparations of FW and SW Eel Tissues

Membranes were incubated for 1hr at 22^{o}C in the absence (total) and presence (non-specific) of 600uM AII. Specific binding was calculated by subtracting NSB from total binding. ^{125}I-AII concentration of the incubation medium was 21.17pM and 22.63pM for the FW and SW preparations respectively. Results indicate duplicate determinations from single preparations which varied by less than 10%.

Protein concentrations (mg/ml) of the membrane preparations were:-

	Liver	Gill	Head kidney	Spleen	Heart	Muscle
FW prep	7.25	0.96	55.0	109.5	0.55	0.24
SW prep	7.5	0.84	300.0	42.5	0.74	0.58

Tissue concentrations (wet weight g/ml) of the membrane preparations were:-

	Liver	Gill	Head kidney	Spleen	Heart	Muscle
FW prep	4.82	15.04	5.73	5.17	5.04	5.17
SW prep	4.81	19.1	6.2	9.12	5.74	7.73

Table 3.2. ^{125}I-AII Binding in Membrane Preparations of FW and SW Eel Tissues

Tissue	FW Eel Binding (fmol/mg protein)		SW Eel Binding (fmol/mg protein)	
	TOTAL	SPECIFIC	TOTAL	SPECIFIC
Liver	1.16	0.56	0.94	0.32
Gill	2.11	0.25	3.94	0.34
Head kidney	1.96	0.47	0.40	0.009
Spleen	1.31	0.14	2.71	0.19
Heart	2.54	0.04	1.47	-
Muscle	3.08	-	3.65	-

Table 3.3. RECEPTOR BINDING AT EACH CENTRIFUGATION STEP DURING FW EEL LIVER MEMBRANE PREPARATION

Table 3.3. **Receptor Binding at each Centrifugation Step During FW Eel Membrane Preparation**

Incubation of ^{125}I-AII (average final conc. 64pM) with the pellets produced after each centrifugal operation, for 1hr at 22^{o} C in the absence (total) and presence (non-specific) of 600uM AII, n=4. Full experimental details are given in the text. Results are expressed as mean $\pm$ S.E.M.

Average protein concentration of pellets :-

Step	g force	[protein] mg/ml
1	1,000	27.27
2	12,000	29.48
3	40,000	8.71
4	40,000	5.00
5	40,000	3.43

Table 3.3. Receptor Binding at each Centrifugation Step During FW Eel Liver Membrane Preparation

Centrifugation Procedure		Total Binding	Non-specific Binding	Specific Binding
Time (min)	g force	Units:- fmol/mg protein		
10	1,000	0.40 ± 0.05	0.46 ± 0.05	-
60	12,000	0.39 ± 0.11	0.43 ± 0.12	-
60	40,000	2.26 ± 0.42	1.33 ± 0.28	0.93 ± 0.18
60 (Wash 1)	40,000	3.57 ± 0.60	2.37 ± 0.23	1.19 ± 0.36
60 (Wash 2)	40,000	5.19 ± 0.64	3.54 ± 0.26	1.65 ± 0.39

Specific binding of ^{125}I-AII, defined as that not displaced by 600uM AII, was not apparent until after the first 40,000g spin which involved precipitation with $NaCl/MgSO_4$. This was proceeded by two washes with 50mM Tris which increased specific binding by 0.26fmol/mg protein in the first case and by 0.46fmol/mg protein after the final spin.

ESTIMATION OF NON SPECIFIC BINDING IN LIVER MEMBRANE PREPARATIONS

Non-specific binding of ^{125}I-AII was estimated using 600uM, 800uM and 1mM AII (fig 3.3.1). Binding was 1.08+0.14, 1.09+0.13 and 1.09+0.11fmol/mg protein for the 3 concentrations of AII respectively in the FW liver preparation, with total binding of 1.58±0.12fmol/mg protein. Total binding was 1.89±0.35fmol/mg protein in the SW liver preparation and binding in the presence of 600uM, 800uM and 1mM AII was 1.12±0.18, 1.18±0.21 and 1.19±0.22fmol/mg protein, respectively.

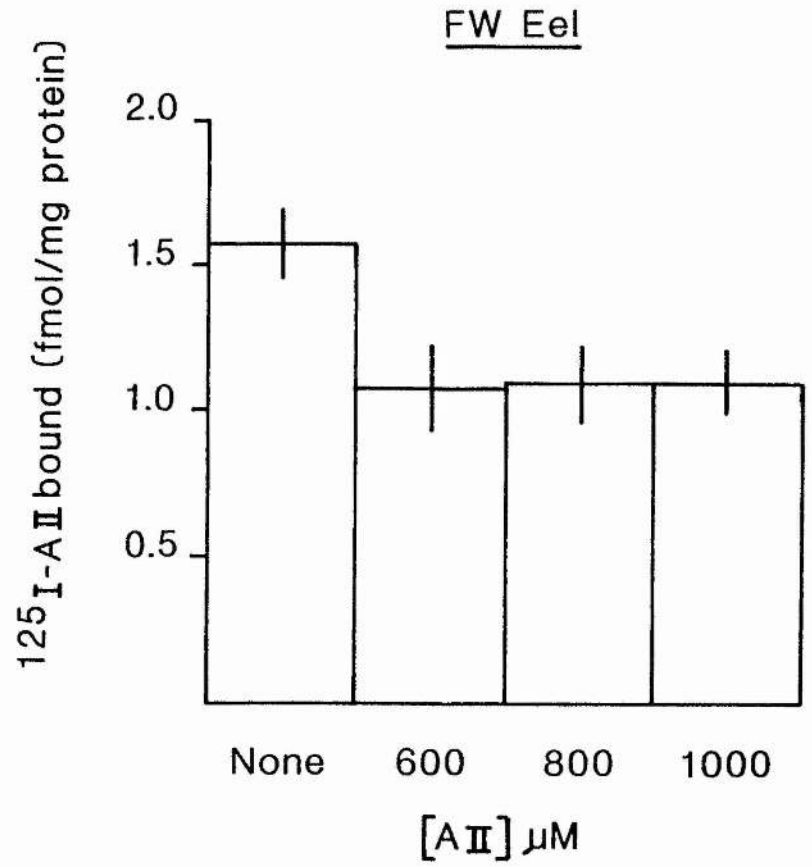
COMPARISON OF ^{125}I-AII BINDING ON INCUBATION OF EEL LIVER MEMBRANE PREPARATIONS AT $4^{o}C$ vs $22^{o}C$

Total and specific binding were both reduced considerably by incubation at $4^{o}C$ compared with $22^{o}C$ in both types of eel (fig 3.3.2), total binding was reduced from 1.85+0.30 to 1.2±0.27fmol/mg protein and specific binding from 0.77±0.11 to 0.2±0.06fmol/mg protein ($p < 0.05$)(in FW eel liver membranes.

Fig 3.3.1 ESTIMATION OF NON-SPECIFIC BINDING IN BOTH FW AND SW EEL LIVER MEMBRANE PREPARATIONS

Fig 3.3.1 Estimation of Non-Specific Binding in Both FW and SW Eel Liver Membrane Preparations

Liver membrane from 5 FW and 3 SW preparations were incubated with ^{125}I-angiotensin II (^{125}I-AII) (average final conc 22.97 pM) in the absence and with the addition of 600uM, 800uM and 1mM concentrations of angiotensin II (AII). Results indicate mean $\pm$ S.E.M.


FW Eel
^{125}I-AII bound (fmol/mg protein)
2.0
1.5
1.0
0.5
None
600
800
1000
[AII] μM

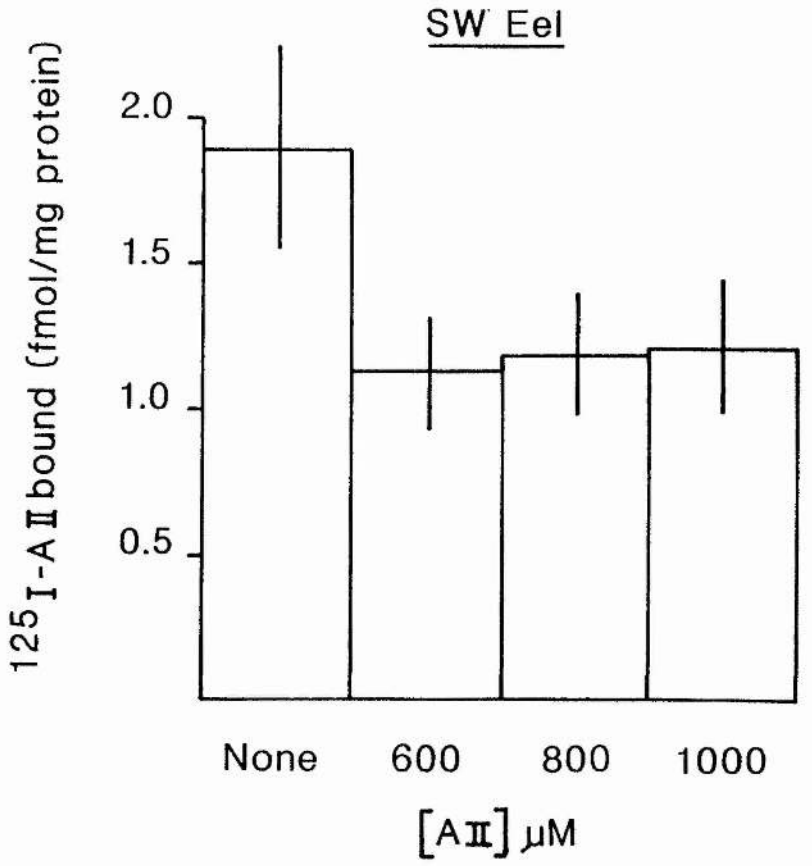
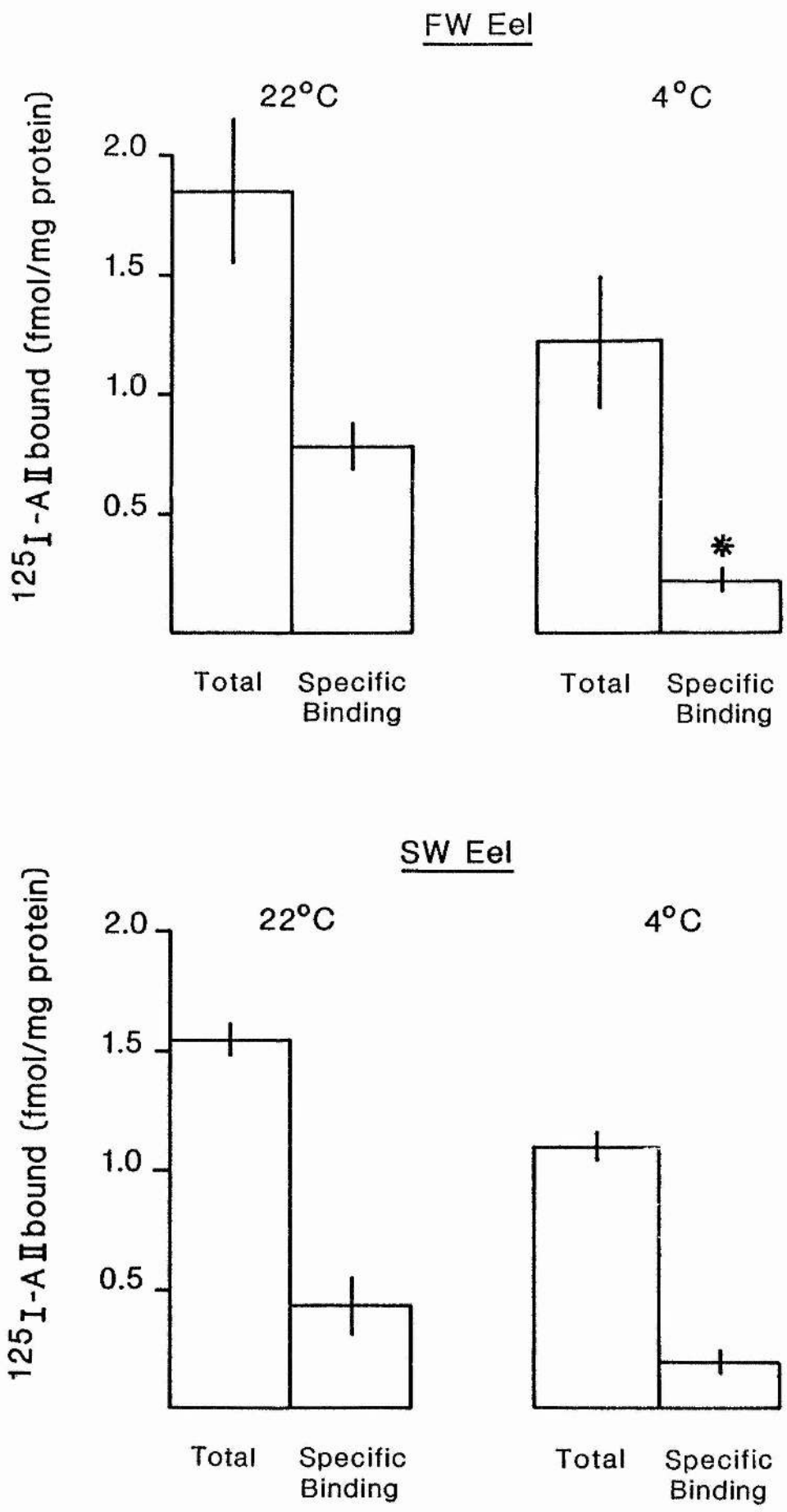

SW Eel
^{125}I-AII bound (fmol/mg protein)
2.0
1.5
1.0
0.5
None
600
800
1000
[AII] μM

Fig 3.3.2 COMPARISON OF ^{125}I-AII BINDING ON INCUBATION OF EEL LIVER MEMBRANE PREPARATION AT 4^{o}C VS 22^{o}C.

Fig 3.3.2 Comparison of ^{125}I-AII Binding on Incubation of Eel Liver Membrane Preparations at 4^{o}C VS 22^{o}C

Incubations were performed at 22^{o}C and 4^{o}C for 1hr in the absence and presence of 600uM AII to estimate specific binding of ^{125}I-angiotensin II (^{125}I-AII) (mean final concentration 22.17pM). Values represent mean $\pm$ S.E.M. from 5FW and 4SW preparations, *$p < 0.05$ as determined by a Mann-Whitney U test.


FW Eel
22°C
4°C
2.0
1.5
1.0
0.5
^{125}I-AII bound (fmol/mg protein)
*
Total
Specific Binding
Total
Specific Binding
SW Eel
22°C
4°C
2.0
1.5
1.0
0.5
^{125}I-AII bound (fmol/mg protein)
Total
Specific Binding
Total
Specific Binding

Total binding decreased from 1.54±0.08 to 1.09±0.07fmol/mg protein and specific binding was reduced from 0.42+0.12 to 0.16±0.05fmol/mg protein on incubation of SW eel liver membrane preparations, at 4°C compared to 22°C. Non-specific binding was relatively constant in both preparations.

^{125}I-AII RECEPTOR BINDING ON DILUTION OF EEL LIVER MEMBRANE PREPARATIONS

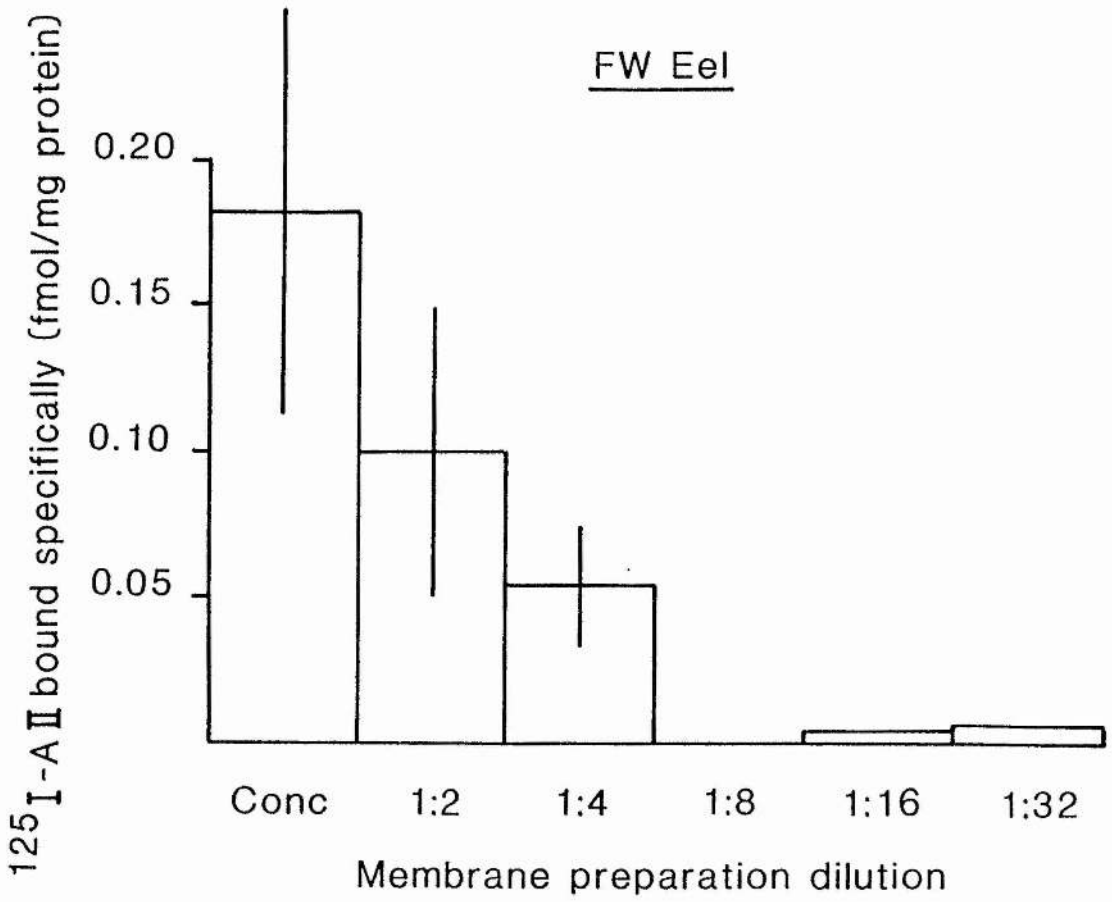
Specific binding of ^{125}I-AII decreased proportionally with decreasing protein concentration of the membrane preparation, $p < 0.05$ (fig 3.3.3). At half protein concentration of FW preparations, specific binding was reduced to 56.9% (0.1±0.05fmol/mg protein) and similarly at quarter of the protein concentration specific binding declined to 28.7% (0.05±0.02fmol/mg protein) that of the concentrated preparation (0.18±0.07fmol/mg protein). A SW preparation behaved comparably producing reductions in specific binding to 36% (0.07fmol/mg protein) and 12.1%(0.023fmol/mg protein) at the equivalent 1:2 and 1:4 dilutions.

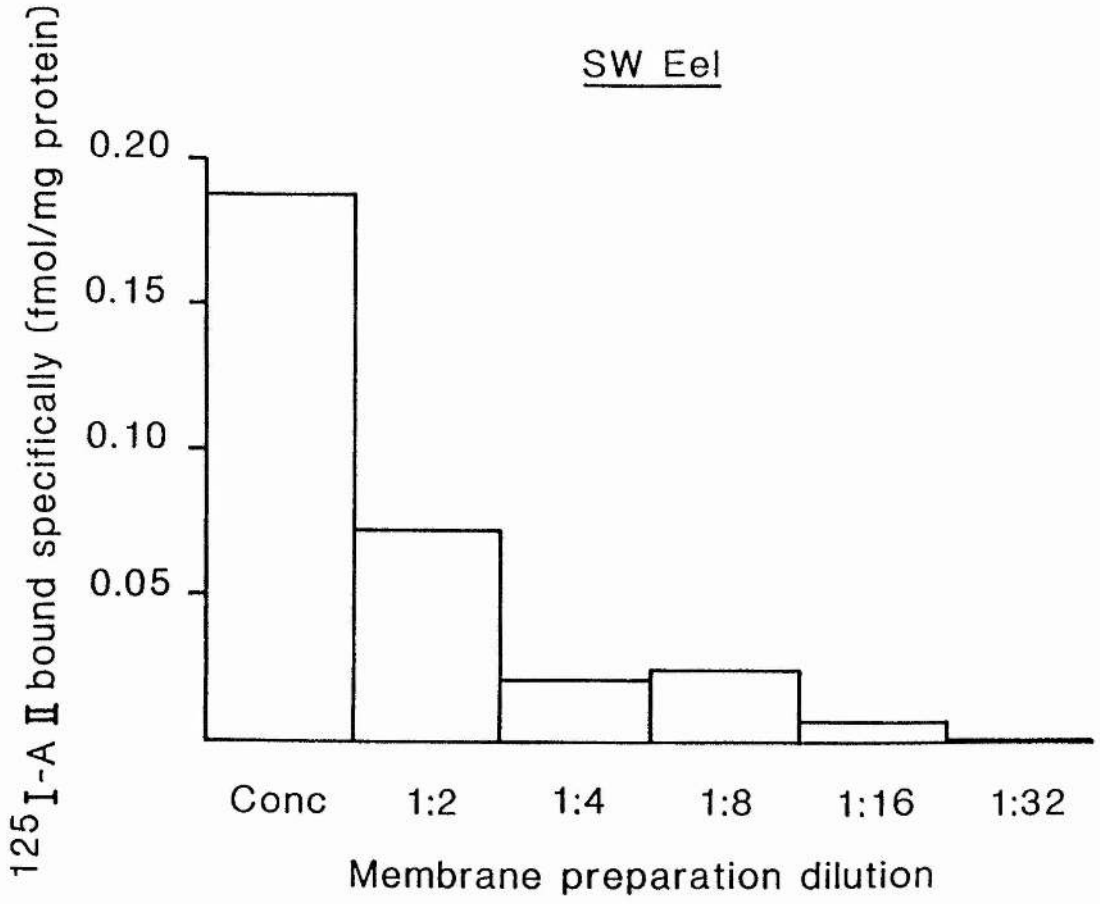
This trend of diminished binding corresponding with membrane dilution is not consistent throughout the experiment. This could be attributed to the fact that at dilutions of 1:8 and greater (less than 0.57mg protein/ml preparation), any apparent specific binding is extremely low in terms of cpm bound as a percentage of total ^{125}I-AII present during incubation. Mean total binding of ^{125}I-AII is 1.64, 1.30 and 1.14% for the 1:8, 1:16, 1:32 dilutions

Fig 3.3.3 ^{125}I-AII RECEPTOR BINDING ON DILUTION OF EEL LIVER MEMBRANE PREPARATION

Fig 3.3.3 ^{125}I-AII Receptor Binding on Dilution of Eel Liver Membrane Preparation

Liver membrane preparations were diluted serially with equivalent volumes of 50mM Tris buffer (pH7.0) in the absence of calcium. Incubations were performed with and without 600uM AII and with an average final ^{125}I-angiotensin II (^{125}I-AII) concentration of 23.63pM. Values represent mean $\pm$ S.E.M. from 3FW and 1SW preparation. Statistical analysis was determined using the one way ANOVA method.


FW Eel
0.20
0.15
0.10
0.05
^{125}I-A II bound specifically (fmol/mg protein)
Conc
1:2
1:4
1:8
1:16
1:32
Membrane preparation dilution


SW Eel
0.20
0.15
0.10
0.05
^{125}I-A II bound specifically (fmol/mg protein)
Conc
1:2
1:4
1:8
1:16
1:32
Membrane preparation dilution

respectively of both eel preparations, compared to 9.11% for the concentrated preparation. Mean ^{125}I-AII binding apparently not displaced by 600uM AII for both eel preparations is 0.064, 0.052 and 0.046% of total ^{125}I-AII available for the 1:8, 1:16 and 1:32 dilutions respectively, the equivalent value for the concentrated preparation being 1.93%.

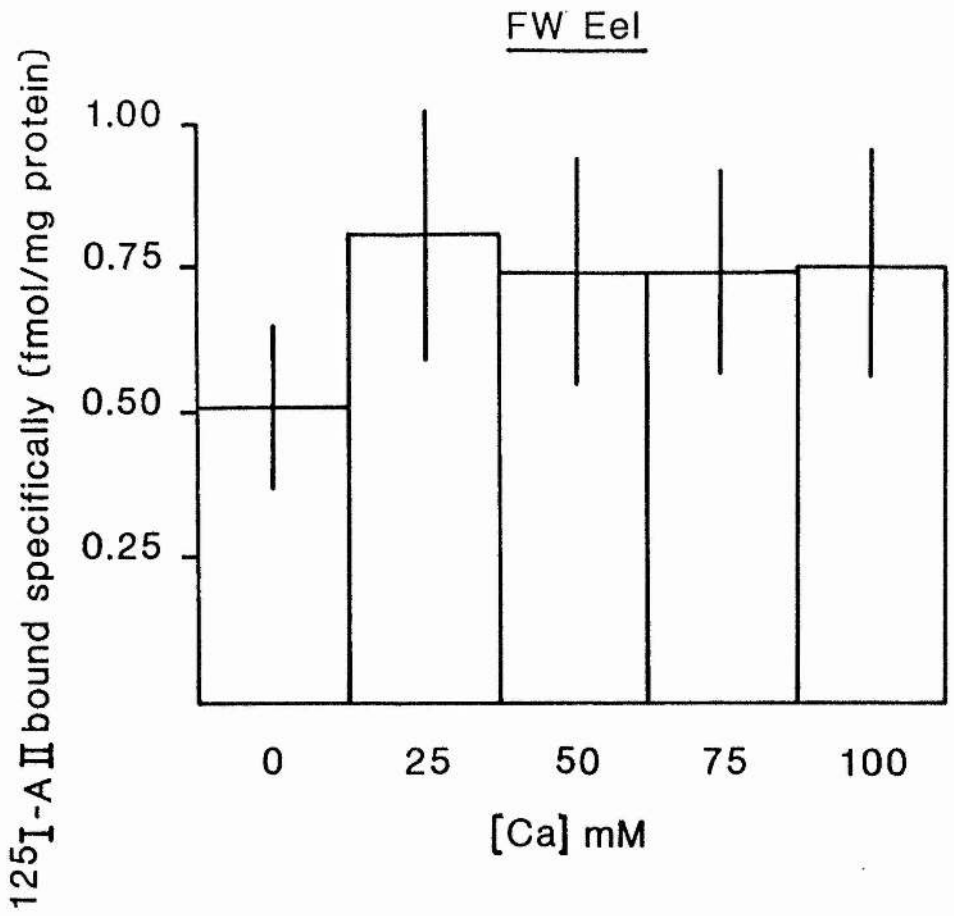
EFFECT OF CALCIUM ON AII RECEPTOR BINDING

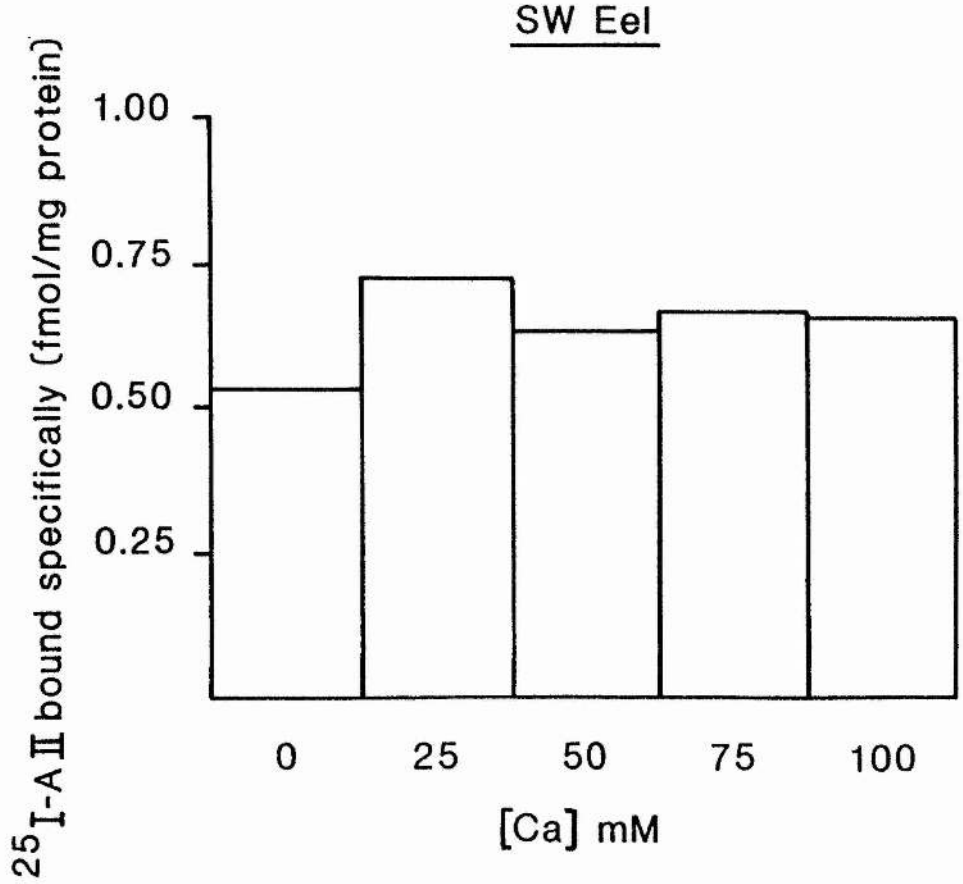
Addition of calcium to both FW and SW eel liver membrane preparations resulted in increased specific binding of ^{125}I-AII (fig 3.3.4a). This binding was maximal at a calcium concentration of 25mM in both cases and was appreciably lower, but relatively consistent over the remaining ion concentrations. 25mM calcium produced an increase of 59% to 0.81±0.21fmol/mg protein over basal binding (0.51±0.14fmol/mg protein) and 50, 75 and 100mM calcium increased binding in the FW preparation to 0.74±0.2, 0.74±0.17 and 0.75±0.19fmol/mg protein respectively. 25mM calcium also produced the greatest binding (0.7fmol/mg protein) in the SW preparation compared to basal (0.53fmol/mg protein). 50, 75 and 100mM calcium also increased ^{125}I-AII binding to 0.63, 0.65 and 0.64 fmol/mg protein, respectively. Non-specific binding was relatively consistent across the four calcium concentrations at 0.72-0.76fmol/mg protein, but was slightly higher in the preparation without calcium at 0.81fmol/mg protein.

Fig 3.3.4a EFFECT OF CALCIUM ON SPECIFIC RECEPTOR BINDING IN EEL LIVER MEMBRANE PREPARATIONS

Fig 3.3.4a **Effect of Calcium on Specific Receptor Binding of Eel Liver Membrane Preparations**

Calcium (0-100mM) was added to the liver membrane preparation immediately prior to incubation in an appropriate volume so as not to exceed the normal incubation volume of 400ul. (Ion concentrations refer to that of the membrane preparation and not of the final incubation cocktail). Membranes were incubated in the absence and presence of 600uM AII to determine specific binding of ^{125}I-angiotensin II (^{125}I-AII). Results signify mean $\pm$ S.E.M. for 3FW membrane preparations and duplicate determinations from 1SW preparation which varied by less than 10%.


FW Eel
1.00
0.75
0.50
0.25
^{125}I-A II bound specifically (fmol/mg protein)
0
25
50
75
100
[Ca] mM


SW Eel
1.00
0.75
0.50
0.25
^{125}I-A II bound specifically (fmol/mg protein)
0
25
50
75
100
[Ca] mM

Calcium also appears to increase total ^{125}I-AII binding in kidney membranes (fig 3.3.4b). 25mM calcium produced an increase of 29% to 0.9±0.4fmol/mg protein compared to basal binding of 0.7±0.28fmol/mg protein. However maximal binding was attained in this preparation at calcium concentrations of between 50 and 75mM at 0.93fmol/mg, which corresponds to an increase of 33% over basal binding. 25mM calcium produced maximal ^{125}I-AII binding of 0.62fmol/mg protein in the SW preparation which is an increase of 75.3% over basal binding of 0.36fmol/mg protein. Binding decreased thereafter with increasing calcium concentration.

The presence of 4M $MgCl_2$ during incubation of receptors with ^{125}I-AII greatly reduced total binding. Addition of 10mM $MgCl_2$ either directly to the membrane preparation or after removal of 4M $MgCl_2$ bore little effect on total ^{125}I-AII binding.

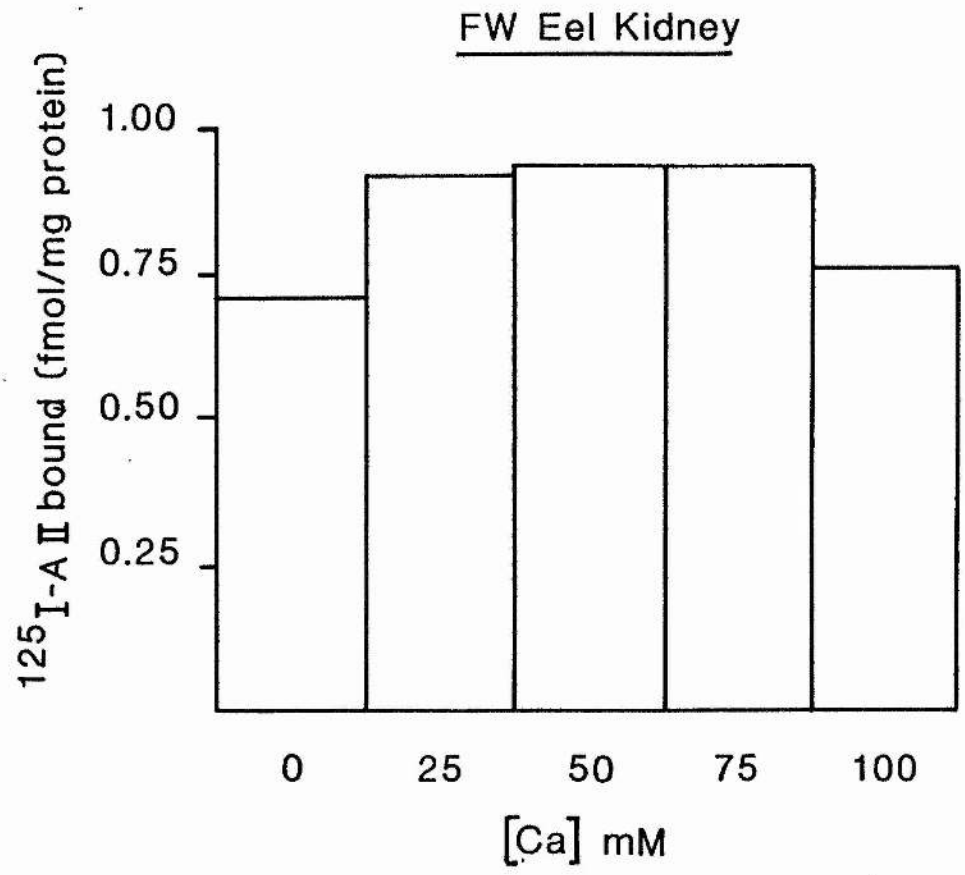
CHANGES IN ^{125}I-AII RECEPTOR BINDING WITH VARYING CONCENTRATION OF TOTAL ^{125}I-AII PRESENT DURING INCUBATION OF EEL LIVER MEMBRANE PREPARATIONS

Specific binding of ^{125}I-AII (fmol/mg protein) was directly proportional to ^{125}I-AII concentration of the incubation medium in both FW and SW eel preparations (fig 3.3.5a-c). Total and non-specific binding of the iodinated peptide also varied directly with total counts present. Results could not be expressed in terms of initial ^{125}I-AII concentration due to variation in the maximum concentration of iodinated peptide available and therefore total counts present on incubation of individual membrane

Fig 3.3.4b Effect of Calcium on Receptor Binding in Eel Kidney Membrane Preparations

Fig 3.3.4b **Effect of Calcium on Receptor Binding in Eel Kidney Membrane Preparations**

Eel kidney membranes were incubated with 0-100mM $CaCl_2$ for 1hr at 22°C. results indicate duplicate determinations for 2FW and 1SW preparation which varied by less than 10%.


FW Eel Kidney
^{125}I-A II bound (fmol/mg protein)
1.00
0.75
0.50
0.25
0
25
50
75
100
[Ca] mM

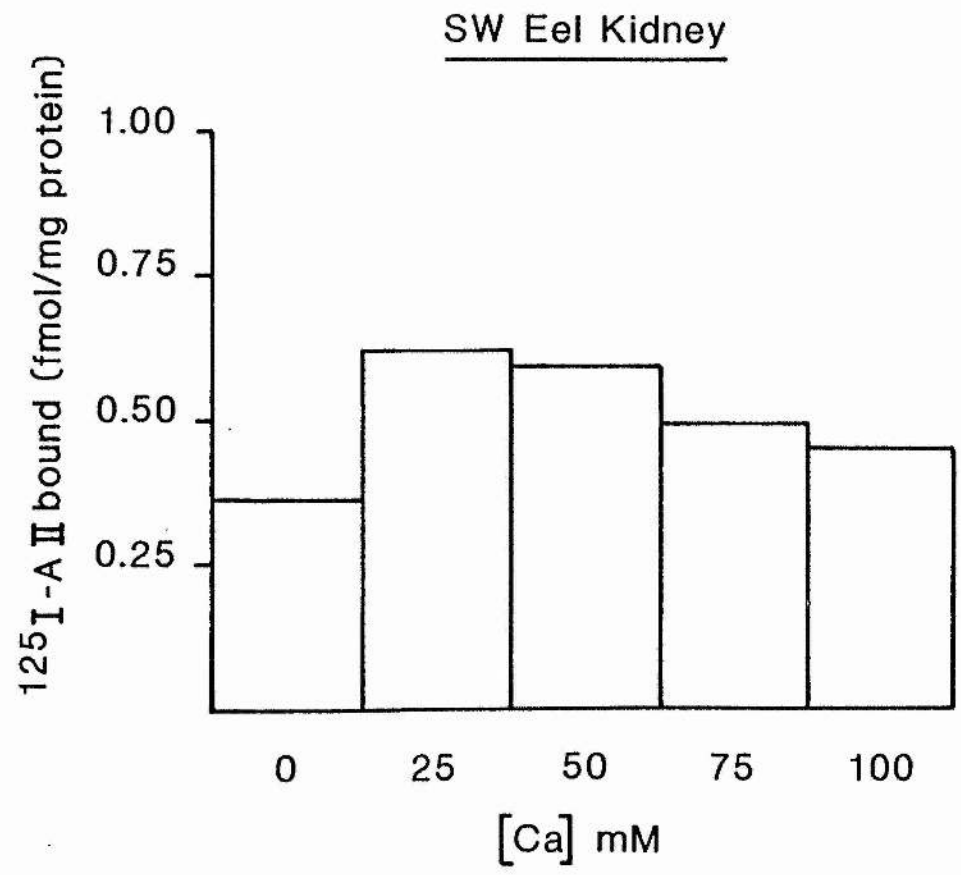

SW Eel Kidney
^{125}I-A II bound (fmol/mg protein)
1.00
0.75
0.50
0.25
0
25
50
75
100
[Ca] mM

Fig 3.3.5a CHANGES IN ^{125}I-AII RECEPTOR BINDING WITH VARYING CONCENTRATIONS OF TOTAL ^{125}I-AII PRESENT DURING INCUBATION OF FW EEL LIVER MEMBRANE PREPARATIONS

Fig 3.3.5a **Changes in ^{125}I-AII Receptor Binding with Varying Concentration of Total ^{125}I-AII Present During Incubation of FW Eel Liver Membrane Preparations**

FW eel liver membranes were incubated with 0.37-403pM ^{125}I-AII (final concentration) for 1hr at 22^{o}C (n=5). results reflect duplicate determinations which varied by less than 10%.

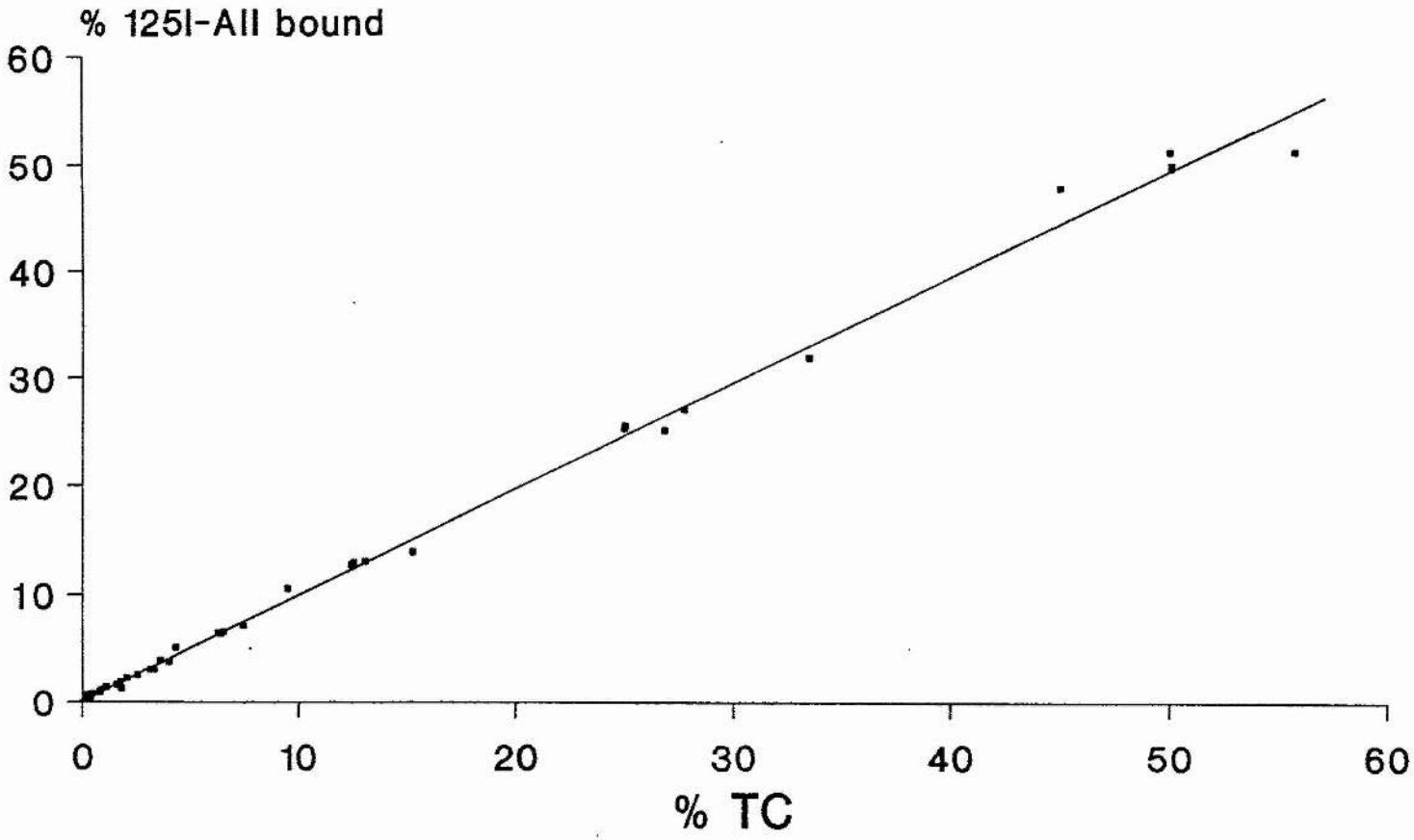

% 125I-AII bound
60
50
40
30
20
10
0
0
10
20
30
40
50
60
% TC

Fig 3.3.5b Changes in Specific ^{125}I-AII Receptor Binding with Varying Concentration of Total ^{125}I-AII Present During Incubation of FW Eel Liver Membrane Preparations

Fig 3.3.5b **Changes in Specific ^{125}I-AII Receptor Binding with Varying Concentration of Total ^{125}I-AII Present During Incubation of FW Eel Liver Membrane Preparations**

FW eel liver membranes were incubated with 15.8-314.2pM ^{125}I-AII in the absence and presence of 600uM AII to estimate specific binding (n=2). Points indicate duplicate determinations which varied by less than 10%.

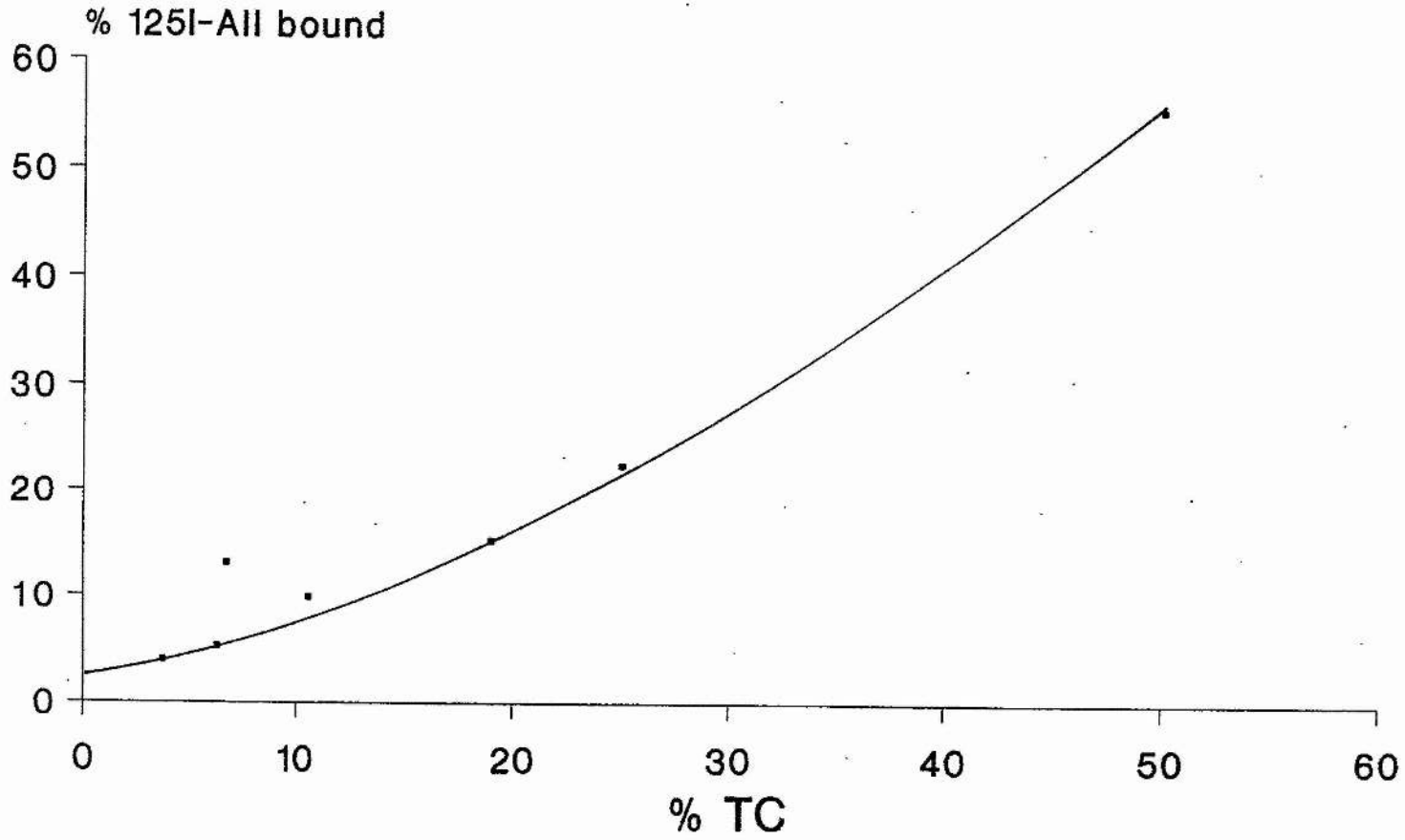
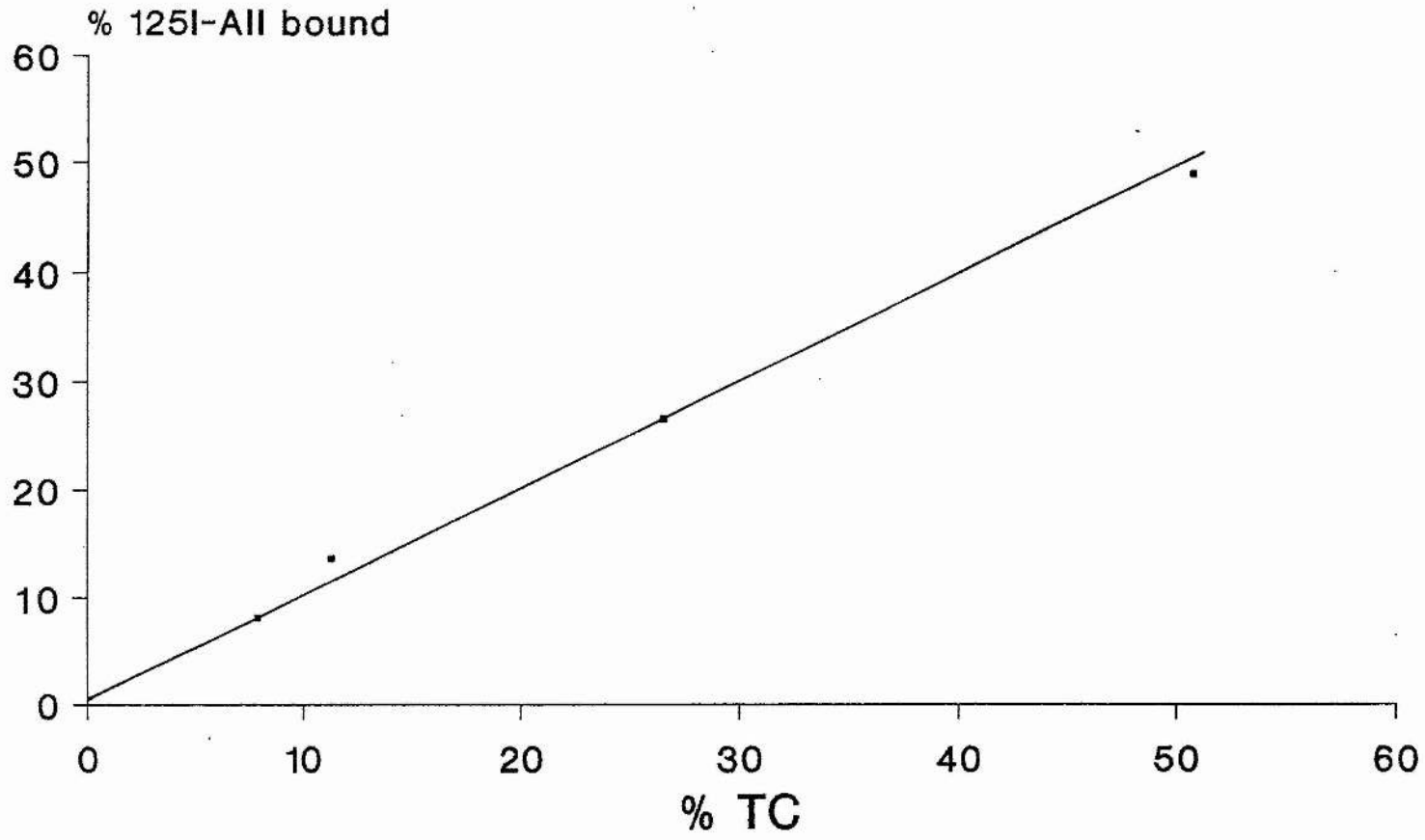

% 125I-AII bound
60
50
40
30
20
10
0
0
10
20
30
40
50
60
% TC

Fig 3.3.5c **Changes in Specific ^{125}I-AII Receptor Binding with Varying Concentration of Total ^{125}I-AII Present during Incubation of a SW Eel Liver Membrane Preparation**

SW eel membranes (n=1) were incubated with 16.6-211.7pM ^{125}I-AII in the absence and presence of 600uM AII. Points indicate replicate samples which differed by 10%.

Fig 3.3.5c CHANGES IN SPECIFIC ^{125}I-AII RECEPTOR BINDING WITH VARYING CONCENTRATION OF TOTAL ^{125}I-AII PRESENT DURING INCUBATION OF A SW EEL LIVER MEMBRANE PREPARATION


% 125I-AII bound
60
50
40
30
20
10
0
0
10
20
30
40
50
60
% TC

preparations. Different binding characteristics of individual membrane preparations also meant that the maximum percentage bound varied, however a reduction in TC present during incubation lead to a corresponding reduction in the percentage of peptide bound by an equivalent factor.

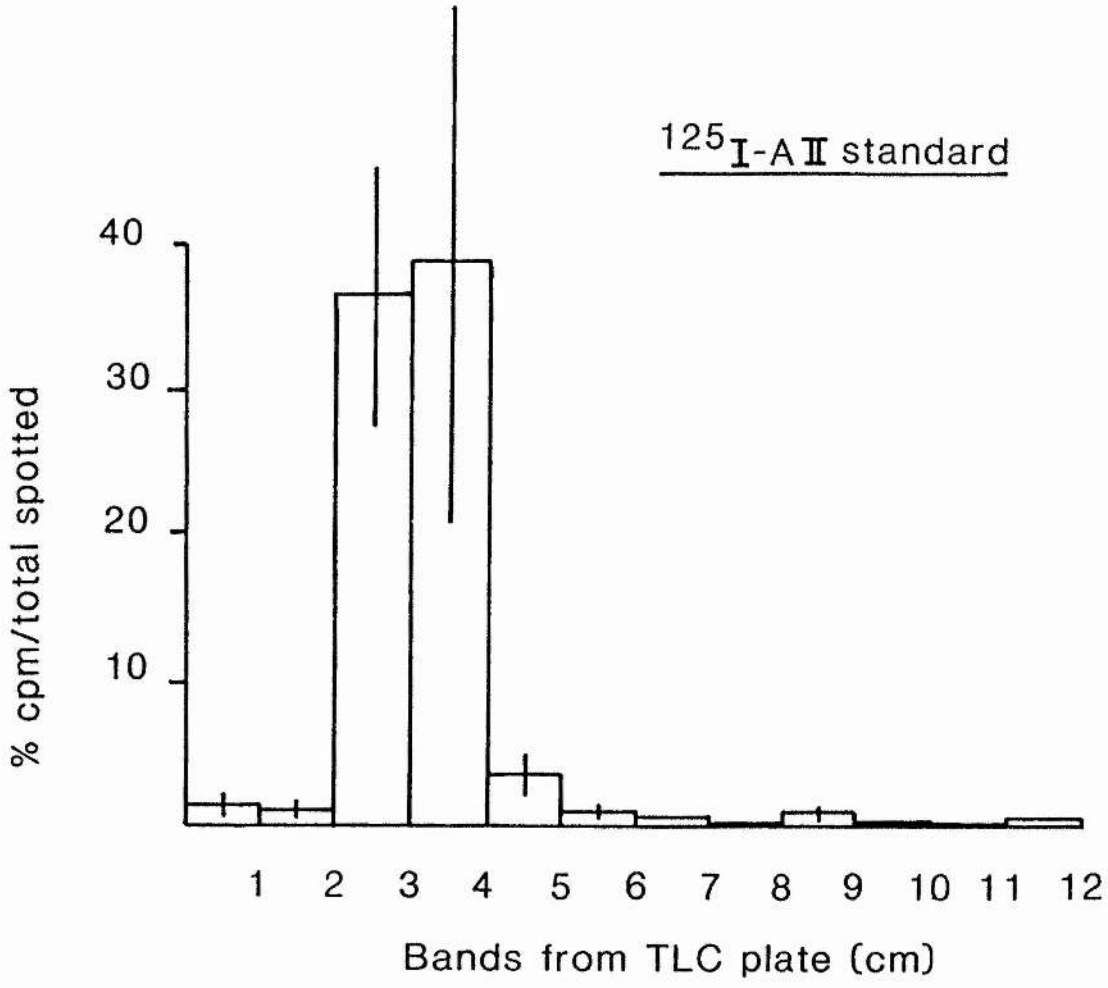
ASSESSMENT OF ^{125}-I-AII DEGRADATION DURING MEMBRANE INCUBATION

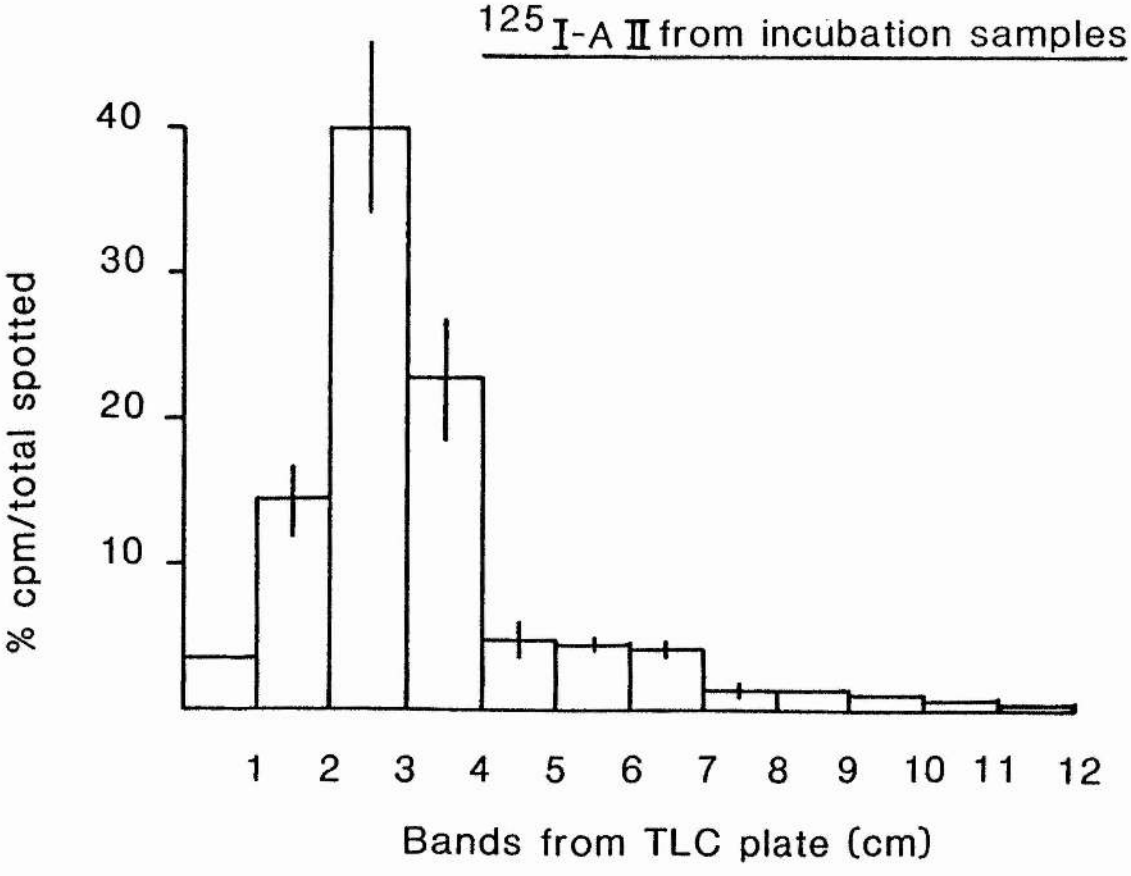
^{125}I-AII standard was spotted onto TLC plates directly from a stock solution stored in acid ethanol at -20^{o}C. Samples consisted of aliquots of supernatant retrieved from separation of membrane pellet bound ^{125}I-AII from free ^{125}I-AII following incubation. These samples were collected from a variety of FW eel liver membrane preparations which had been incubated in the absence and presence of 600uM AII. The supernatants were frozen and stored at -20^{o}C for a period of weeks prior to TLC. Results of TLC (fig 3.3.6) show that the majority of ^{125}I spotted as ^{125}I-AII standard can be traced to the region extending from 2-4cm above origin, 75.1%, whereas ^{125}I spotted from incubation samples covered a greater area of 1-4cm above origin in accounting for 79.6% of the total radioactivity spotted. This slight discrepancy can be attributed to the fact that only 3ul standard volumes were spotted, but sample aliquots were 50ul. This greater sample volume would produce a migrating spot of larger diameter which would occupy a larger area of the TLC column.

Fig 3.3.6 ASSESSMENT OF ^{125}I-AII DEGRADATION DURING MEMBRANE INCUBATION

Fig 3.3.6 **Assessment of ^{125}I-AII Degradation During Membrane Incubation**

Volumes of 3ul stock ^{125}I-angiotensin II (^{125}I-AII) as standard and 50ul incubation samples containing ^{125}I-AII were spotted on individual thin layer chromatography (TLC) columns and allowed to develop until the solvent front reached 12cm. Individual 1cm bands were removed from separate columns and counted for ^{125}I activity. Results indicate determinations from 3 standard and 10 sample columns and are expressed as mean $\pm$ S.E.M.


^{125}I-AII standard
40
30
20
10
% cpm/total spotted
1 2 3 4 5 6 7 8 9 10 11 12
Bands from TLC plate (cm)


^{125}I-AII from incubation samples
40
30
20
10
% cpm/total spotted
1 2 3 4 5 6 7 8 9 10 11 12
Bands from TLC plate (cm)

3.4 STUDIES UTILISING ^{125}I-AII BINDING TO INVESTIGATE ANGIOTENSIN RECEPTOR CHARACTERISTICS

TIME COURSE FOR THE BINDING OF ^{125}I-AII TO FW EEL LIVER MEMBRANE PREPARATION

Total binding of ^{125}I-AII increased with time (fig 3.4.1) on incubation of FW eel liver membrane preparations at 22^{o}C over the 140min time period studied. Total binding (4.83fmol/mg protein) appeared to be nearing saturation at 140mins and non-specific binding reached a maximum of 3.64fmol/mg protein at 80mins and remained constant thereafter.

DISPLACEMENT OF ^{125}I-AII FROM EEL LIVER MEMBRANE PREPARATIONS BY AII

Displacement of ^{125}I-AII from FW eel liver membrane preparations by nanomolar concentrations of AII was achieved using the range 0.05-25.6nM (fig 3.4.2a). The concentration of AII required to produce 50% inhibition of ^{125}I-AII binding (IC50) was 0.9nM and was determined by transformation of the data in fig 3.4.2a (see fig 3.4.2b). Figures derived from Scatchard analysis (fig 3.4.2c) indicate 2 binding sites; one low affinity, high capacity K_d=2.46x10^{-10}M and n=14.0 fmol/mg protein and one high affinity, low capacity with K_d=1.52x10^{-11}M and n=0.211 fmol/mg protein.

Fig 3.4.1 **Time Course for the Binding of ^{125}I-AII to FW Eel Liver Membrane Preparations**

^{125}I-angiotensin II (^{125}I-AII) was incubated with 7 different FW eel liver membrane preparations at 22^{o}C for intervals up to 140mins. Results are for total binding and are expressed as mean $\pm$ S.E.M.

Fig 3.4.1 TIME COURSE FOR THE BINDING OF ^{125}I-AII TO FW EEL LIVER MEMBRANE PREPARATIONS

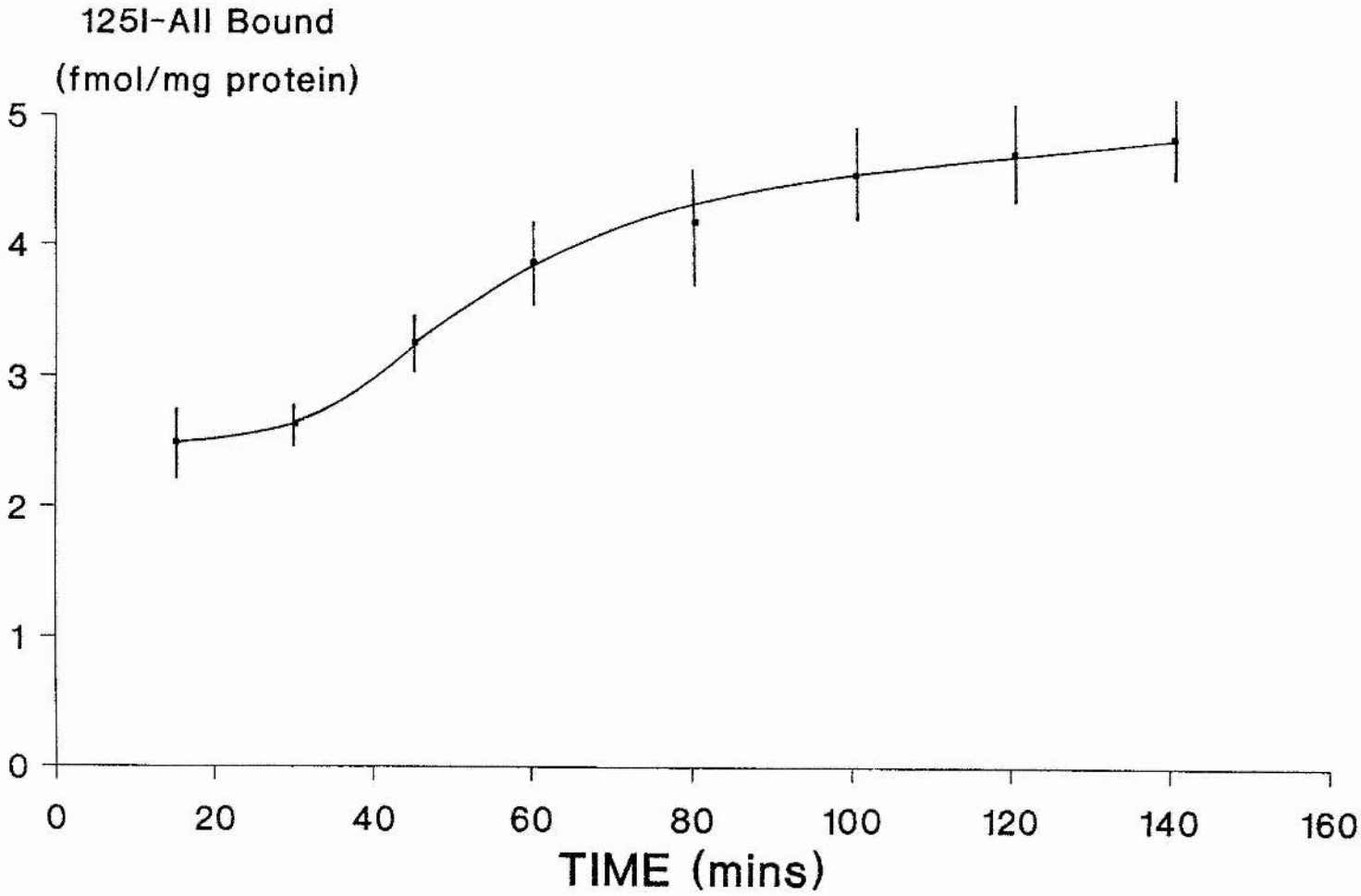

125I-AII Bound
(fmol/mg protein)
5
4
3
2
1
0
0
20
40
60
80
100
120
140
160
TIME (mins)

Fig 3.4.2a **Displacement Curve of ^{125}I-AII by nM concentrations of AII in FW Eel Liver Membrane Preparations**

FW eel liver membranes (8.52mg protein/ml) were incubated at 22^{o}C for 60 mins with 23.19pM ^{125}I-angiotensin II (^{125}I-AII) (final conc.) and a range of AII concentrations (0-25.6nM). NSB was estimated using 10uM AII. Results represent duplicate determinations, which varied by less than 10%, from a single preparation.

DISPLACEMENT OF ^{125}I-AII BY nM CONCENTRATIONS OF AII IN A FW EEL LIVER MEMBRANE PREPARATION

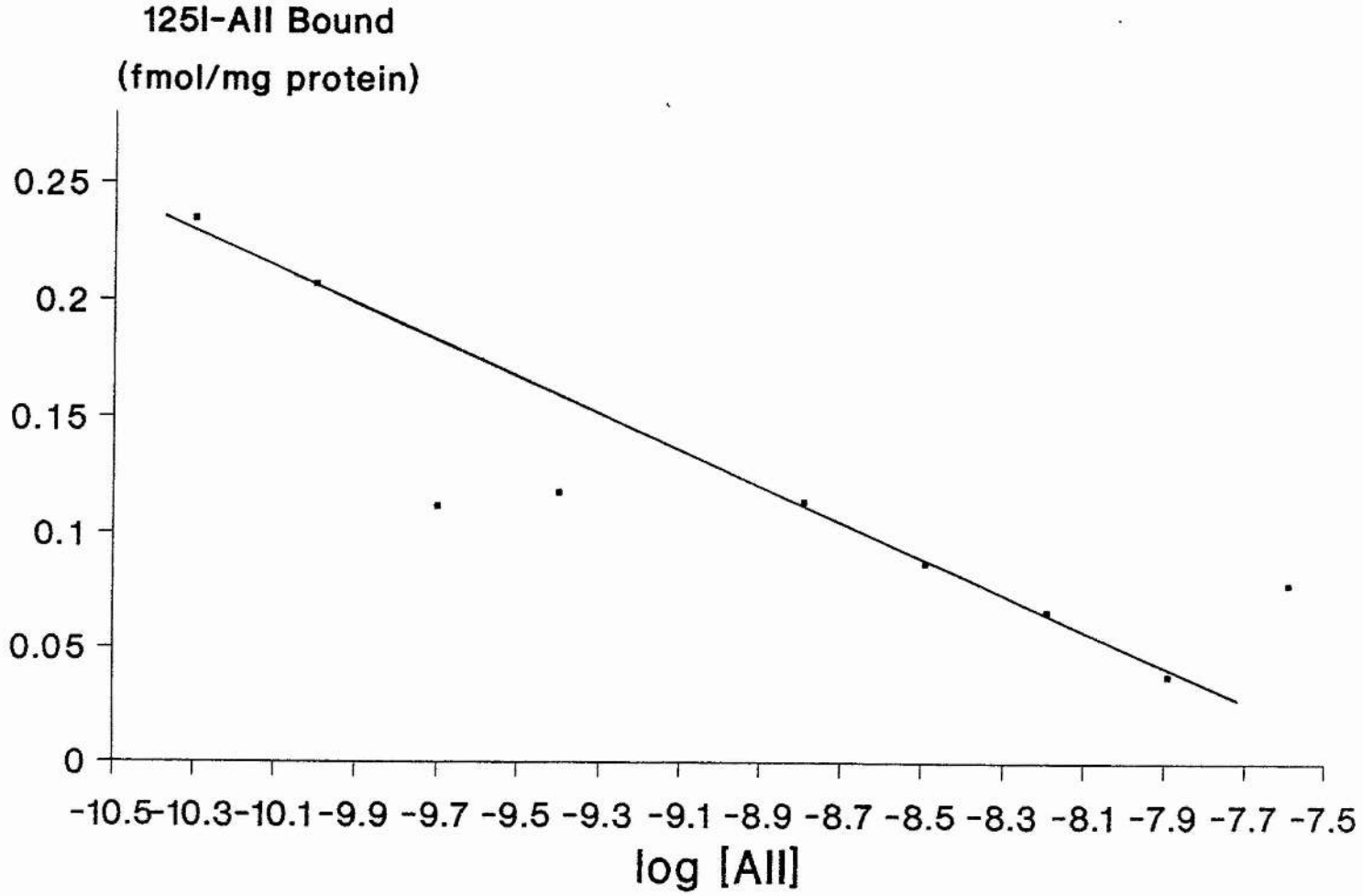

125I-AII Bound
(fmol/mg protein)
0.25
0.2
0.15
0.1
0.05
0
-10.5 -10.3 -10.1 -9.9 -9.7 -9.5 -9.3 -9.1 -8.9 -8.7 -8.5 -8.3 -8.1 -7.9 -7.7 -7.5
log [AII]

Fig 3.4.2b **Dose response Curve for AII (nM) Displacement of ^{125}I-AII from a FW Eel Liver membrane Preparation**

Results shown in fig. 3.4.1a were corrected for NSB and expressed as percentage displacement of ^{125}I-AII binding by angiotensin II (AII). Points represent duplicate determinations, which varied by less than 10%, from a single preparation.

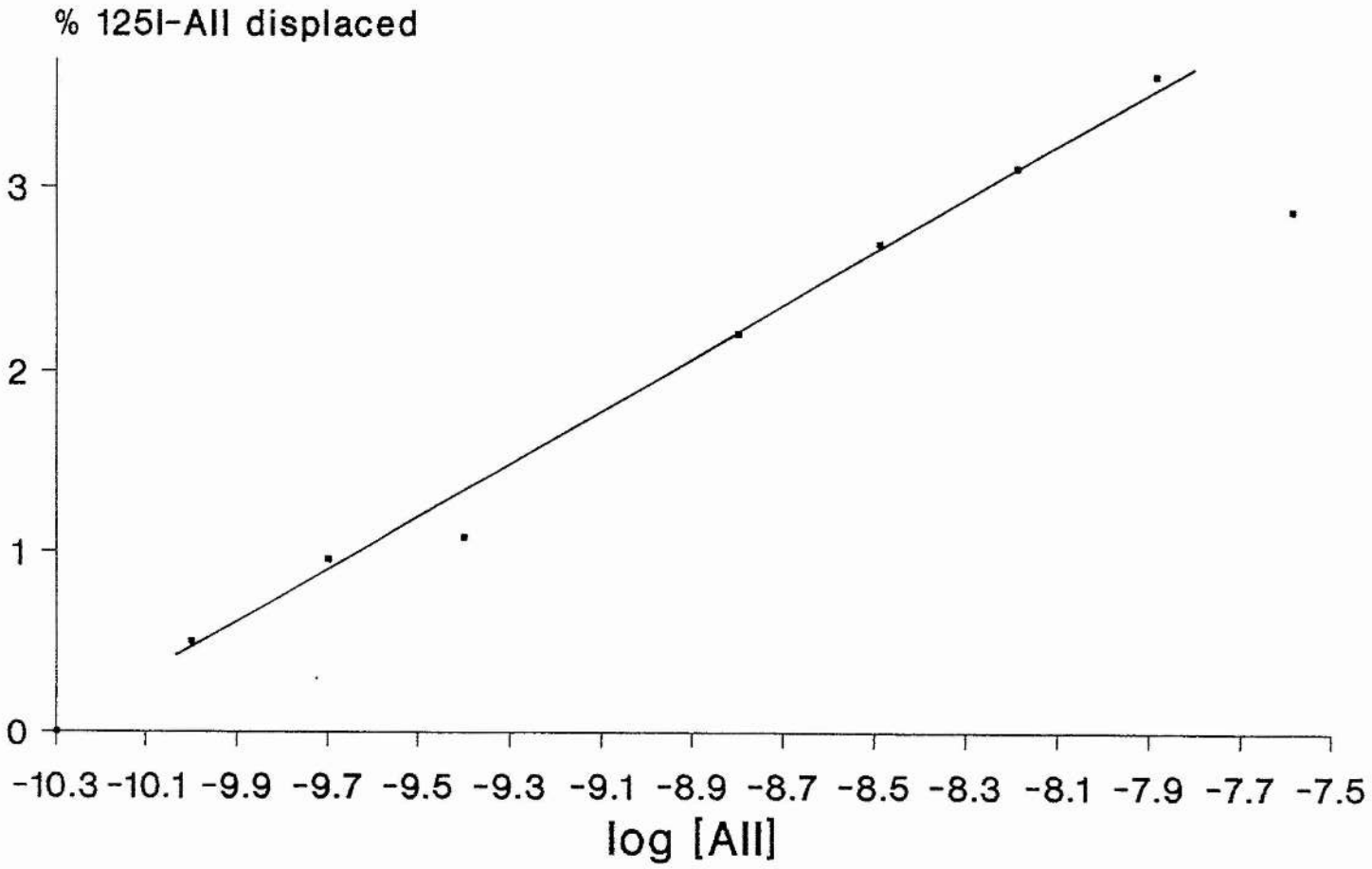
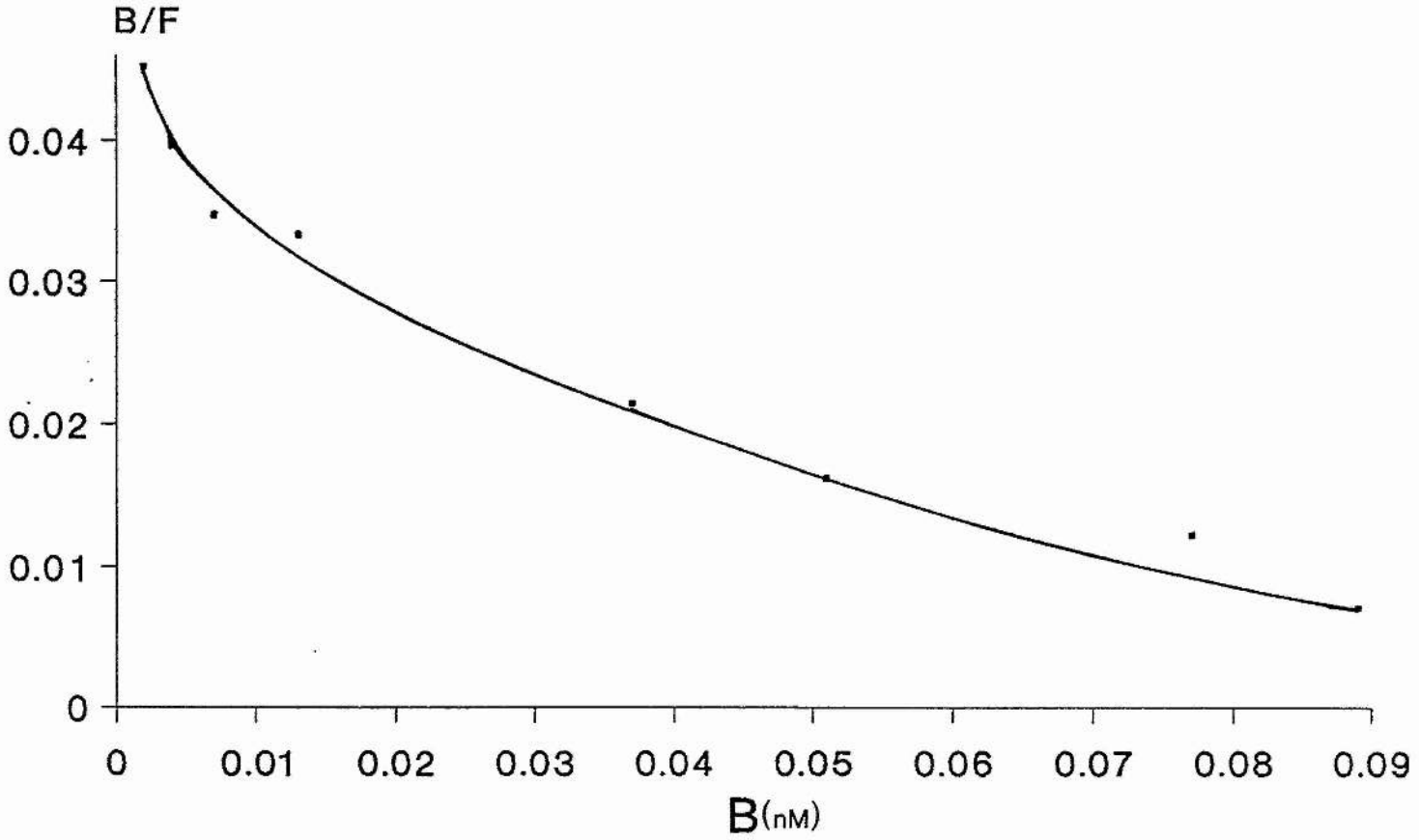

% 125I-AII displaced
3
2
1
0
-10.3 -10.1 -9.9 -9.7 -9.5 -9.3 -9.1 -8.9 -8.7 -8.5 -8.3 -8.1 -7.9 -7.7 -7.5
log [AII]

Fig 3.4.2c A Scatchard plot of the data from fig. 3.4.2b B=bound, F=free. Protein concentration of the membrane preparation was 8.52 mg/ml.


B/F
0.04
0.03
0.02
0.01
0
0
0.01
0.02
0.03
0.04
0.05
0.06
0.07
0.08
0.09
B(nM)

Further experiments using FW eel liver membrane preparations required micromolar concentrations of AII to produce ^{125}I-AII displacement (fig 3.4.3a). The AII concentrations necessary were found to fall in the range 0.4-600uM. Displacement was assessed in a number of preparations (fig 3.4.3b) and an IC50 value of 19.6uM was determined by combination of the data in fig 3.4.3a. This potency of inhibition of ^{125}I-AII binding varied from 14.7 to 31.3uM AII. Figures derived from Scatchard analysis (fig 3.4.3c) reveal a single class of binding sites with K_d=3.31x10^{-8}M and n=0.637nmol/mg protein.

Displacement of ^{125}I-AII from SW eel liver membranes (fig 3.4.4a) also required micromolar concentrations of AII (0.4-600uM) with an IC50 of 15.8uM (fig 3.4.4b). Results derived from Scatchard analysis indicate a single class of receptors with K_d=1.09x10^{-7}M and n=0.241nmol/mg protein.

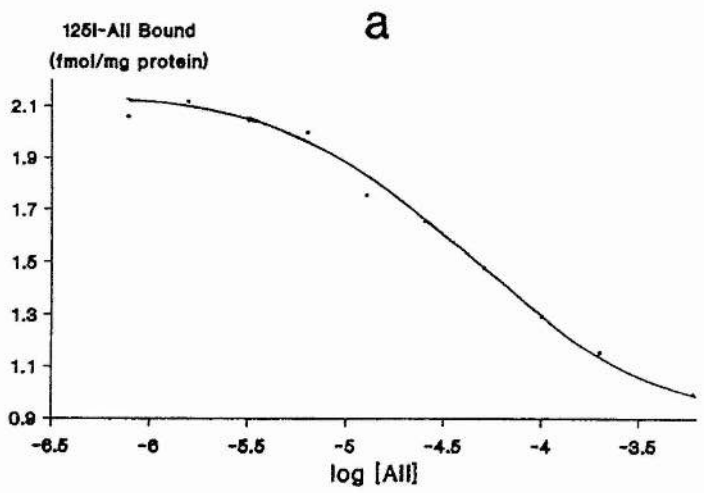
INCUBATION OF LIVER MEMBRANE PREPARATIONS FOR 4 FISH TYPES WITH THE ILE^5 AND VAL^5 SEQUENCES OF AII AND ^{125}I-AII

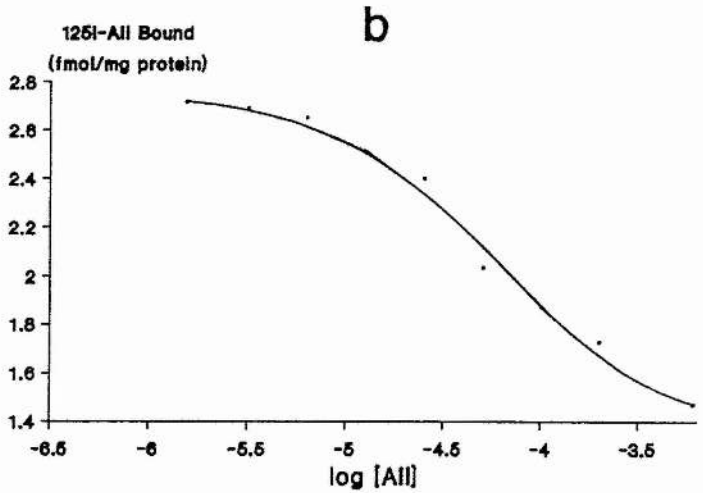
The FW eel liver membrane preparation was apparently more responsive to the ile^5 sequences of ^{125}I-AII and AII (fig 3.4.5a), producing both greater total and specific binding of 7.4 and 3.02fmol/mg protein respectively, than the equivalent incubation with the val^5-AII sequence which resulted in 4.04 and 1.37fmol/mg protein for total and specific binding respectively.

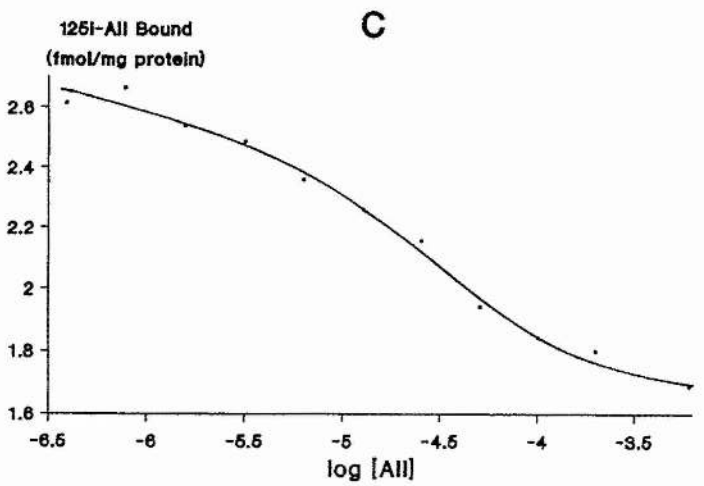
DISPLACEMENT OF ^{125}I-AII FROM EEL LIVER MEMBRANE PREPARATIONS BY MICROMOLAR CONCENTRATION OF AII.

Fig 3.4.3a **Typical Displacement Curves of ^{125}I-AII by uM Concentrations of AII in FW Eel Liver Membrane Preparations**

FW eel liver membranes from 4 individual preparations were incubated at 22^{o}C for 60 min with a range of angiotensin II (AII) concentrations from 0-600uM. ^{125}I-AII concentration of the incubation medium was a) 22.28pM b) 21.33pM c) 25.07pM d)20.82pM for the 4 preparations respectively. Graphical points indicate duplicate determinations which differed by less than 10%.


a
125I-AII Bound
(fmol/mg protein)
2.1
1.9
1.7
1.5
1.3
1.1
0.9
-6.5
-6
-5.5
-5
-4.5
-4
-3.5
log [AII]


b
125I-AII Bound
(fmol/mg protein)
2.8
2.6
2.4
2.2
2
1.8
1.6
1.4
-6.5
-6
-5.5
-5
-4.5
-4
-3.5
log [AII]


c
125I-AII Bound
(fmol/mg protein)
2.6
2.4
2.2
2
1.8
1.6
-6.5
-6
-5.5
-5
-4.5
-4
-3.5
log [AII]

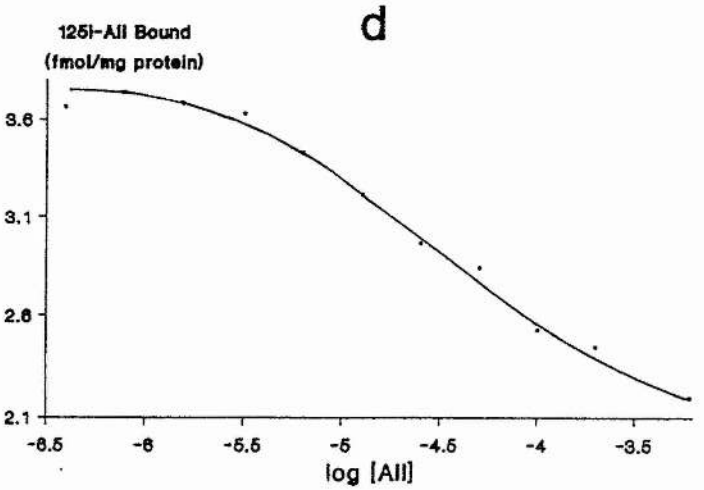

d
125I-AII Bound
(fmol/mg protein)
3.6
3.1
2.6
2.1
-6.5
-6
-5.5
-5
-4.5
-4
-3.5
log [AII]

Fig 3.4.3b **Mean Dose Response Curve for AII (um) Displacement of ^{125}I-AII from FW Eel Liver Membrane Preparations**

Results of experiments shown in fig. 3.4.3a were corrected for NSB and expressed as the percentage displacement ^{125}I-AII binding by angiotensin II (AII). Graphical points represent mean $\pm$ S.E.M.

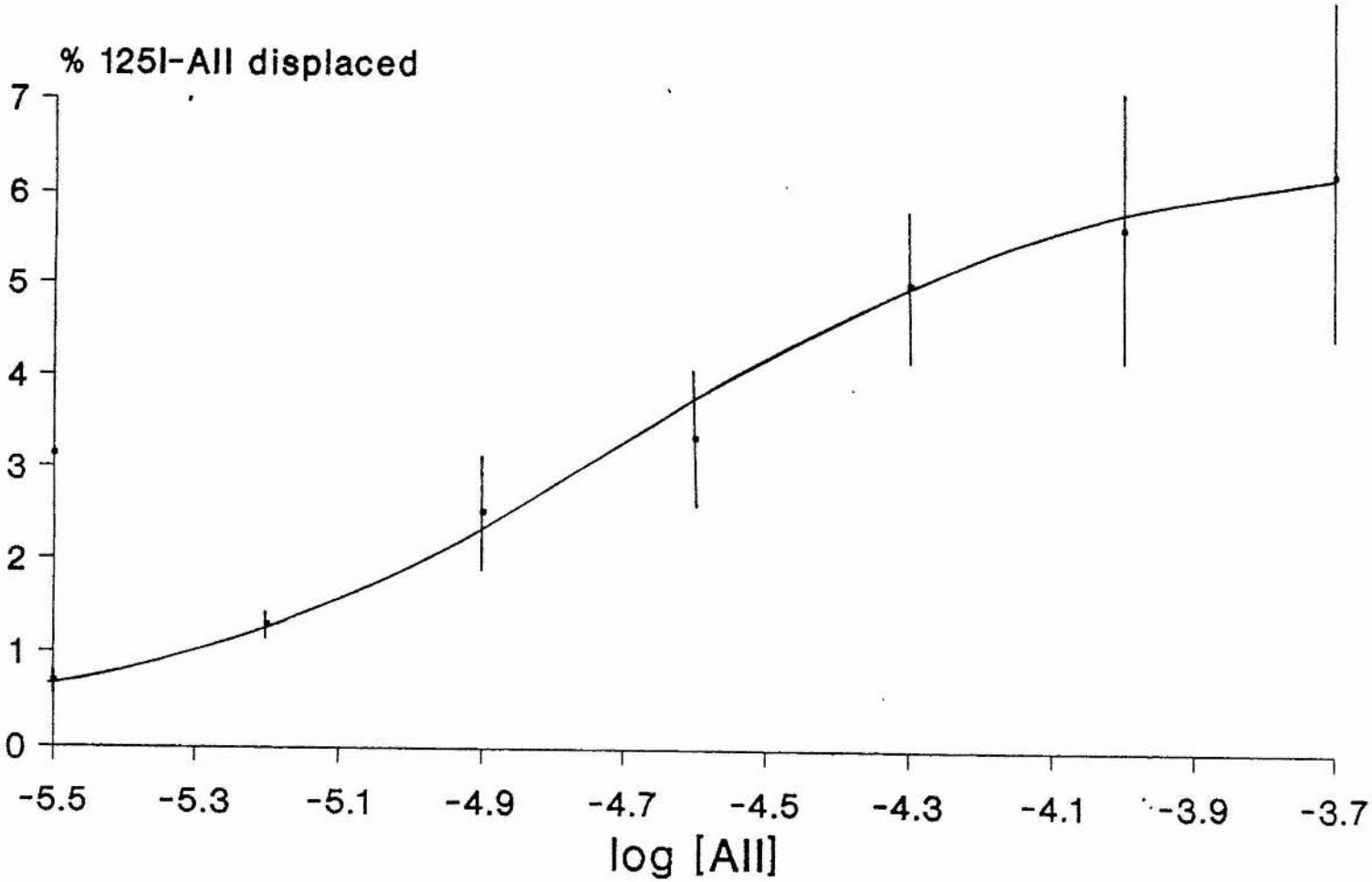

% 125I-AII displaced
7
6
5
4
3
2
1
0
-5.5
-5.3
-5.1
-4.9
-4.7
-4.5
-4.3
-4.1
-3.9
-3.7
log [AII]

Fig 3.4.3c A Scatchard plot of the data shown in 3.4.3b B=bound, F=free, Average protein concentration of membrane preparation was 3.69mg/ml.

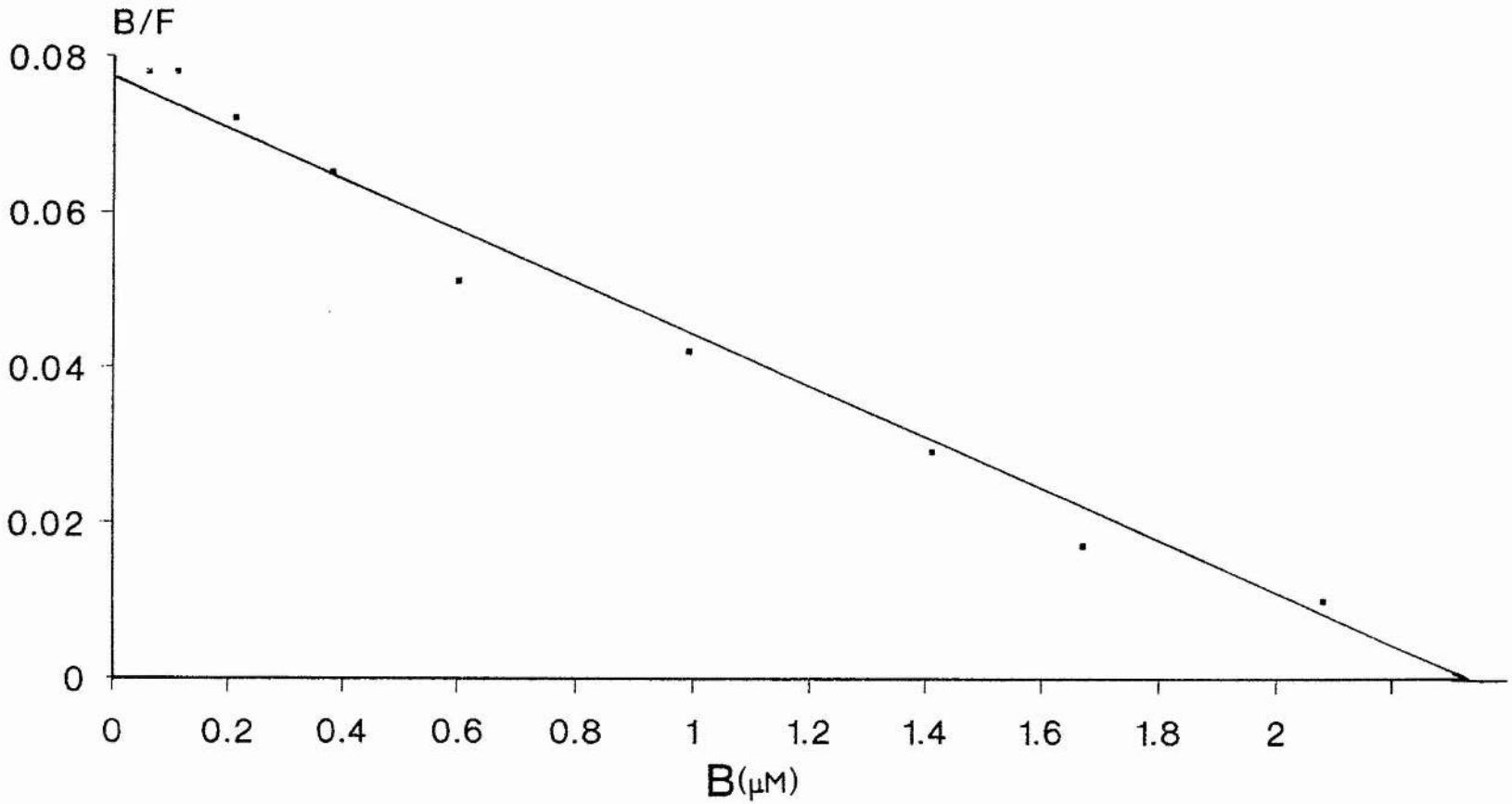
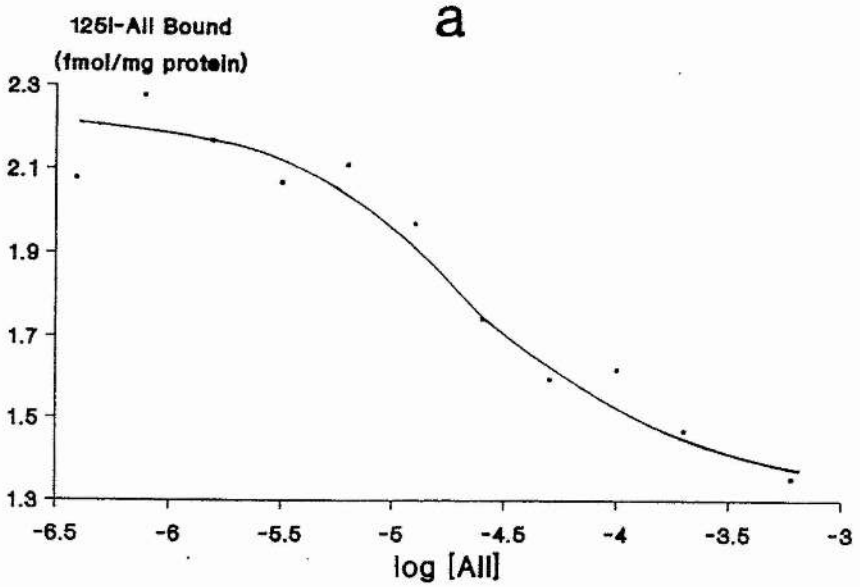
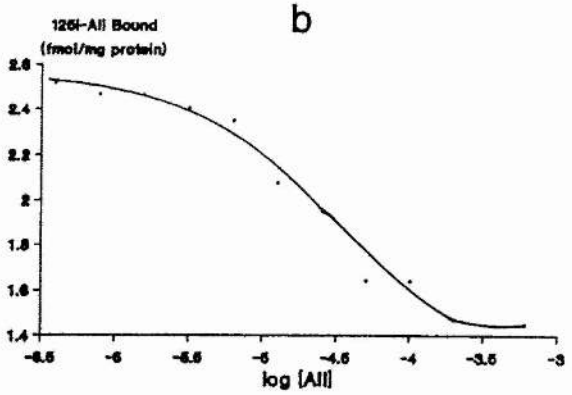

B/F
0.08
0.06
0.04
0.02
0
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
2
B(μM)

Fig 3.4.4a **Typical Displacement Curves of ^{125}I-AII by uM Concentrations of AII in SW Eel Liver Membrane Preparations**

SW eel liver membranes from 3 individual preparations were incubated at 22^{o}C for 60min with a range of angiotensin II (AII) concentrations from 0-600uM. ^{125}I-AII concentration of the incubation medium was a) 23.93pM b) 24.02pM c) 20.93pM for the 3 preparations respectively. Points represent duplicate determinations which varied by less than 10%.


a
125I-All Bound
(fmol/mg protein)
2.3
2.1
1.9
1.7
1.5
1.3
-6.5
-6
-5.5
-5
-4.5
-4
-3.5
-3
log [All]


b
125I-All Bound
(fmol/mg protein)
log [All]

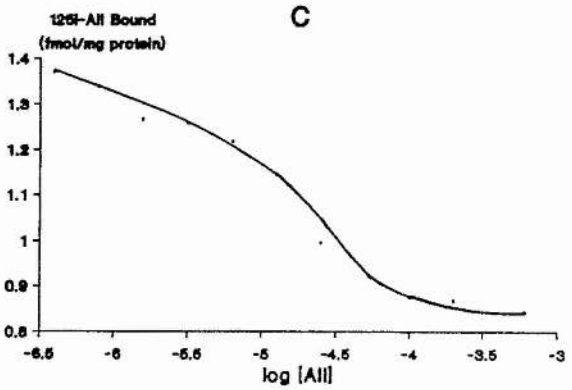

c
125I-All Bound
(fmol/mg protein)
1.4
1.3
1.2
1.1
1
0.9
0.8
-6.5
-6
-5.5
-5
-4.5
-4
-3.5
-3
log [All]

Fig 3.4.4b **Mean Dose Response Curve for AII (um) Displacement of ^{125}I-AII from SW Eel Liver Membrane Preparations**

Results of experiments shown in fig 3.4.4a were corrected for NSB and expressed as the percentage displacement of ^{125}I-AII binding by angiotensin II (AII) graphical points represent mean $\pm$ S.E.M.

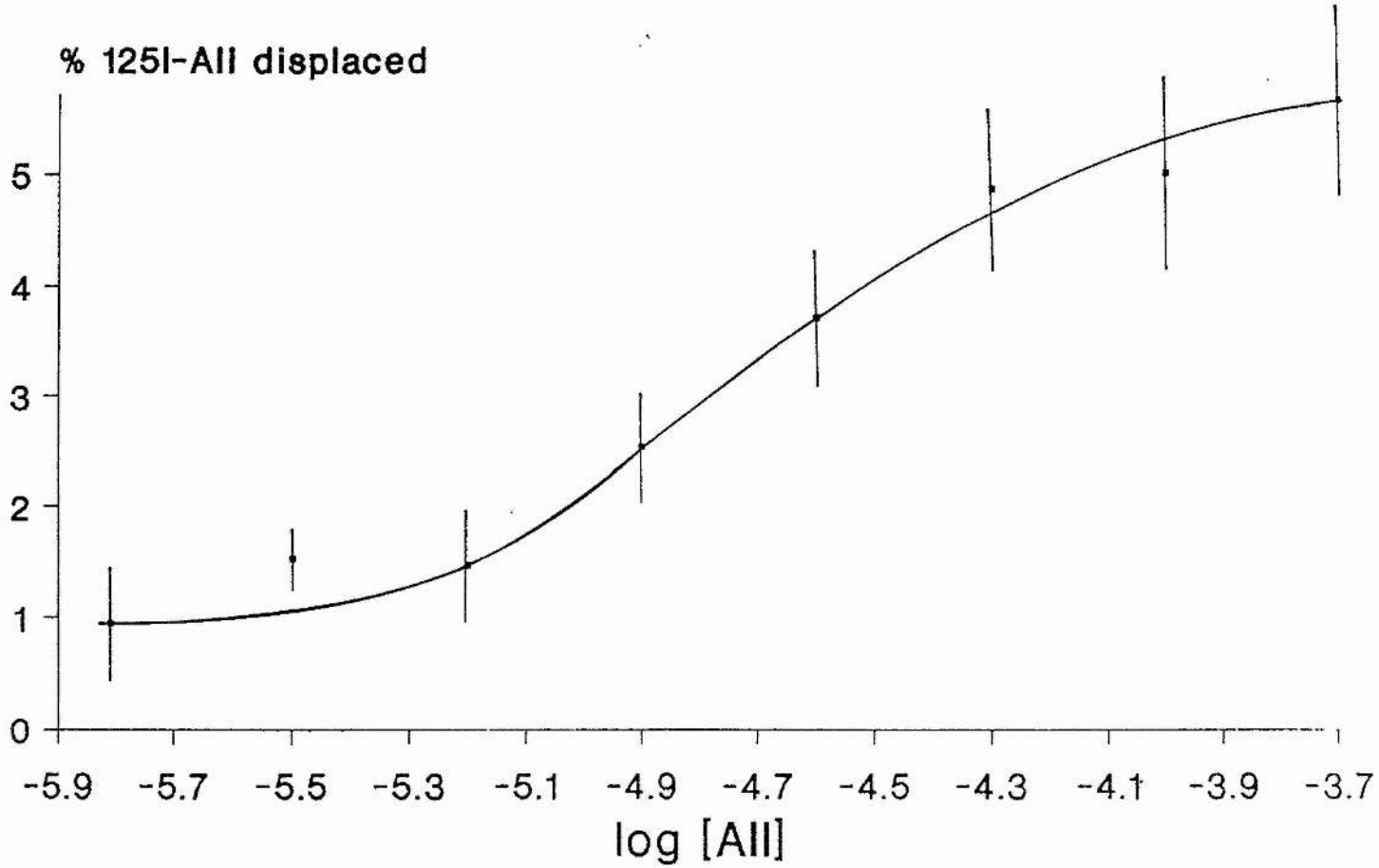
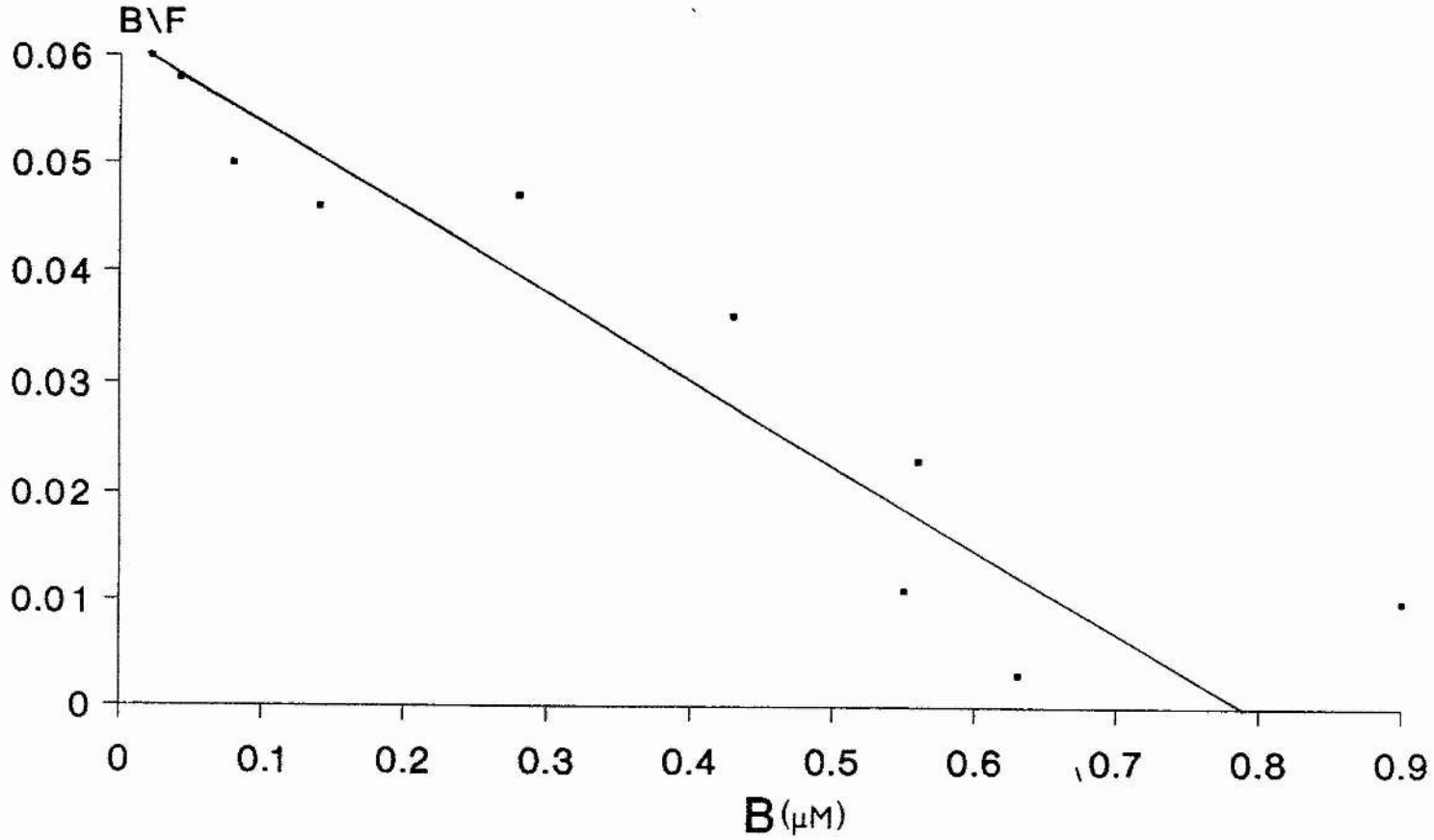

% 125I-AII displaced
5
4
3
2
1
0
-5.9
-5.7
-5.5
-5.3
-5.1
-4.9
-4.7
-4.5
-4.3
-4.1
-3.9
-3.7
log [AII]

Fig 3.4.4c A Scatchard Plot of the Data Shown in 3.4.4b B=bound, F=free. Average protein concentration of membrane preparation was 3.28mg/ml.


B\F
0.06
0.05
0.04
0.03
0.02
0.01
0
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
B (μM)

The preparations from SW eel livers also resulted in greater binding of ^{125}I-ile^5-AII than ^{125}I-val^5-AII (fig 3.4.5a). Total and specific binding of the ile^5 form of the peptide was 3.56 and 1.56fmol/mg protein which contrasts with 2.16 and 0.35fmol/mg protein for total and specific binding respectively, with the val^5 sequence of the peptides. Non-specific binding was similar, at 2.0 and 1.80fmol/mg protein for the ile^5 and val^5 structures respectively.

Since earlier physiological studies had employed the use of the two AII peptide sequences, it was decided to compare binding of the peptides in dab and plaice preparations also.

In plaice liver membranes however, the binding trend was reversed with receptors binding ^{125}I-val^5-AII more readily (fig 3.4.5b). Total binding was 7.76 and 6.32fmol/mg protein for the val^5 and ile^5 sequences of the iodinated peptide respectively. However, non-specific binding was particularly high in this preparation, 6.71 and 5.06fmol/mg protein for the val^5 and ile^5 structures of ^{125}I-AII respectively, therefore decreasing specific binding to 1.06 and 1.26fmol/mg protein.

Dab liver membranes also appear to produce greater binding of ^{125}I-val^5-AII than ^{125}I-ile^5-AII (fig 3.4.5b). Total and specific binding of the val^5 sequence was 5.07 and 1.65fmol/mg protein compared to 3.45 and 0.52fmol/mg protein

Figs 3.4.5a and 3.4.5b

INCUBATION OF LIVER MEMBRANE PREPARATIONS FROM 4 FISH TYPES WITH 2 DIFFERENT BUT HOMOLOGOUS SEQUENCES OF ANGIOTENSIN II AND ^{125}I-ANGIOTENSIN II

Figs 3.4.5a and 3.4.5b

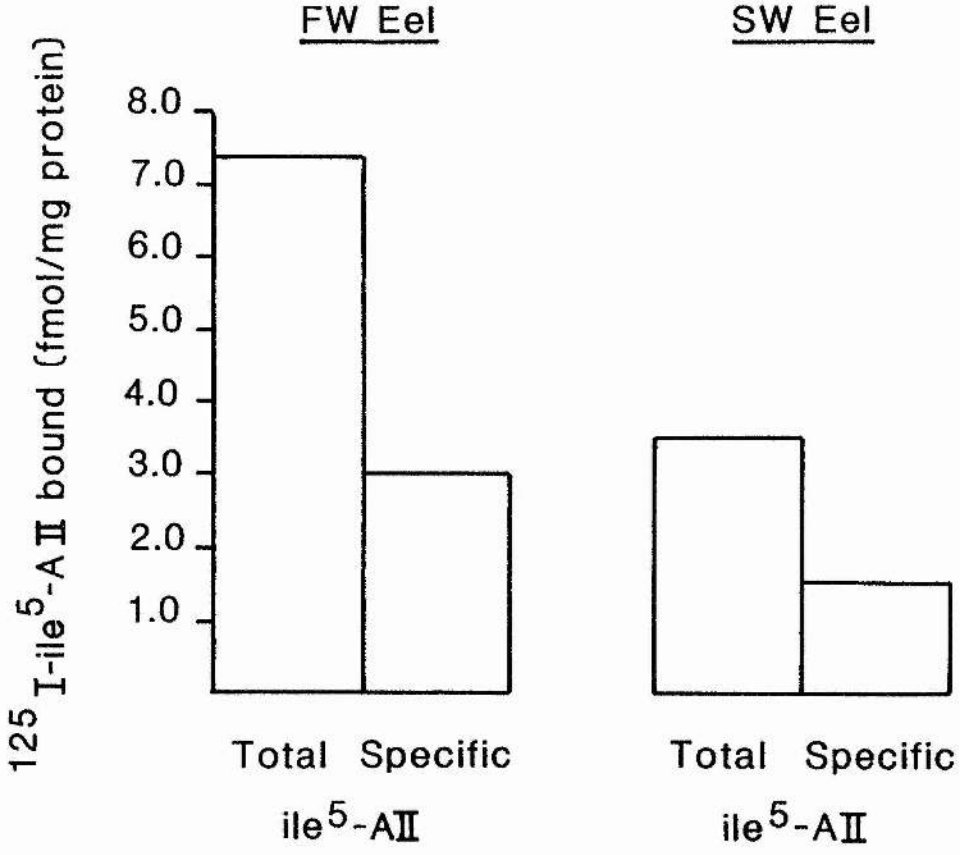
Incubation of Liver Membrane Preparations from 4 Fish Types with 2 Different but Homologous Sequences of Angiotensin II and ^{125}I-Angiotensin II

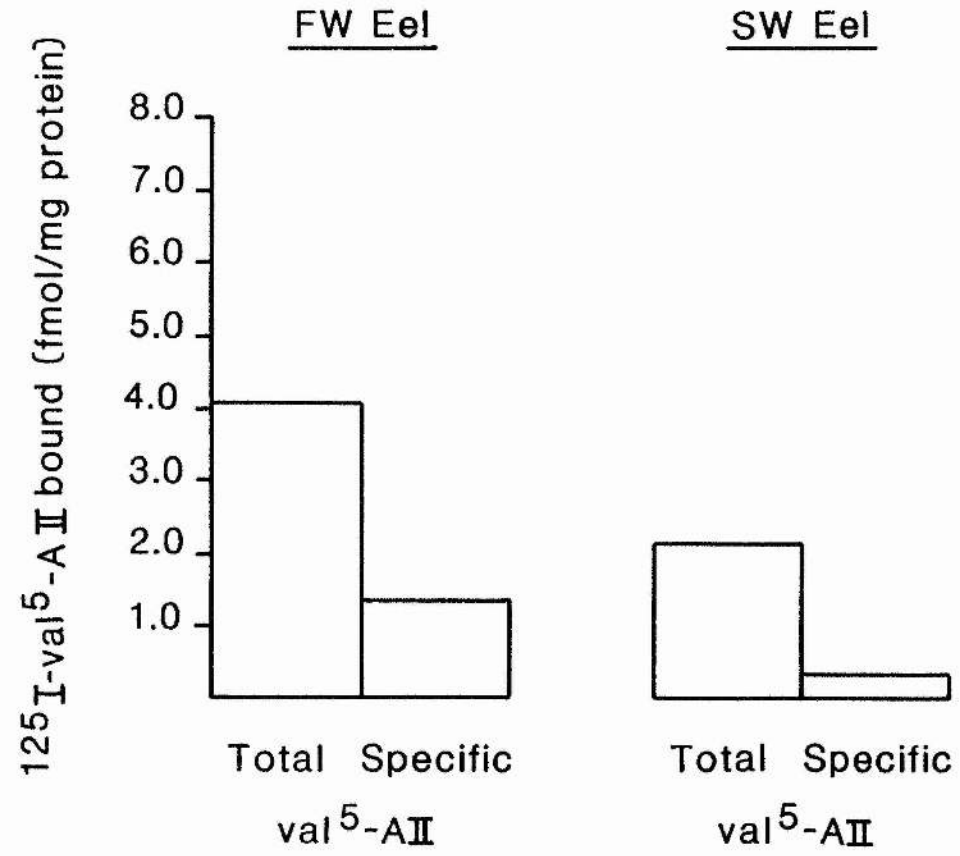
Liver membranes from 4 different individual preparations from 4 different fish were incubated with either ^{125}I-isoleucine^{5}-angiotensin II (^{125}I-ile^{5}-AII) or ^{125}I-valine^{5}-angiotensin II (^{125}I-val^{5}-AII). Displacement of the iodinated peptide was induced using 0-600uM concentrations of the corresponding peptide. Both incubations of each preparation were carried out simultaneously for 1hr at 22^{o}C.

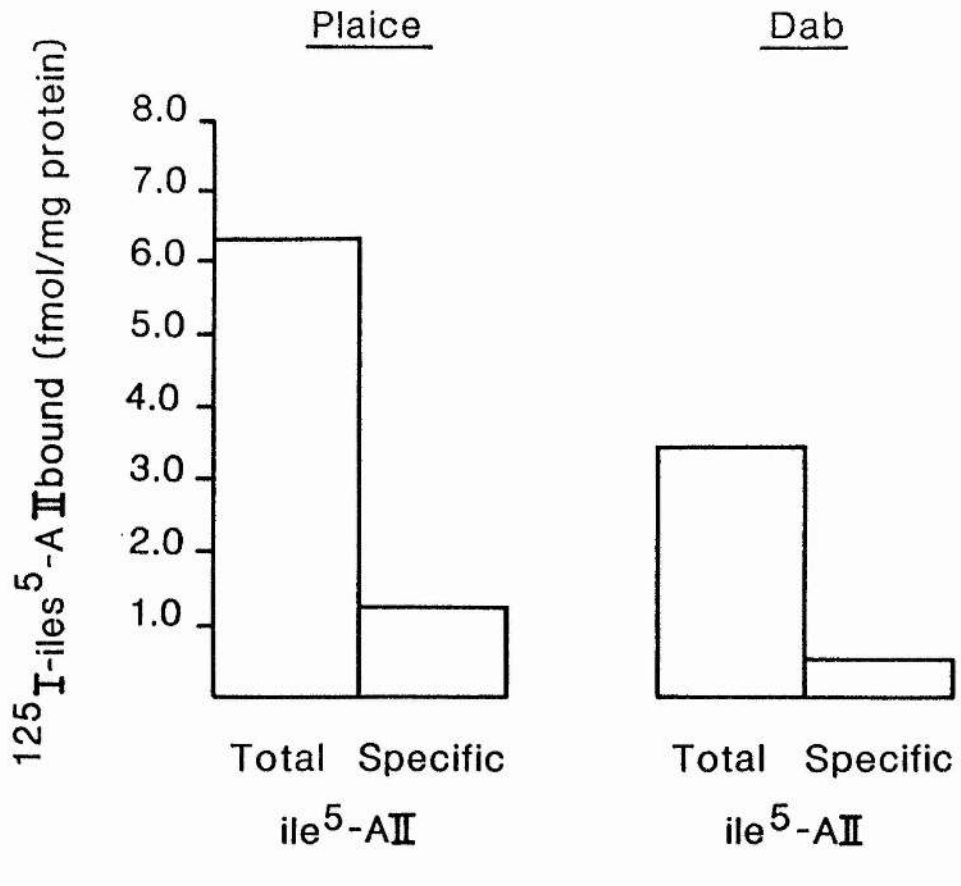
Concentrations of the iodinated peptide (pM) utilised were as follows:-

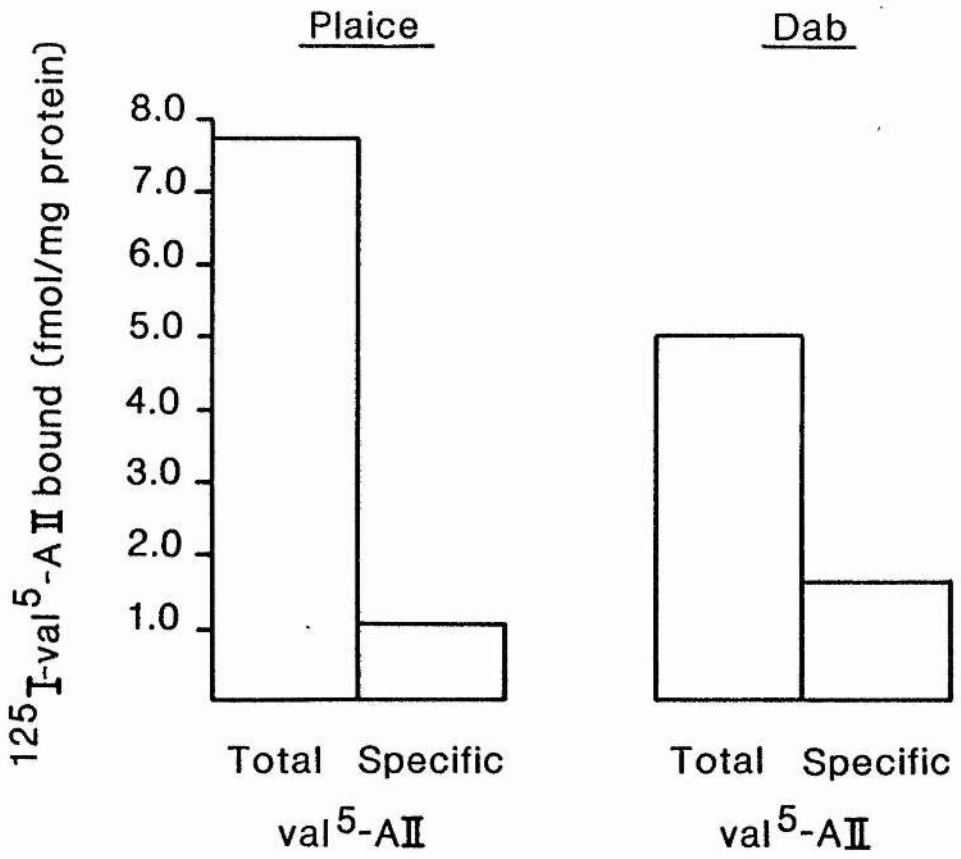
	FW Eel	SW Eel	Plaice	Dab
^{125}I-ile^{5}-AII	56.76	61.72	66.79	61.85
^{125}I-val^{5}-AII	48.75	64.77	63.56	63.24

Values represent duplicate determination which varied by less than 10%.


FW Eel
SW Eel
^{125}I-ile^{5}-AII bound (fmol/mg protein)
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
Total Specific
ile^{5}-AII
Total Specific
ile^{5}-AII


FW Eel
SW Eel
^{125}I-val^{5}-AII bound (fmol/mg protein)
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
Total Specific
val^{5}-AII
Total Specific
val^{5}-AII


Plaice
Dab
^{125}I-iles^{5}-AII bound (fmol/mg protein)
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
Total Specific
ile^{5}-AII
Total Specific
ile^{5}-AII


Plaice
Dab
^{125}I-val^{5}-AII bound (fmol/mg protein)
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
Total Specific
val^{5}-AII
Total Specific
val^{5}-AII

for the equivalent binding of the ile^5 sequence of the iodinated peptide.

It has already been shown that FW eel liver membranes produce greater binding of ^{125}I-ile^5-AII than ^{125}I-val^5-AII, but this study also involves displacement of these iodinated peptides by homologous and heterologous sequences of cold AII (fig 3.4.6). Total binding of ^{125}I-ile^5-AII in this case was 5.79±0.47fmol/mg protein. Individual incubations with 600uM ile^5- and val^5- AII (equivalent to non-specific binding) reduced binding to 3.74±0.21 and 3.83±0.28fmol/mg protein, respectively. These values were not statistically significantly different (Mann-Whitney U test). Total binding with ^{125}I-val^5-AII was again lower than that of ^{125}I-ile^5-AII at 3.96±0.34fmol/mg protein. Incubations with 600uM ile^5 and val^5-AII reduced binding to 3.17±0.24 and 3.13±0.19fmol/mg protein respectively, again results not statistically significant (Mann-Whitney U test). Total binding of the 2 radioactive peptides was statistically significantly different ($p < 0.01$, Mann-Whitney U test).

Plaice liver membranes were again more reactive to ^{125}I-val^5-AII, producing total binding of 3.90fmol/mg protein in contrast to 3.30fmol/mg protein with ^{125}I-ile^5-AII. However, non-specific binding with either 600uM ile^5 or val^5-AII and each iodinated peptide was relatively constant at 2.78 and 2.91fmol/mg protein respectively with ^{125}I-val^5-AII and 2.43 and 2.46fmol/mg protein with ^{125}I-ile^5-AII incubated with the val^5 and ile^5 sequence of the cold peptide.

Fig 3.4.6 COMBINATIONS OF BOTH SEQUENCES (ile^5, val^5)OF THE AII PEPTIDE TO PRODUCE TOTAL AND NON-SPECIFIC BINDING IN FW EEL AND PLAICE LIVER MEMBRANE PREPARATIONS

Fig 3.4.6 **Combination of both Sequences (ile^5, val^5) of the AII Peptide to Produce Total and Non-Specific Binding in FW Eel and Plaice Liver Membrane Preparations**

5 FW eel and 1 plaice preparation were incubated in the absence (total) and presence (non-specific) of either 600uM isoleucine^5-angiotensin II (ile^5-AII) or 600uM valine^5-angiotensin II (val^5-AII) in combination with either ^{125}I-ile^5-AII or ^{125}I-val^5-AII. Concentrations of iodinated peptide used were:-

	FW Eel (mean)	Plaice
^{125}I-ile^5-AII	65.3	56.0
^{125}I-val^5-AII	69.1	59.3

Values indicate duplicate determinations which varied by less than 10% and FW eels result symbolise mean $\pm$ S.E.M.

FW Eel
^{125}I-ile^5-A II bound (fmol/mg protein)
6.0
5.0
4.0
3.0
2.0
1.0
None
600μM ile^5-A II
600μM val^5-A II

FW Eel
^{125}I-val^5-A II bound (fmol/mg protein)
6.0
5.0
4.0
3.0
2.0
1.0
None
600μM ile^5-A II
600μM val^5-A II

Plaice
^{125}I-ile^5-A II bound (fmol/mg protein)
6.0
5.0
4.0
3.0
2.0
1.0
None
600μM ile^5-A II
600μM val^5-A II

Plaice
^{125}I-val^5-A II bound (fmol/mg protein)
6.0
5.0
4.0
3.0
2.0
1.0
None
600μM ile^5-A II
600μM val^5-A II

INVESTIGATION OF ABILITY OF A RANGE OF PEPTIDES TO DISPLACE ^{125}I-AII

A range of small peptides either derived from, or with similar structures to, angiotensin were tested in the receptor assay to assess their ability to displace ^{125}I-AII (table 3.4.). All peptides produced some displacement of ^{125}I-ile^5-AII, although the ability of 1-4 AII tetrapeptide was much lower than the other peptides utilised. Sar^1, ile^5-AII produced the greatest displacement of ^{125}I-ile^5-AII and was more potent than ile^5-AII itself. 5-8 AII tetrapeptide and ile^5-AII produced similar maximum displacement of total iodinated peptide bound. Ile^5 and val^5 sequences of AI behaved comparably in displacing ^{125}I-ile^5-AII, however val^4-AIII was not as potent as the equivalent ile^4-AIII in inhibition of binding. Although bradykinin did displace the radioactive peptide from binding sites it was a poorer competitor than those peptides belonging to the angiotensin family.

Using table 3.4. and individual preparations, the ability of peptides to displace ^{125}I-ile^5-AII from receptors appears to be ranked in the following order:-

Sar-AII>ile^5-AII>5-8AII>ile^5-AI>val^5-AI>ile^4-AIII>val^4-AIII>Bradykinin>1-4AII

Table 3.4. **INVESTIGATION OF ABILITY OF A RANGE OF PEPTIDES, WITH SEQUENCES RELATED TO ANGIOTENSIN II, TO DISPLACE ^{125}I-ANGIOTENSIN II FROM FW EEL LIVER MEMBRANE PREPARATIONS**

INVESTIGATION OF ABILITY OF A RANGE OF PEPTIDES, WITH SEQUENCES RELATED TO ANGIOTENSIN II, TO DISPLACE ^{125}I-ANGIOTENSIN II FROM FW EEL LIVER MEMBRANE PREPARATIONS

Table 3.4.1 indicates maximum displacement of ^{125}I-isoleucine^{5}-angiotensin II (^{125}I-ile^{5}-AII) from FW eel liver membranes (expressed as a percentage) by 8 test peptides. These results were compared with ile^{5}-AII displacement of the iodinated peptide in the same membrane incubation. Results represent mean $\pm$ S.E.M. for 4 incubations of ile^{5}-AII, saralasin^{1}(sar),ile^{5}-AII and bradykinin; 3 incubations of valine^{5}(val)-AI, ile^{4}-AIII and 1-4 AII tetrapeptide; and from 2 incubations each of val^{4}-AIII and the 5-8 AII tetrapeptide.

Table 3.4. Displacement of ^{125}I-AII by angiotensin related peptides

TEST PEPTIDE	MAXIMUM DISPLACEMENT OF ^{125}I-ile^5-AII by Test Peptide (% age)	MAXIMUM DISPLACEMENT OF ^{125}I-ile^5-AII by AII (% age)	EXCESS ^{125}I-ile^5-AII displaced over peptide (% age)
ile^5-AI	37.57±4.6	41.29±3.34	3.72
val^5-AI	29.83±5.42	34.14±3.29	4.31
ile^4-AIII	50.89±5.26	55.44±6.55	4.55
val^4-AIII	45.46±5.44	52.76±14.6	7.3
1-4AII tetrapeptide	22.52±4.43	45.14±2.99	22.62
5-8 AII tetrapeptide	41.97±2.32	42.2±1.85	-0.28
sar^1,ile^5-AII	46.89±4.63	40.66±3.4	-6.23
bradykinin	28.16±4.69	39.33±4.03	11.17

4.0 DISCUSSION

Angiotensin II is known to increase blood pressure in FW trout and FW and SW adapted American eels. (Gray and Brown 1985; Nishimura and Sawyer, 1976). AII has also been shown to increase drinking rates of eels and flounder in both FW and SW (Takei et al, 1979; Carrick and Balment, 1983). From studies on nearly 40 species of fish, Kobayashi et al (1983) found that AII did not induce drinking in fish found exclusively in FW or SW and concluded that a drinking response to AII is characteristic of fish which encounter water more hypertonic than that in which they typically reside.

AII was found to increase blood pressure and drinking rates of the SW adapted euryhaline eel Anguilla anguilla L., with the ile^5-AII sequence of the peptide producing a greater effect than val^5-AII. The same result arose with plasma chloride concentrations with ile^5-AII increasing levels and val^5-AII producing little effect. These results are contrary to those expected since the reported AII structures of Japanese and American eels contain val at position 5 (Hasegawa et al, 1983; Khosla et al, 1985). However, other eel peptides sequenced to date, such as somatostatin have unusual substitutions compared with corresponding peptides from other teleost fish (Conlon et al, 1988) and it is possible that there may be species differences in AII amino acid sequences which may alter binding and activity of this peptide.

Since administration of hormones exogenously produces different results, stimulation of the endogenous RAS using the smooth muscle relaxant papaverine (Balment & Carrick, 1985), should reflect the true eel AII actions. Papaverine initially reduced blood pressure followed by a return to control, increased drinking rates and elevated plasma chloride concentrations. The ACE inhibitor, Captopril, which should prevent endogenous production of AII, however did not affect drinking rates or plasma chloride levels of the SW adapted eel. This may reflect an inability of the inhibitor to act on ACE of eels, due to a different local environment or structure of the enzyme. This is in contrast to inhibition by Captopril of the vasopressor response to an eel angiotensin (presumably AI) and val^5,Ser^9-AI in FW American eels (Nishimura et al 1978). However, Captopril is not specific, but a general inhibitor of the enzyme, and its mechanism of action is unclear. Injection of both the ile^5 and val^5 sequences of AII failed to elicit drinking in FW acclimated eels, however a vasopressor response was produced.

Val^5-AII proved the more potent sequence in elevating blood pressure and increasing drinking rates in plaice and dab. Val^5-AII was the only peptide to affect plasma chloride concentrations and this effect was only seen in plaice. Papaverine lowered blood pressure in both flatfish species followed by a return to control levels and increased plasma chloride levels in plaice. Captopril did not affect

the drinking rates of dab, but did reduce both the drinking rates and plasma chloride concentrations in plaice.

Although there are no publications disclosing the structure of flatfish AII, documentation to date citing fish sequences all contain val at position 5 (Takemoto et al, 1983; Hayashi et al, 1978) and therefore it remains unlikely that the opposite trend of potency of the peptides in eels and flatfish is due to structural differences of the endogenous peptide.

Plasma AII levels are greater in SW than FW adapted European eels (Henderson et al 1986), therefore it can be assumed that AII plays a role in homeostasis of body fluids and electrolytes primarily in SW acclimated fish. In SW, eels lose water osmotically, therefore the hormone stimulates drinking to replace this loss which may also produce increased blood pressure.

AII had a pronounced effect on ANP release from eel myocytes. This increased release was similar in both ventricular and atrial myocytes of FW or SW adapted fish, however the concentration of ANP produced in SW eels was much greater than those of the equivalent FW preparations. These results indicate that both atria and ventricles of eels contain ANP. This is in contrast to mammalian studies where the peptide is stored only in atrial granules (De Bold et al, 1981). ANP has been detected in ventricles of teleost, chondrichthyan and agnathan fishes (Reinecke et al, 1985, 1987a, 1987b). Data also suggests that AII induced ANP release in fish may be a phenomenon of SW adaptation.

This observation is supported by reports of AII plasma levels in eels being higher in SW (Henderson et al, 1986) and AII producing a vasopressor response (Nishimura et al, 1978). ANP may counteract this increased blood pressure through the hormones natriuretic and diuretic properties (De Bold et al, 1981) which have been demonstrated with heart extracts from Gila atraria in a rat bioassay (Westenfelder et al, 1988). Reports of AII receptors on myocytes involve studies on cultured neonatal rat myocytes (Rogers et al, 1986). Receptors were found to bind AII specifically and in a biphasic manner, producing a stimulation of the contractile frequency of spontaneously beating myocytes.

Initial studies indicated that ANP and AII also increased net sodium efflux from SW adapted European eels suggesting that this natriuresis may be produced by AII stimulation of ANP (Hazon, personal communication).

Liver had the greatest tissue to plasma ratio of ^{125}I-AII in SW eels, with high distribution also in the kidney, ventricle and brain. Lower levels were detected in the atria, spleen and gill and very little in the gut muscle and pancreas. This binding may be correlated with AII receptor density of these tissues. Binding in both the ventricle and atria supports the suggestion of receptor sites in both tissues, indicated from the results of ANP release from myocytes.

Liver also had the highest specific binding of ^{125}I-AII of the SW eel membrane preparations and was just slightly lower than the gill of FW membranes. It is not entirely clear why the liver should be able to respond to a hormone like AII, whose main purpose appears to be blood pressure regulation and body fluid and eletrolyte balance. AII is thought to be involved in carbohydrate metabolism by the liver (Campanile et al, 1982), stimulating both gluconeogenesis and glycogenolysis in rats (Sernia et al, 1985). The hormone also inhibits fatty acid synthesis (Hems, 1977) and regulates angiotensinogen production by a positive feedback mechanism, (Freeman and Rostorfer, 1972; Sernia and Reid, 1980). Present data (rat) indicates the liver as the major and perhaps exclusive site of renin substrate biosynthesis (Freeman and Rostorfer, 1972). AII is also thought to influence adenylate cyclase activity (Jard et al, 1981; Crane et al, 1982; Cardenas - Tanus, 1982) and nuclear RNA initiation (Re et al, 1982) in the liver. The liver also has a high blood volume therefore AII could play a role in controlling blood flow and blood vessel resistance.

It is predicted that these actions are exerted by AII through a specific membrane receptor (LaFontaine et al, 1979) and a correlation has been identified between AII binding and phosphorylase activity and between specificity and biological potency in rats (Campanile et al, 1982).

Results from Scatchard analysis of AII displacement curves of FW and SW eel liver membranes suggest there may be

down-regulation of receptors in SW eels. This would be consistent with greater plasma levels of AII in SW (Henderson et al, 1986) and perhaps feedback inhibition of angiotensinogen production in the liver. Another possible role could be vasoconstriction of blood vessels within the liver producing reduced blood perfusion. This could increase venous return of blood at times when blood volume is reduced (i.e. SW acclimation) by reducing the volume of blood which normally pools in the fine vasculature and hepatic blood sinuses. Affinities obtained from Scatchard plots are consistent with those from liver membranes from other species, however, the capacity appears to be around 1000-fold greater (Campanile et al, 1982; Gunther, 1984; McQueen and Semple, 1989; Sernia et al, 1985).

Amongst the other tissues tested for receptor binding of ^{125}I-AII, gill was higher in SW and head kidney greater in FW. This compares with Na^+,K^+-ATPase activity in FW and SW acclimated fish in these organs and AII may play a role in regulation of the enzyme activity. It was unusual that the protein concentration of the SW head kidney was almost 6-fold greater than in FW although both preparations originated from the same tissue weight per ml of preparation. This could be due to hyperplasia of the tissue in SW. This increased protein concentration did not however appear to affect assay binding as NSB was 4-fold greater in the FW preparation.

Protein concentration of spleen preparations was also significantly different, with the FW preparation more

than 2-fold greater from an original tissue preparation concentration (g/ml) of around 50%. However, again total binding was greater in the SW preparation although specific binding was similar in both preparations. The role of angiotensin in spleen of rats and mice is thought to be in regulation of blood volume, blood flow and also modulation of lymphocyte function (Castren et al, 1987).

Specific binding of ^{125}I-AII in the FW eel heart was barely detectable and no specific binding was demonstrated in the SW eel heart or either muscle preparation.

It is difficult to compare the results obtained with tissues from FW fish against those from SW since these preparations arose from different groups of fish kept under separate conditions and were prepared on different days. Binding may vary from day to day in the standard FW liver preparation from the same batch of fish kept in similar conditions. Therefore it is difficult to compare preparations directly which were not prepared assayed, incubated and separated under identical conditions.

Binding of iodinated AII was also measured in dab and plaice membrane preparations. Results also indicated substantial binding in the liver, with spleen slightly greater than kidney for both fish. Binding in the kidney of these SW fish was similar to that of FW eel liver.

Documented location of angiotensin receptors in various tissues in a range of species is summarised in table 4.1. The majority are restricted to adrenal, arterial, renal and

Table 4.1 **Location of Angiotensin Receptors**

Organ/Tissue/Cell		Species	Physiological Response	Reference
Adrenal	Glomerulosa	Human	Aldosterone synthesis	Douglas et al, 1984 Brown et al, 1980b
Adrenal	Glomerulosa	Rat	Aldosterone synthesis	Catt et al, 1979 Maurer and Reubi, 1986
Adrenal	Glomerulosa	Mouse	Aldosterone synthesis	Maurer and Reubi, 1986
Adrenal	Glomerulosa	Hamster	Aldosterone synthesis	Maurer and Reubi, 1986
Adrenal	Glomerulosa	Primate	Aldosterone synthesis	Douglas et al, 1984 Maurer and Reubi, 1986
Adrenal	Glomerulosa	Guinea Pig	Aldosterone synthesis	Maurer and Reubi, 1986
Adrenal	Glomerulosa	Cow	Aldosterone synthesis	Maurer and Reubi, 1986 Carrol et al, 1986 Penhoat et al, 1988
Adrenal	Fasciculata	Cow	Steroid synthesis	Valloton et al, 1987 Maurer and Reubi, 1986 Penhoat et al, 1988
Adrenal	Fasciculata	Human	Steroid synthesis	Douglas et al, 1984
Adrenal	Fasciculata	Primate	Steroid synthesis	Douglas et al, 1984
Adrenal	Reticularis	Cow	?	Maurer and Reubi, 1986
Adrenal	Medulla	Cow	Catecholamine secretion	Lin and Goodfriend, 1970
		Rat		Maurer and Reubi, 1986 Israel et al, 1985 Bianchi et al, 1986
Adrenal	Medulla	Mouse	Catecholamine secretion	Maurer and Reubi, 1986
Adrenal	Medulla	Hamster	Catecholamine secretion	Maurer and Reubi, 1986
Aorta		Rabbit	Contraction	Devynck and Meyer, 1978
Aorta		Fowl	Contraction	Takei et al, 1988
Renal Artery		Cow	Contraction	Lin and Goodfriend, 1970
Mesenteric Artery		Rat	Contraction	Gunther et al, 1980 McQueen et al, 1984
Umbilical Artery		Cow	Contraction	Goodfriend and Simpson, 1981
Uterus		Rat	Contraction	Lin and Goodfriend, 1970 Devynck and Meyer, 1978 Moore and Kwok, 1987
Uterus		Rabbit	Contraction	Bennett and Snyder, 1980
Bladder		Rat	Contraction	Lin and Goodfriend, 1970
Heart		Cow	Positive Inotropy	Lin and Goodfriend, 1970
Heart	Neonatal myocytes	Rat	?	Rogers et al, 1986
Kidney	Glomeruli	Rat		Mendelsohn et al, 1986 Douglas, 1987 Kitamura et al, 1986 Sraer et al, 1974 Sraer et al, 1977
Kidney	Tubules	Rat		Douglas, 1987 Mendelsohn et al, 1986 Mujais et al, 1986 Lewis and Ferguson, 1989
Kidney	Cortex	Cow	Sodium flux	Catt et al, 1979
Kidney	Medulla	Rat		Gehlert et al, 1984
Kidney	Medulla	Dog		Gehlert et al, 1984

Brain	Thalamus	Rat	Drinking	Bennet and Snyder, 1976 Sirett et al, 1977 Mann et al, 1980
Brain	Hypothalamus	Rat	Vasomotion	Saavedra et al, 1986 Sirett et al, 1977 Sumners et al, 1986
Brain	Midbrain	Rat		Sirett et al, 1977
Brain	Forebrain	Rat		Sirett et al, 1977
Brain	Septum	Rat		Sirett et al, 1977
Brain	Medulla	Rat		Sirett et al, 1977
Brain	Cerebellum	Rat		Plunkett et al, 1987
Brain	Subfornical organs	Rat		Saavedra et al, 1986
Brain		Duck		Gerstberger, 1987
Brain		Sheep	Electrolyte balance	McKinley et al, 1986
Brain	Cerebellum	Cow		Bennett and Snyder, 1980
Brain		Dog	Role in C.N.S.	Speth et al, 1985
Anterior Pituitary		Rat	ACTH release	Catt et al, 1979 Gregg and Malvin, 1977 Carriere et al, 1986
Posterior Pituitary		Rat	ACTH release	Gregg and Malvin, 1977
Corticotrope		Goldfish	ACTH release	Weld and Fryer, 1987
Nerve		Rat	?	Lin and Goodfriend, 1970
Liver		Rat	Glycogenolysis	Lafontaine et al, 1979 Sernia et al, 1985 McQueen and Semple, 1989
Spleen		Rat	Regulation of splenic vol, blood flow,	Castren et al, 1987
Spleen		Mice	modulation of lymphocyte function	Castren et al 1987
Colon		Rat	fluid transport	Lin and Goodfriend, 1980 Cox et al, 1986
Jejunum		Rat		Cox et al, 1986
Ovary		Rat		Husain et al, 1987 Pucell et al, 1987
Oesphagus		Rat	?	Lin and Goodfriend, 1970
Trachea		Rat	?	Lin and Goodfriend, 1970
Placenta		Human	?	Cooke et al, 1981
Leukocytes		Human	?	Lin and Goodfriend, 1970 Shimada and Yazaki, 1978
Platelets		Human	Aggregation	Moore and Williams, 1982 Siebers and Goodfriend, 1986 Ding et al, 1985 Ding et al, 1984

brain membranes, where the actions of AII are best defined. The greatest density of binding sites is reported to be the adrenal glomerulosa (up to 2 million sites/cell) while one of the sparsest locations is in smooth muscle. This is in contrast to the much greater response to the hormone in smooth muscle than in the adrenal. Therefore the magnitude of the response of individual tissues is not proportional to receptor concentration, and may depend on the properties of receptors in particular tissues.

Putative angiotensin receptors have been solubilised and purified from adrenal glomerulosa and myometrium and demonstrate a molecular weight (M.Wt) of around 125,000 daltons with the possibility of a 2 subunit structure (Devynck et al, 1974; Capponi and Catt, 1980). The probability of a dimeric entity, is strengthened by evidence that DTT inactivates the receptor (Gunther, 1984) and AII dissociation is enhanced at non-physiological pH values, in 4.5M urea or 1% SDS presence (Sen et al, 1983). The apparent M.Wt of the reduced hepatic receptor has been estimated at 68,000 daltons (Sen et al, 1983) with uterine receptors also 68,000 daltons and that of the adrenal slightly lower at 65,000 daltons (Capponi and Catt, 1980).

Results of specific receptor binding at each centrifugation stage during FW eel liver membrane preparation indicate that $NaCl/MgSO_4$ successfully precipitates the AII binding protein. Although repeated washing of the membrane pellet increased specific binding, there was a combined decrease in protein concentration.

Therefore the procedure was limited to 2 washes. Non-specific binding of ^{125}I-AII to liver membranes was estimated using 600uM AII (standard displacement curves ranged from 0.4-200uM) and the increased concentrations of 800uM and 1mM did not produce any further reduction of binding. In terms of the percentage of total counts present during incubation, NSB amounts to 5-10%. This is similar to reports in rat liver (Campanile et al, 1982). NSB can be accounted for by molecular adhesion to a variety of areas, including other proteins and even the walls of the reaction vessel.

Reducing the incubation temperature from 22^{o}C to 4^{o}C drastically reduced specific binding of ^{125}I-AII in both FW and SW eel liver membrane preparations. This may be due to reduced movement of molecules or a conformational change in either the peptide or the receptor or perhaps receptor degradation. However, studies suggest degradation is unlikely because preincubation of fowl aorta membranes at 2^{o}C for 3hrs did not alter the time course or the maximum of ^{125}I-ile^{5}-AII binding (Takei et al, 1988). Receptors from rat liver membranes have also been successfully stored at -20^{o}C for a week and even for months at -80^{o}C without loss of activity (Sernia et al, 1985).

Dilution of membrane preparation indicates ^{125}I-AII binding and therefore receptor density are directly related to protein concentration. Binding of the peptide to fowl aorta membranes, rat renal brush border membranes, rat liver membranes and also GH in Tilapia liver increased linearly

as a function of protein concentration (Takei et al, 1988; Brown and Douglas, 1982; Gunther, 1984; Fryer, 1979a). Protein concentration of the eel liver membrane preparation required for AII reactivity was approximately 700ug per assay tube. This is greater than the 50-400ug used in rat liver membrane binding assays (Gunther, 1984; Campanile et al, 1982; McQueen and Semple, 1989). Fryer et al (1979a) investigating binding of ^{125}I-labelled Tilapia GH in Tilapia liver membranes found that total binding and NSB became maximal at 400 and 500ug membrane protein, respectively.

Calcium increased specific binding in both FW and SW eel liver membranes with 25mM (12.5mM final incubation concentration) producing maximal binding. The cation also increased binding in kidney membranes. Magnesium (4M $MgCl_2$) however, drastically reduced binding in FW eel liver membranes, but 10mM $MgCl_2$ was without effect. The divalent cations calcium, magnesium and manganese have all increased AII binding to rat liver (2-50mM), mesenteric artery (2-10mM), and isolated renal glomeruli (10uM-5mM) (Sernia et al, 1985; Campanile et al, 1982; McQueen et al, 1984; Blanc et al, 1978), with the chelating agents EDTA, EGTA and DTT all decreasing binding (McQueen et al, 1984; Blanc et al, 1978; Sernia et al, 1984). Lin and Goodfriend (1970), however showed EDTA increased specific binding to rat uterine segments and bovine adrenal particles. This could be attributed to the fact that calcium is necessary for activation of aminopeptidase A which degrades the peptide.

bovine adrenal cortex (Douglas et al, 1982; Douglas et al, 1978; Bennett and Snyder, 1980). The concentration of AII receptor sites was shown to vary with divalent cation concentration in cardiac (Wright et al, 1983; Baker et al, 1984) and uterine smooth muscle (Douglas et al, 1982). In rat liver, calcium increased the proportion of high affinity sites, however strontium, barium and magnesium were less effective (McQueen and Semple, 1989). Guanine nucleotides and some calcium antagonists are also able to modify the proportion of hepatic angiotensin receptors in the high and low affinity states. It is thought that magnesium may delay receptor dissociation and/or protect the receptor from degradation. Campanile et al (1982) postulate that magnesium acts on adenylate cyclase or G-proteins to form a high affinity state rather than directly affecting ligand-receptor interaction. However, McQueen and Semple (1989) found that receptor affinity was reduced 10-fold by Gpp(NH)p, but receptor density still varied with assay calcium concentration. Calcium can alter the physicochemical state of the lipid bilayer (Houslay and Stanley, 1982) and might therefore indirectly affect the angiotensin receptor. However, the influence of divalent cations on receptor density would be a more general phenomenon. McQueen and Semple (1989) also suggest that receptor regulation occurs through masking or inactivation of binding sites which can be reversed by calcium or that these high calcium concentrations <u>in vitro</u> are mimicking a continual transmembrane calcium flux shown <u>in vitro</u> to be necessary for a sustained response to the peptide.

Sodium is another potent cationic regulator of AII binding, due largely to its affect of decreasing plasma AII concentrations. Sodium down-regulates receptors in rat tubular epithelia (Mendelsohn et al, 1986), bovine adrenals (Glossmann et al, 1974), although an increase was observed in the calf adrenal (Bennett and Snyder, 1980), smooth muscle (Catt et al, 1979) although binding was unaffected in rabbit uterine homogenates (Bennett and Snyder, 1980), and in brains of SW acclimated pekin ducks (Gerstberger et al, 1987). Receptor up-regulation by sodium has been detected in rat glomeruli (Mendelsohn et al, 1986), calf cerebellar cortex (Bennett and Snyder, 1980), neuronal cultures of normotensive and hypertensive rats (Feldstein et al, 1986), human platelets (Ding et al, 1985) and rat arterial membranes (McQueen and Semple, 1987). Potassium had no effect on human platelet binding (Ding et al, 1985) or receptor sites in canine adrenal glomerulosa cells (Fredlund et al, 1977).

Other hormonal systems may affect receptor regulation. Down-regulation of AII receptors has been observed by oestradiol in cultured anterior pituitary cells (Carriere et al, 1986; Platia et al, 1986; Schoepp and Bailey, 1987), catecholamines in neuronal cultures (Sumners et al, 1986), corticotropin in cultured bovine fasciculata (Penhoat et al, 1988) and aldosterone, dexamethasone and insulin in rat glomeruli (Douglas, 1987). Deoxycorticosterone and gonadotrophin produced up-regulation in rat brain and ovaries, respectively (Wilson et al, 1986; Pucell et al, 1987).

Binding of ^{125}I-AII increased linearly with increasing concentration of the iodinated peptide present during incubation. This trend was observed in both FW and SW liver membrane preparations. However, saturation was not achieved at the maximal concentrations of ^{125}I-AII available.

^{125}I-AII degradation appears to be negligible during incubation, with the majority of iodine from both standard and samples being traced to the same region of the TLC plate. These low levels of degradation are consistent with high levels of membrane bound peptide, rendering the hormones virtually resistant to degradation (Cox et al, 1986) through a lack of binding to degradative enzymes. (Brown and Douglas, 1982). Campanile et al (1982) reported little degradation of ^{125}I-AII in rat liver plasma membranes in contrast to LaFontaine et al (1979). Takei et al, (1988) found that bacitracin (the inhibitor included in eel liver incubations) suppressed ligand metabolism almost completely in fowl aorta membranes. These results are encouraging since peptide hormones are susceptible to proteolysis and certain tissues such as liver and fat are very rich in such proteases. This could explain why particularly fatty preparations from livers from very large eels or those recently acquired in the aquarium and therefore not starved for any significant period of time, did not produce solid pellets after centrifugation and unresponsive membranes with undetectable specific binding of ^{125}I-AII.

Total binding of ^{125}I-AII to FW eel liver membranes was close to saturation after incubation for 140 minutes, but to avoid any possible tracer degradation a period of 60 minutes was routinely used for incubation of membrane preparations.

Although displacement of ^{125}I-AII from FW eel liver membranes was achieved using nanomolar concentrations of AII, unfortunately it was never repeated using this range (0-25.6nM) of the peptide. Subsequent studies required micromolar (0-600uM) concentrations of AII to displace the iodinated peptide. The protein concentration of the initial preparation was particularly high at 8.52mg/ml compared to an average of 3.6mg/ml in succeeding preparations. Although in terms of wet weight tissue /ml membrane preparation the earlier preparation was much lower at around 1.4g/ml compared to 5.0mg/ml latterly required to produce responsive preparations.

It was considered a problem that these high concentrations of AII, 1.56-600uM were necessary to produce displacement since nM and pM concentrations have been reported to do so by authors in other liver preparations (Gunther, 1984; McQueen and Semple, 1989; Sernia et al, 1985; Campanile et al, 1982) and because the concentration of ^{125}I-AII routinely present during incubation was around 25pM. However plasma AII levels have been measured in European eels and found to be 192nM in fish adapted to distilled water and 3.6uM in those acclimated to SW (Henderson et al, 1986). These AII concentrations are in contrast to circulating levels in the nanomolar range in

mammals. Possible reasons for this anomaly between the concentrations of AII and the iodinated peptide could be due to:

1. preparation too concentrated
2. sequences of peptides utilised
3. inhibitors present during incubation
4. different receptor classes
5. bonding other than ionic
6. alteration of peptide properties by iodination

Taking these points in order:-

1. Dilution of FW eel liver membrane preparation and therefore of the protein concentration had no effect, with ^{125}I-AII displacement only occuring between 6.25 and 600uM for the concentrated preparation and those diluted to 1:2 and 1:4.
2. Replacing ^{125}I-ile^{5}-AII and ile^{5}-AII with the endogenous val^{5} sequence of the peptides did not overcome this dilemma. In fact greater concentrations of the peptide were required to produce displacement of ^{125}I-val^{5}-AII than the equivalent ^{125}I-ile^{5}-AII. In comparative studies iodinated AII appeared to have similar binding characteristics in both plaice and dab liver membranes in contrast to eels, with 12.5-600uM AII and 3.12-600uM AII required to produce displacement of both the ile^{5} and val^{5} sequences of the iodinated peptide, in plaice and dab preparations respectively. In FW eel kidney only a concentration of 600uM AII produced any significant displacement of ^{125}I-AII

although an extensive range of AII concentrations (0.1-200uM) was tested.

3. Assays involving both nanomolar and micromolar concentrations of AII included bacitracin, (to counteract peptide degradation) in the buffer used to make up the angiotensins. However, reports suggest bacitracin may be a weak chelator (Garbutt et al, 1961) which may upset the equilibrium of receptor binding, although the same concentration of calcium was added to all preparations. Another inhibitor, amastatin, was included in the assay buffer instead of bacitracin. Unfortunately, at this time there was a loss of specific binding in the assays containing bacitracin, therefore it was difficult to compare the binding produced with amastatin. However, micromolor concentrations of AII were still needed to produce inhibition of ^{125}I-AII binding, and displacement curves resembled those previously obtained with bacitracin. Neither omission of BSA, routinely used in the buffer nor combination of amastatin and bacitracin or new stocks of bacitracin solved the lack of binding in the presence of bacitracin.

4. Scatchard analysis from the preparations involving nanomolar and micromolar concentrations of AII suggest that there are different classes of receptors present in these preparations. This may be due to loss of one class of receptors during preparation (if there were two) or masking of one class.

The presence of high capacity low affinity receptors could explain why so much AII was necessary to displace the iodinated peptide since all the sites would have to be occupied before displacement of ^{125}I-AII became apparent. This would also explain the low rate of degradation since membrane bound peptides are relatively resistant to enzymes (Cox et al, 1986). These could be "spare" receptors since different biological responses require different degrees of receptor occupancy or "clearance" receptors serving as specific peripheral storage - clearance binding sites (Maack et al, 1987).

Contradictory reports on the number of classes of receptor in other species came from LaFontaine et al (1979) who detected only one class of binding site in rat liver plasma membranes and Campanile et al, (1982) who observed 2 sites. Campanile proposed these differences may have been due to:- a. the procedure of membrane preparation being slightly different b. different sequences of AII used to displace the iodinated peptide which may be degraded by aminopeptidases at different rates (Khairallah et al, 1963; Khairallah and Page, 1967).

Reduction may destroy receptors, inactivate the high affinity site or convert low to high affinity sites (McQueen et al, 1984; Campanile et al, 1982). DTT is known to inactivate the vascular receptor and the high affinity liver site through receptor disulphide bond reduction (Gunther et al, 1980).

It has been suggested that the low affinity site may represent binding to degradative enzymes (Takei et al, 1988). However, although these non-receptor proteins can exhibit high affinity and specificity the specificity for the plasma membrane binding site is much stricter than the substrate specificity for enzymes which metabolise AII. The low affinity site may also represent the resting state of the receptor with AII interaction inducing a portion of the receptor into a high affinity "excited state" (Moore and Kwok, 1987).

Gunther et al (1980) suggest that hormone induced stimulation of glycogen phosphorylase by AII in the liver correlates well with fractional occupancy of the high affinity receptor site. In contrast, binding to the low affinity site correlated well with hormone induced inhibition of adenylate cyclase. The high affinity site is identified as the calcium pathway receptor and the low affinity as the adenylate cyclase coupled receptor.

5. Transient covalent bonding of the tyrosine (tyr) hydroxyl group to the AII receptor has been suggested by Moore and Matsoukas (1985) and it has also been proposed that the tyr hydroxyl proton in AII may form a hydrogen bond with the AII receptor (Hsieh et al, 1979). Chloramine T used in the iodination process has been shown to cause covalent bond formation by oxidising and therefore activating several amino acids, between hormone and receptor, EGF is an example (Linsley et al, 1979).

6. This inconsistency between concentrations of AII (uM) required to produce displacement of iodinated AII (pM) suggests the iodinated peptide is binding the receptor with a much greater affinity than AII or that binding may be covalent or even to some extent irreversible. Most peptides are iodinated with ^{125}I sodium iodide which was the case for the peptides used in the eel membrane assays, along with the oxidising agents chloramine T or iodogen. This rather large electronegative atom can alter both the physical and biological properties of the hormone. Although iodination can be adjusted, usually by pH, to give an average of one iodide atom per hormone molecule, the procedure actually generates a mixture of uniodinated, mono and diiodinated species. Tyrosyl can be converted to monoiodotyrosyl with minimal loss of hormone activity, on the contrary the diiodinated derivative is almost completely inactivated. There are 2 main effects of iodination of a tyrosyl group:- a. the iodine atom being of comparable size to the benzene nucleus may sterically interfere with the amino acids immediate environment b. the pK of the phenolic OH is substantially lowered. The conversion of angiotensin into the monoiodinated version will therefore result in a doubling of the size of the tyr^4 side chain, with a lowering of the pKOH by 2 units. Available data however, suggest the tyrosyl group has little structural relationship to its immediate environment (Kurcbart et al, 1971).

An example of iodination altering the properties of a peptide in a fish preparation is:- ovine PRL displaced iodinated Tilapia PRL, but ^{125}I-ovine PRL demonstrated

negligible specific binding. Observations suggest iodination of ovine PRL alters the protein such that it no longer binds the teleost kidney PRL binding site (Fryer, 1979b).

It appears possible that the binding affinity of AII for its receptor, could be altered by iodination since tyr, the amino acid which is iodinated, at position 4 of AII has been reported to be the key amino acid for binding of AII to the human arteriolar receptor (Kono et al, 1987). This hypothesis arose from studies involving infusion of various fragments of the AII peptide into normal men. All fragments tested (1-5 pentapeptide, 1-4 tetrapeptide, AIII, 3-8 hexapeptide, 4-8 pentapeptide, 2-7 hexapeptide, 1-7 heptapeptide and the 1-6 hexapeptide of AII) except 1-3 tripeptide and 5-8 tetrapeptide of AII produced a pressor action.

Analysis of antagonistic potency of analogues of AII and AIII, in which the tyr and/or phenylalanine (phe) residues were substituted, in a rat isolated uterus assay indicated each peptide had less than 0.1% agonist activity of AII. The significance of the tyr hydroxyl group in angiotensin may be related to its proposed participation in a tyrosine charge relay system (tyr-OH-His-carboxylate interaction) (Matsoukas, et al, 1985). The fact that a minimum of val-tyr-ile/val-his from AII was found to be necessary for direct renin suppressing action in man, also follows this theory (Kono et al, 1987).

Phe at position 8 may also be involved in this system since antagonistic activity of analogues of AII in which the phe residue is substituted appears to result from elimination of a his/phe stacking interaction in AII, where the phe ring shields the his sidechain (Freer and Stewart, 1973). This lack of interaction may affect conformational properties and therefore binding characteristics of the AII analogues.

Early studies on eel liver membrane preparations involved the use of ^{125}I-ile^{5}-AII, which was not entirely satisfactory since the reported sequence of eel AII contains val at position 5 (Hasegawa et al, 1983; Khosla et al, 1985). However, in light of earlier results on physiological actions of the two AII sequences a series of experiments were conducted using the iodinated version of each peptide. Assays including homologous combinations of the native and radioactive sequences of the hormone did not produce the expected results. Both the FW and SW liver membrane preparations were apparently more responsive to the ile^{5} sequences of ^{125}I-AII, producing both greater total and specific binding that the equivalent incubations with the val^{5} sequence. However, again in plaice and dab, the binding trend was reversed with greater binding of the val^{5} than the ile^{5} sequence of ^{125}I-AII. It appears, however that the two sequences of AII had the same ability to displace each radioactive peptide.

Comparisons of binding potencies of non-radioactive angiotensins in the fowl aorta indicate that significantly

more val^5-AII than ile^5-AII is necessary to displace bound ^{125}I-val^5-AII or ^{125}I-ile^5-AII (Takei et al, 1988). This data also appears contradictory as fowl AII contains val at position 5 (Nakayama et al, 1973) and therefore it would be expected that endogenous receptors were specific for this sequence. In contrast, val^5-AII had greater binding potency than ile^5 to the rat hepatic receptor and had greater pressor activity in rats although again to the contrary rat AII includes ile at position 5 (Sernia et al, 1985 Sokabe and Watanabe, 1977). These observations suggest flatfish AII may in fact be of the ile^5 sequence since the non-native sequences, perhaps surprisingly, seem to be more potent in receptor displacement. In man, however the native peptide, ile^5-AII, had stronger pressor and steroidogenic activities, with longer duration of the pressor response than asn^1-val^5-AII (Kono et al, 1985), although this does not necessarily reflect potency of binding.

Studies involving displacement of ^{125}I-ile^5-AII, by a range of angiotensin related peptides are summarised in table 4.2. Investigations involving FW liver membranes revealed sar^1, ile^5-AII as the most potent inhibitor of binding. Sar-AII contains a sarcosine (N-methylglycine) in position 1 of the peptide. Additional reports of sar-AII having greater potency than AII in binding inhibition of AII include studies on rat uterine smooth muscle (Moore and Kwok, 1987), human platelets (Ding et al, 1984) and rat ovarian membranes (Husain et al, 1987). Sar-AII also produced greater pressor and steroidogenic activity in man, than ile^5-AII (Kono et al, 1985). This increased binding

capacity could be due to the position 1 substitution increasing the biological half life of the peptide (Matsoukas et al, 1985). Harding et al (1986) have shown a half life in the circulation of 100s, which is greater than that of AII. Authors also suggest sar^1 could protect the peptide from degradation (Turker et al, 1972) since an angiotensin peptidase found in human plasma and red blood cells hydrolyses val^5- and ile^5-AII and requires the presence of asp or asn in position 1 (Khairallah et al, 1963). Sar^1-AII may also bind receptors more tightly and therefore become more resistant to metabolism (Kono et al, 1985). The rate of internalisation (receptor-mediated endocytosis) and therefore degradation of sar^1-AII was found to be lower than that of AII in adrenocortical cells (Crozat et al, 1986).

In fish preparations sar^1, ile^8-AII was found to have neither agonistic vasopressor nor antagonistic responses in eels against asn^1, val^5-AII or eel angiotensin, although at a higher dose the peptide did increase eel aortic pressure and reduced the vasopressor response to asn^1, val^5-AII. The authors suggest local environment such as pH or ionic composition of the interstitial fluid may differ in eels and thus alter responsiveness to angiotensin derivatives (Nishimura et al, 1978). In goldfish, sarcosine analogues of AII failed to block AII stimulated ACTH release (Weld and Fryer, 1987).

The 5-8 AII tetrapeptide proved as potent, in this study of FW eel liver membranes, as AII in causing receptor

Table 4.2.

TISSUE	ANALOGUE POTENCIES IN RECEPTOR DISPLACEMENT OF ^{125}I-AII	REF
Arterial Arcades of Rat Mesentry	^{125}I-AII=AII>AIII>sar^{1},ile^{8}-AII>sar^{1},gly^{8}-AII>AI	McQueen et al, 1984
Rat Liver Membranes	val^{5}-AII>ile^{5}-AII>AIII>sar^{1},ala^{8}-AII>sar^{1},gly^{8}-AII>AI>des asp^{1}-AI>C4-C8>phe^{1},tyr^{8}-AII>>Neurotensin>LHRH pentapeptide of AII	Sernia et al, 1985
Isolated Rat Glomeruli	AII=AI=ile^{8}-AII=sar^{1},ile^{8}-AII	Sraer et al, 1974
Human Platelets	sar^{1},thr^{8}-AII>AII>AIII>sar^{1},ala^{8}-AII>AI	Ding et al, 1984
Rat Ovarian Membranes	sar^{1},ile^{8}-AII>AII>des asp^{1}-AII>AI>des asp^{1}arg^{2}-AII	Husain et al, 1987
Cultured Rat Brain Glial Cells	AII=sar^{1},ala^{8}-AII>sar^{1},ile^{8}-AII>>AIII>AI	Raizada et al, 1987

binding inhibition. This is in contrast to reports of the fragment having no pressor action in man (Kono et al, 1987) and decreasing chain length of COOH terminal peptides displaying progressive reduction in binding inhibition activity of dog adrenal and uterine receptors (Capponi and Catt, 1979). The 4-8 AII pentapeptide was demonstrated to be the shortest fragment able to stimulate aldosterone activity in the dog adrenal glomerulosa (Capponi and Catt, 1979). The potency of the 5-8 AII tetrapeptide may be attributed to the presence of the phe residue in position 8 which many authors believe is important in eliciting the biological activity of AII. Phe^8 has been shown to be important for aldosterone production (Kono et al, 1987) and for enhanced pressor action of the AII molecule (Kono et al, 1975; Kono et al, 1978; Kono et al 1982; Kono et al 1986). Removal of phe^8 impaired binding activity of the molecule and almost completely abolished its biological activity in the dog adrenal cortex and uterus (Capponi and Catt, 1979). Replacement of the c-terminal phe residue by non-aromatic hydrophobic amino acids produces antagonists (Matsoukas et al, 1985). Ile^8, thr^8 (Khosla et al, 1974), ala^8 and ile^8-AII were all competitive inhibitors of AII (Turker et al, 1972). This antagonistic activity is thought to result from elimination of a his/phe ring stacking interaction in AII (Matsoukas et al, 1985).

The fact that bradykinin produced inhibition of ^{125}I-AII binding, although it only has sequence homology of 2 amino acids (pro^7 and phe^8) could be due to the occupancy of

position 8 by phenylalanine. However ^{125}I-ile^{5} and ^{125}I-val^{5}-AII were not competitively displaced by bradykinin in the fowl aorta (Takei et al, 1988).

Ile^{4}- and val^{4}- AIII produced displacement of ^{125}I-AII from FW eel liver membranes, however neither were as potent as AII and val^{4}-AIII did not produce the same extent of inhibition as ile^{4}-AIII. This last fact compares with ile in position 5 of AII producing greater displacement of the iodinated peptide than the equivalent peptide containing val. Analysis of specific receptors for AIII is controversial with reports of a specific receptor in rat adrenals (Devynck et al, 1977), while other observations suggest AII and AIII bind the same receptor with the same affinity and biological activity in rat adrenal glomerulosa (Douglas et al, 1985). Kono et al (1987) found no specific AIII receptors in human arterioles. Differences in the biological properties of AII and AIII analogues substituted at the tyr residue, suggest different binding/conformational requirements for the two endogenous hormones at AII receptors in smooth muscle (Matsoukas et al, 1985). The AIII molecule is more tolerant to chemical modification in general than AII, which also suggests a strict conformational requirement of AII for binding to its receptor. A turn has been reported to be the preferential configuration of AII (Fermandjian et al, 1972). Ackerly et al (1977) suggest that smooth muscle preparations resistant to tachyphylaxis reflect the capacity of the tissue to convert AII to AIII. This would indicate that AIII either no longer binds the AII receptor or at least is unable to

trigger the intracellular events normally elicited by AII.

The ability of AI to displace ^{125}I-AII from the eel preparation does not appear to depend to the same extent on the residue at position 5 for potency, with both human (ile^5) and eel AI (val^5) producing inhibition of binding. The fact that the AI's produced similar if not greater displacement than AIII's from the receptor is contrary to studies cited in table 4.2. AIII produced greater binding inhibition than AI in rat mesenteric arterial arcades (McQueen et al, 1984), rat liver membranes (Sernia et al, 1985), human platelets (Ding et al, 1984) and cultured rat brain glial cells (Raizada et al, 1987), with AI having little effect on binding of tracer AII except in isolated rat glomeruli where the four peptides tested were equally potent as competitive inhibitors of ^{125}I-AII binding (Sraer et al, 1974). McQueen et al (1984) postulate that the discrimination between AI and AII by the AII receptor of the rat mesenteric artery is related to pressor activities of the peptides in the rat, with AI having little intrinsic activity (Bumpus et al, 1961). This compares to the lack of inhibition of the drinking response in eels by Captopril and the possibility that the AII receptor also recognises AI. In the goldfish corticotrope, Captopril was also unable to block salmon AI stimulated ACTH release, indicating that the angiotensin receptor could not discriminate between AI and AII (Weld and Fryer, 1987).

There are several lines of evidence that receptors are evolutionarily more conserved than the peptide hormones that

bind them. 1. kinetically receptors frequently retain an ominibus binding site that can recognise many members of a hormone family. 2. antibodies to the insulin, GH and PRL receptors have been shown to cross react with the peptides of many species. (Muggeo et al, 1979; Drake and Friesen 1981).

In summary, an osmoregulatory role for the RAS in eels can be postulated from results presented in this thesis and other documented data. Plasma AII concentrations increase in eels transferred from FW to SW. The peptide in turn increases drinking rates, ingesting water and excess sodium, possibly contributing to increased blood volume/pressure. AII may increase ANP release which may produce a short-term natriuretic effect at the gill. It remains a possibility that AII, perhaps along with ANP, may also stimulate cortisol secretion which could have a long-term natriuretic effect during SW acclimation (see section 1.4.8). Unfortunately it was difficult to compare receptor studies in different tissues of FW and SW acclimated eels and an asmoregulatory role for the liver in fish - the model used in the majority of receptor studies in this thesis - is as yet unclear although it could possibly be involved in maintenance of blood volume.

Clearly more studies are required to elucidate fully the hormonal control of osmoregulation in euryhaline teleost fish and further experiments following investigations presented here include:-

1. sequencing of eel, plaice and dab AII.

2. determination of plasma AII concentrations in FW and SW adapted fish.
3. determination of plasma ANP levels in FW and SW acclimated fish and also following AII administration.
4. development of a specific radioreceptor assay in a key osmoregulatory tissue, such as gill or glomerular preparations.
5. second messenger studies especially following binding of both sequences of AII.
6. determination of a role for AII in teleost liver.

REFERENCES

Acher, R. (1974). Chemistry of the neurohypophysial hormones: An example of molecular evolution. In "Handbook of Physiology" section 7 "Endocrinology"; vol 4, part 1 (Eds. E. Knobil and, W.H. Sawyer). pp.119-130. Amer. Physiol. Soc., Washington.

Acher, R. (1988).The neurohypophysial hormone - neurophysin precursors: Composite biosynthesis and composite evolution. In "Progress in Endocrinology" (Eds. H. Imura et al). pp.1505-1506. Elsevier Science Publ., Amsterdam.

Acher, R. and Chauvet, J. (1988). Structure, processing and evolution of the neurohypophysial hormone - neurophysin precursors. Biochimie 70: 1197-1207.

Ackerly, J.A., Moore, A.F. and Peach, M.J. (1977). Demonstration of different contractile mechanisms for angiotensin II and des-asp^{1}-angiotensin II in rabbit aortic strips. Proc. Natl. Acad. Sci. USA. 74:5725-5728.

Ando, M., (1981). Effects of ouabain on chloride movements across the seawater eel intestine, J. Comp. Physiol 145:73-79.

Ando, M., (1983). Potassium-dependent chloride and water transport across the seawater eel intestine. J. Membr. Biol. 73:125-130.

Ando, M. (1985). Relationship between coupled Na^{+}-K^{+}-Cl^{-} transport and water absorption across the seawater eel intestine. J. Comp. Physiol 155:311-317.

Atarashi, K., Mulrow, P.J., Franco-Saenz, R., Snajdar, R. and Rapp, J. (1984). Inhibition of aldosterone production by an atrial extract. Science 224:992-994.

Babiker, M.M. and Rankin, J.C. (1978). Neurohypophysial hormonal control of kidney function in the European eel (Anguilla, anguilla L.) adapted to sea-water or fresh water. J. Endocr. 76:347-358.

Bailey, J.R. and Randall, D.J. (1981). Renal perfusion pressure and renin secretion in the rainbow trout, Salmo gairdneri. Can. J. Zool. 59:1220-1226.

Baker, K.M., Campanile C.P., Trachte, G.J. and Peach, M.J., (1984). Identification of the rabbit angiotensin II myocardial receptor. Circ. Res. 54:286.

Balm, P.H.M. (1986). Osmoregulation in teleosts by cortisol and prolactin. Adaptation to low pH environments. Thesis, University of Nijmegen.

Balment, R.J. and Carrick, S. (1985). Endogenous renin-angiotensin system and drinking behaviour in flounder. Am. J. Physiol. 248:R157-R160.

Balment, R.J. and Henderson, I.W. (1987). Secretions of Endocrine glands and their relationship to ormoregulation. In Fundamentals of Comparative Vertebrate Endocrinology". (Eds. I. Chester Jones, P.M. Ingleton, J.G. Phillips). pp.413-508 Plenum Press, New York and London.

Balment, R.J., Hazon, N. and Perrott, M.N. (1987). Control of corticosteroid secretion and its relation to osmoregulation in lower vertebrates. In "Comparative Physiology of Environmental Adaptations vol. 1. 8th ESCP Conf. Strasbourg 1986, (Eds. R. Kirsch and B. Lahlou). pp.92-102 Karger, Basel.

Baranowski, R.L. and Westenfelder, C. (1989). Secretion of atrial natriuretic peptide (ANP) from fish atrial and ventricular myocytes in tissue culture. Life Sci. 44:187-191.

Barnett, D.B., Rugg, E.R. and Nahorski, S.R. (1978). Direct evidence of two types of adrenoceptor binding site in lung tissue. Nature 273:166-168.

Batten, T.F.C. and Ingleton, P.M. (1987). The structure and function of the hypothalamus and pituitary gland. In "Fundamentals of Comparative Vertebrate Endocrinology". (Eds. J. G. Phillips, I. Chester-Jones, P. M. Ingleton). Plenum Press, New York and London. pp.285-409.

Beasley, D., Schier, D.N., Malvin, R.L. and Smith, G., (1986). Angiotensin stimulated drinking in marine fish. Am. J. Physiol. 250:R1034-R1038.

Bennet, J.P.and Snyder, S.H. (1976). Angiotensin II binding to mammalian brain membranes. J. Biol. Chem. 251:7423-7430.

Bennett, J.P. and Snyder, S.H. (1980). Regulation of receptor binding interactions of ^{125}I-angiotensin II and ^{125}I-[sarcosine^{1},leucine^{8}], angiotensin II, an angiotensin antagonist by sodium ion. Eur. J. Pharmacol. 67:1-10.

Bentley, P.J. (1971.) Failure to detect amphibian-like levels of vasotocin in the plasma of the eel Anguilla rostrata. J. Endocr. 49:183-184.

Bentley, P.J. (1973). Role of the skin in amphibian sodium metabolism. Science N.Y. 181:686-687.

Berlind, A. Lacanilao, F., and Bern H.A. (1972). Teleost caudal neurosecretory system: Effects of osmotic stress on urophysial proteins and active factors. Comp. Biochem. Physiol. 42A:345-352.

Bern, H. A. (1975). Prolactin and osmoregulation. Am. Zool. 15:937-948.

Bern, H.A. (1985). The elusive urophysis-Twenty years in pursuit of caudal neurohormones. Am. Zool. 15:763-769.

Bern, H.A. and Nishioka, R.S. (1979). The caudal neurosecretory system and osmoregulation. Gunma. Symp. Endocrinol. 16:9-17.

Bern, H.A., Nishioka, R.S., Chester-Jones I, Chan D.K.O., Rankin J.C. and Ponniah, S. (1967). The urophysis of teleost fish. J. Endocr. 37, xL-xLi (abstr).

Bianchi, C., Gutkowska, J., Charbonneau, C., Ballak, M., Anand-Srivastava, M.B., De Lean, A., Genest, J., and Cantin, M. (1986). Internalisation and lysosomal association of [^{125}I] angiotensin II in norepinehrine-containing cells of the rat adrenal medulla. Endocrinology 119:1873-1875.

Blanc, E., Sraer, J., Sraer, J.D., Baud, L. and Ardaillou, R. (1978). Ca^{2+} and Mg^{2+} dependence of angiotensin II binding to isolated rat renal glomeruli. Biochem. Pharmacol. 27:517-524.

Bolander, F.F. (1989). "Molecular Endocrinology" pp61-118 San Diego, California.

Bolton, J.P. and Henderson, I.W. (1987). Water uptake by Rana temporaria effects of diuretics, the renin-angiotensin system and nephrectomy. Gen. Comp. Endocr 66:155-162.

Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilising the principle of protein-dye binding. Analytical Biochemistry 72:248-254.

Broadhead, C.L., Deacon, C.F., O'Sullivan, U.T. and Henderson, I.W. (1989). Specific receptors and general characteristics of atrial natriuretic peptide (ANP) in the eel, Anguilla anguilla L.: Influences of environmental salinity. Arch. Int. Phys. Biochem. 97:C9 [Abstr.].

Brown, P.S. and Brown S.C., (1987). Osmoregulatory actions of prolactin and other adenohypophysial hormones. In "Vertebrate Endocrinology: Fundamentals and biomedical implications. Regulation of water and electrolytes. (Eds. P.K.T. Pang and M. P. Schreibman) pp.45-73. Academic Press, London.

Brown, G.P. and Douglas, J.G. (1982.) Angiotensin II binding on isolated rat renal brush border membranes. Endocrinology 1830-1836.

Brown, G., Douglas, J. and Bravo, E. (1980b). Angiotensin receptors and in vitro aldosterone responses of aldosterene producing adenoma, adjacent non-tumorous tissue, and normal human adrenal glomerulosa. J. Clin. Endocrinol. Metab. 51:718-723.

Brown, J.A., Oliver, J.A., Henderson, I.W., and Jackson, B.A. (1980a). Angiotensin and single nephron glomerular function in the trout Salmo gairdneri. Am. J. Physiol. 239:R509-R514.

Bulger, R.E. (1965). The fine structure of the aglomerular nephron of the toadfish Opsanus tau. Am. J. Anat. 117:171-192.

Bulger, R.E. and Trump, B.F. (1965). Effects of fixatives on tubular ultrastructure of the aglomerulor midshipman, Porichthys notatus, and the glomerular flounder, Parophrys vetulus. J. Histochem. Cytochem. 13:719.

Bumpus, F.M., Khairallah, P.A., Arakawa, K., Page, I.M., and Smeby, R.R. (1961). The relationship of $_5$structure to pressor and oxytocic actions of isoleucine^5 angiotensin octapeptide and various analogues. Biochim. Biophys. Acta 46:38-46.

Butler, D.G. and Carmichael, F.J. (1972). (Na^+ - K^+) - ATPase activity in eel (Anguilla anguilla) gills in relation to changes in environmental salinity. Role of adrenocortical steroids. Gen. Comp. Endocr. 19:421-427.

Campanile, C.P., Crane, J.K., Peach, M.J. and Garrison, J.C. (1982). The hepatic angiotensin II receptor. J. Biol. Chem. 257:4951-4958.

Campbell, W.B., Currie, M.G. and Needleman, P. (1985). Inhibition of aldosterone biosynthesis by atriopeptins in rat adrenal cells. Circ. Res. 57:113-118.

Capelli, J.P., Wesson, L.G. and Aponte, G.E. (1970). A phylogenetic study of the renin-angiotensin system. Am. J. Physiol. 218:1171-1178.

Capponi, A.M. and Catt, K.J. (1979). Angiotensin II receptors in adrenal cortex and uterus. J. Biol. Chem. 254:5120-5127.

Capponi, A.M. and Catt, K.J. (1980). Solubilization and characterization of adrenal and uterine angiotensin II receptors after photoaffinity labeling. J. Biol. Chem. 255: 12081-12086.

Cardenas-Tanus, R.J., Huerta-Bahena, J., and Garcia-Sainz, J.A. (1982). Angiotensin II inhibits the accumulation of cyclic AMP produced by glucagon but not its metabolic effects. FEBS letters 143:1-4.

Carrick, S. and Balment, R.J. (1983). The renin angiotensin system and drinking in the euryhaline flounder, Platichthys flesus. Gen. Comp. Endocr. 51: 423-433.

Carriere, P.D., De Lean, A., Gutkowska, J. and Cantin, M. (1986). Estrogens directly down-regulate receptors for angiotensin II in anterior pituitary cell culture without decreasing target cell responsiveness. Endocrinology 119:429-431.

Carroll, R. G. and Opdyke, D.F. (1982). Evolution of angiotensin II induced catecholamine release. Am. J. Physiol. 243:R65-R69.

Carroll, J.E., Landry, A.S., Elliot, M.E. and Goodfriend, T.L. (1986). Effects of pH on adrenal angiotensin receptors and responses. J. Lab. Clin. Med. 108:23-29.

Castren, E., Kurihara, M. and Saavedra, J.M., (1987). Autoradiographic localisation and characterisation of angiotensin II binding sites in the spleen of rats and mice. Peptides 8:737-742.

Catt, K.J. and Dufau, M.L. (1977) Peptide hormone receptors. Ann. Rev. Physiol. 39:529-57.

Catt, K.J., Aguilera, G., Capponi, A., Fujita K., Schirar, A. and Fakunding J. (1979.) Angiotensin II receptors and aldosterone secretion. J. Endocr. 81:37P-48P.

Chan, D.K.O. (1975). Cardiovascular and renal effects of urotensins I and II in the eel Anguilla rostrata. Gen. Comp. Endocr. 27:52-61.

Chartier L., Schiffrin, E., Thibault, G. and Garcia, R. (1984). Atrial natriuretic factor inhibits the stimulation of aldosterone secretion by angiotensin II, ACTH and potassium in vitro and angiotensin II induced steroidogenesis in vivo. Endocrinology 115:2026-2028.

Chester Jones, I., Chan D.K.O. and Rankin, J.C. (1969). Renal function in the European eel Anguilla anguilla L. Effects of the caudal neurohypophysial peptides and vasoactive substances. J. Endocr. 43:21-31.

Chester Jones, I., Henderson, I.W., Wales, N.A.M. and Garland H.O. (1971). Effects of arginine vasotocin in fish and amphibians. In "Neurohypophysial Hormones", Ciba Foundation Study Group No. 39. (Eds. G. E. W. Wolstenholme and J. Birch) pp. 29-44. Churchill Livingstone, Edinburgh and London.

Chester Jones, I., Bellamy, D., Chan, D.K.O., Follet, B.K., Henderson, I.W., Phillips, J.G. and Snart, R.S. (1972). Biological actions of steroid hormones in nonmammalian vertebrates. In "Steroids in Nonmammalian Vertebrates". (Ed. D. R. Idler) pp.414-480 Academic Press, New York.

Chester Jones, I., Henderson, I.W., Chan, D.K.O., Rankin, J.C. Moseley, W. Brown J.J., Lever A.F., Robertson J.I.S. and Tree M. (1966). Pressor activity in extracts of the Corpuscles of Stannius from the European eel. (Anguilla anguilla L.). J. Endocr. 34:393-408.

Christensen, J.A., Knutsson, S., and Bohle, A., (1982). Granular epitheloid cells in the kidney of the Atlantic salmon (Salmo salar). Kidney Int. Suppl. 12:5179-5184.

Claiborne, J.B. and Evans, D.H. (1980). The isolated perfused head of the marine teleost fish Myoxecephalus octodecimspinosus : Hemodynamic effects of epinephrine. J. Comp. Physiol 138:79-85.

Clarke W.C. and Bern, H.A. (1980). Comparative endocrinology of prolactin. In "Hormonal Proteins and Peptides" (Ed. C. H. Li) pp.105-197. Academic Press, New York.

Clarke, W.C., Farmer, S.W. and well, K.U. (1977). Effects of teleost pituitary growth hormone on growth of Tilapia mossambica and on growth and sea water adaptation of sockeye salmon (Oncorhynchus nerka). Gen. Comp. Endocr. 33:174-178.

Collie, N.L., Bolton, J.P., Hirano, T. and Kawauchi, H. (1985). The effects of salmon growth hormone on seawater adaptation of rainbow trout. Zool. Sci. 2:973 (abstract).

Conlon, J.M., Deacon, C.F., Hazon, N., Henderson, I.W. and Thim, L (1988). Somatostatin-related and glucagon-related peptides with unusual structural features from the European eel Anguilla anguilla. Gen. Comp. Endocr. 72:181-188.

Conte, F.P. (1969). Salt secretion. In "Fish Physiology" vol. 1 (Eds. W. S. Hoar and D. J. Randall) pp.241-292. Academic Press, New York.

Cooke, S.F., Craven, D.J. and Symonds, E.M. (1981). A study of angiotensin II binding sites in human placenta, chorion, and amnion. Am. J. Obstet. Gynecol. 140:689-692.

Cox, H.M., Munday, K.A. and Poat, J.A., (1986). Identification of selecive, high affinity ^{125}I-Bradykinin (Bk) binding sites in rat intestinal epithelia. Br. J. Pharmacol. 87:201-209.

Crane, J.K., Campanile, C.P. and Garrison, J.C. (1982). The hepatic angiotensin II receptor. J. Biol. Chem. 257:4959-4956.

Crozat, A., Penhoat, A. and Saez, J.M. (1986). Processing of angiotensin II (A-II) and (sar^1,ala^8) AII by cultural bovine adrenocortical cells. Endocrinology 118:2312-2318.

Cuatrecasas, P. (1972.) Isolation of the insulin receptor of liver and fat cell membranes. P.N.A.S. 69:318-322.

Dange, A.D. (1986). Branchial Na^+-K^+-ATPase activity in freshwater or saltwater acclimated tilipia, Oreochromis (Sarotherodon) mossambicus. Effects of cortisol and thyroxine. Gen. Comp. Endocr. 62:341-343.

Darnell, J., Lodish, H. and Baltimore, D. (1990). Molecular Cell Biology 2nd Ed. Scientific American Books distributed by W.H. Freeman and Co. N.Y.

De Bold, A.J., Borenstein, H.B., Veress, A.T. and Sonnenberg, H. (1981). A rapid and potent natriuretic response to intravenous injection of atrial myocardial extracts in rats. Life Sci. 28:89-94.

Decourt, C. and Lahlou, B. (1987). Evidence for the direct intervention of AII in the release of cortisol in teleost fishes. Life Sciences. 41:1517-1524.

Degnan, K.J. and Zadunaisky, J.A. (1980). Passive sodium movements across the opercular epithelium: The paracellular shunt pathway and ionic conductance. J. Membr. Biol. 55:175-185.

Degnan, K.J., Karnaky, K.J. Jr. and Zadunaisky, J.A. (1977). Active chloride transport in the in vitro opercular skin of a teleost (Fundulus heteroclitus) a gill-like epithelium rich in chloride cells. J. Physiol. London. 271:155:191.

Devynck, M.A. and Meyer, P. (1978). Angiotensin receptors, Biochem. Pharmacol. 27:1-5.

Devynck, M.A., Pernollet, M.G., Meyer, P., Fermandjian, S., Fromageot, P. and Bumpus, F.M., (1974). Sulubilisation of angiotensin II receptors in rabbit aortae membranes. Nature 249:67-68.

Devynck, M.A., Pernollet, M.G., Matthews, P.G., Khosla, M.C., Bumpus, F.M. and Meyer, P. (1977). Specific receptors for des asp^1-angiotensin II ("angiotensin III") in rat adrenals. Proc. Natl. Acad. Sci. 74: 4029-4032.

Dharmamba, M and Maetz, J. (1972). Effects of hypophysectomy and prolactin on the sodium balance of Tilapia mossambica adapted to sea water. Gen. Comp. Endocr. 21:179-187.

Dharmamba, M and Maetz, J. (1976). Branchial sodium exchange in seawater adapted Tilapia mossambica: Effects of prolactin and hypophysectomy. J. Endocr. 70:293-299.

DiBona, G.F. (1983). Catecholamines and neuroadrenergic control of renal function. In "Renal Endocrinology" (Ed. M. J. Dunn). pp.323-366. Williams and Wilkins, Baltimore, Mayland.

Ding, Y.A., Kenyon, C.J. and Semple, P.F. (1984). Receptors for angiotensin II on platelets from man. Clin. Sci. 66:725-731.

Ding, Y.A., Kenyon, C.J. and Semple, P.F. (1985). Regulation of platelet receptors for angiotensin II in man. J. Hypertension 3:209-212.

Doneen, B.A. (1976). Water and ion movements in the urinary bladder of the Gobiid teleost Gillichthys mirabilis in response to prolactins and to cortisol. Gen. Comp. Endocr. 28:33-41.

Douglas, J.G. (1987). Angiotensin receptor subtypes of the kidney cortex. Am. J. Physiol 253:F1-F7.

Douglas, J.G., Brown, G. and White, C., (1982). Influence of cations on kinetics of angiotensin II binding to adrenal, renal and smooth muscle receptors. Hypertension (Suppl. III) 4:111-79.

Douglas, J.G., Brown, P. and White, C., (1984). Angiotensin II receptors of human and primate adrenal fasciculata and glomerulosa: Correlations of binding and steroidogenesis. Metabolism 33:685-688.

Douglas, J.C., Khosla, M.C. and Bumpus, F.M. (1985). Efficacy of octa- and heptapeptide antagonists of angiotensin II as inhibitors of angiotensin III binding in the rat adrenal glomerulosa. Endocrinology 116:1598-1602.

Douglas, J., Aguilera, G., Kondo, T.O. and Catt, K. (1978). Angiotensin II receptors and aldosterone production in rat adrenal glomerulosa cells. Endocrinology 102:685.

Doyle, W.L. and Gorecki, D. (1961). The so-called chloride cell of the fish gill. Physiol. Zool. 34:81-88.

Doyle, W.L. and Epstein, F.H. (1972). Effects of cortisol treatment and osmotic adaptation on the cytology of chloride cells in the eel Anguilla rostrata. Cytobiologie 6:58-73.

Drake, R.G. and Friesen, H.G. (1981). Antibodies to prolactin receptors and growth hormone receptors. In "Methods in Enzymology" (Eds. J.J. Langonen, and H.V. Vunakis,) Vol. 74, pp.380-405. Academic Press, New York.

Duff, D.W. and Olson, K.R. (1986). Trout vascular and renal responses to atrial natriuretic factor and heart extracts. Am. J. Physiol. 251:R639-R642.

Edery, M., Young, G., Bern, H.A. and Steiny, S. (1984). Prolactin receptors in tilapia (Sarotherodon mossambicus) tissues: Binding studies using ^{125}I-labeled ovine prolactin. Gen. Comp. Endocr. 56:19-23.

Elger, M., and Hentschel, H. (1983). Effect of adrenergic blockade with bretylium and phentolamine on glomemular filtration rate in the rainbow trout Salmo gairdineri adapting to saline water. Comp. Biochem. Physiol. C75C: 253-258.

Ellory, J.C., Lahlou, B. and Smith, M.W. (1972). Changes in the intestinal transport of sodium induced by exposure of goldfish to a saline environment. J. Physiol. (London) 222:497-509.

Epstein, F.H., Cynamon, M. and McKay, W. (1971). Endocrine control of Na-K-ATPase and seawater adaptation in Anguilla rostrata. Gen. Comp. Endocr. 16:323-328.

Epstein, F.H., Maetz, J. and Renzis, G. (1973). Active transport of chloride by the teleost gill: inhibition by thiocyanate. Am. J. Physiol. 224:1295-1299.

Epstein, F.H., Silva, P. and Kormanik, G. (1980). Role of $Na^+ - K^+$ - ATPase in chloride cell function. Am. J. Physiol. 238:R246-R250.

Epstein, F.H., Manitius, A., Weinstein, E., Katz, A.I. and Pickford, G.E. (1969). Sodium and potassium-activated adenosine triphosphatase in kidneys of Fundulus heteroclitus adapted to fresh and saltwater. Yale. J. Biol. Med. 41:388-393.

Evans, D.H. and Mallery, C.H. (1975). Time course of seawater acclimation by the euryhaline teleost. Dormitator maculatus: Correlation between potassium stimulation of sodium efflux and Na^+/K^+ activated ATPase activity. J. Comp. Physiol. 96:117-122.

Evans, D.E., Chipoura, E. and Payne, J.A. (1989). Immunoreactive atriopeptin in plasma of fishes: its potential role in gill haemodynamics. Am. J. Physiol 257:R939-R945.

Feldstein, J., Sumners, C. and Raizada, M.K. (1986). Sodium increases angiotensin II receptors in neuronal cultures from brains of normotensive and hypertensive rats. Brain. Res. 370: 265-272.

Fermandjian, S., Greff, B. and Fromageot, P. (1972). In "Chemistry and Biology of Peptides". Proceedings of the third American peptide symposium (Ed. J. Meienhofe), pp545-562, Ann. Arbor Science Publishers, Ann Arbur.

Fitzsimons, J.T. (1975) The renin-angiotensin and drinking behaviour. In Hormones, Homeostasis and the Brain, Progress in Brain Research" (Eds. W.H. Gispen, T.B. Van Wimersma, Griedanus Jr., B. Bonus and D. De Wied), Vol. 42, pp215-233. Elsevier, Amsterdam.

Fitzsimons, J.T. and Kaufman, S. (1977). Cellular and extracellular dehydration, and angiotensin as stimulus to drinking in the common iguana Iguana iguana. J. Physiol. (London) 265:443-463.

Flik, G., Wendelaar Bonga, S.E. and Fenwick, J.C. (1984). Ca-dependent ATPase activities in plasma membranes of eel gill epithelium-II. Evidence for transport high affinity Ca ATPase. Comp. Biochem. Physiol. 79B:9-16.

Folmar, L.C. and Dickhoff, W.W. (1979). Plasma thyroxine and gill Na^+/K^+-ATPase change during SW acclimation of coho salmon Oncorhynchus kisutch. Comp. Biochem. Physiol. 63A:329-332.

Folmar, L.C. and Dickhoff, W.W. (1981). Evaluation of some physiological parameters as predictive indices of smoltification. Aquaculture 23:309-324.

Fontaine, M. (1964). Corpuscles de Stannius et regulation ionique (Ca,K,Na) du milieu interieur de l'anguille (Anguilla anguilla L.). C.R. Acad. Sci. 259:875-878.

Forrest, J.N., Cohen, A.D., Schon, D.A. and Epstein, F.H. (1973). Na transport and Na^+K^+-ATPase in gills during adaptation to seawater : effects of cortisol. Am. J. Physiol. 224:709-713.

Foskett, J.K. and Scheffey, C. (1982). The chloride cell: Definitive identification as the salt-secretory cell in Teleosts. Science 215:164-166.

Foskett, J.K, Hubbard, G.M., Machen, T. and Bern, H.A. (1982). Effect of epinephrine, glucagon and vasoactive intestinal peptide on chloride secretion by teleost opercular membrane. J. Comp. Physiol 146:27-34.

Foskett, J.K., Bern, H.A., Machon, T.E. and Conner, M. (1983). Chloride cells and the hormonal control of teleost fish osmoregulation. J. Exp. Biol. 106:255-281

Foster, R.C. (1975). Changes in urinary bladder and kidney function in the starry flounder (Platichthys stellatus) in response to prolactin and freshwater transfer. Gen. Comp. Endocr. 27:153-161.

Fouchereau-Peron, M., Arlot-Bonnemains, Y., Moukhtar, M.S. and Milhaud, G. (1986). Adaptation of rainbow trout (Salmo gairdneri) to sea water. Changes in calcitonin levels. Comp. Biochem. Physiol. A 83A:83-87.

Fredlund, P., Saltman, S., Kondo, T., Douglas, J. and Catt, K.J. (1977). Aldosterone production by isolated glomerulosa Cells: Modulation of sensitivity to angiotensin II and ACTH by extracellular potassium concentration. Endocrinology 100:481-486.

Freeman, R.H. and Rostorfer, H.H. (1972). Hepatic changes in renin substrate biosynthesis and alkaline phosphate activity in the rat. Am. J. Physiol. 223:364-370.

Freeman, R.H., Davis, J.O., Lohmeier, T.E. and Spielman, W.S. (1977). Des-Asp^{1} angiotensin II: mediator of the renin-angiotensin system. Fed. Proc. 36:1766-1770.

Freer, R.J. and Stewart, J.M. (1973). Synthesis and pharmacology of position 6 analogs of angiotensin II. J. Med. Chem. 16:733-735.

Fromm, P.O. (1968). Some quantitative aspect of ion regulation in teleosts. Comp. Biochem. Physiol, 27:865-869.

Fryer, J.N., (1979a). Prolactin-Binding Sites in Tilapia (Sarotherodon mossambicus) kidney. Gen. Comp. Endocr. 39:397-403.

Fryer, J.N., (1979b). A radioreceptor assay for purified teleost growth hormone. Gen. Comp. Endocr.39:123-130.

Gaitskell, R.E. and Chester-Jones, I. (1970). Effects of adrenalectomy and cortisol injection on the in vitro movement of water by the intestine of the FW European eel (Anguilla anguilla L.). Gen. Comp. Endocr. 15:491-493.

Galardy, R., Podhasky, P. and Olson, K.R. (1984). Angiotensin-converting enzyme activity in tissues of the rainbow trout. J. Exp. Zool. 230:155-158.

Galli, S.M., Evans, D.H., Kimura, B. and Phillips, M.I. (1988). Changes in plasma and brain levels of atrial natriuretic peptide in fish adapting to fresh water and sea water. (Abstract) FASEB J. 2:A524.

Gallis, J.L., Belloc, F., Lasserre, P. and Boisseau, J. (1979). Freshwater adaptation in the euryhaline teleost Chelon labrosus II. Effects of continuance of adaptation, cortisol treatment, and environmental calcium on water influx in isolated gill. Gen. Comp. Endocr. 38:11-20.

Garbutt, J.T., Morehouse, A.L. and Hanson, A.M. (1961). Metal binding properties of bacitracin. Agr. Food Chem. 9:285.

Gehlert, D.R., Speth, R.C. and Wamsley, J.K. (1984). In vitro autoradiographic localisation of ^{125}I-angiotensin II binding sites in the rat and dog kidney. Peptides 5:1043-1048.

Gerstberger, R., Healy, D.P., Hammel, H.T. and Simon, E. (1987). Autoradiographic localisation and characterisation of circumventricular angiotensin II receptors in duck brain. Brain Res. 400:165-170.

Gilloteaux, J. (1969). Note sur l'innervation des branchies chez Anguilla anguilla L. Experientia 25:270-271.

Gilman, A.G. (1984). G-proteins and dual control of adenylate cyclase. Cell 36:577.

Girard, J.P. and Payan, P. (1976). Effect of epinephrine on vascular space of gills and head of rainbow trout. Am. J. Physiol. 230:1555-1560.

Girard, J.P. and Payan, P. (1980). Ion exchange through respiratory and chloride cells in fresh water and sea water adapted teleosteans. Am. J. Physiol. 238:R260-267.

Glossmann, H., Baukal, A., and Catt, K.J. (1974). Angiotensin II receptors in bovine adrenal cortex. Modification of angiotensin II binding by Guanyl Nucleotides. J. Biol. Chem. 249:664-666.

Goodfriend, T. L., and Simpson, R.U. (1981). Angiotensin receptors in bovine umbilical artery and their inhibition by non steriod anti-inflammatory drugs. Br. J. Pharmac. 72:247-255.

Grafflin, A.L. (1931). The Structure of the renal tubule of the toadfish. Bull. Johns. Hopkins Hosp. 48:269-271.

Grafflin, A.L. (1937). Observations upon the aglomerular nature of certain teleostean kidneys. J. Morphol. 61:165-169.

Grau, E.G., Nishioka, R.S., Specker, J.L. and Bern H.A. (1985). Endocrine involvement in the smoltification of salmon. In "Comparative Endocrinology" (Eds. B. Lofts and W.N. Holmes), pp.491-493. Hong Kong Univ. Press, Hong Kong.

Gray, C.Y. and Brown, J.A. (1985). Renal and cardiovascular effects of angiotensin II in the rainbow trout, Salmo gairdneri. Gen. Comp. Endocr. 59:375-381.

Gregg, C.M. and Malvin, R.L. (1977). Binding of angiotensin by neurohypophysis and adenohypophysis in vivo and in vitro. Proc. Soc. Exp. Biol. Med. 155:400-404.

Guibbolini, M.E. and Lahlou, B. (1987a). Adenylate cyclase activity in fish gills in relation to salt adaptation. Life Sci. 41:71-78.

Guibbolini, M.E. and Lahlou, B. (1987b). Neurohypophysial peptides inhibition of adenylate cyclase activity in fish gills. The affect of environmental salinity. FEBS Lett. 220:98-102.

Guibbolini, M.E. and Lahlou, B. (1987c). Basal and stimulated adenylate cyclase activity in the gill epithelium of the rainbow trout. Gen. Comp. Endocr. 67:178-188.

Guibbolini, M.E. and Lahlou, B. (1990). Evidence for presence of a new type of neurohypophysial hormone receptor in fish gill epithelium. Am. J. Physiol. 258:R3-R9.

Guibbolini, M.E., Henderson, I. W., Mosley, W. and Lahlou, B. (1988). Arginine vasotocin binding to isolated branchial cells of the eel: effect of salinity. J. Mol. Endocr. 1:125-30.

Gunther, S. (1984). Characterisation of angiotensin II receptor subtypes in rat liver. J. Biol. Chem. 259:7622-7629.

Gunther, S., Gimbrone, M.A. and Alexander, R.W. (1980). Identification and characterization of the high affinity vascular angiotensin II receptor in rat mesenteric artery. Circ. Res. 47:278-286.

Hall, J.E. (1986). Regulation of glomerular filtration rate and sodium excretion by angiotensin II. Fed. Proc. 45:1431-1437.

Harding, J.W., Yoshida, M.S., Dilts, R.P., Woods, T.M. and Wright, J.M. (1986). Cerebroventricular and intravascular metabolism of [^{125}I] angiotensins in rat. J. Neurochem. 46:1292-1297.

Hasegawa, Y., Nakajima, T. and Sokabe, K. (1983). Chemical structure of angiotensin formed with kidney renin in the Japanese eel, Anguilla japonica. Biomed. Res. 4:417-420.

Hayashi, T., Nakayama, T., Nakajima, T. and Sokabe, H. (1978). Structure of angiotensin formed by the kidney of Japanese goosefish and its identification by Dansyl method. Chem. Pharm. Bull. 26:215-219.

Hayes, M.E., Guilland-Cumming, D.F., Russell, R.G.G. and Henderson, I.W. (1986). Metabolism of 25-hydroxycholecalciferol in a teleost fish, the rainbow trout (Salmo gairdneri). Gen. Comp. Endocr. 64:143-150.

Haywood, G.P., Isaia, J. and Maetz, J. (1977). Epinephrine effect on branchial water and urea flux in rainbow trout. Am. J. Physiol. 232:R110-R115.

Hazon, N. and Henderson, I.W. (1985). Factors affecting the secretory dynamics of 1 -hydroxycorticosterone in the dogfish, Scyliorhinus canicula. Gen. Comp. Endocr. 59:50-55.

Hazon, N., Balment, R.J., Perrott, M. and O'Toole, L.B. (1989). The renin-angiotensin system and vascular and dipsogenic regulation in elasmobranchs. Gen. Comp. Endocr. 74:230-236.

Hegab, S.A. and Hanke, W. (1984). Hormonal mechanisms in saltwater acclimation of freshwater teleosts. Gen. Comp. Endocr. 445 (abstracts).

Hems, D.A. (1977). Short-term hormonal control of hepatic carbohydrate and lipid catabolism. FEBS Letters 80:237-245.

Henderson, I.W. and Wales, N.A.M. (1974). Renal diuresis and antidiuresis after injections of arginine vasotocin in the freshwater eel (Anguilla anguilla L.). J. Endocr. 61:487-500.

Henderson, I.W. and Brown, J.A. (1978). Hormonal actions on single nephron function in teleosts. Proceedings of the memorial symposium to Jean Maetz, villefranche-sur-mer, France. June 26-27, p163-170.

Henderson, I.W., Hazon, N. and Hughes, K. (1986). Hormones, ionic regulation and kidney function in fishes. In "Physiological adaptation of marine mammals". (Ed. M. Laverack, S.). Soc. Exp. Biol. Symp. 39:245-265. The Company of Biologists Ltd., Cambridge.

Henderson, I.W., Jotisankasa, V., Mosley, W. and Oguri, M. (1976). Endocrine and environmental influences upon plasma cortisol concentrations and plasma activity of the eel, Anguilla anguilla L. J. Endocr. 70:81-95.

Henderson, I.W., Oliver, J.A., McKeever, A. and Hazon, N. (1981). Phylogenetic aspects of the renin-angiotensin system. Adv. Physiol, vol.20. Advances in Animal and Comparative Physiology (Eds. G. Pethes and V.L. Frenyo), pp.355-363.

Hickman, C.P. Jr. (1965). Studies on renal function in freshwater teleost fish. Trans. R. Soc. Can. 3:213-236.

Hickman, C.P. and Trump, B.F. (1969). The kidney. In "Fish Physiology" vol. 1. (Eds. W. S. Hoar and D. J. Randall). pp.91-230. Academic Press, New York.

Hirano, T., (1967). Effect of hypophysectomy on water transport in isolated intestine of the eel Anguilla japonica. Proc. Jpn. Acad. 43:793-796.

Hirano, T. (1977). Prolactin and hydromineral metabolism in the vertebrates. Gunma Symp. Endocr. 14:45-59.

Hirano, T. (1986). The spectrum of prolactin action in teleosts. Comparative Endocrinology: developments and directions. Ed. Charles L. Ralph. p.53-74. Alan R. Liss, Inc., New York.

Hirano, T. and Utida, S. (1968). Effects of ACTH and cortisol on water movement in isolated intestine of the eel. Anguilla anguilla L. Gen. Comp. Endocr. 11:373-380.

Hirano, T. and Mayer-Gostan, N. (1976). Eel oesophagus as an osmoregulatory organ. Proc. Nat. Acad. Sci., U.S.A. 73:1348-1350.

Hirano, T., and Mayer-Gostan, N. (1978). Endocrine control of osmoregulation in fish. In "Comparative Endocrinology", (Eds. P. J. Gaillard and H. H. Boer). pp.209-212. Elsevier, Amsterdam.

Hirano, T., and Hasegawa, S. (1984). Effects of angiotensins and other vasocative substances on drinking in the eel, Anguilla japonica. Zool. Sci. 1: 106-113.

Hirano, T., Takei, Y. and Kobayashi, H. (1978). Angiotensin and drinking in the eel and the frog. In: Proceedings of the Alfred Benzon Symposium XI. Osmotic and Volume Regulation (Eds. C.B. Jorgensen and E. Skadhauge), pp.123-136. Munksgard, Copenhagen. Academic Press, New York.

Hirano, T., Johnson, D.W., Bern, H.A. and Utida, S. (1973). Studies on water and ion movements in the isolated urinary bladder of selected fresh water, marine and euryhaline teleosts. Comp. Biochem. Physiol. 45A:529-540.

Hirano, T., Morisawa, M., Ando, M. and Utida, S., (1976). Adaptive changes in ion and water transport mechanism in the eel intestine. In "Intestinal Ion Transport". (Ed. J. W. L. Robinson). pp.301-317. M. T. P. Lancaster.

Hirano, T., Prunet, P., Kawauchi, H., Takahashi, A., Kubota, J., Nishioka, R.S., Bern, H.A., Takada, K., and Ishii, S. (1985). Development and validation of a salmon prolactin radioimmunoassay. Gen. Comp. Endocr. 59:266-276.

Holder, F.C. (1969). Separation chromatogophique et dosage des hormones de type ocytocique. (arginine-vasotocine et isotocine de l'hypophyse et du noyau preoptique de l'anguille europeenne (Anguilla anguilla L.) Anguilles d'Alsace (Auenheim) et de loire Atlantique (Grande-Briere). C.R. Hebd, Seances Acad. Sci (Paris). 269:1304-1307.

House, C.R. and Maetz, J. (1974). On the electrical gradient across the gill of seawater adapted gill. Comp. Biochem. Physiol A47:917-924.

Houslay, M.D., and Stanley, K.K. (1982). Dynamics of Biological Membranes: Influence on Synthesis, Structure and Function. p.133. Wiley, Chichester.

Howe, D. and Gutknecht, J. (1978). Role of urinary bladder in osmoregulation in the marine teleost Ospanus tau. Am. J. Physiol. 235:R48-R54.

Hughes, G. and Grimstone, A. (1965). The fine structure of the secondary lamellae of the gills of Gadus pollachius Q.J. Micosc. Sci. 106:343-353.

Hsieh, K., Kiraly-Olah, I.C. and Jorgenson, E.C. (1979). Angiotensin II analogues 13. Role of the hydroxyl group of position 4. Tyrosine in pressor activity. J. Med. Chem. 22:1044-1047.

Husain, A., Bumpus, F.M., De Silva, P. and Speth, R.C. (1987). Localization of angiotensin II receptors in ovarian follicles and the identification of angiotensin II in rat ovaries. Proc. Natl. Acad. Sci. U.S.A. 84:2489-2493.

Isaia, J. (1979). Non electrolyte permeability of trout gills. Effect of temperature and adrenaline. J. Physiol. (London) 286:361-373.

Israel, A., Niwa, M., Plunkett, L.M. and Saavedra, J. M. (1985). High-affinity angiotensin receptors in rat adrenal medulla. Regul. Peptides 11:237-243.

Jampol, L.M. and Epstein, F.H. (1970). Sodium-potassium-activated adenosine triphosphate and osmotic regulation by fishes. Am. J. Physiol. 218:607-611.

Jard, S., Contau, B. and Jakobs, K.H. (1981). Angiotensin II and -adrenergic agonists inhibit rat liver adenylate cyclase. J. Biol. Chem. 256:2603-2606.

Johnson, A.K. and Schwob, J.E. (1975). Cephalic angiotensin receptors mediating drinking to systemic angiotensin II. Pharm. Biochem. Behav. 3:1077-1084.

Johnson, D.W., Bern, H.A. and Hirano, T. (1972). Water and sodium movements and their hormonal control in the urinary bladder of the starry flounder. Platichthys stellatus. Gen. Comp. Endocr. 19:115-128.

Kamiya, M. (1972). Hormonal effect of Na-K-ATPase activity in the gill of Japanese eel, Anguilla japonica with special reference to seawater adaptation. Endocrinology (Japan) 19:489-493.

Kamiya, M. and Utida, S. (1969). Sodium-potassium-activated adenosinetriphosphatase activity in the gills of freshwater, marine and euryhaline teleosts. Comp. Biochem. Physiol, 31:671-674.

Kangawa, K., and Matsuo, H. (1984). Purification and complete amino acid sequence of -human atrial natriuretic polypeptide (-hANP) Biochem. Biophys. Res. Commun. 118:131-139.

Karnaky, K.J. Jr. (1980). Ion-secreting epithelia: chloride cells in the head region of Fundulus heteroclitus Am. J. Physiol. 238:R185-R198.

Karnaky, K.J. Jr., Kinter, L.B., Kinter, W.B. and Stirling, C.E. (1976). Teleost chloride cell II. Autoradiographic localization of gill Na,K-ATPase in killifish Fundulus heteroclitus adapted to low and high salinity environments. J. Cell Biol. 70:157-177.

Kawauchi, H., Moriyama, S., Yasuda, A., Yamaguchi, K., Shirahata, K., Kubota J. and Hirano, T. (1986). Isolation and characterisation of chum salmon growth hormone. Arch. Biochem. Biophys 244:542-552.

Kelly, P.A., Leblanc, G. and Djane, J. (1979). Estimation of total prolactin-binding sites after in vitro desaturation. Endocrinology (Baltimore) 104:1631-1638.

Kenyon, C.J., McKeever, A., Oliver, J.A. and Henderson, I.W. (1985). Control of renal and adrenocortical function by the renin-angiotensin system in two euryhaline teleost fishes. Gen. Comp. Endocr. 58:93-100.

Kessel, R. and Beams, H. (1960). An electron microscope study of the mitochondria rich "chloride cells" from the gill filaments of fresh water and sea water adapted Fundulus heteroclitus. Biol. Bull. 119-322.

Kessel, R. and Beams, H. (1962). Electron microscopic studies on the gill filaments of Fundulus heteroclitus from the sea water and fresh water with special reference to the ultrastructure organisation of the "Cl" cell. J. Ultrastruct. Res. 6:77-87.

Keys, A.B. and Bateman, J.B. (1932). Branchial response to adrenaline and to pitressin in the eel. Biol. Bull. (Woods Hole, Mass) 63:327-336.

Keys, A.B. and Wilmer, E.N. (1932). Chloride secreting cells in the gills of fishes with special reference to the common eel. J. Physiol. London 76:368-378.

Khairallah, P.A., and Page, I.H. (1967). Plasma angiotensinases. Biochem. Med. 1:1-8.

Khairallah, P.A., Bumpus, F.M., Page, I.H. and Smeby, R.R. (1963). Angiotensinase with a high degree of specificity in plasma and red cells. Science 140:672-674.

Khosla, M.C., Smeby, R.R. and Bumpus, F.M. (1974). Structure activity relationship in angiotensin II analogs. In Handbook of Experimental Pharmacology: Angiotensin. (I.H. Page and F.M. Bumpus, Eds) (Vol.37), pp126-161. Springer, Heidelberg.

Khosla, M.C., Nishimura, H., Hasegawa, Y. and Bumpus, F.M. (1985). Identification and synthesis of [1-asparagine, 5-valine, 9-glycine] angiotensin I produced from plasma of the American eel, Anguilla rostrata. Gen. Comp. Endocr. 57:2230233.

King, R.J.B. (1987). Structure and function of steroid receptors. J. Endocr. 114:341-349.

Kirsch, R. (1972). The kinetics of peripheral exchanges of water and electrolytes in the silver eel (Anguilla anguilla L.) in fresh water. J. Exp. Biol. 57:489-512.

Kirsch, R. (1978). Role of the esophagus in osmoregulation in teleost fishes. In "Proceedings of the Alfred Benzon Symposium XI Osmotic and volume regulation (Eds. C. B. Jorgensen and E. Skadhauge). pp. 138-154 Munksgaard, Copenhagen. Academic Press, New York.

Kirsch, R. and Laurent, P. (1975). L'oesophage, organe effecteur de l'osmoregualtion chez un teleosteen euryhalin, l'anguille (Anguilla anguilla L.). C.r. hebd. Seanc. Acad. Sci. Paris 280D:2013-2015.

Kirsch, R. Guinier, D. and Meens, R. (1975). L'equilibre hydrique de l'Anguille europeenne (Anguilla anguilla L.) Etude du role de l'oesophage dan l'utilisation de l'eau de boisson et etude de la permeabilite osmotique branchiale. J. Physiol., (Paris) 70:605-626.

Kirschner, L.B., Greenwald, L. and Sanders, M. (1975). On the mechanism of sodium extrusion across the irrigated gill of seawater adapted rainbow trout (Salmo gairdneri). J. Gen. Physiol 64:148-165.

Kitamura, E., Kikkawa, R., Fujiwara, Y., Imai, T. and Shigeta, Y. (1986). Effect of angiotensin II infusion on glomerular angiotensin II receptor in rats. Biochimica et Biophysica Acta 885:309-316.

Kloas, W. Flugge, G., Fuschs E. and Stolte, H. (1988). Binding sites for atrial natriuretic peptide in the kidney and aorta of the hagfish (Myxine glutinosa). Comp. Biochem. Physiol., 91A:685-688.

Knoeppel, S.J., Aitkins, D.L. and Packer, R.K. (1982). The role of the thyroid gland in osmotic and ionic regulation in Fundulus heteroclitus acclimated to fresh water and seawater. Comp. Biochem. Physiol., A 73A:25-29.

Kobayashi, H., Uemura, H., Wada, M. and Takei, Y. (1979). Ecological adaptation of angiotensin II induced thirst mechanisms in tetrapods. Gen. Comp. Endocr. 38:93-104.

Kobayashi, H., Uemura, H., Takei, Y., Hatsu, N., Ozawa, M. and Ichinhoe, K. (1983). Drinking induced by angiotensin in fishes. Gen. Comp. Endocr. 49:295-306.

Kono, T., Kiso, Y., Khosla, M.C. and Sakura, N. (1987). Tyrosine in position 4 is the key amino acid for the binding of angiotensin II to human arteriolar receptor. Endocrinology (Japan). 34:737-743.

Kono, T.F., Oseko, S., Shimpo, M. Nanno and Endo, J. (1975). Biological activity of des-asp^{1}-angiotensin II (angiotensin III) in man. J. Clin. Endocrinol. Metab. 41:1174-1177.

Kono, T., Taniguchi, T. A., Imura, H. Oseko, F. and Khosla, M.C$_{5}$ (1985). Relative biological activities of asn^{1}, val^{5}-angiotensin II, ile^{5}-angiotensin II and sar^{1}-angiotensin II in man. Life Sci. 37:365-369.

Kono, T., Taniguchi, T.A., Imura, H., Oseka, F. and Khosla, M.C. (1986). Biological activities of angiotensin II-(1-6)-hexapeptide and angiotensin II-(1-7)-heptapeptide in man. Life Sci. 38:1515-1519.

Kono, T., Ikeda, F., Oseko, F., Nanno, M., Imura, H. and Endo, J. (1978). Inhibition of angiotensin III action be des-asp^{1}-,ileu^{8}-angiotensin II in man. Acta endocr. (Kbh) 87:359-366.

Kono, T., Ikeda, F., Oseko, F., Ohmori, Y., Nakano, R., Muranaka, H., Taniguchi, T. A., Imura, H., Khosla, M.C. and Bumpus, F.M. (1982). Biological activity of des-asp^{1}-, des-arg^{2}-angiotensin II in man. Acta endocr. (Kbh) 99:577-584.

Kriebel, R.M. (1980). Ultrastructural changes in the urophysis of Mollienesia sphenops, following adaptation to seawater. Cell Tissue res. 207:135-142.

Kurcbart, H., Coli, A., Vancheri, L. and Rosa, U. (1971). Chemical and biological effects associated with the iodonation of angiotensin II. Biochim. Biophys. Acta 230:160-169.

LaFontaine, J.J., Nivez, M.P. and Ardaillou, R. (1979). Hepatic binding sites for angiotensin II in the rat. Clin. Sci. 56:33-40.

Lahlou, B. (1970). La fonction renal des teleosteens et son role dans l'osmoregulation Bull. Inf. Sci. Tech. Commis. Energ. At. Fr. 144:17-32.

Lahlou, B. and Giordan, A. (1970). Le controle hormonal des exchanges et de la balance de l'eau douce Carassius auratus, intact et hypophysectomise. Gen. Comp. Endocr. 14:491-509.

Lahlou, B., Henderson, I.W.and Sawyer, W.H. (1969). Renal adaptation, by Opsanus tau, a euryhaline aglomerular teleost, to dilute media. Am. J. Physiol 216:1266-1272.

Lam, T.J. (1972). Prolactin and hydromineral regulation in fishes. Gen. Comp. Endocr. Supplement 3:328-338

Leatherland, J.F. (1982). Environmental physiology of the teleostean thyroid gland. Environmental Biology of Fishes 7:83-110.

Lefkowitz, R.J., Roth, J. and Pastan, I. (1970). Radioreceptor assay of adrenocorticotropic hormone: New approach to assay of polypeptide hormones in plasma. Science 170:633-635.

Leibush, B.N. (1984). Cerebral insulin receptors in vertebrate evolution. J. Evol. Biochem. Physiol. 19:293-298.

Lewis, N.P. and Ferguson, D.R. (1989). [^{3}H] Angiotensin II binding to basolateral membranes from rat proximal renal tubule: effect of sodium intake and captopril. J. Endocr. 122:499-507.

Lin, S.Y. and Goodfriend, T.L. (1970). Angiotensin receptors. Am. J. Physiol. 218:1319-1328.

Linsley, P.S., Blifeld, C., Wrann, M., and Fox, C.F. (1979). Direct linkage of epidermal growth factor to its receptor. Nature (London). 278:745-748.

Loretz, C.A. (1983a). Ion transport by the intestine of the Goby, Gillichthys mirabilis. Comp. Biochem. Physiol. 75A:205-210.

Loretz, C.A. (1983b). Control of ion transport by Gillichthys mirabilis urinary bladder. Am. J. Physiol 14:R45-52.

Loretz, C.A. and Bern, H.A. (1980). Ion transport by the urinary bladder of the gobiid teleost, Gillichthys mirabilis. Am. J. Physiol. 239:R415-R423.

Loretz, C.A. and Bern, H.A. (1981). Stimulation of sodium transport across the teleost urinary bladder by urotensin II. Gen. Comp. Endocr. 43:325-330.

Loretz, C.A. and Bern, H.A. (1983). Control of ion transport by Gillichthy mirabilis urinary bladder. Am. J. Physiol. 245:R45-R52.

McFarland, W.N. (1965). The effect of hypersalinity on serum and muscle ion concentrations in the striped mullet Mugil cephalus L. Publ. Inst. Mar. Sci. Univ. Tex 10:179-186.

MacKay, W.C. and Janicki, R. (1979). Changes in the eel intestine during seawater adaptation. Comp. Biochem. Physiol. 62A:757-761.

McKinley, M.J., Allen, A.M., Clevers, J., Denton, D.A. and Mendelsohn, F.A.O. (1986). Autoradiographic localisation of angiotensin receptors in sheep brain. Brain. Res. 375:373-376.

McQueen, J. and Semple, P.F. (1987). Cation-dependent and cation-independent stages in regulation of the vascular angiotensin receptor after alteration of sodium balance in the rat. Endocrinology 121:1256-1264.

McQueen, J. and Semple, P.F. (1989). Divalent cation-induced interconversion of hepatic angiotensin receptor subtypes. Molecular Pharmacology 35:809-817.

McQueen, J., Murray, G.D. and Semple, P.F. (1984). Identification of the angiotensin II receptor in rat mesenteric artery. Biochem. J. 223:659-671.

Ma, S.W.Y. and Copp, D.H. (1978). Purification, properties and action of glycopeptide from the corpuscles of Stannius which affects calcium metabolism in the teleost. In "Comparative Endocrinology". (Eds. P.J. Gaillard and H. H. Boer), pp.283-286. Elsevier/North Holland Biomedical Press, Amsterdam.

Maack, T., Suzuki, M., Almeida, F.H., Nussenzreig, D., Scarborough, R.M., McEnroe, G.A. and Lewicki, J.A. (1987). Physiological role of silent receptors of atrial natriuretic factor. Science 238:675-678.

Maetz, J. (1970). Mechanism of salt and water transfer across membranes in teleosts in relation to the aquatic environment. In "Hormones and the Environment". (Eds. G.K. Benson and J.G. Phillips) p.3-29. Cambridge University Press, London and New York.

Maetz, J. (1971). Fish gills: Mechanisms of salt transfer in freshwater and seawater. Phil. Trans. R. Soc. Lond. (Biol) 262:209-251.

Maetz, J. and Bornancin, M. (1975). Biochemical and biophysical aspects of salt excretion by chloride cells in teleosts. Fortschr. Zool. 23:322-362.

Maetz, J., Bourguet, J. and Lahlou, B. (1964). Urophyse et osmoregulation chez Carassius auratus. Gen. Comp. Endocr. 4:401-414.

Maimer, H., Heim, J.M., Weil, J. and Gerzer, R. (1988). The ANF test-cyclic GMP reponses to injected ANF. In "Advances in Atrial Peptide Research"; American Society of Hypertension - Symposium Series 2:265-268.

Mainoya, J.R. (1982). Water and NaCl absorption by the intestine of tilapia Sarotherodon mossambicus adapted to fresh water or seawater and the possible role of prolactin and cortisol. J. Comp. Physiol. B146:1-8.

Mainoya, J.R. and Bern, H.A. (1984). Influence of vasocative intestinal peptide and urotensin II on the absorption of water and NaCl by the anterior intestine of the tilapia, Sarotherodon mossambicus. Zool. Sci. 1:100-105.

Maki, M., Takayanagi, R., Misonon, K.S., Pandey, K.N., Tibbet, C.I. and Inagami, T. (1984). Structure of rat atrial natriuretic factor precursor deduced from cDNA sequence. Nature 309: 722-724.

Malvin, R.L. and Vander, A.J. (1967). Plasma renin activity in marine teleosts and cetacea. Am. J. Physiol. 213:1582-1584.

Malvin, R.L., Schiff, D. and Eiger, S. (1980). Angiotensin and drinking rates in the euryhaline killifish. Am. J. Physiol. 239:R31-R34.

Mann, J. F. E., Schiffrin, E.L., Schiller, P.W., Rascher, W., Boucher, R. and Genest, J. (1980). Central actions and brain receptor binding of angiotensin II. Influence of sodium intake. Hypertension. 2:437-443.

Marshall E.K. Jr. and Smith H.W. (1930). The glomerular development of the vertebrate kidney in relation to habitat. Biol. Bull. 59:135-153.

Marshall, W.S. and Nishioka, R.S. (1980). Relation of mitochondria-rich cells to active chloride transport in the skin of a marine teleost. J. Exp. Zool. 214:147-156.

Marshall, W.S. and Bern, H.A. (1981). Active chloride transport by the skin of a marine teleost is stimulated by urotensin I and inhibited by urotensin. Gen. Comp. Endocr. 43:484-491.

Matsoukas, J.M., Goghari, M.H., Scanlon, M.N., Franklin, K.J. and Moore, G.J. (1985). Synthesis and biological activities of analogues of angiotensins II and III containing O-methyltyrosine and D-tryptophan. J. Med. Chem. 28:780-783.

Maurer, R. and Reubi, J.C. (1986). Distribution and coregulation of three peptide receptors in adrenals. Eur. J. Pharmacol. 125:241-246.

Mayer, N. and Maetz, J. (1967). Axe hypophyso-interrenalien et osmoregulation chez l'anguille en eau de mer. Etude de l'animal intact avec notes sur les pertubations de l'equilibre mineral produites par l'effect de "choc", C.R. Hebd, Seances Acad. Sci., Ser. D. 264:1632-1635.

Mayer-Gostan, N., and Maetz, J. (1980). Ionic exchanges in the opercular membrane of Fundulus heteroclitus adapted to seawater. In "Epithelial transport in the lower vertebrates" (Ed. B. Lahlou) pp.233-248. Cambridge Univ. Press London and New York.

Mayer-Gostan, N., Wendelaar Bonga, S.E. and Balm, P.H.M. (1987). Mechanisms of hormone actions on gill transport. In "Vertebrate Endocrinology: Fundamentals and Biomedical Implications" vol. 2. (Eds. P.K.T. Pang and M.P. Schreibman). pp.211-238. Academic Press, London and New York.

Mendelsohn, F.A.O., Dunbar, M. Allen, A., Chou, S.T., Millan, M.A., Aguilera, G. and Catt, K.J. (1986). Angiotensin II receptors in the kidney. Fed. Proc. 45:1420-1425.

Michell, R.H., Kirk, C.J. and Billah, M.M. (1979). Hormonal stimulation of phosphatidylinositol breakdown with particular reference to the hepatic effects of vasopressin. Biochem. Soc. Trans. 7:861-865.

Middler, S.A., Kleeman, C.R. and Edwards, E. (1968). The role of the urinary bladder in salt and water metabolism of the toad Bufo marinus. Comp. Biochem. Physiol. 26:57-68.

Milet, C., Hillyard, C.J., Martelly, E., Girgis, S., MacIntyre, I. and Lopex, E. (1980). Similitudes structurales entre l'hormone hypocalcemiante des corpuscles de stannius de l'anguille (Anguilla anguilla) et l'hormone parathyroidienne mammalienne. C. R. Hebd. Sceances Acad. Sci. Ser. D 977-980.

Miwa, S. and Inui, Y. (1983). Effects of thyroxine and thiourea on the parr-smolt transformation of amago salmon (Onchoryncus rhodunus). Aquaculture 4:41-52.

Miwa, S. and Inui, Y. (1985). Effects of L-thyroxine and ovine growth hormone on smoltification of amago salmon (Oncoryhynus rhodurus). Gen. Comp. Endocr. 58:436-442.

Mizogami, S., Aguri, M., Sokabe, H.I. and Nishimura, H. (1968). Presence of renin in the glomerular and aglomerular kidney of marine teleosts. Am. J. Physiol. 215:991-994.

Mizuhira, V., Amakawa, T., Tamashina, S., Shiria, N. and Utida, S. (1970). Electron microscopic studies on the localization of sodium ions and sodium-potassium-activated adenosinetriphosphatase in chloride cells of the eel gills. Expl. Cell. Res. 59:346-348.

Mohsen, T. and Tourette, C. (1962). Role de la glande thyroide dans, l'equilibre hydromineral chez. Lebistes reticulatus Peters (Cyrpinodontiformes, Pociliidae C.R. Sceances Soc. Biol. Ses. Fil 156:1338-1341.

Moore, T.J. and Williams, G.H (1982). Angiotensin II receptors on human platelets. Circ. Res. 51:314-320.

Moore G.J. and Matsoukas J.M. (1985). Angiotensin as a model for hormone:receptor interactions. Bio. Sci. Rep. 5:407-416.

Moore, G.J. and Kwok, Y.C. (1987). Angiotensin receptors in resting smooth muscle are the low affinity sites observed in binding studies. Life Sci. 41:505-511.

Morley, M., Chadwick, A. and El, Tounsy, E.M. (1981). The effect of prolactin on water absorption by the intestine of trout Salmo gairdneri. Gen. Comp. Endocr. 44:64-68.

Motais, R. (1961). Cinetique des echanges de sodium chez un teleosteen euryhalin (Platichthys flesus L.) au cours de passages successifs eau de mer, eau douce, eau de mer, en function du temps de sejour en eau douce. Compt. Rend. Acad, Sci. 253:2609-11.

Motais, R. (1967). Les mecanismes d'echanges ioniques branchiaux chez les teleosteens Ann. Inst. Oceanogr. Paris 45:1-83.

Motais, R. and Garcia-Romeu, F. (1972). Transport mechanisms in the teleostean gill and amphibian skin. Annu. Rev. Physiol. 34:141-176.

Muggeo, M., Ginsberg, B.H., Roth, J., Neville, D.M., De Meyts, P. and Kahn, C.R. (1979). The insulin receptor in vertebrates is functionally more conserved during evolution than insulin itself. Endocrinology 104:1393-1402.

Mujais, S.K., Kauffman, S. and Katz, A.I. (1986). Angiotensin II binding sites in individual segments of the rat nephron. J. Clin. Invest. 77:315-318.

Murray, T.F. and Siebenaller, J.F. (1987). Composition of the binding properties of A_1 adenosine receptors in brain membranes of two congeneric marine fishes living at different depths. J. Comp. Physiol. B. 157:267:277.

Musch, M.W. Orellana, S.A., Kimberg, L.S., Field, M., Halm, D.R., Kransy, E.J. Jr. and Firzzell, R.A. (1982). Na^+-K^+-Cl^- cotransport in the intestine of a marine teleost. Nature 300:351-353.

Nahorski, S.R. (1981). Identification and significance of beta-adrenoceptor subtypes In "Towards Understanding Receptors". (Ed. J. W. Lamble). pp.71-77. Elsevier/North Holland, Amsterdam.

Nakayama, T., Nakajima, T. and Sokabe, H. (1973). Comparative studies on angiotensins. III. Structure of fowl angiotensin and its identification by DNS method. Chem. Pharm. Bull. 21:2085-2087.

Namoto, S. and Yamada, K. (1987). Effects of forskolin, isoproterenol and lithium ions on leucophores of a teleost, Oryzias latipes: Evidence for involvement of adenylate cyclase in pigment-dispersion response. Comp. Biochem. Physiol 86C:91-95.

Nash, Y. (1931). The number and size of glomeruli in the kidneys of fishes with observations on the morphology of the renal tubules of fishes. Am. J. Anat. 47:425-445.

Nicoll, C.S., Wilson, W.S., Nishioka, R.S. and Bern, H.A. (1981). Blood and pituitary prolactin levels in tilapia (Sarotheredon mossambicus. Teleostei) from different salinities as measured by a homologous radioimmunoassay. Gen. Comp. Endocr. 44:356-373.

Nilsson. S. (1984). Innervation and pharmacology of the gill. In "Fish Physiology" (Eds. W.S. Hoar and D.J. Randall). vol. 10. part 17 pp.185-229. Academic Press, Orlando, Florida.

Nishimura, H. and Sawyer, W.H. (1976). Vasopressor, diuretic and natriuretic responses to angiotensin by the american eel, Anguilla rostrata. Gen. Comp. Endocr. 29: 337-348.

Nishimura, H., Sawyer, W.H. and Nigrelli, R.F. (1976). Renin, cortisol and plasma volume in marine teleost fishes adapted to dilute media. J. Endocr. 70:47-59.

Nishimura, H., Norton, V.M. and Bumpus, F.M. (1978). Lack of specific inhibition of angiotensin II in eels by angiotensin antagonists. Am. J. Physiol 235:H95-H103.

Nishimura, H., Lunde, L.G., and Zucker, A. (1979). Renin response to haemorrhage and hypotension in the aglomerular toadfish Opsanus tau. Am. J. Physiol. 237:H105-H111.

Nishimura, H., Oguri, M., Ogawa, M., Sokabe, H. and Imai, M. (1970). Absence of renin in kidneys of elasmobranchs and cyclostomes. Am. J. Physiol. 218:317-325.

Ogawa, M. (1961). Comparative study of the external shape of the teleostean kidney with relation to phylogeny. Sci. Rept. Tokyo Kyoiku Daigaku B10:61-68.

Ogawa, M. (1962). Comparative study on the internal structure of the teleostean kidney. Sci. Rept. Saitama. Univ. B4:107-129.

Ogawa, M. (1975). The effects of prolactin, cortisol and calcium free environment on water influx in isolated gills of Japanese eel, Anguilla japonica. Comp. Biochem. Physiol. 52:539-543.

O'Grady, S.M., Field, M., Nash, N.T. and Rao, M.C. (1985). Atrial natriuretic factor inhibits Na-K-Cl cotransport in teleost intestine. Am. J. Physiol. 249: C531-C534.

Oide, M. and Utida, S. (1967). Changes in water and ion transport in isolated intestine of the eel during seawater adaptation and migration. Mar. Biol. 1:102-106.

Oide, M. and Utida, S. (1968). Changes in intestinal absorption and renal excretion of water during adaptation to seawater in the Japanese eel. Mar. Biol. 1:172-177.

Oikawa, S., Imai, M., Ueno, A., Tanaka, S., Noguchi, T., Nakazato, H., Kangawa, K., Fukuda, A. and Matsuo, H. (1984). Cloning and sequence analysis of cDNA encoding a precursor for human atrial natriuretic polypeptide. Nature 309:724-726.

Okawara, Y., Karakida, T., Aihara, M., Yamaguchi, K. and Kobayashi, H. (1987). Involvement of angiotensin II in water intake in the Japanese eel, Anguilla japonica. Zool. Sci. 4:523-528.

Olson, K.R. and Meisheri, K.D. (1989). Effects of atrial natriuretic factor on isolated arteries and perfused organs of trout. Am. J. Physiol. 256:R10-R18.

Owada, K., Kawata, M., Takagi, A., Moriga, M. and Kobayashi, H. (1985). Urotensin II immunoreactive neurons in the caudal neurosecretory system of freshwater and seawater fish. Cell Tissue Res. 239:349-354.

Pang, P.K.T., Pang, P.K., Liu, V.K.Y. and Sokabe, H. (1981). Effect of fish angiotensins and angiotensin-like substances on killifish calcium regulation. Gen. Comp. Endocr. 43:292-298.

Parmalee. J.T. and Renfro. J.L. (1983). Oesphageal desalination of seawater in flounder: role of active sodium transport. Am. J. Physiol. 245:R888-R893.

Parry, G. (1966). Osmotic adaptation in fishes. Biol. Rev. 42:392-444.

Parsons, J.A., Gray, D., Rafferty, B., and Zanelli J.M. (1978). Evidence for a hypercalcemic factor in the fish pituitary, immunologically related to mammalian parathyroid hormone. In "Endocrinology of calcium metabolism" (Eds. D. H. Copp and R. V. Talmage), pp.111-114. Exerpta Medicca, Amsterdam.

Patino, R., Bradford, C.S. and Schreck, C.B. (1986). Adenylate cyclase activators and inhibitors, cyclic nucleotide analogues, and phosphatidyl inositol: Effects on interrenal function of Coho salmon (Oncoryhynchus kisutch) in vitro. Gen. Comp. Endocr. 63:230-235.

Payan, P. and Girard, J.P. (1978). Mise en evidence d'un echange Na^+/NH^+4 dans la branchive de la truite adaptee a'l'eau de mer: Controle adrenergique C.R. Hebd. Sceances Acad. Sci. Ser. D. 286:335-338.

Payan, P., Matty, A. J. and Maetz, J. (1975). A study of the sodium pump in the perfused head preparation of the trout Salmo gairneri in fresh water. J. Comp. Physiol. 104:33-48.

Peart, W.S. (1969). A history and review of the renin-angiotensin system. Proc. R. Soc. London B173:317-325.

Penhoat, A. Jaillard, C., Crozat, A. and Saez, J.M. (1988). Regulation of angiotensin II receptors and steroidogenic responsiveness in cultured bovine fasciculata and glomerulosa adrenal cells. Eur. J. Biochem. 172:247-254.

Perrott, M.N. and Balment, R.J. (1985). Drinking behaviour and the renin-angiotensin system (RAS) in euryhaline and stenohaline fish. J. Endocr. :107 suppl. no.93.

Perry, S.F., Payan, P., and Girard, J.P. (1984). Adrenergic control of branchial chloride transport in the isolated, saline perfused head of freshwater trout (Salmo gairdneri J. Comp. Physiol. 154, 269-274.

Philpott, C.W. and Copeland, D.E. (1963). Fine structure of chloride cells. J. Cell. Biol. 18:389-404.

Pic, P., Mayer-Gostan, N. and Maetz, J. (1974). Branchial effects of epinephrine in the seawater-adapted mullet II. Na^+ and Cl^- extrusion. Am. J. Physiol. 228:441-447.

Pickford, G.E. and Phillips, J.G. (1959). Prolactin, a factor promoting the survival of hypophysectomized killifish in freshwater. Science 130:454-455.

Pickford, G.E., Griffith, R.W., Torretti, J. Hendler, E. and Epstein, F.H. (1970a). Branchial reduction and renal stimulation of (Na^+,K^+)-ATPase by prolactin in hypophysectomized killifish in fresh water. Nature 228:378-379.

Pickford, G.E., Pang, P.K.T., Weinstein, E., Torretti, J., Hendler, E. and Epstein, F.H. (1970b). The response of the hypophysectomized cyprinodont, Fundulus heteroclitus, to replacement therapy with cortisol. Effects on blood serum and sodium-potassium activated adenosine triphosphatase in the gills, kidney and intestinal mucosa. Gen. Comp. Endocr. 14:524-534.

Pillai, A.K. and Terner, C. (1974). Studies of metabolism in Embryonic Development. VI. Cortisol-binding proteins of trout embryos. Gen. Comp. Endocr. 24:162-167.

Pisam, M., Caroff, A. and Rambourg, A. (1987). Two types of chloride cells in the gill epithelium of a freshwater-adapted euryhaline fish, Lebistes reticulatus: their modifications during adaptation to saltwater. Am. J. Anat. 179:40-50.

Platia, M.P., Catt, K.J. and Aguilera, G. (1986). Effects of 17 -Estradiol on angiotensin II receptors and prolactin release in cultured pituitary cells. Endocrinology 119:2768-2772.

Plunkett, L.M., Shigematsu, K., Kurihara, M. and Saavedra, J.M. (1987). Localisation of angiotensin II receptors, along the anteroventral third ventricle area of the rat brain. Brain Res. 405:205-212.

Potts, W.T.W. (1968). Osmotic and ionic regulation. Ann. Rev. Physiol. 30:73-104.

Pucell, A.G., Bumpus, F.M. and Husain, A. (1987). Rat ovarian angiotensin II receptors: Characterisation and coupling to oestrogen secretion. J. Biol. Chem. 262:7076-7080.

Raizada, M.K., Phillips, M.I., Crews, F.T. and Sumners, C. (1987). Distinct angiotensin II receptor in primary cultures of glial cells from rat brain. Proc. Natl. Acad. Sci. U.S.A. 84:4655-4659.

Rankin, J.C. and Maetz, Z.J. (1971). A perfused teleostean gill preparation: Vascular actions of neurohypophysial hormones and catecholamines. J. Endocr. 51:621-635.

Re, R., Fintel, D., Bryan, S., Haber, E., Labiche, R. and Parab, M. (1982). Studies on two novel angiotensin II actions. Clin. and Exper. Hyper. - Theory and Practice, A4:1649-1660.

Redding, J. M. Schreck, C.B., Birk, E.K. and Ewing, R.D. (1984). Plasma cortisol, thyroxine (T4) triiodothyronine (T3) concentrations increased during SW acclimation of yearling coho salmon, Oncorhynchus kisutch. Gen. Comp. Endocr. 56:146-155.

Refstie, T. (1982). The effect of feeding thyroid hormones on saltwater tolerence and growth rate of atlantic salmon. Can. J. Zool. 60:2700-2712.

Reinecke, M., Nehls, M. and Forssmann, W.G. (1985). Phylogenetic aspects of cardiac hormones as revealed by immunocytochemistry, electron microscopy, and bioassay. Peptides 6:321-331.

Reinecke, M., Betzler, D. and Forssmann, W.G. (1987a). Immunocytochemistry of cardiac polypeptide hormones (cardiodilatin/atrial natriuretic polypeptide) in brain and hearts of Myxine glutinosa (cyclostomata). Histochemistry 86:233-239.

Reinecke, M., Betzler, D., Forssman, W.G., Thorndyke, M., Askensten, U. and Falkmer, S. (1987b). Electronmicroscopal, immunohistochemical and biological evidence for the occurence of cardiac hormones (ANP/CDD) in chondrichthyes. Histochemistry 87:531-538.

Richards, B.D. and Fromm, P.O. (1969). Patterns of blood flow through filaments and lamellae of isolated-perfused rainbow trout (Salmo gairdneri) gills. Comp. Biochem. Physiol. 29:1063-1070.

Richards, B.D. and Fromm, P.O. (1970). Sodium uptake by isolated-perfused gills of rainbow trout (Salmo gairdneri). Comp. Biochem. Physiol. 33:303-310.

Richman, N.H. and Zaugg, W.S. (1987). Effects of cortisol and growth hormone on osmoregulation in pre and desmoltified coho salmon (Oncorhynchus kisutch). Gen. Comp. Endocr. 65:189-198.

Rogers, T.B., Gaa, S.T. and Allen, I.S. (1986). Identification and characterisation of functional angiotensin II receptors on cultured heart myocytes. J. Pharmocol. Exp. Ther. 236:438-444.

Saavedra, J.M., Israel, A., Plunkett, L.M., Kurihara, M., Shigematsu, K. and Correa, F.M.A. (1986). Quantitative distribution of angiotensin II binding sites in rat brain by autoradiography. Peptides 7:679-687.

Sandor, T., Fazekas, A.G. and Robinson, B.H. (1976). The biosynthesis of corticosteroids throughout the vertebrates. In "General Comparative and Clinical Endocrinology of the Adrenal Cortex". vol. 1. (Eds. I. Chester-Jones and I. W. Henderson). pp.25-142. Academic Press, London.

Sandor, T., DiBattista, J.A. and Mehdi, A.Z. (1984). Glucocorticoid Receptors in the gill tissue of fish. Gen. Comp. Endocr. 53:353-364.

Sawyer, W.H. (1972). Neurohypophysial hormones and water and sodium excretion in the African lungfish. Gen. Comp. Endocr. Suppl. 3. pp.345-349.

Scatchard, G. (1949). "The attractions of proteins for small molecules and ions." Ann. N.Y. Acad. Sci. 51:660-672.

Scheer, B.T. and Langford, R.W. (1976). Endocrine effects on the cation-dependent ATPase of the gills of European eels (Anguilla anguilla L.) efflux of Na^+. Gen. Comp. Endocr. 30:313-326.

Scheffey, C., Foskett, J.K. and Machen, T.E. (1983). Localisation of ionic pathways in the teleost opercular membrane by extracellular recording with a vibrating probe. J. Membr. Biol. 75:193-203.

Schmidt-Neilsen B. (1960). The salt secreting gland of marine birds. Circulation 21:955-967.

Schmidt-Nielsen, B. and Renfro, J.L. (1975). Kidney function of the American eel Anguilla rostrata Am. J. Physiol. 228:420-431.

Schoepp, D.D. and Bailly, D.A. (1987). Oestrogen modulation of angiotensin-stimulated phosphoinositide hydrolysis in slices of rat anterior pituitary. Neurochem. Int. 11:149-154.

Schultz, S.G. and Curran, P.F. (1970). Coupled transport of sodium and organic solutes. Physiol. Rev. 50:637-718.

Schwob, J.E. and Johnson, A.K. (1977). Angiotensin-induced dipsogenesis in domestic fowl (Gallus gallus) J. Comp. Physiol. Psychol. 91:182-188.

Sen, I., Jim, K.F. and Soffer, R.L. (1983). Solubilization and characterization of an angiotensin II binding protein from liver. Eur. J. Biochem. 136:41-49.

Sernia, C. and Reid, I.A. (1980). Stimulation of angiotensinogen production: a dose-related effect of angiotensin II in the conscious dog. Am. J. Physiol. 239:E442-E446.

Sernia, C., Sinton, L., Thomas, W.G. and Pascoe, W. (1985). Liver angiotensin II receptors in the rat: binding properties and regulation by dietary Na^+ and angiotensin II. J. Endocr. 106:103-111.

Severs, W.B. and Summy-Long, J. (1975). The role of angiotensin in thirst. Life Sci. 17:1513-1526.

Shehadeh, Z. and Gordon, M. (1969). The role of the intestine in salinity adaptation of the rainbow trout Salmo gairdneri. Comp. Biochem. Physiol. 30:397-418.

Shimada, K. and Yazaki, Y. (1978). Binding sites for angiotensin II in human mononuclear leucocytes. J. Biochem. 84:1013-1015.

Shirai, N. (1972). Electron microscope localization of sodium ions and adenosine-triphosphatase in chloride cells of the Japanese eel. Anguilla japonica J. Fac. Sci. Univ. Tokyo Sect 412:385-403.

Shirai, N. and Utida, S. (1970). Development and degeneration of the chloride cell during seawater and freshwater adaptation of the Japanese eel, Anguilla japonica J. Fac. Sci. Univ. Tokyo Sect. 412:385-403.

Shivakumar, K. and Jayaraman, J. (1984). Salinity adaptation in fish. Effect of thyroxine on mitochondrial status. Arch. Biochem. Biophys, 223:728-735.

Siebers, M.J. and Goodfriend, T.L. (1986). Platelet angiotensin receptors in young and old humans. J. Gerontol. 41:574-578.

Silva, P., Solomon, R., Spokes, K. and Epstein, F.H. (1977). Ouabain inhibition of the gill Na-K-ATPase: relationship to active chloride transport J. Exp. Zool. 199:419-426.

Simpson, C.F. and Sargent, J.R. (1985). Inositol lipid turnover and adenosine 3,5 cyclic monophosphate in the salt-secreting rectal gland of the dogfish (Scyliorhinus canicula). Comp. Biochem. Physiol. 82B:781-786.

Sirett, Nancy E., McLean, A.S., Bray, J.J. and Hubbard, J.I. (1977). Distribution of angiotensin II receptors in rat brain. Brain Res. 122:299-312.

Skadhauge E. (1969). The mechanism of salt and water absorption in the intestine of the eel (Anguilla anguilla) adapted to waters of various salinities. J. Physiol. Lond. 204:135-158.

Smith, H.W. (1930). The absorption and excretion of water and salts by marine teleosts. Am. J. Physiol. 93:480-505.

Smith, H.W. (1932). Water regulation and its evolution in the fishes. Quart. Rev. Biol. 7:1-26.

Sokabe, H. (1968). Comparative physiology of the renin-angiotensin system. J. Japan. Med. Assoc. 59:502-512.

Sokabe, H. and Watanabe T.X. (1977). Evolution of the chemical structure of angiotensins and their physiological roles. Gunma. Symposium 14:83-97.

Sokabe H., Mizogami, S., Murase, T. and Sakai, F. (1966). Renin and euryhalinity in the Japanese eel., Anguilla anguilla japonica. Nature 212:952-953.

Sokabe, H., Nishimura, H., Ogawa, M. and Oguri, M. (1970). Determination of renin in the Corpuscles of Stannius of the teleost. Gen. Comp. Endocr. 14:510-516.

Specker, J.L., Ingleton, P.M. and Bern, H.A. (1985). Comparative physiology of the prolactin cell. In: "Frontiers and Perspectives of Prolactin Secretion". (Eds. F. Mena and C. M. Valverde). pp.17-30. Academic Press, New York and London.

Speth, R. C., Wamslay, J.K., Gehlert, D.R., Chernicky, C.L., Barnes, K.L. and Ferrario, C.M. (1985). Angiotensin II receptor localisation in the canine C.N.S. Brain Res. 326:137-143.

Sraer, J.D., Sraer, J., Ardaillou, R. and Mimoune, O. (1974). Evidence for renal glomerular receptors for angiotensin II. Kidney Int. 6:241-246.

Sraer, J., Baud, L., Cosyns, J.P., Verroust, P., Nivez, M.P. and Ardaillou, R. (1977). High affinity binding of ^{125}I-angiotensin II to rat glomerular basement membranes. J. Clin. Invest. 59:69-81.

Sullivan, C.V., Darling D.S. and Dickhoff, W.W. (1987). Nuclear receptors for L-triiodothyronine in trout erythrocytes. Gen. Comp. Endocr. 65:149-160.

Sumners, C., Watkins, L.L. and Raizada, M.K. (1986). Alpha$_1$ adrenergic receptor-mediated downregulation of angiotensin II receptors in neuronal cultures. J. Neurochem. 47:117-1126.

Takasugi, N. and Bern, H.A. (1962). Experimental studies on the caudal neurosecretory system of Tilapia mossambicus. Comp. Biochem. Physiol. 6:289-303.

Takei, Y. (1977a). Angiotensin and water intake in the Japanese quail (Coturnix coturnix japonica). Gen. Comp. Endocr. 31:364-372.

Takei, Y. (1977b). The role of the subfornical organ in drinking induced by angiotensin in the Japanese quail, Coturnix coturnix japonica. Cell Tissue Res. 185:175-181.

Takei, Y., Hirano, T. and Kobayashi, H. (1979). Angiotensin and water intake in the Japanese eel. Gen. Comp. Endocr. 38:466-475.

Takei, Y., Stallone, J.N., Nishimura, H. and Campanile, C.P. (1988). Angiotensin II receptors in the fowl aorta. Gen. Comp. Endocr. 69:205-216.

Takei, Y., Takahashi, A., Watanabe, T.X., Nakajima, K. and Sakakibara, S. (1989). Amino acid sequence and relative biological activity of eel natriuretic peptide. Biochem. Biophys. Res. Commun. 164:537-543.

Takemoto, Y., Nakajima, T., Hasegawa, Y., Watanabe, T.X., Sokabe, H., Kumagore, S. and Sakakibara, S. (1983). Chemical structures of angiotensins formed by incubating plasma with the kidney and the Corpuscles of Stannius in the chum salmon, Oncoryhynchus keta. Gen. Comp. Endocr. 51:219-227.

Taylor, A.A. (1977). Comparative physiology of the renin-angiotensin system. Fed. Proc. 36:1776-1980.

Thomson, A.J. and Sargent, J.R. (1977). Changes in the levels of chloride cells and Na-K-dependent ATPase in the gills of yellow and silver eels adapting to seawater. J. Exp. Zool. 200:33-70.

Threadgold, L. and Houston, A. (1964). An electron microscope study of the "chloride cell" of Salmo salar L. Exptl. Cell. Res. 34:1-23.

Turker, R.K., Hall, M.M., Yamamoto, M., Sweet, C.S. and Bumpus, F.M. (1972). A new, long-lasting competitive inhibitor of angiotensin. Science 177:1203-1204.

Urist, M. R. (1976). In "Handbook of physiology". (Eds. R. O. Greep, E. B. Astwood and G. D. Aurbachs). vol. 7. pp.183-213. Williams and Wilkins, Baltimore.

Utida, S. and Hirano, T. (1972). Effects of changes in environmental salinity on salt and water movement in the intestine and gills of the eel, Anguilla japonica. In "Responses of Fish to Environmental Changes". (Ed. W. Chavin). Charles C. Thomas, Springfield, III.

Utida, S., Kamiya, M. and Shirai, N. (1971). Relationship between the activity of Na^+-K^+-activated adenosine triphosphatase and the number of chloride cells in eel gills with special reference to seawater adaptation. Comp. Biochem. Physiol. A38:443-447.

Utida, S., Kamiya, M., Johnson, D.W. and Bern, H.A. (1974). Effects of fresh water adaptation and of prolactin on sodium-potassium-activated adenosine triphosphatase activity in the urinary bladder of two flounder species J. Endocr. 62:11-14.

Vallotton M.B., Capponi, A.M., Grillet, C., Knupfer, A.L., Hepp, R. Khosla M.C. and Bumpus, F.M. (1981). Characterization of angiotensin receptors on bovine adrenal fasciculata cells. Proc. Natl. Acad. Sci. USA. 78:592-596.

Wada, M., Kobayashi, H. and Farner, D.S. (1975). Induction of drinking in the white crowned sparrow, Zonotrichia leucophrys gambelii, by intercranial injection of angiotensin II. Gen. Comp. Endocr. 26 192-197.

Weisbart, M., Youson, J.H. and Weibe, J.P. (1978). Biochemical, histochemical and ultrastructural analysis of presumed steroid producing tissues in the sexually mature sea lamprey Petromyson marinus L. Gen. Comp. Endocr. 34 26-37.

Weld, M.M. and Fryer, J.N. (1987). Stimulation by angiotensins I and II of ACTH release from goldfish pituitary cell columns. Gen. Comp. Endocr. 68:19-27.

Westenfelder, C., Birch, F.M., Baranowski, R.L., Rosenfeld, M.J., Shiozawa, D.K. and Kablitz, C. (1988). Atrial natriuretic factor and salt adaptation in the teleost fish Gila atraria. Am. J. Physiol 255:F1281-F1286.

Wilson, K.M., Sumners, C., Hathaway, S. and Fregly, M. (1986). Mineralocorticoids modulate central angiotensin II receptors in rats. Brain Res. 382:87-96.

Woo, N.Y.S. and Tong, W.C.M. (1981). Urophysial control of glucose metabolism and osmoregulation in the snakehead, Ophiocephalus macalatus. In "Neurosecretion molecules, Cells, Systems" (Eds. D.S. Farmer and K. Lederis). pp.512-513. Plenum, New York.

Wood, C.M. (1975). A pharmacological analysis of the adrenergic and cholinergic mechanisms regulating branchial vascular resistance in the rainbow trout (Salmo gairdneri) Can. J. Zool. 53:1569-1577.

Wood, C.M. and Randall, D.J. (1973). The influences of swimming activity on water balance of rainbow trout (Salmo gairdneri). J. Comp. Physiol 82:257-276.

Wright, G.B., Alexander, R.W., Ekstein, L.S. and Gimbrone, M.A. (1983). Characterisation of the rabbit ventricular myocardial receptor for angiotensin II. Mol. Pharmacol 24:213.

Yamanaka, M., Takayanagi, R., Johnson, L., Seilhamer, J., Brewer, M., Friedman, T., Muller, J., Atlas, S., Laragh, J., Lewicki, J. and Fiddes, Y. (1984). Cloning and sequence analysis of the cDNA for the rat atrial natriuretic factor precursor. Nature 309:719-722.

Yasuda, A., Itoh, H. and Kawauchi, H. (1986). Primary structure of chum salmon prolactins. Occurrence of highly conserved regions. Arch. Biochem. Biophys. 244:528-541.

Young, G. (1986). Cortisol secretion in vitro by the interrenal of coho salmon (Oncorhynchus kisutch) during smoltification: Relationship with plasma thyroxine and plasma cortisol. Gen. Comp. Endocr., 63:191-200.