THE UNIVERSITY OF SOUTHAMPTON

CONTROL OF INTESTINAL FLUID TRANSPORT IN NORMOTENSIVE AND SPONTANEOUSLY HYPERTENSIVE RATS

BY

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A thesis presented for the degree of Doctor of Philosophy in the Faculty of Science of the University of Southampton

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Erratum

1. P192 Para 7 Insert:-
"Carey, R.M., Darracott Vaughan, E. Jr., Peach, M.J. and Ayers, C.R. (1977)".
Activity of (des-aspartyl')-angiotensin II and angiotensin II in man. Differences in blood pressure and adrenocortical response during normal and low sodium intake.
5. Clin Invest 61 20-31.

2. For "segements" read "segments"

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Finally the greatest thanks are due to my parents who have provided continual support throughout my education.

In Broken Images

He is quick, thinking in clear images;
I am slow, thinking in broken images.

He becomes dull, trusting in his clear images;
I become sharp, mistrusting my broken images.

Trusting his images, he assumes their relevance;
Mistrusting my images, I question their relevance.

Assuming their relevance, he assumes the fact;
Questioning their relevance, I question the fact.

When the fact fails him, he questions his senses;
When the fact fails me, I approve my senses.

He continues quick and dull in his clear images;
I continue slow and sharp in my broken images.

He in a new confusion of his understanding;
I in a new understanding of my confusion.

Robert Graves.

PUBLICATIONS

DOREY, P.G., MUNDAY, K.A., PARSONS, B.J., POAT, J.A. and UPSHER, M.E., (1980)

The effect of pentolinium on angiotensin-stimulated fluid transport.
J. Physiol. 308, 153P

DOREY, P.G., POAT, J.A., and MUNDAY, K.A., (1980)

A simple in vivo closed sac preparation for the measurement of net fluid transport in the rat small intestine.
Gastroenterologie Clinique et Biologique.
(Procedings of European Intestinal Symposium, April, 1980)

DOREY, P.G., MUNDAY, K.A., PARSONS, B.J., POAT, J.A., and UPSHER, M.E., (1981)

Effect of chemical sympathectomy on angiotensin-stimulated fluid absorption.
J. Endocr. (In the press)

DOREY, P.G., MUNDAY, K.A., and POAT, J.A., (1981)

Increased intestinal transport in spontaneously hypertensive rats (SHR).
(In the press)

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ABBREVIATIONS

3-H	Tritium
5-HT	5-hydroxytryptamine
6-OHDA	6-hydroxydopamine
18-OH-DOC	18,21- dihydroxy- 4-pregnene-3,20, dione
A	amp
A II	angiotensin II
ACE	angiotensin converting enzyme
ACh	acetylcholine
ACTH	adrenocorticotrophic hormone
ADH	antidiuretic hormone (vasopressin)
AIP	alodsterone induced protein
ATP	adenosine 5' triphosphate
ATPase	adenosine 5' triphosphate phosphorylase
BP	blood pressure
C	carbon
c-AMP	3' 5' cyclic adenosine monophosphate
c-GMP	3; 5' cyclic guanosine monophosphate
Ca	calcium
Cl	chlorine
cm	centimeter
CNS	central nervous system
CO_2	carbon dioxide
COMT	catechol-O-methyl transferase
DBH	dopamine B hydroxylase
DOC	deoxycorticosterone
DOCA	dehydrocorticosterone acetate
L-DOPA	L-dihydroxyphenylalanine
DMPP	1,1-dimethyl-4-phenylpiperazinium
dpm	disintegrations per minute
DPX	distrene/tricresyl phosphate/xylene
ECF	extracellular fluid
ECFV	extracellular fluid volume
fl	fluorescence
g	gram
GFR	glomerular filtration rate
GI	gastrointestinal
H	hydrogen
HCO_3	bicarbonate
hr	hour
ICF	intracellular fluid
ID50	inhibitory dose for 50% inhibition of maximum response
i.p.	intra peritoneal
Isc	short circuit current
i.v.	intra venous
JG	juxtaglomerular
K	potassium
l	litre
M	molar

MAO	monoamine oxidase
Mg	magnesium
m	metre
mm Hg	millimetres of mercury
mM	milimolar
mho	unit of conductance
NA	noradrenaline
NTR	normotensive rats
Na	sodium
O_2	oxygen
OsM	osmolarity units
(butyl) PBD	2(4'-t-butylphenyl)-5-(4"-biphenyl)-1,3,4-oxadiazole
p.d.	potential difference
PEG	polyethylene glycol
PMSF	para-methyl-sulphonyl-fluoride
PNMT	phenylethanolamine-N-methyl-transferase
PRA	plasma renin activity
RAS	renin angiotensin system
s	second
S.E.M.	standard error of the mean
SH	spontaneously hypertensive
SHR	spontaneously hypertensive rat
Sig	statistical significance
SO_3	sulphate
STWS	Scott's tapwater substitute
TCA	trichloroacetic acid
V	volts
VIP	vasoactive intestinal polypeptide
w/v	weight per volume
wt.	weight

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UNIVERSITY OF SOUTHAMPTON

ABSTRACT

FACULTY OF SCIENCE

PHYSIOLOGY AND PHARMACOLOGY

Doctor of Philosophy

CONTROL OF INTESTINAL FLUID TRANSPORT IN NORMOTENSIVE AND SPONTANEOUSLY HYPERTENSIVE RATS

by Paul Geoffrey Dorey

A study was conducted on the role of both angiotensin and the nervous system in the control of intestinal fluid transport in normotensive Wistar animals.

1. The possibility that dopamine might be involved in the control of intestinal fluid transport was studied. Dopamine was found to stimulate intestinal fluid transport, at a dose 50 times higher than that for noradrenaline, but this action was sensitive to α-blockade and refractory to blockade by the specific dopamine receptor antagonist sulpiride.

2. The pharmacological nature of the receptor involved in the control of intestinal fluid transport was studied. Curiously, in line with recent studies on vascular receptors, the stimulation of intestinal fluid transport was shown to be at least partially mediated by receptors of α_2-type pharmacological specificity.

3. The possibility that the action of angiotensin II on intestinal fluid transport is mediated by release of noradrenaline from the nervous system was tested. Both chemical sympathectomy with 6-hydroxydopamine and ganglion blockade with pentolinium tartrate were found to abolish the action of angiotensin.

The Okamoto spontaneously hypertensive rat (SHR) was shown to have a markedly increased intestinal fluid transport and altered electrical parameters compared with Wistar controls.

4. This elevation of transport was not related to blood pressure.

5. Animals had an altered sensitivity to exogenous noradrenaline but neither α-blockade nor ganglion blockade reduced transport.

6. Similarly neither nephrectomy nor treatment with captopril reduced transport.

7. The possible involvement of an endocrine inbalance or membrane defect in these animals is discussed.

Chapter 1

INTRODUCTION

Section 1.1

SALT AND WATER HOMEOSTASIS

1.1.1) Total Body Fluid.

Water is essential to life, and consequently forms a large proportion of all living organisms. In mammals approximately 65% of total body weight consists of fluid (Gamble, 1954). This body fluid may be divided up into two major compartments, fluid within cells (intracellular fluid, ICF) and fluid in the blood plasma and intercellular spaces (extracellular fluid, ECF). In electrolyte solution in this body fluid are a complex balance of ions, such as sodium and potassium, which must remain within close tolerances for cellular processes to occur. So important is this water and electrolyte balance that a number of homeostatic mechanisms exist to control intake and loss from the body compartments. Figure 1.1 illustrates these compartments, together with the major routes for fluid gain and loss. Whilst table 1.1 shows the relative concentration of various ions between ECF and ICF, it is primarily this ion balance (particularly that of extracellular sodium) which determines the relationship between the volumes of the compartments.

1.1.2) Intracellular Fluid Homeostasis.

The major regulation of ICF volume is classically thought to be a response to a shrinkage of cells that occurs on cellular dehydration (Gillman, 1937). In particular two groups of cells in the hypothalamus are excited by a reduction in their volume. Firstly osmoreceptors in the supraoptic nucleus respond to this event by stimulating the release of antidiuretic Hormone (ADH) from the neurohypophysis (Verney, 1947), thus reducing water loss via the kidneys. Secondly, in the lateral pre-optic region cell shrinkage produces a drinking response to make up the water deficit (Blass and Epstein, 1971; Peck and Novin, 1971). Simple reduction of cell volume may not be the whole story, however, as

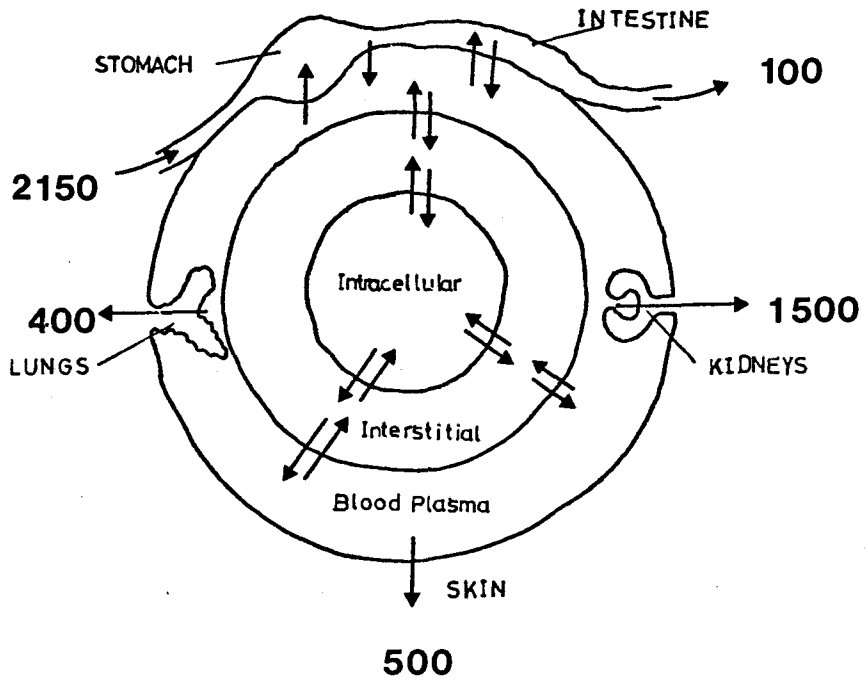


Figure 1.1 Human body fluid compartments with sites of water gain and loss. Approximate values of daily fluid intake and loss given in ml . (Bell et al, 1978). Intestinal secretions amount to approximately 8000 ml per day. Thus total intestinal absorption amounts to 10,050 ml/day.

	Plasma	Interstitial Fluid	Intracellular Fluid
Cations			
Na	142	145	10
K	4	4	160
Ca	2	1	1
Mg	1	1	13
Anions			
Cl	101	114	3
HCO_3	27	31	10
HPO_4	1	1	50
SO_3	0.5	0.5	10
Organic anions	6	7	8
Protein	2	<0.01	-

Table 1.1 Approximate values of solute concentration in various body fluid compartments. Values in mmol/l for normal fasting adult human. ECF = Interstitial fluid + plasma = 4200 mOsM. ICF = 8400 mOsM. Figures from Bell, Emslie-Smith and Paterson (1978).

more recently Andersson (1977) and other workers have suggested that actual ion concentrations may be more important, and that receptors may be capable of responding to changes in the sodium concentration of cerebrospinal fluid. Evidence now also exists for an osmoreceptor mechanism lying ouside the blood brain barrier that may promote drinking and ADH release (Thrasher, Brown, Kiel and Ramsay, 1980; Thrasher, Jones, Kiel, Brown and Ramsay, 1980). Similarly, McKinley, Denton and Weisinger (1978) have further demonstrated the complexity of the control mechanism by their suggestion of a dual 'ventricular sodium receptor-peripheral osmoreceptor' system.

1.1.3) Extracellular Fluid Homeostasis.

Exchanges between ECF and ICF occur as a form of controlled osmosis where a change in the amount of electrolyte in one compartment is followed by predictable changes in the composition of the other (Gamble, 1942). This, coupled with the fact that cells are continuously bathed in intercellular fluid make the maintainance of ECF volume and composition as important as that of the cells themselves. Stretch receptors sensitive to volume changes can be found at a number of sites on the low pressure side of the circulatory system in regions including the walls of the atria and the large veins (Gauer and Henry, 1963). Afferent fibres from these receptors run as part of the vagus and , in general, have been well documented (Gauer and Henry, 1963). This is in contrast to the efferent pathways which are complicated and remain unclear. Much evidence exists for a joint neuro-humoral mechanism on the effector side of the reflex consisting predominantly of the sympathetic nervous system coupled with the hormones ADH, aldosterone and angiotensin (Gauer, 1978; Gauer and Henry, 1976; Goetz, Bond and Bloxham, 1975). The hormonal control of ECF volume is further discussed

in sections 1.1.6-1.1.9 .

1.1.4) Thirst and Salt Appetite.

Thirst can be classified as either primary or secondary thirst depending on whether or not the thirst results from an actual water lack or as a response in anticipation of future water requirement. The majority of drinking occurs at a level above that actually needed and so is classified as secondary. The more urgent demands of primary thirst may be produced by changes in both ICF and ECF.

ICF depletion thirst is stimulated by water deprivation or administration of a high sodium diet (osmotically equivalent to intracellular water depletion) and as previously discussed is mediated by osmoreceptors (Blass and Epstein, 1971; Peck and Novin, 1971).

The control of ECF depletion thirst is more complicated, involving salt appetite as well as thirst. In various mammalian species a deficiency of body sodium has been shown to result in a specific appetite for sodium (Richter, 1936; Denton and Sabine, 1961). This salt appetite can also be obtained by means that reduce ECF volume without changing total body sodium, such as a subcutaneous formalin injection (Wolf and Steinbaum, 1965). Furthermore, isotonic saline rather than water is required during hypovolemia to restore plasma volume, and hypovolemic rats will preferentially select saline solutions to drink (Smith and Strickler, 1969). Stimuli that alter ECF to produce thirst include :- haemorrhage, vomiting, diarrhoea and sodium depletion. Changes resulting from this stimuli are detected by the arterial baroceptors and the low pressure volume receptors which relay the information to the central nervous system. This proposed mechanism (Fitzsimmons, 1976), together with the separate sensing apparatus of the renin-angiotensin system are illustrated in figure 1.2.

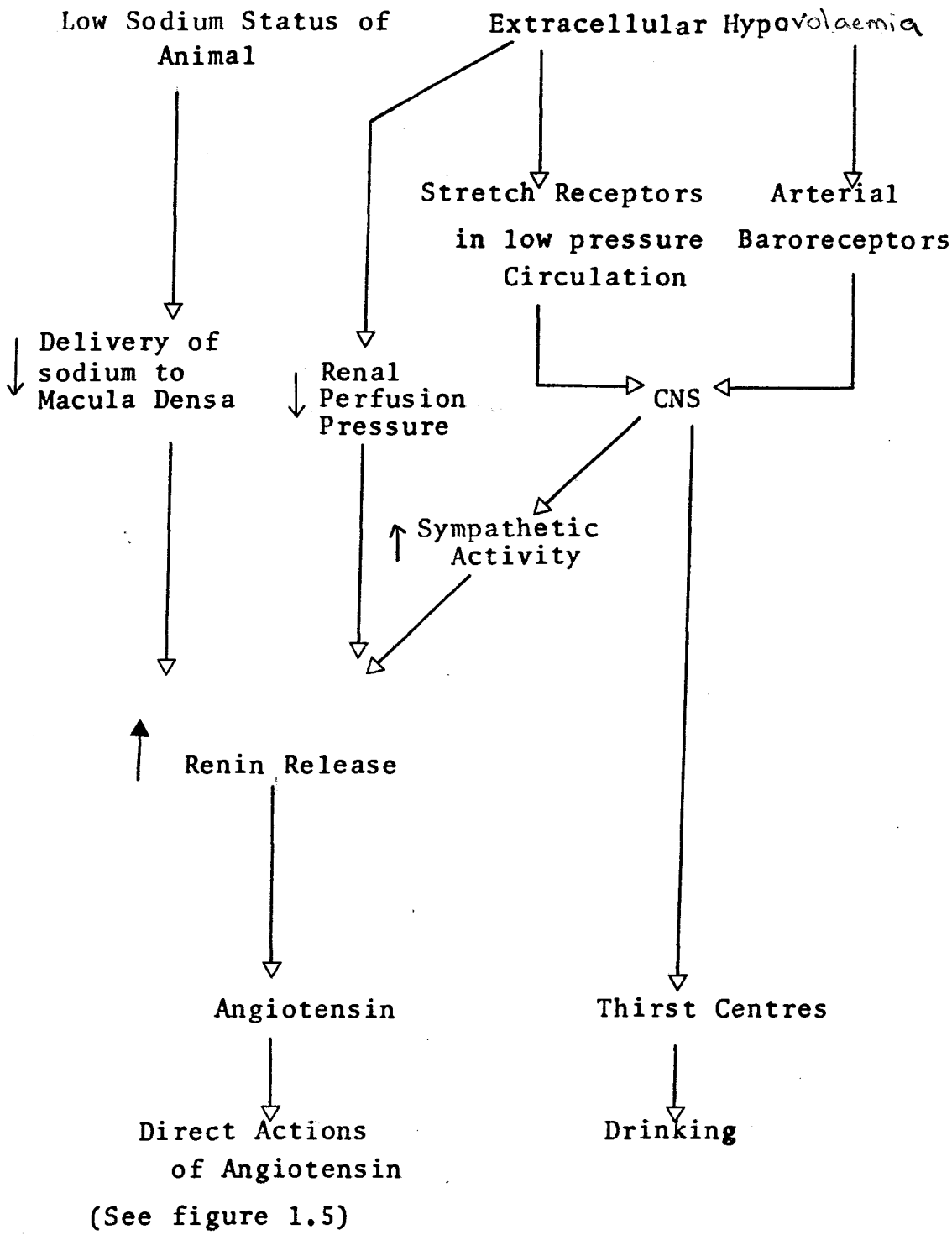


Figure 1.2 Proposed model for the control of extracellular fluid volume and composition. Based on Fitzsimmons (1976)

1.1.5) Importance of the Intestine in Salt and Water Balance.

Control of the excretion of water and electrolytes by the kidneys is often considered to be the most important area of water and electrolyte homeostasis. However, a simple study of general water balance soon reveals the intestine as a likely secondary site for control. Typical figures for an adult human give a total fluid absorption of approximately 10 litres per day (ingested fluid plus re-absorbed secretions) compared with 350 ml. produced by body metabolism (Bell, Emslie-Smith and Paterson, 1978). This volume is of such a size that a close control correlating ingested water with absorption is essential.

The fact that sodium depletion can result in increased sodium reabsorption in the dog small and large intestine (Clark, Miller and Shields, 1967) and in the rat colon (Dolman and Edmonds, 1975) led early workers to suggest that the rate of intestinal fluid transport may be affected by the sodium status of the animal. This is further supported by the findings of Martinson and Ingraham (1974) who showed that haemorrhage in the dog produced a blood borne factor that stimulated sodium absorption in the rat ileum; also Mariscotti, Munday, Parsons and Poat (1977) demonstrated both that fluid absorption from the *in vivo* rat jejunum is increased following sodium depletion, water depletion and haemorrhage and that absorption is decreased following the ECF expansion produced by sodium loading. Furthermore, the characteristics of a number of transport processes have been shown to vary in the colon of the adult hen when the amount of dietry NaCl is changed (Lind, Munck, Olsen and Skadhauge, 1980). A number of circulating hormones are involved in these mechanisms , the most important being antidiuretic hormone, aldosterone and angiotensinII.

1.1.6)Antidiuretic hormone (Vasopressin).

The control of antidiuretic hormone (ADH) release has been demonstrated to be regulated by a number of stimuli (Share and Claybaugh, 1972; Schrier and Berl, 1975) of which the most important appear to be plasma hyperosmolarity and volume depletion. Both types of stimuli act rapidly by a neural afferent—CNS—efferent reflex.

Initial work in this field produced the observation that there was a reduction in urine flow in a change from the supine to the upright position (Brun, Knudson and Raaschau, 1945). These findings began to be explained when plasma ADH concentration was shown to be increased from supine to erect (Segar and Moore, 1968). It was proposed that, although not altering total blood volume, the intrathoraxic blood volume is reduced on standing and this volume reduction produces the stimulus for ADH secretion. Contrary to these findings some workers (Goetz et al., 1975) dismiss the evidence for a blood volume influence as being purely indirect, and favour osmotic stimuli as the primary regulatory influence. In all likelihood both sensory mechanisms are involved in the control of ADH secretion in accordance with the findings of Robertson and Athar (1976) that responses following osmotic changes are the most senstive, although changes in blood volume can alter the set of the osmoreceptors towards higher or lower levels of osmolarity. Schrier, Berl and Anderson (1979) further discuss these interactions between osmotic and nonosmotic control mechanisms.

Earlier work that suggested a possible negative feedback control loop between the renin-angiotensin system and ADH secretion, namely the findings by Bonjour and Malvin (1970) that angiotensin could stimulate ADH secretion, and that ADH can inhibit renin release (Bunag, Page and McCubbin, 1967; Tagawa, Vander and Bonjour, 1971), has provoked much

controversy. Claybaugh, Share and Shimizu (1972) failed to confirm that angiotensin could stimulate ADH release, although the findings of Bonjour and Malvin are supported by most workers when using pressor doses of angiotensin-II (Reviewed by Share, 1979). It therefore appears that although the inhibition of renin secretion by circulating ADH is well documented, any stimulation of ADH secretion by peripheral angiotensin will be dependant on physiological conditions. Centrally administered angiotensin has been shown to be able to stimulate ADH release (Mouw, Bonjour, Malvin and Vander, 1971) but the physiological significance of this is not yet clearly established.

The physiological function of ADH is the homeostatic control of ICF volume and composition, achieved by control of water handling in the kidney (Share and Claybaugh, 1972). ADH generally acts on epithelia with a high integral resistance (200-2,000 ohm/cm2) and therefore in mammals, ADH predominantly acts on the collecting tubule of the renal cortex and on the medullary collecting duct (Andreoli and Schafer, 1976; Hays and Levine, 1974). On reaching the epithelia of these target tissues ADH increases the water reabsorption by reducing the limiting permeability barrier of the apical membranes of the cells to water (Orloff and Handler, 1962). These processes occur through the mediation of c-AMP (reviewed by Strewer and Orloff, 1977). ADH has also been shown to have effects on spontaneous p.d. and sodium transport across epithelia such as the bladder (Hays and Levine, 1974). Although these effects are minor in the collecting tubule (Frindt and Berg, 1972).

1.1.7)Aldosterone.

The mineralocorticoid aldosterone is one of a number of steroid hormones produced by the zona glomerulosa of the adrenal cortex.

Physiologically it generally acts on transporting epithelia to enhance sodium retention either alone or in conjunction with excretion of potassium.

The biosynthesis, regulation and actions of aldosterone have been extensively reviewed by a number of workers (Blair-West, Coghlan, Denton, Goding, Wintow and Wright, 1963; Ganong, Biglieri and Mulrow, 1966; Melby, 1974; Davis, 1974; Blair-West, Coghlan, Denton and Scoggins, 1974; Gross, 1974). Despite this large amount of attention on the subject there still remain some aspects, in particular that of the regulation of aldosterone secretion, which are unclear (Reid and Ganong, 1977; Frazer, Brown, Lever, Mason and Robertson, 1979). In general the major regulatory factors appear to be plasma ACTH level, plasma sodium and potassium and the renin-angiotensin system.

Adrenocorticotrophic hormone (ACTH) has been shown to stimulate aldosterone release in a number of species, both in vivo and in vitro but its exact day to day role in control is uncertain. Conditions such as salt restriction produce aldosterone levels that parallel plasma renin activity rather than plasma ACTH and cortisol. This suggests that the renin-angiotensin system is the more important control mechanism (Vagnucci, McDonald, Drash and Wong, 1974). However, ACTH control seems to be dominant in stress-induced aldosterone release, such as surgical trauma (Espiner and Hart, 1978), and also in the control of episodic diurnal secretion of aldosterone (James, Tunbridge and Wilson, 1976).

In vitro preparations both of glomerulosa cells (Lobo, Marusic and Aguilera, 1978) and of perfused adrenals (Ganong et. al. 1966) have been used to demonstrate that an increase in potassium or decrease in sodium can directly stimulate the release of aldosterone. The physiology of this response is not too clear for although increases in plasma potassium are sometimes observed, changes in plasma sodium

levels are rare (Carey, Vaughan, Peach and Ayers, 1977; Nicholls, Tree, Brown, Douglas, Fraser, Hay, Lever, Morton and Robertson, 1978). In fact some workers consider that, under physiological conditions, the response to sodium may be secondary to the renin-angiotensin system (Brown, Frazer, Lever, Love, Morton and Robertson, 1972).

Under most conditions encountered the renin-angiotensin system predominates over the other regulatory systems for aldosterone release. Angiotensin II can stimulate aldosterone production both in vivo (Laragh, Angers, Kelly, and Leiberman, 1960) and in vitro (Ganong, Mulrow, Boryczka and Cera, 1962). This system for control is discussed by a number of workers (Davis, 1974; Peach, 1977; Brown, Frazer, Lever, Morton and Robertson, 1977), and involves a full reflex pathway. Stimuli promoting neural stimulated release of renin, such as aortic constriction (Freeman, Davis and Spielman, 1977), sodium depletion (Lowenstein and Steele, 1977) or electrical stimulation of specific brain areas (Jenkins, Frankel, Wright and Khan, 1976) can promote aldosterone release. In each experimental procedure this concomitant increase in plasma aldosterone can be abolished by preventing renin release by nephrectomy, β blockade or by use of specific angiotensin antagonists (McCaa, 1977). The exact mechanism by which angiotensin has its aldosterone releasing actions does not appear to be a simple stimulation of release. The adrenals have very little reserve store of aldosterone so a physiological role for the observed stimulation of aldosterone biosynthesis by angiotensin is much more likely (reviewed by Davis, 1975).

Aldosterone is considered to have its major actions on the distal regions of the kidney nephron, where it enhances Na^+ retention and K^+ excretion (Fimognari, Fanestil and Edelman, 1967). However, aldosterone has also been shown to have actions on a number of other tissues

involved in water and electrolyte handling :- colon (Edmonds and Marriott, 1970; Shields, Mulholland and Elmslie, 1966; Edmonds, 1976; Frizzell and Schultz, 1978) rat jejunum and ileum (Hill and Clarke, 1969; Crocker and Munday, 1969) and also on sweat and salivary glands (Reviewed by Sato, 1977). The mechanism of action of aldosterone has mainly been studied using the *in vitro* toad urinary bladder preparation (Sharp and Leaf, 1966), figure 1.3 shows the proposed mechanism of action, discussed by Feldman, Funder and Edelman (1972) and Pelletier, Ludens and Fanestil (1972), which follows the classical scheme for most steroid hormones; and explains the 30-60 minute time lag observed in the response. Aldosterone enters the cell and binds to a cytoplasmic receptor, the hormone—receptor complex then enters the nucleus and stimulatesthe transcription of an effector protein; in this case aldosterone induced protein (AIP). The aldosterone response is inhibited by actinomycin D, puromycin and cycloheximide (reviewed by Sharp and Leaf, 1966a; 1966b).

The ion transport changes produced by AIP have been investigated. *In vivo* mammalian studies in the colon by Edmonds and Marriott (1970) and in the renal tubule by Barrter and Fourman (1962) demonstrated that an aldosterone induced increase in sodium absorption is associated with an increase in the rate of potassium and/or (more controversially) H^+ secretion. These changes are considered to occur by both an apical and a basolateral mechanism having respectively short and long timecourses of action. The shorter timecourse effect was studied by Frizzel and Schultz (1978) using an *in vitro* short circuit current (Isc) technique. This study showed that there were no changes in transepithelial K^+ movement, suggesting that the K^+secretion observed *in vivo* is entirely due to an increase in paracellular diffusion, this occuring purely secondary to the electrogenic sodium absorption. These workers further

(A) Short-term (30-60 minute) response.

? (B) Long-term (several hours) response.

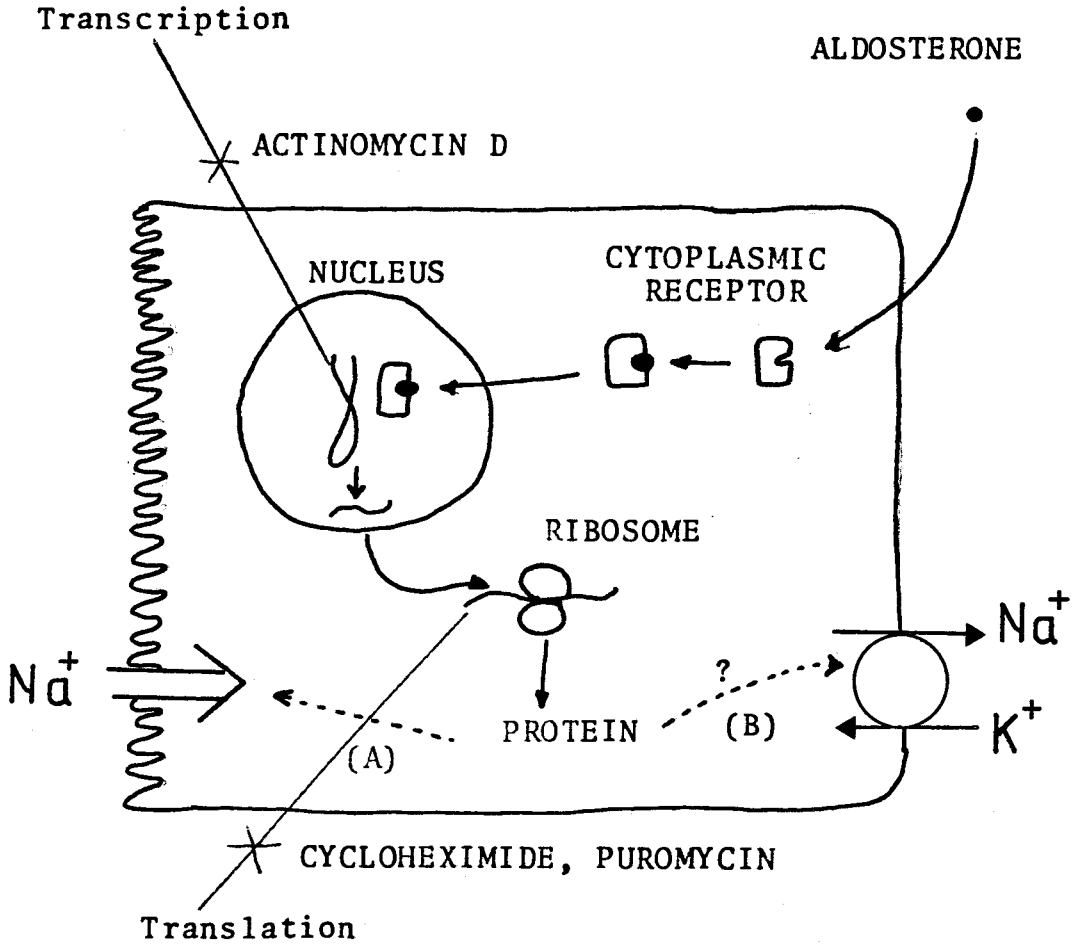


Figure 1.3 The mechanism of action of aldosterone (see text).

observed that the aldosterone enhanced unidirectional flux of Na^+ into the cell was not additive with Amphoterin B (an antibiotic known to increase brushed-border permeability), therefore implying that both AIP and Amphoterin B are acting on the same membrane site. The second, longer time scale, effect has been observed to occur several hours after aldosterone administration and in this case evidence points to an enhancement of activity of the basolateral pump (Crabbe, 1974; Spooner and Edelman, 1975).

Recently both the apical and basolateral mechanisms have been demonstrated by the technique of cell puncture (Nagel and Crabbe, 1980).

1.1.7.1) 18-OH-DOC.

Other steroids produced by the adrenals that may very well have alodosterone-like actions generally have too weak an activity or too low a plasma concentration to be physiologically important. One exception could be the steroid 18,21—dihydroxy-4-pregnene-3,20—dione (18-OH-DOC) which has been shown to have mild mineralocorticoid activity, causing sodium retention and potassium loss in adrenalectomised rats (Kagawa and Pappo, 1962) or simple sodium retention with no effect on potassium excretion (Birmingham, McDonald and Rochefort, 1968; Hall, Gomez-Sanchez, Holland, Nasseth and Hall, 1978). In the rat 18-OH-DOC only has an affinity for mineralocorticoid receptors of 1/75th that of aldosterone (Feldman and Funder, 1973). However the rat has circulatory levels of 18-OH-DOC 1000 times greater than aldosterone, making this major adrenocorticoid secretory product (Peron, 1961; Birmingham and Ward, 1961) of likely physiological importance.

The mechanisms of action and of control of 18-OH-DOC secretion

remain to be fully elucidated but evidence suggests that the renin-angiotensin system plays a much lesser role than is true for aldosterone, with ACTH being the most important controlling agent. (Reviewed by May, 1980)

1.1.8) Renin Angiotensin System.

In a series of now classical experiments Tigerstedt and Bergman (1898) injected a crude saline extract of rabbit kidney into anaesthetised rabbits and obtained a pressor response. Initially named renin, the active component was isolated and found to be the polypeptide angiotensin (Braun-Mendez, Fasciolo, Leloir and Munoz, 1940; Page and Helmer, 1940). With the greater availability arising from the purification and then the synthesis of angiotensin, a large number of studies were conducted on the actions of this hormone. Soon revealed was the significant endocrine role of angiotensin, along with the complexity of the renin-angiotensin system of which it formed a part.

Figure 1.4. shows the major elements of this system, which basically involves the degradation of the large polypeptide precursor angiotensinogen, secreted by the liver (Skeggs, Dorer, Kahn, Lentz and Levine, 1974) to the decapeptide angiotensin I. Subsequently further degradation produces the active angiotensin II and metabolite angiotensin III. Angiotensin II is the most active product although both angiotensin I and angiotensin III have some action in physiological systems (Davis and Freeman, 1977). Both angiotensinogen and angiotensin converting enzyme are normally present in excess and therefore the amount of angiotensin II produced will depend on the amount of active renin available. Control of the renin-angiotensin system is therefore much dependent on the availability of active renin.

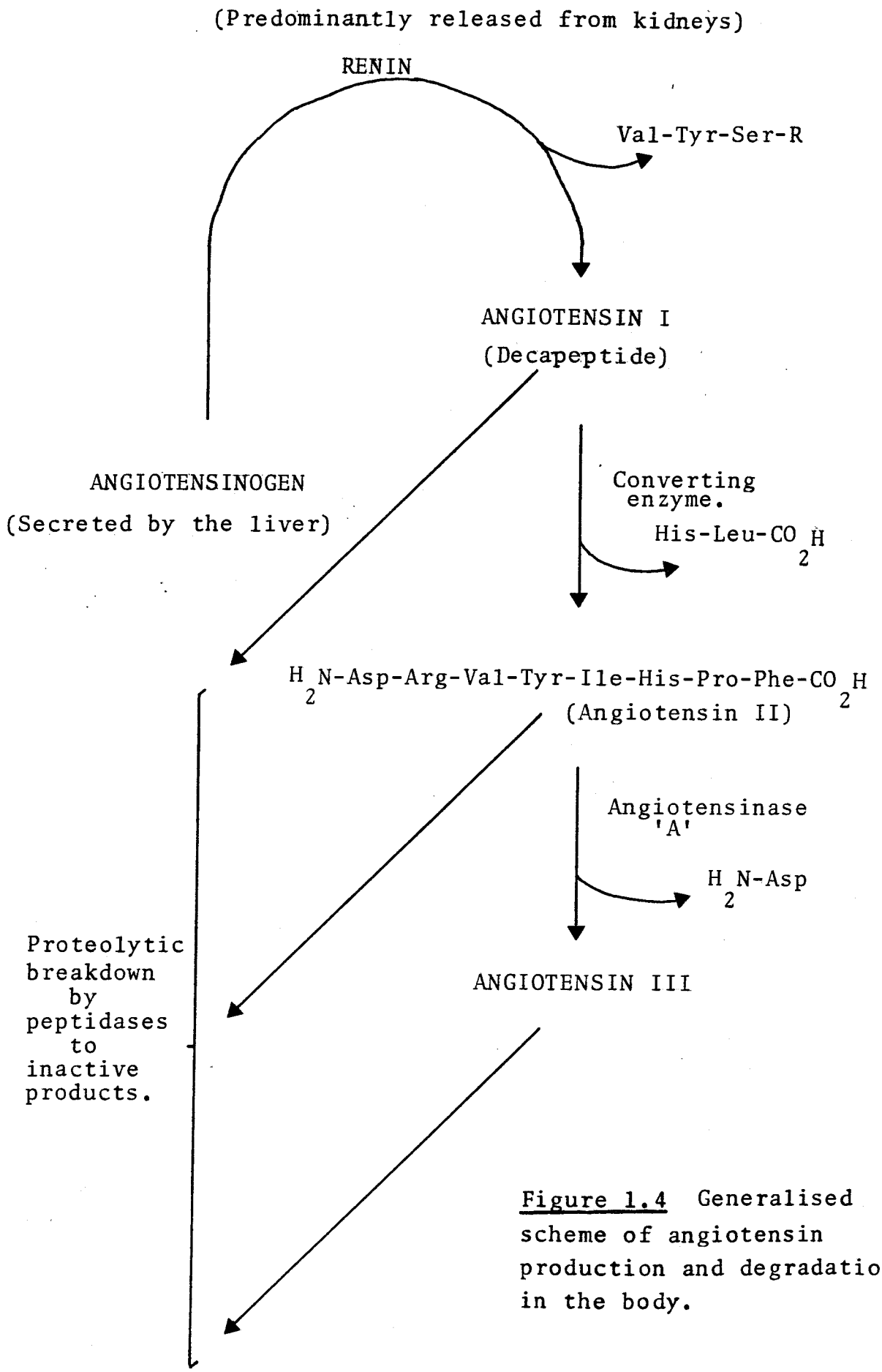


Figure 1.4 Generalised scheme of angiotensin production and degradation in the body.

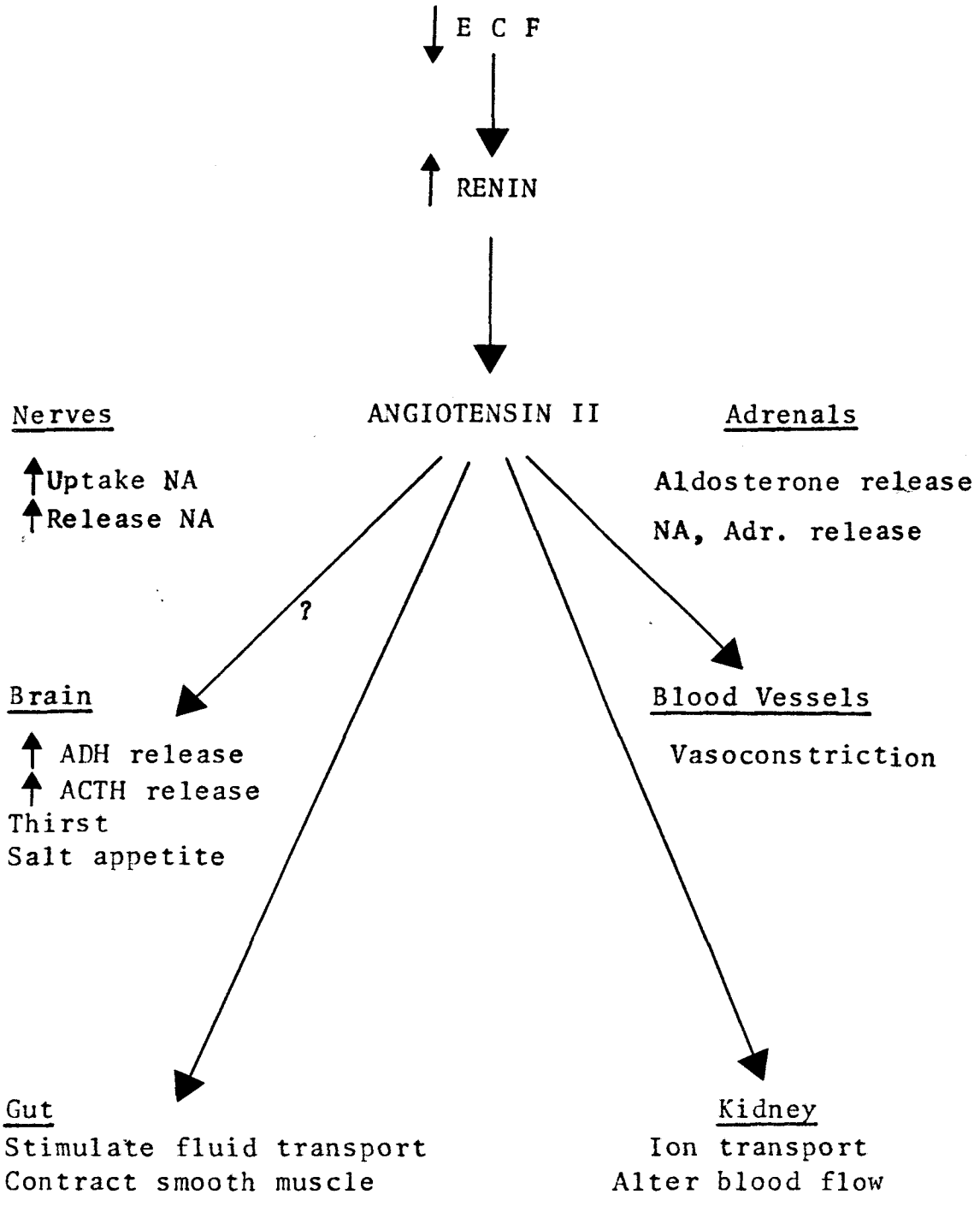


Figure 1.5 Extracellular fluid homeostasis and other actions of the hormone angiotensin II, expressed in terms of target organs.

1.1.8.1)Control of Renin Secretion.

The majority of actions of the effector hormone angiotensin II (figure 1.5.) appear to be directed towards the control of salt and water homeostasis and maintenance of the ECF (Reviewed by Page and Bumpus, 1974; Regoli, Park and Rioux, 1974; Peach, 1977). Interestingly enough the control of renin secretion by the kidney is triggered by stimuli effected by ECF conditions.

Renin is a protein with a molecular weight of approximately 40,000 which is released from the granular juxtaglomerular cells of the kidney. The control of renin release by the kidney has been reviewed by a number of workers (Vander, 1967; Freeman and Davis, 1979; Peart, 1978; Yun, 1979) and can be divided into the two categories of intra- and extra-renal control. Intrarenal control is generally thought to occur by two major mechanisms. The first mechanism follows from the baroreceptor theory of Tobian (1960) who considered that the location of the granular cells of the juxtaglomerular (JG) apparatus in the wall of the afferent arteriole allowed them to respond to stretch, and therefore haemodynamic changes caused by alterations in renal perfusion pressure (Tobian, Tomboulian and Janecek, 1959; Blaine and Zimmerman, 1979). An increase in intravascular pressure will thus inhibit renin release whilst a decrease will induce release. Tobian's theory has had much support since the development of the non-filtering kidney model (Blaine, Davis and Witty, 1970) which has allowed the baroreceptors to be studied in isolation (Blaine and Davis, 1971).

The second mechanism for the intrarenal control of renin release was originally proposed by Vander and Miller (1964) in a series of studies using diuretics. These workers postulated a mechanism whereby renal renin release is modulated by changes in the amount of sodium

present at the macula densa, a concept which has had a fair amount of experimental support (Davis, 1973).

Extra-renal control of renin release is well documented, occurring to some extent by means of circulating hormone levels but predominantly by stimulation from the nervous supply to the kidney. The kidney has a nerve supply from both the parasympathetic and sympathetic nervous systems, with the sympathetic fibres predominant as a rich innervation which have been demonstrated supplying the JG cells (Wagermark, Ungerstedt and Ljungquist, 1968). The evidence for sympathetic stimulation producing renin release is good and extensive: Electrical stimulation of brain regions (Vander, 1965), postural changes (Davies and Slater, 1976), exercise (Bozovic and Castenfors, 1967) as well as psychological stimuli such as learning (Young, Longford and Blanchard, 1976) all produce release of renin by a neurogenic mechanism. This mechanism has been further characterised as being mediated by β-receptors (Winer, Chokski, Yoon and Freedman, 1969; Schrier, 1974; Thames, Jarecki and Donald, 1978). Lee (1965) suggested that the role of the sympathetic nervous system may be for instantaneous minute—by-minute adjustments associated with small blood pressure changes whereas the intrarenal events such as sodium sensitivity provide a much longer timescale for control.

The hormonal control of renin secretion, although probably of less importance than the sympathetic nervous system, is still very much under study. Exogenous angiotensin II inhibits renin release without effecting renal blood flow or aldosterone secretion (Vander and Geelhoed, 1965; Bunag et al., 1967), suggesting that endogenous angiotensin exhibits a negative feedback control. ADH, which inhibits renin secretion (section 1.1.6), is just one of a large number of agents that influence renin release (reviewed by Davis and Freeman,

1976).

1.1.8.2) Peripheral Actions of AII.

The first observed action of angiotensin II was not surprisingly its highly potent pressor activity (Braun-Menendez et. al., 1940). This was later found to be due to its actions causing contraction of smooth muscle of vascular tissue and further divided into both direct and indirect actions. Angiotensin's direct actions on smooth muscle are reviewed by Regoli et. al. (1974), and have been shown to be in particular directed towards the smooth muscle of peripheral resistance vessels (Dyer, 1970).

Angiotensin's indirect actions causing smooth muscle contractions involve stimulation of the adrenal medulla to release catecholamines (Feldberg and Lewis, 1964), liberation of acetylcholine from parasympathetic nerves as shown in the guinea pig intestine (Khairallah and Page, 1961) and release of noradrenaline from adrenergic ganglia cells (see section 1.2.9) into the neuromuscular junction of vascular smooth muscle (Aiken and Reit, 1968; Farr and Grupp, 1971).

At much lower concentrations than the pressor doses, angiotensin has been found to have a large number of separate actions concerned with its aforementioned primary role of salt and water homeostasis. Firstly, stimulating the release of salt and water and water retaining hormones, aldosterone (section 1.1.7) and ADH (section 1.1.6), and secondly acting directly on organ systems involved with salt and water homeostasis. Freeman and Davis (1979) have reviewed the actions of angiotensin on the kidney. The actions of angiotensin are dose dependent and biphasic; high doses increasing urine flow and sodium excretion and low, more physiological doses, having an antinaturetic and antidiuretic effect (Navar and Langford, 1974; Thurau, 1974). The mechanism of action being both to effect the renal vasculature changing

GFR and the filtered sodium load (Freeman and Davis, 1979) and to have a direct action on the tranporting epithelia.

Studies on the direct action of angiotensin II on transporting epithelia arose from earlier work investigating such actions of aldosterone. Aldosterone was found to increase sodium and water absorption from the small intestine of sodium depleted dogs (Clarke, Miller and Shields, 1967), these findings being confirmed in the rat by Crocker and Munday (1967; 1969). Crocker and Munday, however, observed that aldosterone did not always produce an increase in intestinal fluid transport from in vitro everted sacs. Further observations showed that it was in the sacs with an already abnormally elevated basal transport level that this was the case. These workers postulated that the elevation in transport rate was due to another factor. Sodium depletion had been shown to raise plasma renin levels (Gross, Schaerchtelin, Brunner and Peters, 1963; Crocker et al., 1967) so the possibility that angiotensin II was the factor was studied. It was found that angiotensin, at close to physiological levels, caused an increase in the absorption of Na^+ and water from everted jejunal sacs of rats that had been sensitized by prior nephrectomy and adrenalectomy (Crocker and Munday, 1970). Subsequent studies showed that these findings held true for low doses of angiotensin II on everted sacs of jejunum, ileum and colon, with the further observation that high doses produced an inhibition of transport (Davies, Munday and Parsons, 1970; 1972) similar to the dose dependant biphasic action found in the kidney (Barraclough, Jones and Marsden, 1967; Bonjour and Malvin, 1969). The physiological significance of these findings was studied in a preparation of in vivo rat jejunum (Bolton, Munday, Parsons and York, 1975). These workers also found biphasic actions and demonstrated that the transport effects were observed with no changes in intestinal blood

flow or blood flow distribution.

Water transport has been shown to occur as a direct consequence of active ion movement (section 1.1.17). However the in vivo and everted sac intestinal models are not suitable for studies of ion movements. Further studies on the mechanism of action of angiotensin were therefore conducted using a kidney cortex slice preparation (Munday, Parsons and Poat, 1971). These workers found that low physiological doses of angiotensin II stimulate active sodium extrusion both in the presence or absence of extracellular potassium or ouabain. Further ion replacement studies suggested that Cl^- is an essential ion linked with the transport of sodium (Munday, Parsons and Poat, 1976) and that the stimulatory action of angiotensin was likely to be by a mechanism involving the second sodium extrusion pump proposed by Whittembury (1968;1970). This hypothesis was further supported by the findings of Bolton et al. (1975) that the effect of angiotensin in vivo is indeed the stimulation of an electroneutral transport process.

Other work has shown that angiotensin's stimulatory action in kidney cortex slices (Munday et al., 1976) and in vitro colon sacs (Munday, Parsons, Poat and Smith, 1973; 1979) is dependant on calcium ions . With $Ca^{2+} > Str^{2+} > Ba^{2+} >>> Mg^{2+}$ in effectiveness of replacement.

The exact mechanism of action by which angiotensin promotes the activity of the NaCl pump is as yet unclear. The classical secondary messenger c-AMP has been shown to be ineffective in studies in both the kidney cortex (Munday, Parsons and Poat, 1971) and the in vitro colon (Davies, Munday and Parsons, 1972). The action of angiotensin can be blocked by inhibitors of translation such as puromycin and cycloheximide, but not by transcription inhibitors such as actinomycin D. This indicates that angiotensin is having its effects on protein synthesis at the translational level (reviewed by Vesely, 1981). This

mechanism is likely for the *in vitro* kidney (Munday, Parsons and Poat, 1977), *in vitro* intestine (Davies, Munday and Parsons, 1972) and the *in vivo* intestine (Bolton, Munday and Parsons, 1977) preparations.

Further complications to the mechanism of action are introduced by the findings that c-GMP has angiotensin II—like effects (Evans, Munday and Parsons, 1976) and the findings by Levens, Munday, Parsons, Poat and Stewart (1977a; 1977b; 1979), that angiotensin II may be having its actions via the release of noradrenaline (section 1.2.10).

1.1.8.3) Central Actions.

Much debate has continued over the relative importance of systemic angiotensin verses a cerebral isorenin- angiotensin system. The central actions of the system are reviewed by Philips (1978) and Fitzsimmons (1980) and can be summarised as :-

1. Increased Thirst (Fitzsimmons, 1978; 1980, Andersson, 1978).
2. Increased Salt Appetite (Chieraviglio,1976; but compare with Fitzsimmons and Strickler, 1971).
3. Rise in blood pressure (Bickerton and Buckley, 1961).
4. Release of ADH (Andersson and Westbye, 1970).
5. Release of ACTH (Severs and Daniels-Severs, 1973).
6. Increased sodium excretion (Olsson and Kolmodi, 1974).

1.1.9)Neural Factors.

As discussed earlier, the sympathetic nervous system has a role in many areas of ECF homeostasis. An increase in sympathetic firing will occur on the loss of extracellular fluid volume (Folkow, 1955) which will then have a number of actions, promoting renin release from the kidney (Lee, 1965; Winer, Chokshi, Yoon and Freedman, 1969), and producing generalised vasoconstriction. Furthermore, the sympathetic nervous system may have direct actions on the sodium and water transporting systems of the kidney and the intestine (Schrier, 1974; Hermansson, Larson, Kallskog and Wolgast, 1981; Kim, Linas and Schrier, 1980; Levens, Munday and Stewart, 1977; Brunsson, Eklund, Jodal, Lundgren and Sjovall, 1979). These actions are further discussed in section 1.2.10.

1.1.10)Routes of Solute and Water Transport.

Two definitions of types of transporting epithelial tissue exist i.e. 'tight' and 'leaky' epithelia. Many tissues lie in between these two extremes. Typical 'tight' epithelia include frog skin and mammalian urinary bladder, tissues having a high transepithelial p.d. of between 50 - 100 mV and a high tissue resistance (typically 5,000 - 20,000 ohms/cm2). The functional 'tightness' of these tissues allows transport of ions across steep solute gradients. 'Leaky' epithelia are generally concerned with the bulk transport of water and solutes, examples are the mammalian renal proximal tubule or small intestine having a spontaneous p.d. of only a few mV and a tissue resistance of between 5 - 100 ohms/cm2.

Observations by a number of workers have shown that a large proportion, of some 75%, of the tissue conductance of 'leaky' epithelia is due to conductance along a shunt pathway between cells (reviewed by

Schultz, 1979). Thus, although 'tight' epithelia have a predominantly transcellular route for solute and water transport, 'leaky' epithelia such as the intestine have both a transcellular and a significant paracellular—shunt pathway (Smyth and Wright, 1966; Clarkson, 1967). The morphology and properties of the junctions between cells (zonae occludens) is therefore of great importance to the physiology of transport processes and has been the subject of much study. Farquhar and Palade (1963) made some initial observations on these structures. This was followed by Staehelin (1974) who showed that the zona occludens is a system of fibres connecting adjacent cells (figure 1.7). Much work has now been carried out in order to investigate and describe zona occludens properties in terms of ion selectivity (Claude, 1978; Wright and Diamond,1977) and these studies are well reviewed by a number of workers (Schultz, Frizzell and Nelans, 1974; Parsons, 1976; Erlij, 1976; Schultz, 1979; Bentzel and Hainau, 1979; Nellans, 1979).

1.1.11) Methods of Measuring Fluid and Ion Transport.

The study of intestinal water and electrolyte transport, as in the study of all physiological systems, has necessitated the development of a number of *in vivo* and *in vitro* preparations to enable a more detailed investigation of function and mechanism. The majority of these are reviewed by Parsons (1968). The *in vivo* studies enable the more physiologically relevant parameters to be assessed and for this two major preparations are in common use. Firstly the chronic fistula preparation where a loop of intestine is exteriorised and can be perfused, and secondly acute experiments involving anaesthetised animals in which closed intestinal loops (Bolton et al., 1975) or perfused intestinal loops (Winne, 1973) are used.

In vivo experiments, although being useful for basic physiological

studies often provide little information about the mechanism of transport processes. Most bulk fluid absorption studies cannot distinguish between reduced absorption or increased secretion and studies involving ion movements or the effect of metabolic inhibitors are very difficult to conduct. For this reason a large number of in vitro preparations have been developed, ranging from vascular perfusion (Windmeuller and Spaeth, 1977) through isolated everted sacs (Wilson and Wiseman, 1954) to electrical measurements across epithelial sheets (Ussing and Zehran, 1951). These electrical measurements, especially those in tissue having a single cell type, are of particular interest for it is these studies, combined with isotopic flux measurements,that have enabled a number of ionic and water transport mechanisms to be postulated.

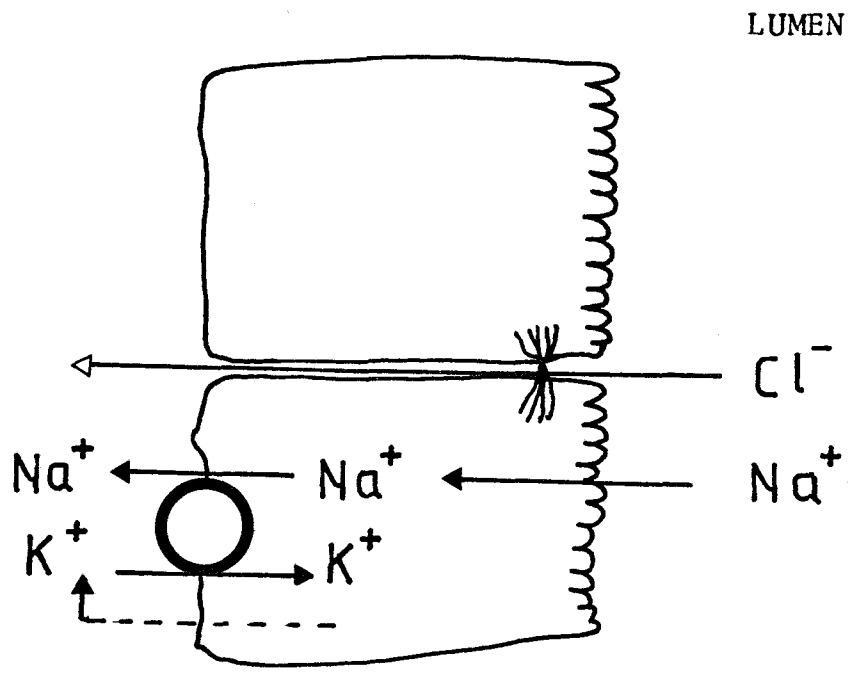
1.1.12) Intracellular Transport.

Absorptive transport processes involving water movement require an entry process into the cell at the brush-border membrane, and then an extrusion from the cell at the basolateral membrane. These processes can be passive, where they simply move along osmotic and/or electrical gradients; facilitated, where they are saturable and involve some form of membrane carrier; or active where a movement of ions can occur against an electrochemical gradient. For active transport energy is consumed in the movement of the ions against electrochemical gradients and thus metabolic inhibitors will attenuate this process.

1.1.13) Sodium-Potassium Exchange Pump.

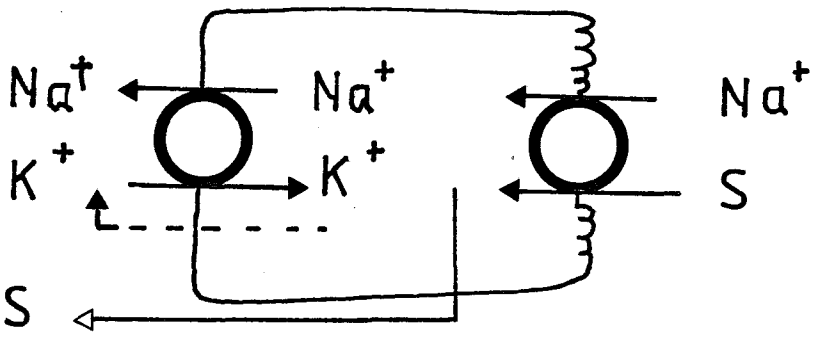
Koeford-Johnsen and Ussing (1958) in a series of studies on frog skin became the first workers to propose a mechanism for active intracellular sodium transport. Figure 1.6a illustrates the mechanism

a



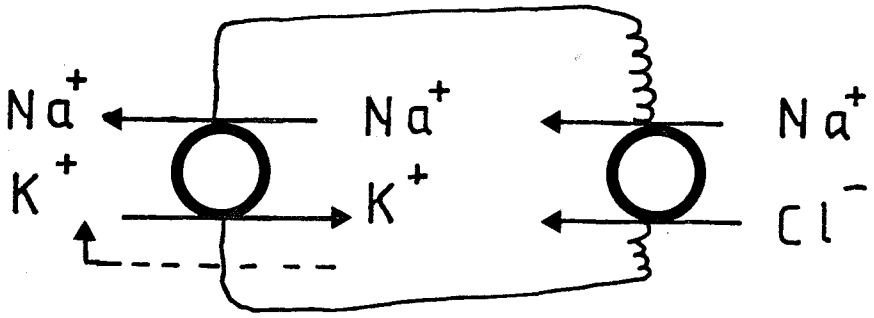
Koefoed-Johnsen and Ussing model for active sodium transport

b



Nellans, Frizzel and Schultz model for organic solute (S) coupling

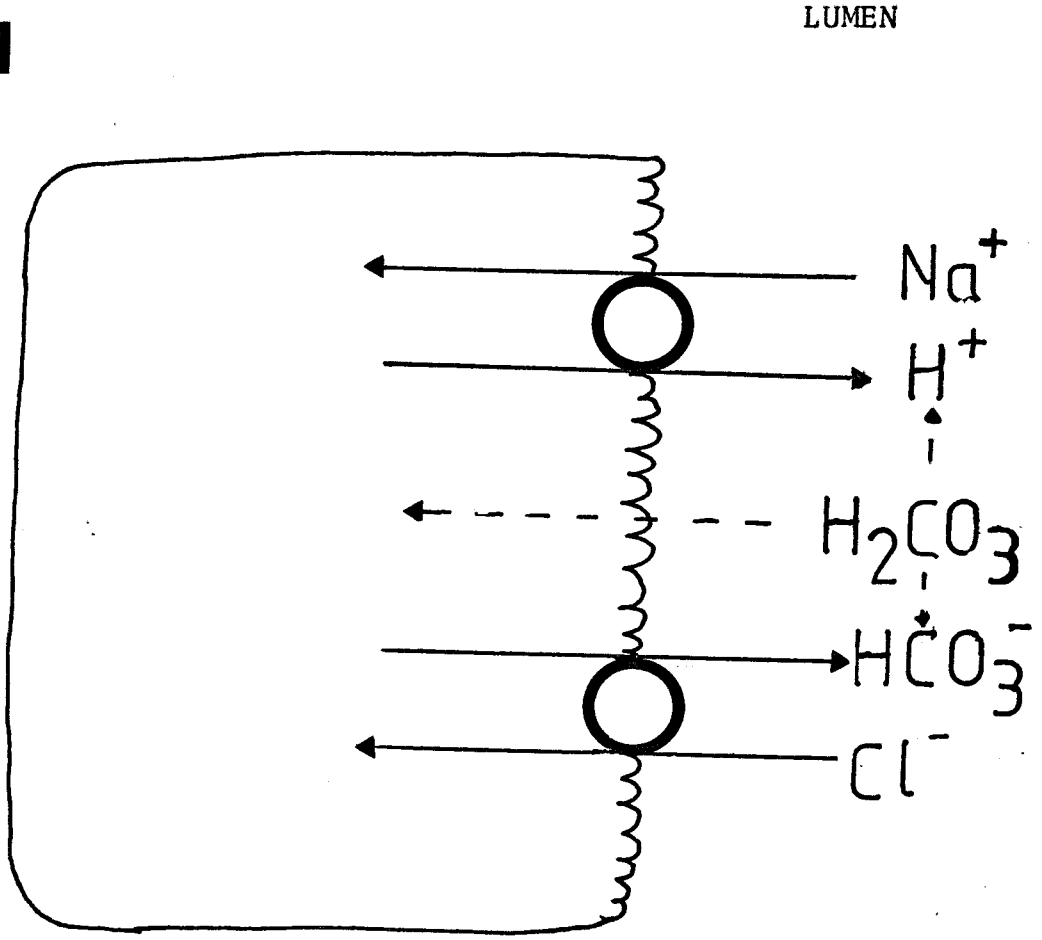
c



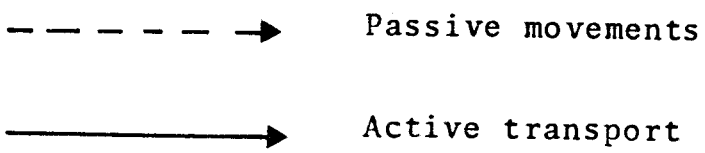
Whittembury and Proverbio electroneutral ion transport model

Figure 6 Models of intestinal ion transport

d



Na/H, Cl/HCO_3 coupled transport proposed by Field (1974)



of transport in the cell which consists of a two step mechanism. Firstly sodium enters the cell by a passive mechanism along an electrochemical gradient (Cereijido and Rotunno, 1967; Rose and Schultz, 1971) and then the ion is actively pumped out in exchange for potassium. In this model sodium transport is therefore transcellular whilst chloride ions move through the paracellular pathway (Civian and Dibona, 1978).

Although the exact inter-membrane mechanism of this pump is unknown (Post, 1968) this now classical model, inhibited by ouabain (Macknight, Civian and Leaf, 1975) and low extracellular potassium, has had much support. Skou (1965) found that the energy source for the pump activity came from ATP hydrolysis, with the pump being located on the basolateral membrane of transporting epithelia (Mills, Ernst and Dibona, 1977; Dibona and Mills, 1979) with the sodium sensitive side orientated inside the cell (Hoffman, 1962; Whittam, 1962). In fact current ideas of this Na^+-K^+—sensitive-ATPase remain very much as Koeford-Johnsen and Ussing originally envisaged it. The only major change has been being brought about by the evidence that Na influx into the cell is saturable and therefore likely to be a facilitated entry-carrier mediated mechanism (Frazier and Leaf, 1964; Frazier, 1964). For further details the reader is refered to Bia and DeFronzo (1981) who have recently reviewed Na^+/K^+ pumps in a number of systems.

1.1.14) Sodium-Chloride Pumps.

The Koeford-Johnson and Ussing mechanism for sodium and chloride transport is reviewed by Macknight, Dibona and Leaf (1980) along with other 'tight' epithelia transport mechanisms. 'Leaky' epithelia have low transepithelial resistances, due to high conductance shunt pathways between the cells (Frizzell and Schultz, 1972; Fromter and Diamond, 1972), and do not have the high spontaneous p.d.. They therefore have

different transport processes from 'tight' epithelia.

Sodium and chloride movement has been demonstrated to be linked in a manner similar to general sodium-coupled solute transport (Schultz, 1977). In a study in stripped isolated rabbit ileum with a low (10mM) luminal bicarbonate concentration Nellans, Frizzel and Schultz (1974) found that if chloride ions are removed from the lumen then sodium transport is reduced by about 50%. This observation is in accordance with their earlier findings that an electroneutral Na^+/Cl^- entry system is present (Nellans, Frizzell and Schultz, 1973). Figure 1.6b illustrates such an entry mechanism which is thought to be driven by ion gradients established by the basolateral ATPase.

Much evidence now also exists for the existence of a sodium/chloride co-transport system for extrusion of sodium from the cell. Observations by Whittembury (1968) and by Macknight (1969) on kidney cortex slices led Whittembury and Proverbio (1970) to describe this second sodium pump, this pump being in addition to the sodium-potassium exhange pump of Koeford-Johnsen and Ussing (1958). Figure 1.6c illustrates this pump system which has been found to be insensitive to ouabain but inhibited by the diuretic ethacrynic acid (Proverbio, Robinson and Whittembury, 1970). Further properties include the pump not requiring K^+ for its operation and being inhibited by low temperature. Indirect evidence for the pump has come from studies using angiotensin, where it has been shown that angiotensin-stimulated efflux from kidney cortex cells functions both in the absence of potassium and the presence of ouabain (Munday et al., 1972; 1976). Bolton et al. (1975) found that angiotensin-stimulated transport was non-electrogenic in their in vivo preparation. Furthermore, ion replacement studies by the same workers and by Smith (1976) and Stewart (1979) have demonstrated that Cl^- is the co-transported ion. A number of mechanisms

have been proposed to interpret the effect on intestinal chloride transport by this mechanism and these are further reviewed by Frizzel, Field and Schultz (1979).

1.1.15) Other Sodium Pumps.

A number of other brushed-border sodium transport mechanisms exist both in the intestine and epithelia in general. The majority of these reported mechanisms involve co-transport of sodium and another solute into the cell. It is thought that the energy for this process is obtained from the electrochemical gradient for sodium set up by the basolateral pump systems. Schultz (1977) has reviewed sodium-coupled solute transport which includes such mechanisms as amino acid transport (Chez, Palmer, Schultz and Curran, 1967), sugar transport (Barry, Smyth and Wright, 1965) and transport of phosphate and calcium (Reviewed by Wasserman, 1981).

1.1.16) Na^+-H^+ and Cl^--HCO_3^-.

When Nellans and coworkers (1974; section 1.1.14) carried out their studies on the interdependence of sodium and chloride transport in the rabbit ileum they used buffers having a low luminal bicarbonate concentration. Studies on *in situ* perfused human ileum showed that under these low bicarbonate conditions Na^+/Cl^- was indeed absorbed and bicarbonate was secreted. However, when the bicarbonate concentration was increased to 60mM then Cl^- secretion and bicarbonate absorption was observed (Turnberg, Bieberdorf, Moranski and Fordtran, 1970). These observations have now been postulated to be due to an active mechanism (Frizzel, Nellans, Rose, Markschield-Kaspi and Schultz, 1973; Turnberg, Fordtran, Carter and Rector, 1970) which has been observed in a number of tissues including *in vivo* rat ileum (Hubel, 1969), *in vivo* and *in*

vitro colon (Devroede and Philips, 1969; Frizzel, Koch and Schultz, 1976) as well as guinea pig gall bladder (Heintz, Petersen, Olles, Saverymuttu and Wood, 1979).

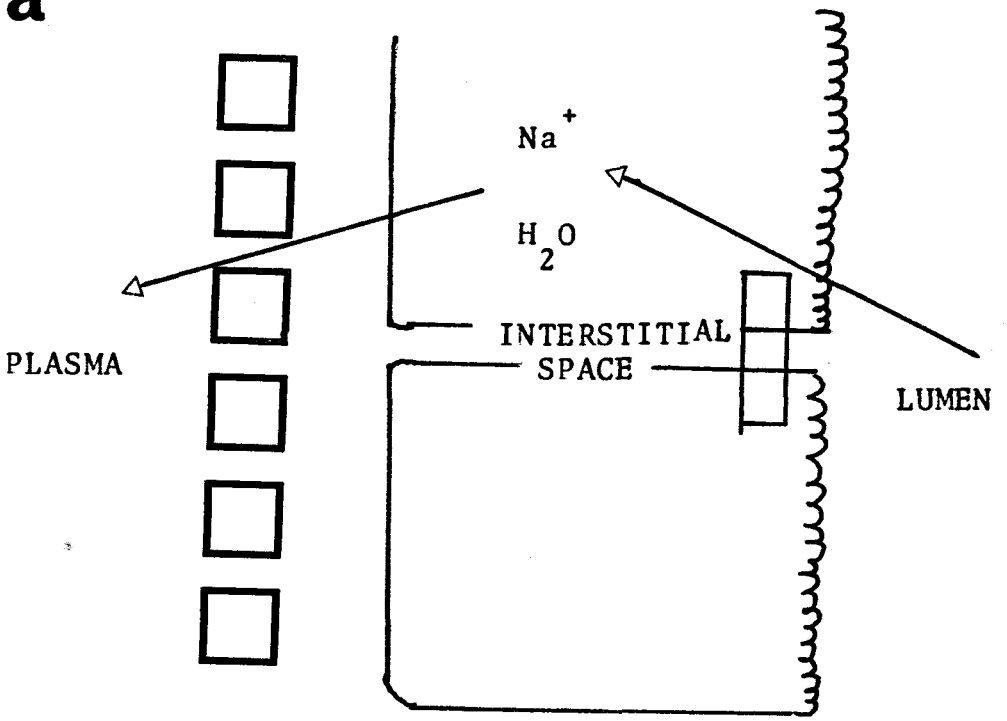
The transport of sodium and H^+ has been postulated to be a neutral transport mechanism although it is not known if it is an active or simply facilitated mechanism (Murer, Hopfer and Kinne, 1976) or even if it exists at all (Schultz, 1979). Nonetheless many workers consider that these mechanisms may be important in sodium transport, and in particular in explaining some of the observed electrical phenomena. Figure 1.6d illustrates both the Na^+/H^+ and $Cl^-/HCO3^-$ transport mechanisms, presenting them as being linked according to the scheme of Field (1974).

1.1.17) Mechanisms of Water Transport.

Initial observations by Parsons and Wingate (1961) in the rat intestine demonstrated that water transport can take place against osmotic gradients. This, coupled with the observation that water transport was inhibited by metabolic inhibitors (Smyth and Taylor, 1957), led workers to believe that water transport was an active process. Further investigation, however, showed that water transport in intestine (Curran and Schwartz, 1960) and in kidney (Whittembury, 1968) is directly proportional to net sodium flow. Curran (1960) therefore proposed a model (see figure 1.7a) in which water transport occurred secondary to active sodium movement.

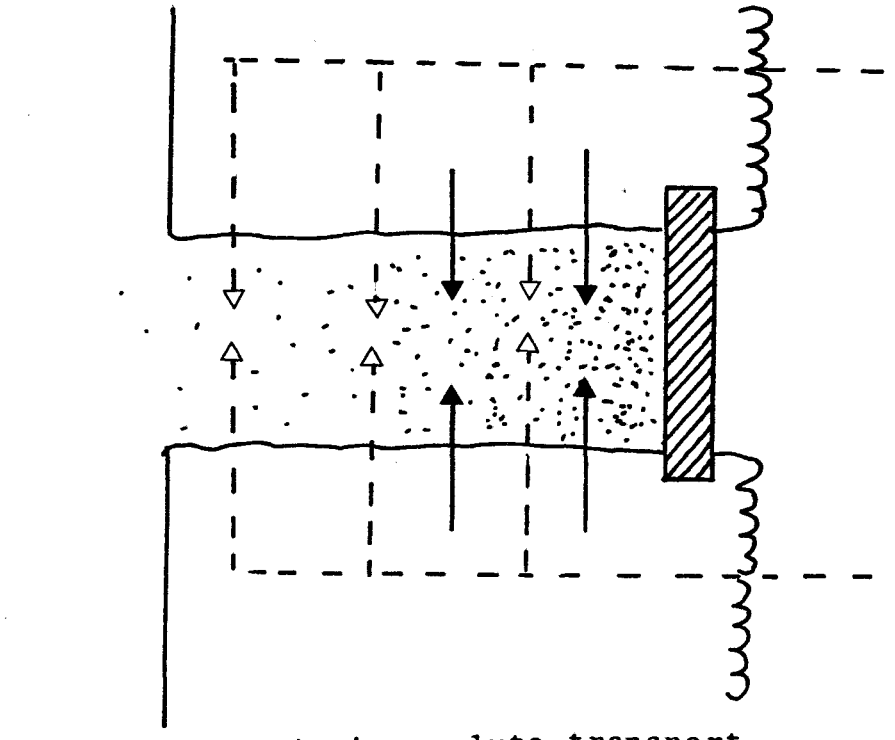
Despite some modification by later workers, mainly in the light of investigations into paracellular pathways (Reviewed by Schultz, 1977; Schafer, 1979), Curran's model largely remains the basis for all water absorption models.

a



Curran Model for water transport

b



Active solute transport

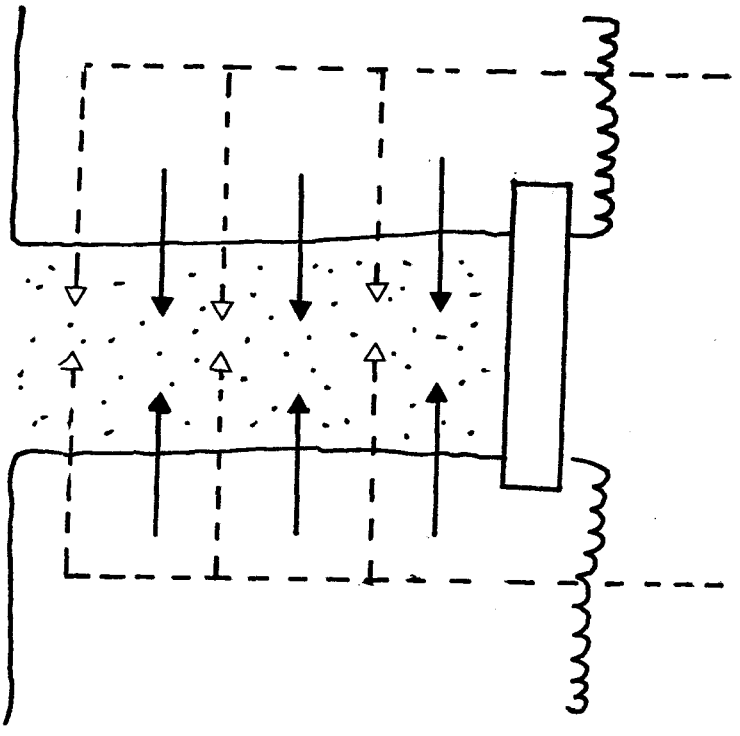
Passive water movement

Frequency of dots denotes solute concentration.

Diamond and Bossert model

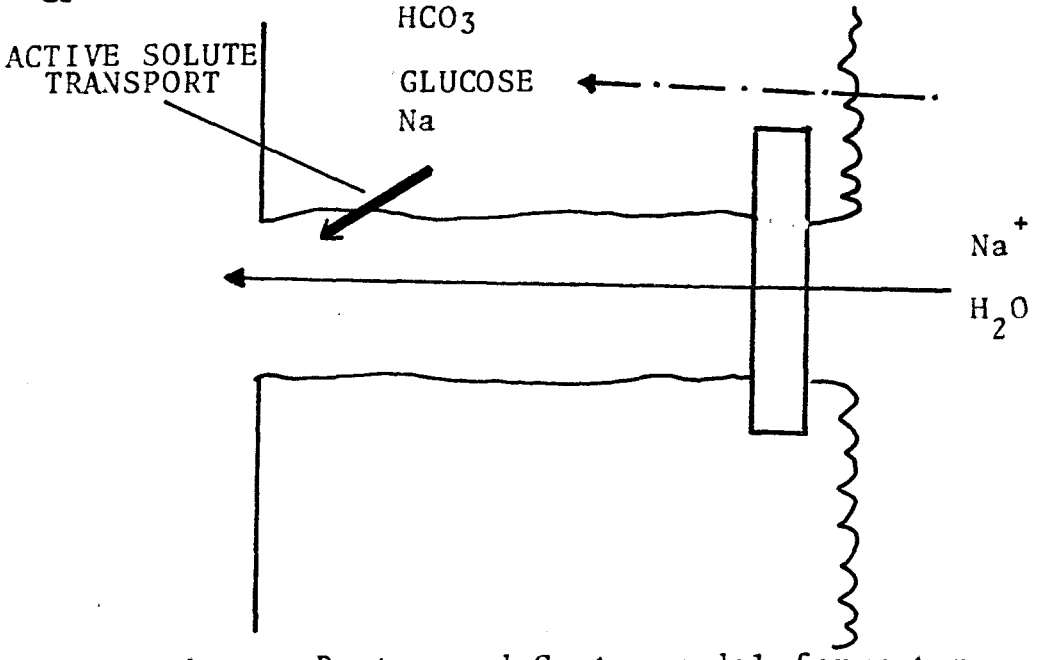
Figure 1.7 Models of solute and water transport

c

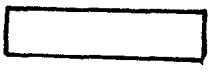


Sackin and Boulpaep model for solute and water transport

d



Fordtran, Rector and Carter model for water and solute transport



Indicates zona occludens, degree of shading denotes 'tightness' of junction

1.1.18)Diamond and Bossert.

The discovery by Tormey and Diamond (1967) using electron microscopy that water transport in gallbladder is via the long and narrow lateral intercellular spaces, made the existence of standing osmotic gradients seem inevitable. Diamond and Bossert (1967) therefore proposed their mechanism for water movement in which a standing gradient flow is set up (figure 1.7b). Important assumptions are made in this model which a number of observations appear to contradict:

Firstly, the intercellular space must be closed at the luminal end, this is contradictory to electrophysiological evidence from studies on the small intestine (Frizzel and Schultz, 1972) and gallbladder (Fromter, 1972).

Secondly, the required uneven pump distribution has been shown to be unlikely by the discovery of the Na^+-K^+-stimulated-ATPase on the basolateral membrane (Dibona and Mills, 1979), and the findings of Stirling (1972) that tritiated-ouabain binds with a uniform distribution.

In a recent review Diamond (1979) discusses the problems of the standing gradient hypothesis, and outlines the difficulties existing with current techniques that make the final acceptance or dismissal of the hypothesis, as yet equivocal.

1.1.19)Sackin and Boulpaep.

To overcome some of the problems raised by the model of Diamond and Bossert, both Huss and Marsh (1975) and Sackin and Boulpaep (1975) proposed models for 'leaky' epithelia. The model of Sackin and Boulpaep is illustrated in figure 1.7c. The model of Huss and Marsh involves transepithelial hydrostatic pressure differences, whilst that of Sackin and Boulpaep depends on a continous osmotic system. In the

model of Sackin and Boulpaep no standing gradients are assumed, suggesting that the salt concentration profile along the intercellular space is uniform. The consequence of this will be a slight hypertonicity of fluid, relative to interstitial fluid, at the serosal end of the intercellular space. This idea is supported by findings in rat ileum (Powell and Malaver, 1968) and rabbit gallbladder (Wheeler, 1963). Further evidence consistent with non-standing gradient models has been obtained by Gupta, Hall and Naftalin (1978) using an X-ray microprobe analysis of Na^+, K^+ and Cl^- concentration along the intercellular space of rabbit ileum.

1.1.20) Fordtran, Rector and Carter.

The work of Fordtran and coworkers has largely involved the study of sodium and bicarbonate movements (see section 1.1.16). Figure 1.7d illustrates their model for water transport proposed in 1968 (Fordtran, Rector and Carter, 1968). In this model glucose moves through the cell along with sodium and bicarbonate, whilst sodium and chloride move by the solvent drag from water passing through the tight junctions. In addition to a number of other observations, glucose stimulation of sodium absorption can be explained by the linkage of sodium to solvent drag.

1.1.21) Mechanisms of Secretion.

The study of secretion is a complex one, for although the primary physiological role of the intestine can be thought to be absorptive, the intestine has numerous structures and processes for the secretion of digestive enzymes, mucus and fluid. Furthermore it appears that a simple pathological or even physiological alteration can quickly switch the intestine from net absorption to net secretion. It is therefore

important to understand some of the mechanisms of secretion in order to conduct a proper study on absorptive processes.

Field (1971) demonstrated that the net secretion of ions and water appears to occur as a consequence of raised tissue c-AMP levels. A variety of secretory agents such as theophylline, cholera toxin, bacterial endotoxins, prostaglandins and vasoactive intestinal peptide(VIP) stimulate intestinal adenylate cyclase (Field, 1974). More recently, however, the importance of calcium has been demonstrated using calcium ionophores (Bolton and Field, 1977; Hubel and Callanan, 1980). Both Ca^{2+} and c-AMP mechanisms are illustrated in figure 1.8.

1.1.22)Transport in the Small Intestine.

Water and electrolyte transport in the small intestine follows the generalised scheme for 'leaky' epithelia (section 1.1.10). For example the transepithelial potential difference across jejunum is approximately 2-3 mV, indicating the importance of the paracellular pathway. These observations are further confirmed by the findings that the jejunum is indeed capable of the rapid absorption of large volumes of fluid and electrolytes (Hill and Clarke, 1969). When considering absorption from intestinal tissue in vivo it becomes important to consider the full absorptive system as a whole combining, cellular ionic mechanisms, transepithelial water transport, as well as the kinetics of capillary uptake, hydrostatic pressure and blood flow.

1.1.23)Transport Kinetics and Blood Flow.

The kinetics for the absorption of water and solute from the intestine can be considered in a series of three compartments. Firstly, the absorption rate will be influenced by physical properties at the

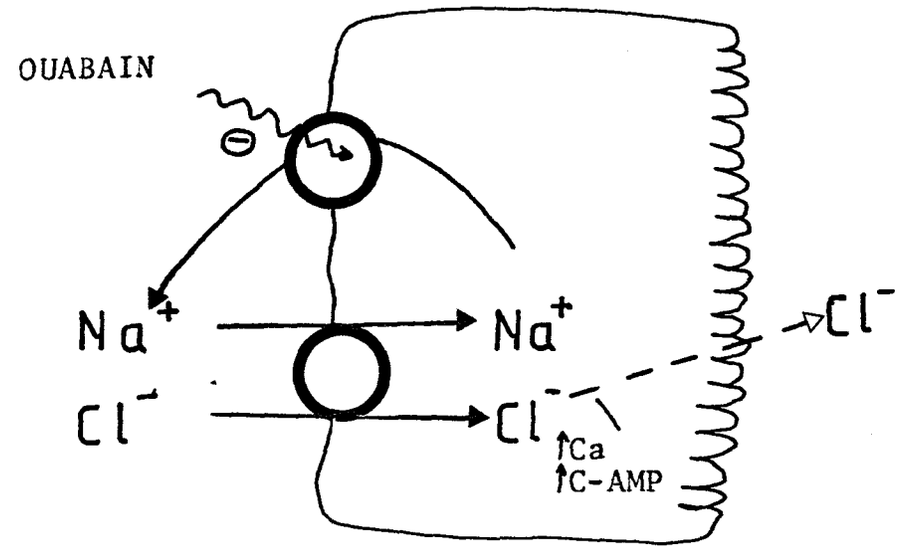


Figure 1.8 Model of intestinal secretion showing two conditions which favour secretion. Frizzel and Schultz (1979).

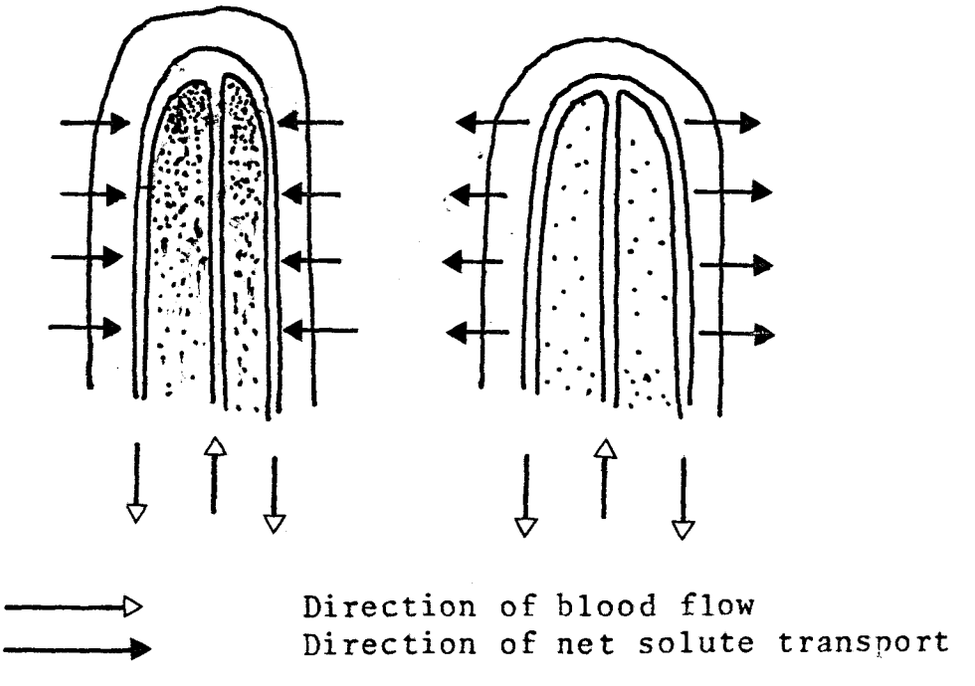


Figure 1.9 Intestinal counter-current multiplier. Frequency of dots denotes osmolarity of villus. Right hand side shows the proposed conditions for secretion and the left hand side shows the proposed conditions for absorption.

absorptive site, such as the area of absorptive surface and the extent of any unstirred layer (Thomson, 1979). Secondly, the activity of active or facilitated transport mechanisms as well as the electrochemical gradient (Kimmich and Carter-Su, 1978) and state of tissue hydrostatic pressure will determine entry into the epithelia (Granger, Mortillardo Kvietys, Rutili, Parker and Taylor, 1980; Hallback, Jodal and Lundgren, 1980). Thirdly the status of the systems concerned with transport away from the tissue, namely that of the capillaries (Starling, 1896; Renkin and Curry, 1979) and the lymphatics (Barrowman and Roberts, 1967), will also be important.

The importance of intestinal blood flow and regional blood flow distribution has been the cause of much debate, and is reviewed by Winne (1978; 1979). Some agents capable of stimulating intestinal fluid absorption, such as angiotensin (Bolton et al., 1975; Mandel and Sapirstein, 1962), do so without effecting intestinal blood flow; whereas others, such as morphine, appear to have their effects by increasing intestinal blood flow (Mailman, 1980), and indeed blood flow to the small intestine has been shown to be increased during digestion in the conscious dog (Gallavan, Chou, Kvietys and Sit, 1980).

Workers from the University of Goetberg have been particularly interested in the role of intestinal blood flow in intestinal fluid transport, having made observations that the villus-tissue osmolality (Jodal, Hallback and Ludgren, 1978) and villus anatomy may very well form the basis for an intestinal countercurrent multiplier (Haljamae, Jodal and Lundgren, 1973; Hallback, Jodal, Sjoqvist and Lundgren, 1979; Hallback, Jodal and Lundgren,1979; Hallback et al., 1980). Figure 1.9 illustrates this multiplier system which, although proposed for the cat, may not be as likely in mammals with different vascular anatomy (Winne, 1975).

In addition to the normal physiological range, in pathophysiological conditions such as severely low blood flow, the intestine may suffer ischaemic damage which can impair its absorptive function (Cassuto, Cedgard, Haglund, Redfors and Lundgren, 1979; Chiu, McArdle, Brown, Scott and Gurd, 1970). This is thought to be important in spite of the large shunt pathways which act to protect the intestine over a large range of blood flow changes (Varro, Blaho, Csernay, Jung and Szarvas, 1965).

Section 1.2

WATER HOMEOSTASIS AND THE NERVOUS SYSTEM

1.2.1) The Autonomic Nervous System.

From as early as the second century A.D. the "involuntary" or "autonomic" nervous system has been the focus of much attention by physiologists (reviewed by Day, 1979). However, it is largely due to the work of the anatomist W.H. Gaskell (1916) and the physiologist W.N. Langley (1898) that we hold our present day view of the structure and function of the autonomic nervous system (ANS). Gaskell and Langley's primary anatomical division of the ANS into sympathetic and parasympathetic was made on the basis of the site at which the cell body of the post-ganglionic axon is located.

1.2.2) Anatomy of the Parasympathetic System.

The parasympathetic system consists of preganglionic fibres from the medulla oblongata and the sacral regions of the spinal cord, together with their respective ganglia and post-ganglionic axons. These are shown in figure 2.1. The preganglionic nerve fibres are generally long, with the ganglia being located near or adjacent to the effector tissues. The post-ganglionic nerves are therefore correspondingly short.

1.2.3) Anatomy of the Sympathetic System.

In the sympathetic system preganglionic fibres pass from the spinal cord, where they branch as myelinated trunks and join the chain of sympathetic ganglia (shown in figure 2.2). Not every nerve leaving the cord immediately connects with the adjacent ganglion. They may run for some distance alongside the vertebral column before synapsing with a ganglion. Furthermore, some preganglionic fibres pass through the sympathetic chain without synapsing and then directly innervate the adrenal medulla.

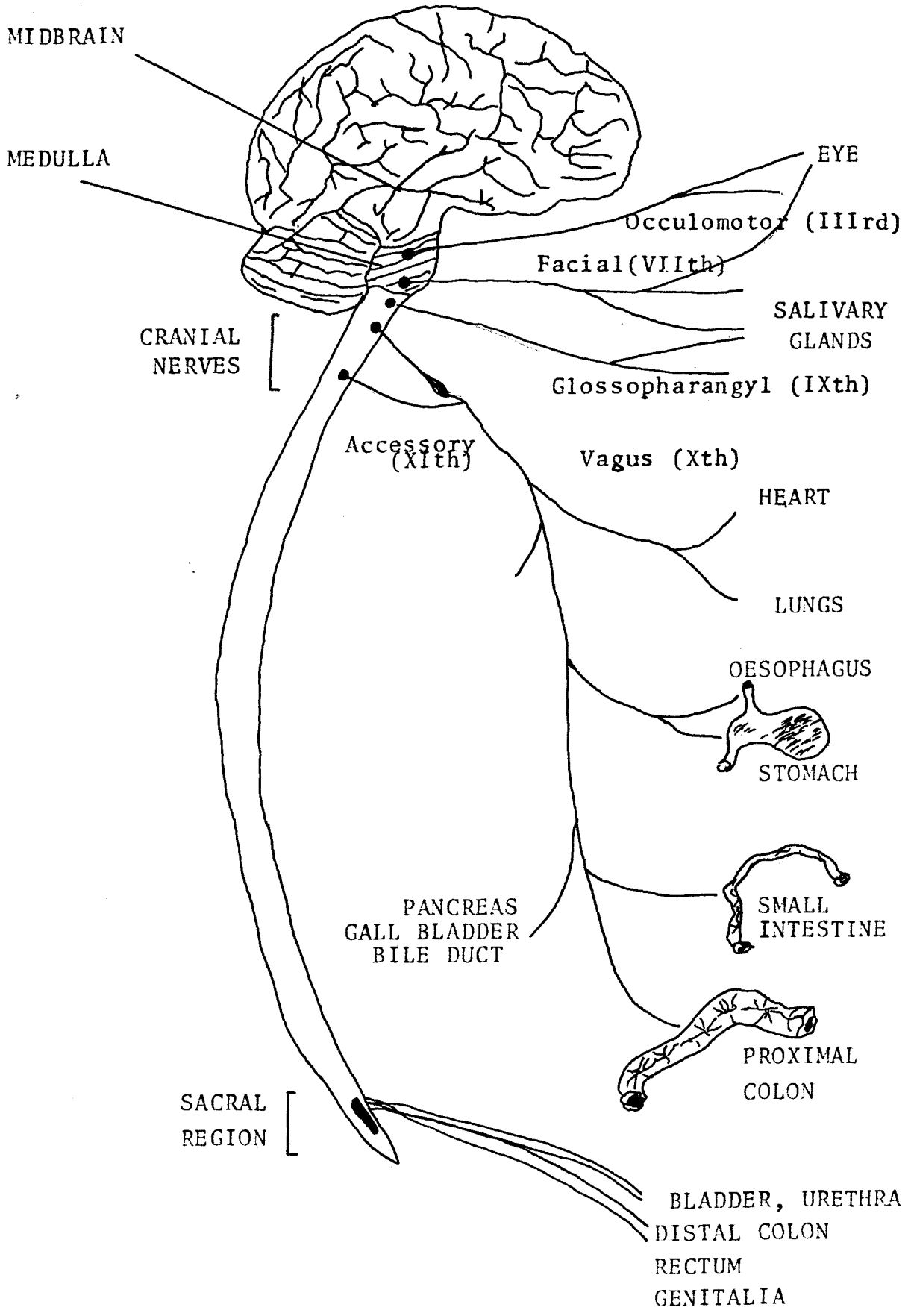


Figure 2.1 Generalised scheme for the mammalian parasympathetic nervous system. Illustrating both cranial and sacral outflows.

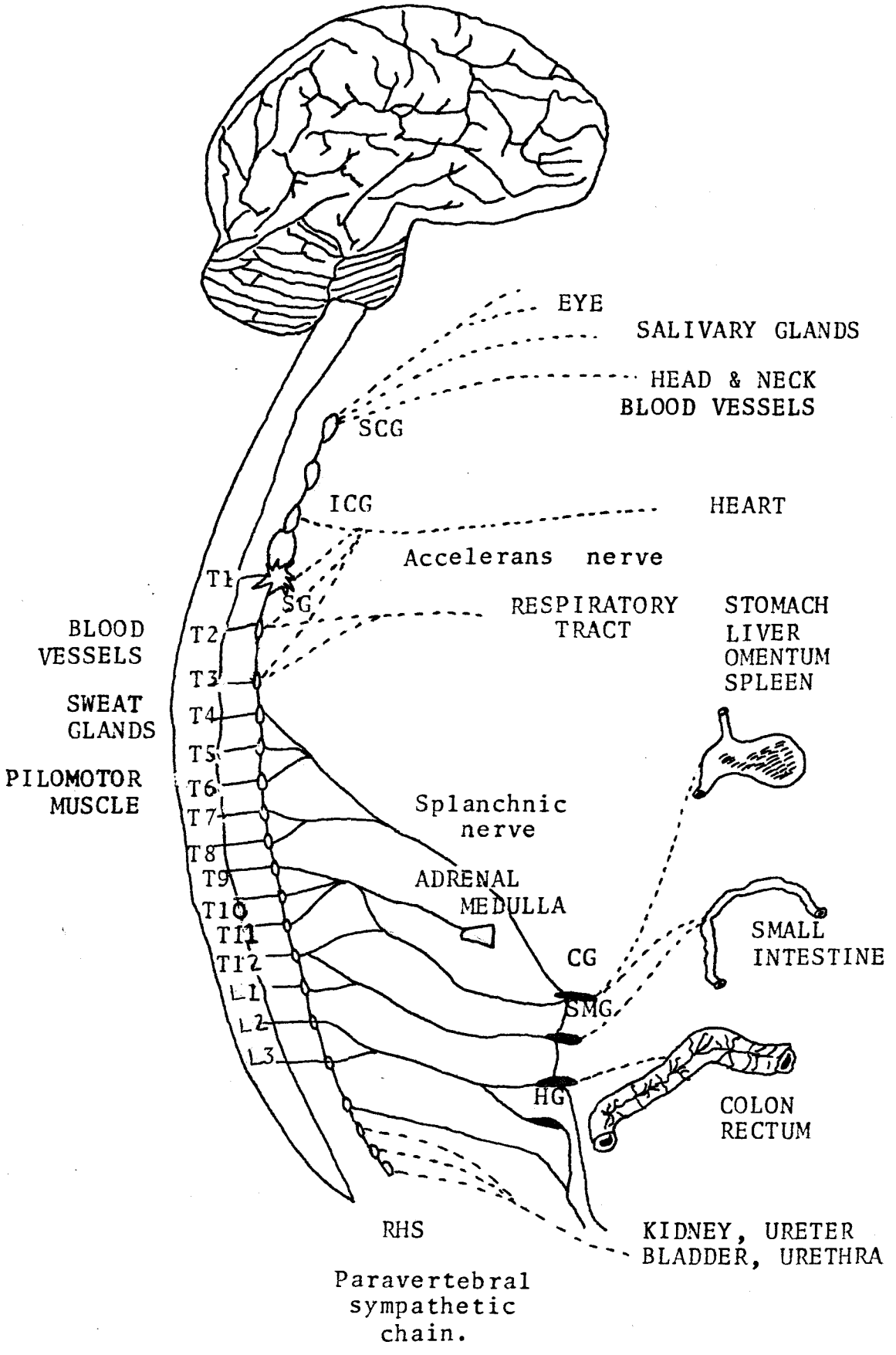


Figure 2.2 Anatomy of the mammalian sympathetic innervation. SCG = superior cervical ganglion. ICG = inferior cervical ganglion. SG = stellate ganglion. CG = coeliac ganglion. SMG = superior mesenteric ganglion. HG = hypogastric ganglion.

1.2.4) Physiology and Pharmacology of the ANS.

The approaches used in unravelling function and mechanism in the ANS has depended very much on the interplay between synthetic chemists, pharmacologists and physiologists. The early work developing theories of synaptic transmission and discoveries of the nature of neurotransmitters has precipitated the production of a vast array of pseudo-transmitters, blockers and inhibitors which have been used as vital research tools. Neurotransmitters have been defined by the criteria of Eccles (1964; reviewed by Day, 1979). Namely:-

(1) The proposed transmitter and enzymes for its synthesis must be present in the nerve.

(2) The proposed transmitter must be released from the nerves when they are stimulated.

(3) The proposed transmitter when added exogenously must mimic the effects of nerve stimulation.

(4) An enzyme or enzyme system capable of inactivating the proposed transmitter must be present in the tissue.

(5) Drugs which alter the response to nerve stimulation must also alter the response to the proposed transmitter in the same way.

Figure 2.3 shows the general scheme for the anatomy of the ANS, together with the related neurotransmitters. Acetylcholine is the predominant transmitter in the system, acting at a number of sites. It is released by post-ganglionic nerves onto muscarinic receptors on the effector tissue (Bebbington and Brimblecombe, 1965). Noradrenaline is more commonly the end-effector neurotransmitter in the sympathetic system although the innervation of some tissues, such as the sweat glands, is the exception having cholinergic muscarinic receptors. These

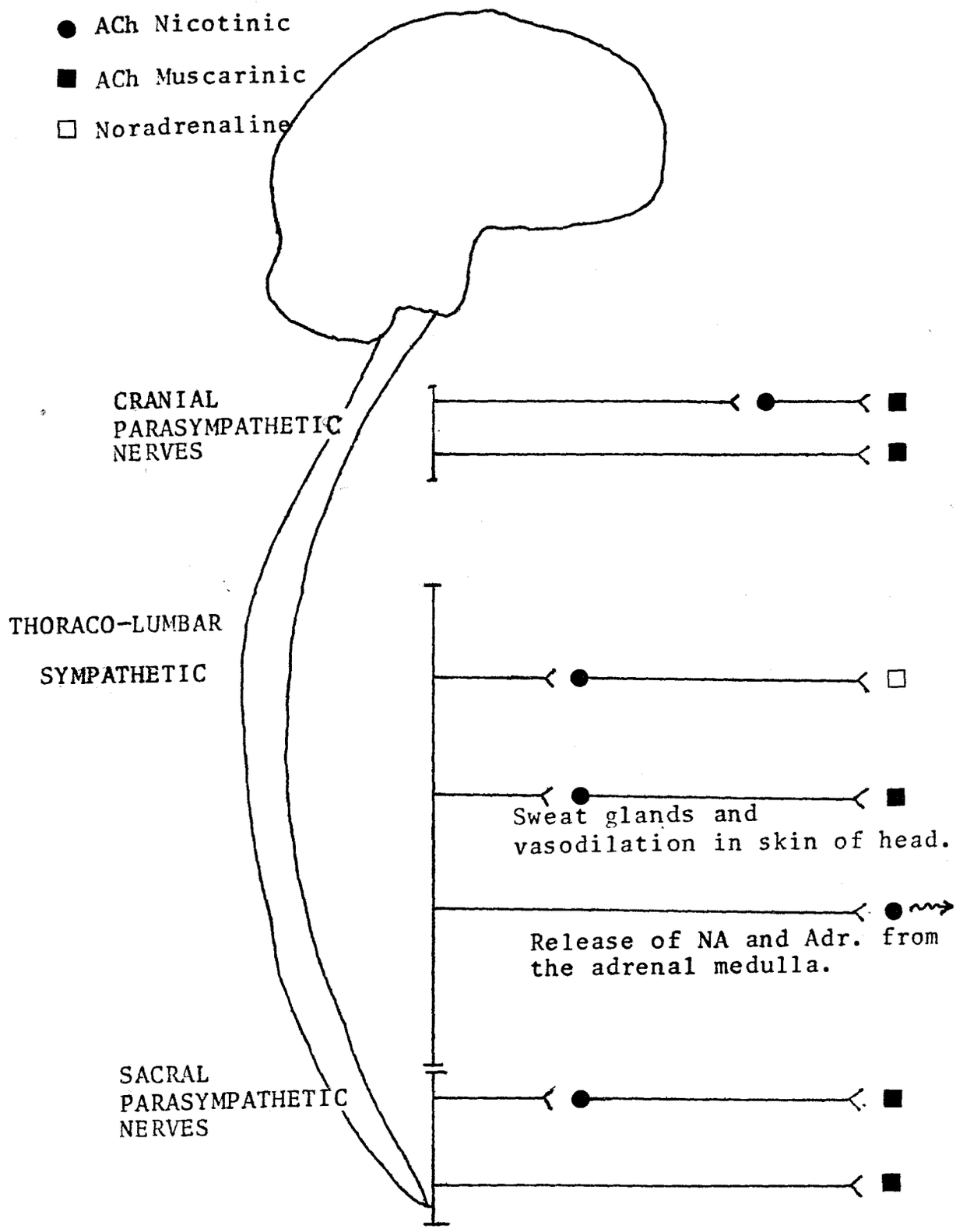


Figure 2.3 Sites of synapses in the autonomic nervous system and the type of neurotransmitter at each site.

anomolous findings have led Burn and Rand to form the hypothesis that acetylcholine is the normal post-ganglionic transmitter and that noradrenaline is only released subsequent to acetylcholine release (reviewed by Burn, 1975). This idea is subject to criticism, however, as the evidence is indirect and the data can equally be explained by considering that the nerves are a mixture of noradrenergic and cholinergic fibres.

Figure 2.3 also shows that acetylcholine is the predominant neurotransmitter in the autonomic nervous system ganglia. In these ganglia the classical view is that the neurotransmitter acts upon nicotinic receptors. Although this may be a simplification as more recent work implicates both muscarinic and α-adrenoreceptors in neurotransmission through ganglia(reviewed by Volle and Koelle, 1975).

1.2.5)Physiology and Pharmacology of the Sympathetic Nervous System.

Cannon (1929) produced the dramatic definition of the actions of the sympathetic nervous system (SNS) as being concerned with 'fight or flight'. This definition is useful but tends to ignore the importance of the moment by moment homeostatic adjusting role of the SNS. Furthermore, the concepts involved lend support to the idea of the sympathetic and parasympathetic systems working in opposition to one another. Current ideas of the autonomic nervous system might better describe the two systems as acting in concert rather than antagonistically. Innes and Nickerson (1975; reviewed by Day, 1979) have defined 5 major actions of the sympathetic and sympathomimetic drugs.

(1) Peripheral excitatory actions such as those on smooth muscle of blood vessels and spleen.

(2) Peripheral inhibitory actions on smooth muscle such as

skeletal blood vessels or smooth muscle of the intestine and respiratory tract.

(3) Cardiac excitation producing an increase in force or rate.

(4) Metabolic actions such as an increase in the rate of glycogenolyis and liberation of free fatty acids.

(5) CNS excitation effects such as increased respiration rate and increased awareness.

These effects are considered to occur via catecholamines acting on specific receptors (reviewed by Levitzki, 1978) which have now been classified according to their pharmacological specificity (discussed by Furchgott, 1972; Jenkinson, 1973).

Ahlquist (1948) studied the actions of the sympathomimetic agents adrenaline, noradrenaline and isoprenaline and made a division of adrenoreceptors into 2 classes. Firstly α-receptors having an order of potency: adrenaline > noradrenaline > isoprenaline, and secondly β—receptors with a potency: isoprenaline > adrenaline > noradrenaline. This α/β classification was further studied by Furchgott (1972) who gave a fuller description that:

"An α-receptor is one that mediates a response pharmacologically characterised by a relative potency series of noradrenaline > adrenaline > phenylephrine >> isoprenaline with a susceptibility to specific blockade by phentolamine, dibenzamine or phenoxybenzamine at low concentrations. " α-receptors (excluding their inhibitory actions on the GI tract) generally initiate excitatory events such as vasoconstriction.

"A β-receptor is one which mediates a response pharmacologically characterised by a relative potency series of isoprenaline > adrenaline

> noradrenaline > phenylephrine with a susceptability to specific blockade by pindolol, propranolol or alprenolol." With the notable exception of the inotropic action on the heart, β-receptors generally mediate inhibitory responses.

β-adrenergic receptors have been further divided into β_1- and β_2- from a pharmacological study using β-agonists (Lands, Arnold McArliff, Luduena and Brown, 1967). Similarly, α-receptors have been subclassified into α_1- and α_2- which arose from initial observations by Brown and Gillespie (1957) that blockade by some α-antagonists at low concentrations would increase the amount of noradrenaline released from cat spleen on nerve stimulation. The findings of these workers have been explained by the hypothesis that noradrenaline released on nerve stimulation is able to act on presynaptic α-receptors located on the nerve terminal. Negative feed-back of noradrenaline onto these receptors then inhibit noradrenaline release. α-blockade by antagonists more specific for this presynaptic α_2-receptor than for postsynaptic α-receptors will therefore cause more noradrenaline to be released and produce a larger effect (see section 1.2.7 on presynaptic receptors). These findings have now been confirmed by a large number of workers (for example, Starke, Endo and Taube, 1975; Borowski, Starke, Ehrl and Endo, 1977; reviwed by Langer, 1974; Starke, 1977; Westfall, 1977).

The interest generated by α-receptor sub-types has promoted the production and characterisation of a large number of relatively specific α_1- and α_2-agonists and antagonists (Davey, 1980; Starke & Docherty, 1980; Timmermans and van Zwieten, 1980). These new pharmacological tools have enabled workers to demonstrate receptors with α_2-type pharmacological specificity on other than presynaptic

locations (Berthelsen and Pettinger, 1977). For example on blood platelets (reviewed by Starke, 1981) and on vascular smooth muscle (Drew and Whiting, 1979). This pharmacological characterisation of α1- and α2-receptors has been largely based on results with currently available anatgonists which seem to be more specific than agonists (Reviewed by Starke and Docherty, 1980; Starke, 1981). α1-receptors therefore have an antagonist potency series in the order: prazosin > corynanthine >> yohimbine > rauwolscine, and agonists act in the series phenylephrine > noradrenaline > clonidine, guanabenz (Reid and Hamilton, 1980). α2-receptors have an antagonist potency series: rauwolscine, yohimbine >> corynanthine, prazosin with a high affinity for the agonists clonidine, guanabenz and α-methyl noradrenaline.

Much work in the area of α/β and sub-receptor classification still needs to be conducted; such studies are especially difficult as a small change in receptor environment may very well change the nature of the receptor (Belleau, 1963; Kunos and Nickerson, 1976).

1.2.6) The Synthesis, Release and Inactivation of Noradrenaline.

Prior to any actions on the effector receptors, noradrenaline must first be synthesised, stored and released from the nerve. In an impressive piece of insight Blaschko (1939) suggested a possible pathway for the synthesis of adrenaline and noradrenaline from the esssential amino acid L-tyrosine. It was then not until 20 years later that the techniques were produced to demonstrate the existence of this pathway (figure 2.4). Essentially, tyrosine is taken up into the adrenergic axon or cell body from the extracellular fluid. The amino acid is then converted by the enzyme tyrosine hydroxylase into L-DOPA. This conversion is the rate limiting step for the pathway and is therefore the point of control for modulators of noradrenaline

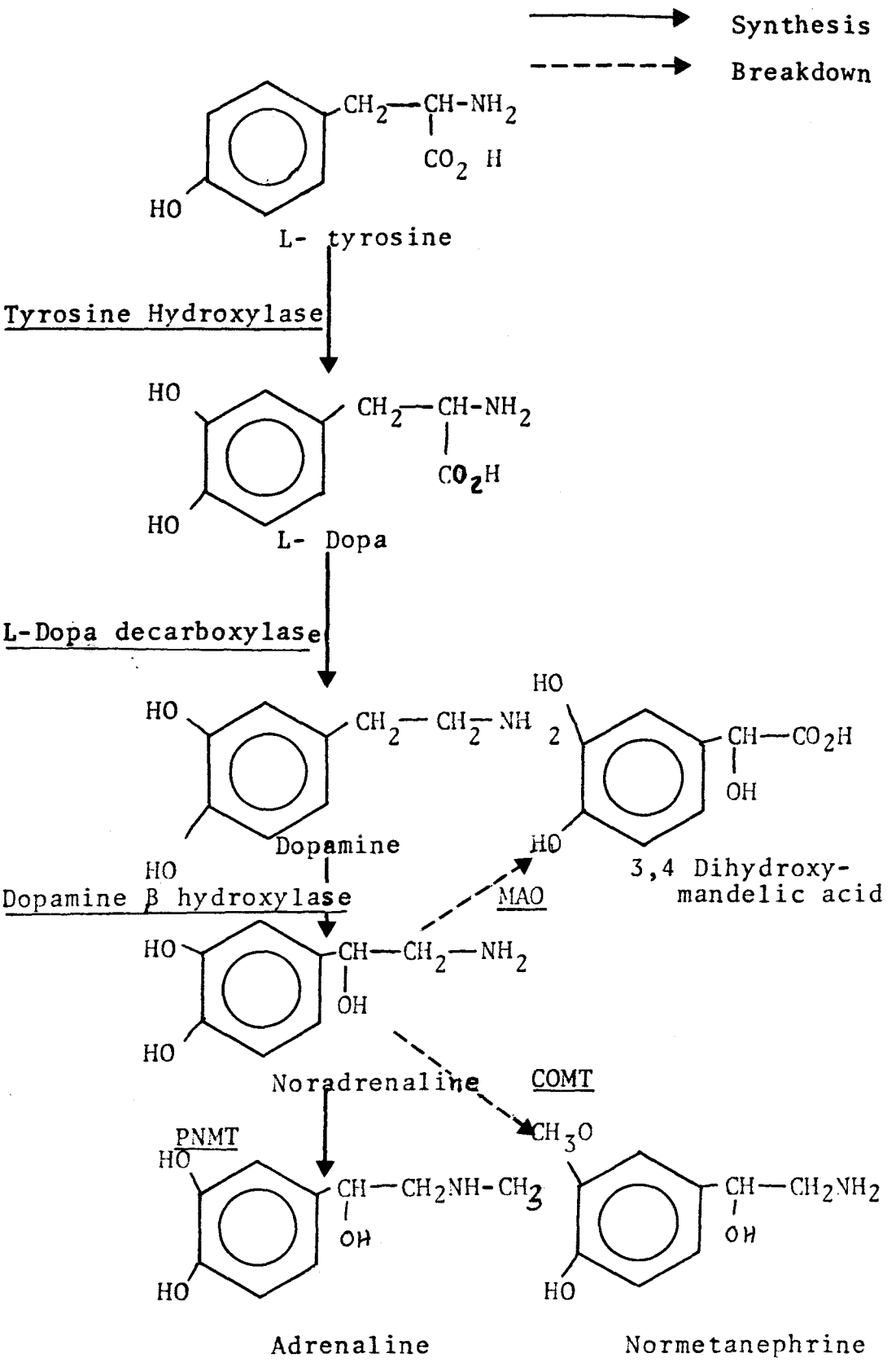


Figure 2.4 The biosynthetic pathway and breakdown of noradrenaline and adrenaline from tyrosine.

production (e.g. noradrenaline and angiotensin). L-DOPA is then converted to dopamine which is taken up into the nerve vesicles where the enzyme dopamine-β-hydroxylase carries out the conversion to noradrenaline. Unlike anuran amphibians (Burnstock and Costa, 1975), the enzyme PNMT is not present in the peripheral nerves of mammals and so adrenaline is not produced, although some evidence suggests that adrenergic fibres may exist in the brain of mammals (Cooper, Bloom and Roth, 1978) and the periphery of some avarians (Komori, Oshaski, Okada and Takewaki, 1979). The pathway for the synthesis of noradrenaline and adrenaline is discussed in detail by Weiner (1970).

Little is known about the events which promote release of noradrenaline from the terminal vesicles of noradrenergic nerves. Figure 2.5 shows the generalised mechanism for release, where an action potential passes along the nerve causing many vesicles to exocytotically release the noradrenaline they contain into the synaptic cleft. This exocytosis has been shown to depend on an influx of calcium which appears to be required for vesicles to attach to the plasma membrane (reviewed by Zimmerman, 1979).

Following release by the nerve, noradrenaline undergoes a number of processes to remove it from the synaptic cleft (see figure 2.5). These processes of removal involve both enzymic and active uptake mechanisms. Many early workers were surprised by the difficulty found in isolating significant levels of enzymes capable of inactivating noradrenaline. They expected a similar rapid metabolism system to the cholinesterase found acting on acetylcholine. Gillespie (1973) pointed out that low noradrenaline metabolising activity may very well reflect the physiological functions of noradrenaline which is also a hormone released by the adrenal medulla and not simply a neurotransmitter as is

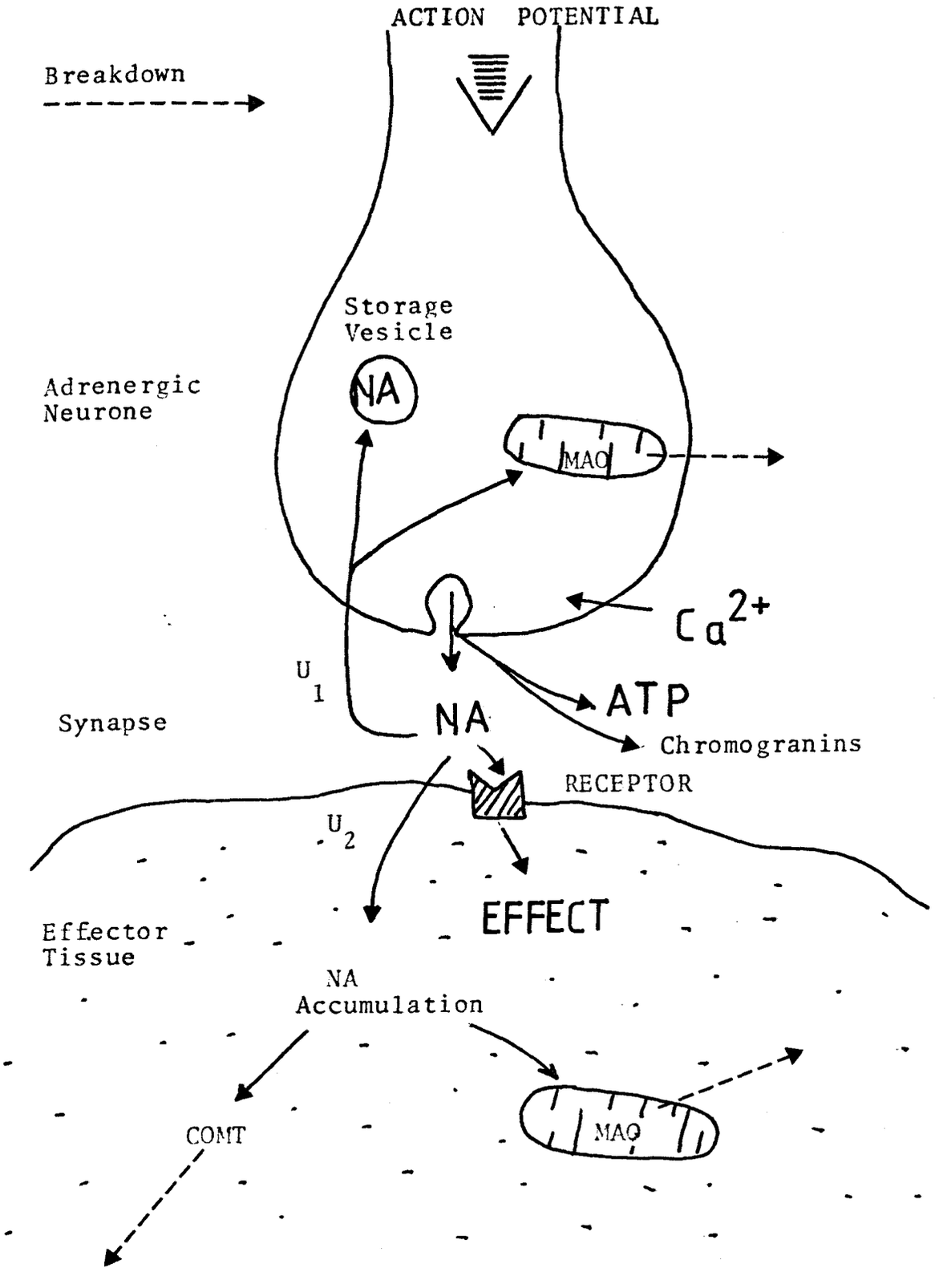


Figure2.5 Scheme for the release and subsequent breakdown and uptake of noradrenaline from the adrenergic nerves of the peripheral nervous system.

the case for acetylcholine. More sensitive techniques have in fact shown two enzymes or enzyme systems to be important in the breakdown of noradrenaline (see figure 2.4). Firstly catechol—O—methyl transferase (COMT) which is found in many tissues, and secondly monoamine oxidase (MAO) which appears to be widely distributed in a number of tissues, and is found in high concentrations in the mitochondria of noradrenergic neurones. MAO is thought to occur in a number of forms, for example the A an B forms isolated in the brain (Yang and Neff, 1973; Johnson, 1968).

The existance of other processes removing noradrenaline was suspected by a number of workers. Crout (1961) demonstrated that combined MAO and COMT inhibition in anaesthetised cats did not greatly increase either the magnitude or the duration of the pressor response. It is now clear that this finding reflected the importance of two noradrenaline uptake processes present in the ANS. Neuronal uptake (uptake-1) was confirmed by the demonstration of uptake of radioactively labelled noradrenaline into richly innervated tissues in the cat, such as the heart (Whitby, Axelrod and Weil-Malherse, 1961). It has since been shown to be an energy dependent process. It can operate against a large concentration gradient and is both saturable and highly specific for noradrenaline (reviewed by Vanhoutte, 1978).

Uptake-2 is the term given by Iversen (1965) to the extra-neuronal uptake system for catecholamines. Uptake-2 processes occur in a variety of tissues including smooth muscle of blood vessels and the intestine. They have a higher maximum capacity for catecholamines than uptake-1 systems, acting when concentrations become high, but have a lower affinity for noradrenaline and adrenaline (reviewed by Iversen, 1973).

1.2.7)Presynaptic Receptors.

As mentioned earlier in section 1.2.5 there is now considerable evidence for receptors at sites other than the classically studied "postsynaptic effector". Receptors have been described on both the nerve cell body and the nerve terminals, the latter receptors being known as "presynaptic" receptors. These presynaptic receptors form part of the neuro-effector release mechanism controlling the release of catecholamines from autonomic nerves (reviewed by Westfall, 1980).

By far the best established of these receptors is the "α_2" autoreceptor whereby noradrenaline feeds-back to inhibit its own release (reviewed by Langer, 1974; 1977; 1981; Starke, 1977; Westfall, 1977). Many other hormones and neurotransmitters have been shown to have either stimulatory or inhibitory actions on autonomic neuroeffector transmission (generally reviewed by Rand, Majewski, Medgett, McCulloch and Story, 1980; Langer, Starke and Dubocovich, 1979; Starke, 1978). Modulators, other than noradrenaline, thought to inhibit release include :- adenosine, ATP (Snyder, Bruns, Daly and Innis, 1981), prostaglandin-E, acetylcholine (muscarinic), dopamine (Enero and Langer, 1975; Hope, Majewski, McCulloch, Rand and Story, 1980), 5HT and enkephalins. Modulators that are thought to have their control by enhancing neurotransmitter release include :- β-adrenergic agonists, acetylcholine (nicotinic), prostaglandin-F and angiotensin (section 1.2.8).

1.2.8)Actions of Angiotensin on the Sympathetic Nervous System.

Angiotensin has been shown to have a variety of effects both centrally and on the peripheral autonomic nervous system (reviewed by Khairallah, 1972; Zimmerman, Gomer and Liao, 1972; Severs and Daniels-Severs, 1973; Regoli, Park and Rioux, 1974; Starke, 1977;

Westfall, 1977).

The major action of angiotensin is to enhance noradrenergic neuroeffector transmission (Zimmerman, 1962; McCubbin and Page, 1963; Benelli, Bella and Gandini, 1964), although it may have a direct postsynaptic action increasing the sensitivity of effector cells to noradrenaline (Panisset and Bourdois, 1968; Day and Moore, 1976).

The presynaptic actions of angiotensin on the sympathetic nervous system have been demonstrated by Zimmerman and Whitmore (1967), who showed increased stimulus evoked release of noradrenaline in the presence of angiotensin. This increase in release could be achieved by all or several of a number of mechanisms:

(1) Inhibition of neuronal uptake of noradrenaline (Khairallah, 1972).

(2) Stimulation of de novo noradrenaline biosynthesis (Boadle-Biber, Hughes and Roth, 1972; Roth, 1972).

(3) Increasing the amount of noradrenaline released per nerve impulse (McCubbin and Page, 1963; Benelli et al., 1964; Zimmerman et al., 1972).

The weight of evidence, in fact, suggests that the enhancement of stimulus evoked release is the more important mechanism (Starke, 1977).

Angiotensin can also release noradrenaline and adrenaline from the adrenal medulla by action on chromaffin cells (Reit, 1972). The release of adrenal catecholamines by angiotensin II is calcium-dependent (Poisner and Douglas, 1966) and is blocked by specific angiotensin antagonists such as the 8-substituted analogues (Peach and Ober, 1974).

1.2.9) Intestinal Parasympathetics and the Action of Acetylcholine on the Intestine.

The gross anatomy of the intestinal innervation is shown in figure 2.6. Parasympathetic preganglionic axons run in the vagus and are readily identified as anatomically distinct structures until they enter the wall of the stomach. From this point on the axons of the parasympathetic system run through the intramural plexus and form synaptic connections with ganglion cells of Auerbach's plexus (discussed by Bowman and Rand, 1980). The action of the parasympathetic system has been studied, along with the action of cholinergic agents, in connection with several aspects of intestinal function. The role of increased vagal activity and the action of acetylcholine in promoting intestinal motility is well documented (Schatzmann, 1968). However, the effect of these agents on water and electrolyte transport in the intestine is far from clear. From initial observations that atropine can inhibit some forms of induced secretion (Florey, Wright and Jennings, 1941), a number of cholinergic agents have been shown to stimulate net intestinal secretion. This has been shown *in vivo* in a number of preparations including the dog (Tidball, 1961) and the rat (Hubel, 1976; 1977). *In vitro* studies able to distinguish ion movements have shown that both acetylcholine and carbachol produce an increase in chloride secretion, with a corresponding increase in short circuit current (Isc) and transepithelial potential difference (Hardcastle and Eggenton, 1973). These studies on rabbit ileum have been confirmed in rat colonic mucosa (Browning, Hardcastle, Hardcastle and Sanford, 1977) and acetylcholine has been shown to stimulate chloride secretion with little effect on sodium movement in human ileum (Isaac, Corbett, Riley, Hawker and Turnberg, 1976).

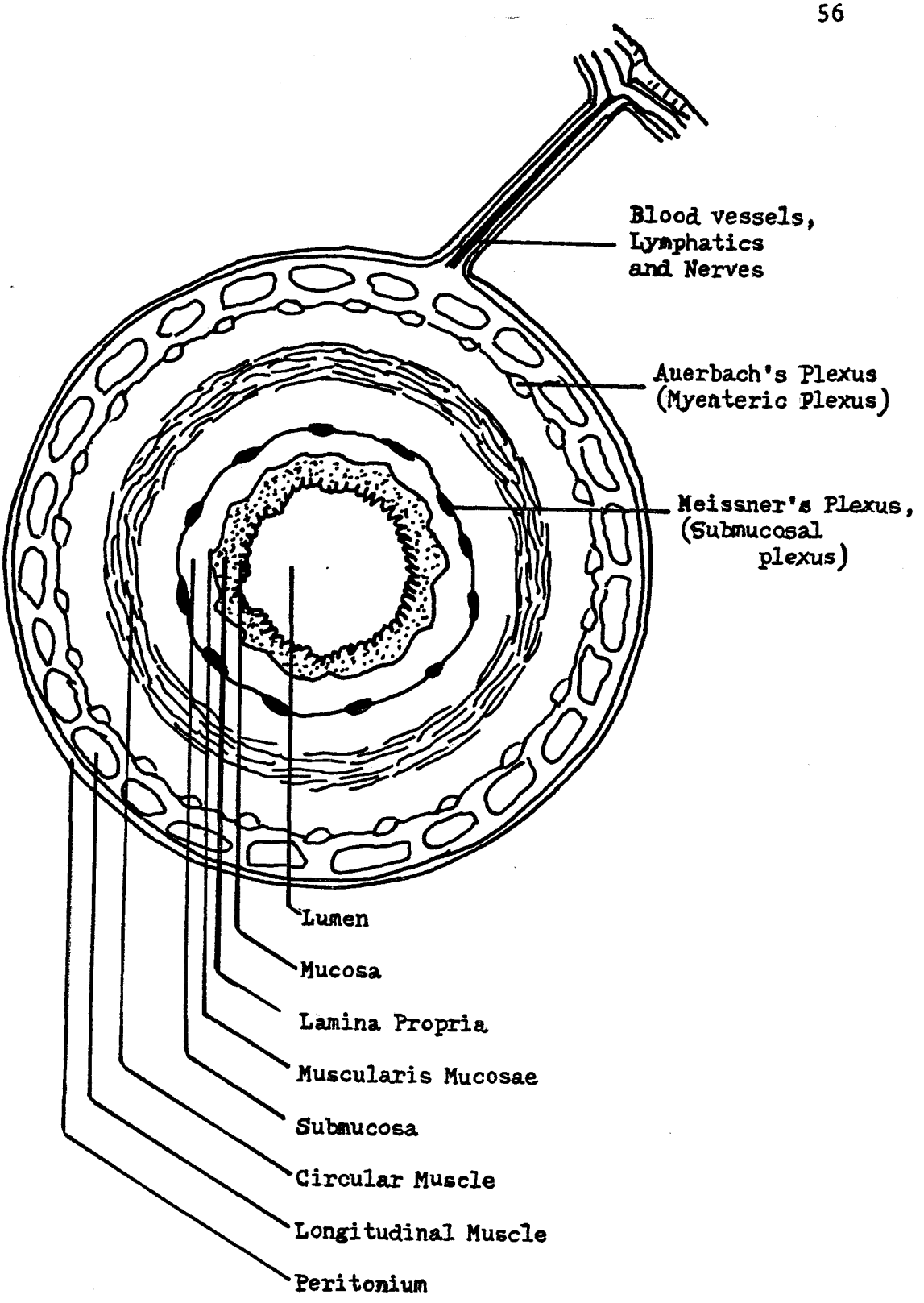


Figure 2.6 Diagram showing the major innervation and plexi in a typical transverse section of small intestine. Parasympathetic nerves run through the intramural plexus and synapse with cells in Auerbach's plexus. Sympathetics enter along the blood vessels.

In contrast to this Morris, Pimblett, Holt and Turnberg (1977) were unable to demonstrate changes in either ileal or jejunal ion transport in human subjects given cholinergic or anticholinergic drugs. Repeated studies, either stimulating or blocking the vagus have also shown no changes in intestinal secretion (Wright, Jennings, Florey and Lium, 1940; Burch and Schields, 1973; Read, Cooper and Fordtran, 1978).

1.2.10) Intestinal Sympathetics and Action of Catecholamines on the Intestine.

Florey et al. (1941) have reviewed the work of Bernard (1859) and Wright et al. (1940) who found that disruption of the sympathetic nerve supply to the intestine produced secretion. More recently, Lundgren and coworkers (Brunsson, Eklund, Jodal, Ludgren and Sjoval, 1976; 1979) have demonstrated that stimulation of the sympathetic splanchnic nerve supply in the cat will increase net fluid absorption. Apart from these nerve studies the majority of work performed on this aspect of sympathetic nervous function has been attempted using adrenergic agents such as noradrenaline.

Aulsebrook (1965a; 1965b - reviewed by Powell and Tapper, 1979) showed that both adrenaline and noradrenaline are able to stimulate the absorption of glucose and sodium in everted segments of rat small intestine. These findings were confirmed in an isolated preparation of rabbit ileal mucosa by Field and McColl (1973), who found that both adrenergic agents produced a rapid fall in Isc together with an increase in mucosal to serosal flux of both sodium and chloride. This effect is likely to be α-adrenergic in nature as these workers found that the effects were partially blocked by phentolamine, unaffected by propranolol whilst the β-agonist isoproteronol had no effect. The evidence for α-receptor mediated stimulation of intestinal absorption

of fluid and electrolytes is now well established. These catecholamine effects have been shown in a number of preparations including the in vitro rabbit colon (Albin and Gutman, 1980) and everted sacs of rat jejunum (Parsons, personal communication). Studies by Racusen and Binder (1979), however, have indicated that β-receptors may still play a role in the colon of the rat: Studies with both α-and β-agonists and antagonists have suggested that both α-and β-receptors were involved in the observed changes in Isc and p.d. Clearly species and antatomical diferences make any general statements difficult, in particular the comparison between the small and large intestine.

Aulsebrook (1965a) considered that the observed catecholamine effects may very well be mediated by c-AMP. However, Field (1974) and Sheerin and Field (1975) have found that, in contrast to the effects of catecholamines, c-AMP increases short circuit current, inhibits active sodium absorption, and produces active chloride secretion. When c-AMP levels have been raised by administration of theophylline these effects can be partly reduced by adrenergic agents, but not if the effects are produced by exogenously administered c-AMP (Field, Sheerin, Henderson and Smith, 1975). Furthermore, adrenergic agonists produce no reduction in basal c-AMP levels.

In vivo studies using kidney cortex slices have shown that the cyclic nucleotide c-GMP may stimulate the extrusion of sodium and chloride from the cell in a similar manner to angiotensin (Evans et al., 1976). Both α-agonists and high doses of the cholinergic agent carbachol are able to increase c-GMP levels in the intestine (Brasitus, Field and Kimberg, 1976; Tapper, Powell and Morris, 1978). But, curiously, exogenous c-GMP has been shown to stimulate secretion rather than absorption in some in vitro preparations (Field, 1971; Brasitus et

al., 1976). (This cholinergic-adrenergic interaction described by Tapper and Powell is more fully discussed in section 1.2.11).

In vivo experiments conducted using catecholamines have confirmed the earlier in vitro findings of Field and McColl (1973). Hubel (1976) demonstrated that noradrenaline augments the net absorption of sodium, chloride and water from the intestine with no change in potential difference. Levens, Munday, Parsons, Poat and Stewart (1979) also showed this absorptive effect to occur in the in vivo rat jejunum, found no alteration in c-AMP levels but a slight alteration in the transepithelial potential difference occured on noradrenaline administration.

Taken together, these findings indicate that noradrenaline released by the sympathetic nervous system promotes intestinal absorption of fluid and electrolytes, either alone or in balance with the parasympathetic nervous system.

1.2.11) Cholinergic - Adrenergic Interactions and the Intrinsic (Enteric) Nervous System.

Noradrenaline and the sympathetic nervous system are known to have an inhibitory role on intestinal motility. Acting directly on intestinal smooth muscle, but also inhibiting the parasympathetic, cholinergic, innervation of the intestinal plexi. In a similar manner to this situation, Powell and Tapper have hypothesised some degree of cholinergic - adrenergic interaction in the control of intestinal secretion and absorption (Tapper et al., 1978; Powell and Tapper, 1979). In the rabbit ileum Ussing chamber preparation low concentrations of exogenously added carbachol produce secretion.

However, higher concentrations of carbachol (10^{-3} M) or nicotinic agonists such as DMPP produce a stimulation of net absorption (Tapper and Lewand, 1981), this being similar to that seen with α-adrenergic agonists. The model for cholinergic——adrenergic interaction requires a nicotinic presynaptic receptor on the sympathetic adrenergic nerve fibre. The nicotinic agents then act upon this receptor, releasing noradrenaline, with a concomitant increase in net absorption.

These workers further discuss the interaction between these two systems and their possible interaction with the intrinsic non-adrenergic, non-cholinergic nerves in the intestine - the enteric nervous system (reviewed by Furness and Costa, 1980). The importance and extent of the enteric nervous system is only just beginning to become apparent, thus opening up the possibilities of a large number of neurotransmitters and neurohumoral modulators including ATP, enkephalins, substance P, VIP and many other biological peptides (Fahrenkrug, Haglund, Jodal, Lundgren, Olbe and Schaffalitzky de Muckadell, 1978; Burnstock, 1979; Hubel, 1979). These are thought to act in a series of complex efferent - afferent reflex networks (Szurszweki, 1978; Morrison, 1978).

1.2.12) The Effects of Angiotensin on Transport Possibly Mediated by the Sympathetic Nervous System.

Angiotensin II is able to enhance the release of neurotransmitters from autonomic nerves (section 1.2.8) including release of acetylcholine from cholinergic nerves (Khairallah and Page, 1961) and noradrenaline from both adrenergic nerve endings (Starke, 1977) and the adrenal medulla (Peach, 1971). Both angiotensin II and adrenergic agonists have been shown to stimulate intestinal fluid transport both *in vitro* (Davies, Munday and Parsons 1970; Parsons, personal

communication; Aulsebrook, 1965) and in vivo (Bolton et al., 1975; Levens, Munday and Stewart, 1977; Levens, Munday, Parsons and Stewart, 1979). Levens and coworkers (1979) noticed a striking similarity between the actions of these two agents and investigated the possibility that angiotensin may be having its actions on intestinal transport by the release of noradrenaline. Their findings showed that the stimulatory action of both agents was able to be blocked by the protein synthesis inhibitor cycloheximide. Similarly the α-antagonists phentolamine and dihydroergotamine were able to inhibit both the angiotensin and noradrenaline stimulatory effects, whilst the β-antagonist propranolol had no action. Further studies demonstrated that depleting endogenous noradrenaline with reserpine will inhibit the response to angiotensin II but not noradrenaline (Levens, Munday, Parsons, Poat and Stewart, 1978) and that the actions of angiotensin will continue after adrenalectomy (Mariscotti, 1980).

Physiologically it is attractive to hypothesise a close interaction between angiotensin and the sympathetic nervous system, mainly because of their major roles in the control of extracellular fluid volume. Splanchnic sympathetic nerve firing rate is known to increase on haemorrhage (Folkow, 1955) as is the plasma renin activity and the rate of intestinal fluid transport (Mariscotti et al., 1977; Mariscotti, 1980).

If angiotensin is indeed having its action on intestinal fluid transport through noradrenaline release (for the evidence so far is indirect), a large number of questions still remain to be answered. Little is known of the exact site of action of angiotensin II upon the sympathetic nerves, and the nature and degree of angiotensin's modulation of the nerve-invoked release of noradrenaline.

Section 1.3

FLUID TRANSPORT AND THE OKAMOTO RAT

1.3) The Spontaneously Hypertensive Rat.

Hypertension has been simply defined as a blood pressure 'higher than normal'. This high blood pressure can be secondary to another disorder, such as renovascular disease, when it is described as 'secondary hypertension', or as an individual disorder in its own right (primary hypertension). The vast majority of cases of primary hypertension are described as 'essential' as very little is know about the causative mechanisms. Hypertension is a serious disorder in the western world having an prevalence of approximately 20%. The widespread nature of its occurrence has promoted the development of a large number of experimental hypertensive models.

1.3.1) Experimental Models of Hypertension.

The first experimental model of hypertension to be developed was that of Goldblatt, Lynch, Hanzal and Summerville (1934). This model hypertension was produced in rats by constriction of a single renal artery, resulting in a serious elevation in the renin-angiotensin system. In later models mineralocorticoids were implicated. For example, Sturtevant (1958) produced hypertension in rats by administration of deoxycorticosterone acetate (DOCA) in conjuction with a saline load.

The above models are only variations on secondary hypertension, and are therefore only of limited use as primary hypertension is the more common human complaint. Models of primary hypertension, which involve genetic factors, have been developed in rats by a number of workers, the three most important being the models of Smirk and Hall (1958), Dahl, Heine and Tassinari (1962a; 1962b) and Okamoto and Aoki (1963). The two strains of rat developed by Dahl and coworkers (1962a) were respectively found to either develop hypertension when fed a salt

loaded diet (salt sensitive) or to remain unaffected (salt resistant). Later investigations have indicated that this defect is located on between 2 to 4 gene sites (Rapp, Knudsen, Iwai and Dahl, 1973). One gene site controls the secretion of 18-OH-DOC from the adrenals and salt sensitive rats have been shown to have double the secretion of 18-OH-DOC compared to the resistant strain. The salt sensitive rats also have a reduction in the secretion of corticosterone (Rapp and Dahl, 1971). Both the models of Smirk and Hall (1958) and Okamoto and Aoki (1963) have been developed from inbreeding normal wistar rats. Rats with a high conscious blood pressure were selected by tail-cuff measurements and these were then inbred to produce hypertensive offspring. These offspring have a more usefully multigenic hypertension (Louis, Tabei, Sjoerdsma and Spector,1969; Phelan, Clark, Gresson and Jones, 1972).

1.3.2)The Okamoto Spontaneously Hypertensive Rat.

The hypertensive strain bred by Okamoto and Aoki were found to be 100% hypertensive when maintained by brother-sister matings. The blood pressure development profile for our colony of these animals shown in figure 3.1 is typical for the Okamoto spontaneously hypertensive rat (SHR). The animals are born normotensive and then blood pressure increases between the ages of 4 to 13 weeks until a hypertensive plateau of around 210 mmHg is reached. The age-weight profile for these animals shown in figure 3.2 provides an example of the genetic divergence that is beginning to occur between different stocks. Okamoto and co-workers observed no weight differences between age-matched wistar and SHR animals (Okamoto and Aoki, 1963). Weight differences have now been found by later workers (Fregly, 1975; Moll, Dale and Melby, 1975; Trippodo and Frohlich, 1981). Similarly, genetic

Figure 3.1

Change of conscious systolic blood pressure with age. Results for spontaneously hypertensive rats (SHR, n=10) and wistar normotensive rats (NTR, n=10). Results expressed as mean ± S.E.M.

Figure 3.2

Change of total body weight of animals with age. Results for spontaneously hypertensive rats (SHR, n=10) and wistar normotensive rats (NTR, n=10). Results expressed as mean± S.E.M.

Results reproduced from C. N. May (1980) with permission .

Figure 3.1

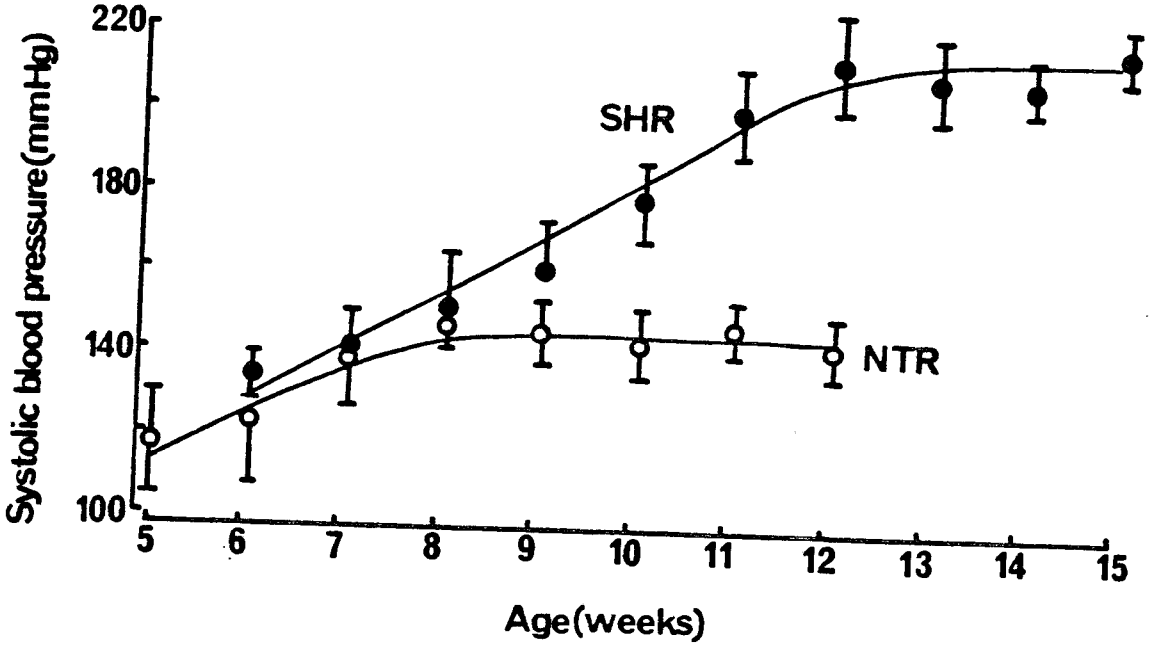
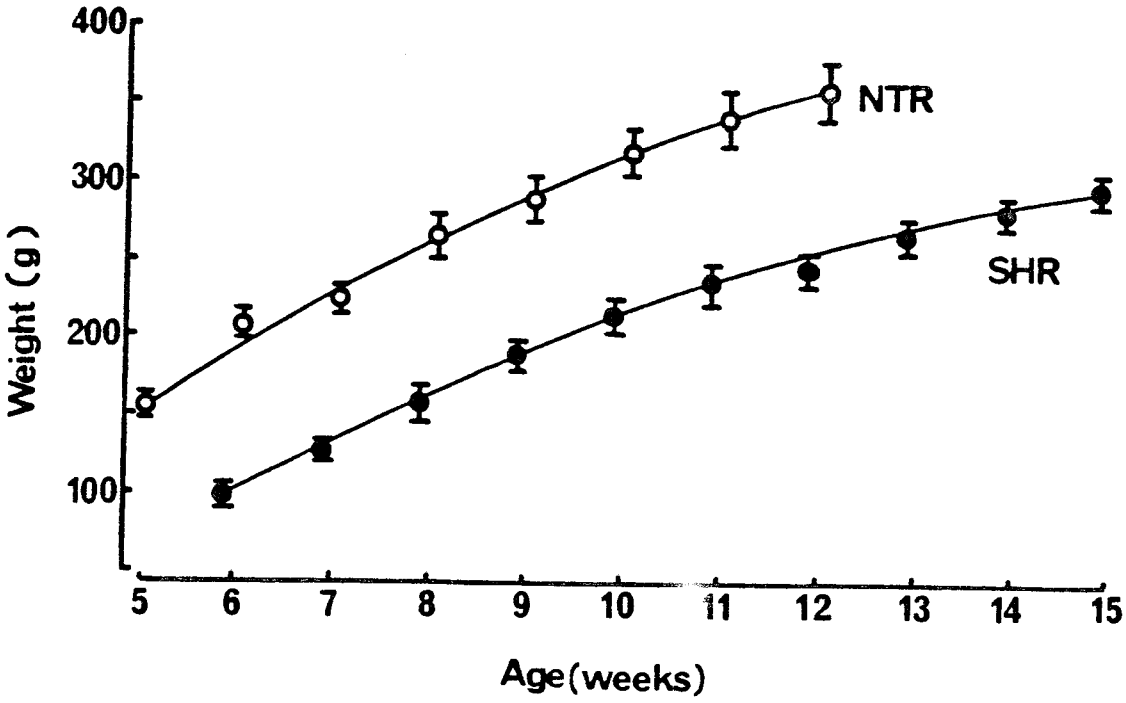


Figure 3.2



divergence has been shown in the heterogenicity of kidney function (Mullins and Banks, 1976) and response to stress (McMurtry and Wexler, 1981; Mcmurtry, Wright and Wexler, 1981) of different SHR stocks. Despite these criticisms the observed mechanisms of SHR hypertension are similar in many respects to those of essential hypertension (Yamori, 1977) and the SH rat has consequently been the basis of a considerable amount of study. Recently many of these experiments have been discussed in two reviews discussing the usefulness of the SHR as a model for human hypertension (Trippodo and Frohlich, 1981; McGiff and Quilley, 1981).

1.3.3) Salt and Fluid Intake in the SHR.

In a choice test between water and various saline concentrations SHR have been shown to have a greater intake of fluid and saline than normotensive control animals (Catalanotto, Schecter and Henkin, 1972; Fregly, 1975). Furthermore the hypertensive animals also demonstrate a greater preference than controls for potassium salt solutions, although not for bitter solutions of HCl or quinine. McConnell and Henkin (1973) have shown that even pre-hypertensive 6 week old SHRs have a higher saline preference than age matched controls, and that this appetite increases with age and increasing blood pressure. Although a high salt diet is neccessary for the development of some forms of hypertension (Dahl. et al. 1962a) this is not true in the case of the SHR which will develop hypertension, albeit less rapidly, even on a low salt diet (Dahl and Tuthill, 1974). As salt intake is not producing the hypertension it is interesting to consider if this increased saline preference occurs secondarily to hypertension or as an expression of a genetic change which independently increases both blood pressure and salt appetite.

Some discrepancies exist when considering actual volume changes and alteration in fluid compartment composition that may occur in the SHR. Trippodo, Walsh and Frohlich (1978) found a small but significant increase in total body water over control animals in 18 - 43 day old SHRs in the initial phase of hypertension. No such differences were seen in 10-14 day SHRs. ECFV and plasma volume remained unchanged in both cases, a finding similar to that of Willis and Bauer (1978) who studied 12 week old male SHRs and found unchanged ECFV, plasma potassium and plasma creatinine. However, these workers found no changes in total body water but discovered that the SHRs had higher levels of plasma sodium. Generally the work in this area remains unclear, with many considering that the 'neurogenic variant' SHR has no standard fluid volume variation (Folkow, 1979) as does the 'volume variant' Milan rat strain (Bianchi, Baer, Fox, Duzzi, Pagetti and Giovanetti, 1975). Therefore ECFV and plasma sodium concentration in the SHR would be expected to remain normal (Baer, Knowlton and Laragh, 1972).

1.3.4) Renal Function in the SHR.

Norman, Enobakhare, DeClue, Douglas and Guyton (1978) observed a parallel shift in the arterial pressure - urinary output relationship of the kidney. This they suggested could be explained by elevated sympathetic activity causing increased renal vascular resistance. Interestingly enough both functional (Beierwaltes and Arendshorst, 1978; Arendshorst and Beierwaltes, 1979) and structural (Gotheberg, Hallback, Lundin, Ricksten and Folkow, 1976; Folkow, Gotheberg, Lundin and Ricksten, 1977; Collis and Vanhoutte, 1977) increased renal vascular resistance has been demonstrated in the SHR. Studies have also shown that a neural mechanism may be involved in altered renal function

in these animals. Not only do SHRs have increased renal sympathetic nerve activity (Okamoto, Nosaka, Yamori and Matsumoto, 1967; Judy, Watanabe, Murphy, Aprison and Yu, 1979) but the onset of hypertension can be delayed by renal denervation (Liard, 1977; Kline, Kelton and Mercer, 1978).

The renal function in the SHR has generally been studied by investigating the effects of an acute saline load on urinary excretion. Farman and Bonvalet (1975) reported a reduced natriuretic response in both young and old anaesthetised female SHR compared with wistar controls. This observation was later confirmed in male and female SHR under various conditions (Vandewalle, Wanstok, Farman and Bonvalet, 1977). Contrastingly, Willis, McCallum and Higgins (1976) found an increased natriuresis in SHR after an intragastric isotonic saline load, and Fregly (1975) found increased sodium excretion in SHR after intraperitoneal injection of hypertonic but not isotonic saline. The apparent contradictions between the findings of these and other workers (Dibona and Sawin, 1978; Dibona and Rios, 1978; Beierewaltes and Arendshorst, 1978) may be explained both by the heterogeneity of SHR kidney function (Mullins and Banks, 1976) and from differences produced by using different species of rat as controls (Vandewalle, Farman and Bonvalet, 1978).

1.3.5) Neural Alterations in the SHR.

SH rats have been shown to have increased sympathetic firing in a number of nerves including the splanchnic (Okamoto et al., 1967; Judy et al., 1979). In contrast to these studies Lais and Brody (1975) were unable to show any increase in activity, although this could have been due to the age of the SHR (over 6 months in this study) and the level and type of anaesthesia used in this study (Yamori, 1976a: discussion).

Another index of sympathetic activity is the tissue and circulating levels of catecholamines. Catecholamine synthesis and turnover is increased in the heart of young SHR (Louis, Spector, Tabei and Sjoerdsma, 1969; Louis, Kraus, Kopin and Sjoerdsma, 1970; Yamori, 1974). Furthermore young SHR have increased circulating levels of noradrenaline (Grobecker, Roizen, Weisse, Saavedra and Kopin, 1975) and increased dopamine-β-hydroxylase (DBH) activity in both serum and mesenteric vessels (Nagatsu, Ikatu, Numata, Kato, Sano, Nagatsu, Umezawa, Matsuzaki and Takeuchi, 1976). Lower activities of DBH, tyrosine hydroxylase and phenylethanolamine-N-methyltransferase (section 1.2.6) have generally been found in the adrenals of young SHR (Grobecker et al., 1975), although some investigators have found a slight increase in adrenal DBH activity in these animals (Nagatsu, Kato, Numata, Ikuta, Sano, Nagatsu, Umezawa, Matsuzaki and Takeuchi, 1977). In older animals with established hypertension DBH activity is normal in serum, but elevated in the adrenals (Nagatsu et al., 1977). This suggests that the increase in neural activity may play some role in the genesis of hypertension in the SHR.

Certainly, sectioning the splanchnic nerves (Iriuchijima, 1973) or generalised reduction of sympathetic activity by surgery or pharmacological blockers causes a much greater than normal reduction in blood pressure in SHR compared with controls (Okamoto, Hazama, Takeda, Tabei, Nosaka, Ichijima and Suzuki, 1966; Folkow, Hallback, Lundgren, and Weiss, 1972; Numao and Iriuchijima, 1974; Yamori, 1976a; Igarashi, Nakajima and Ohtake, 1977). Perhaps more interesting is the fact that neonatal immunosympathectomy (Cutilleta, Erinoff, Heller, Low and Oparil, 1977), chemical sympathectomy with 6-hydroxydopamine (Provoost and de Jong, 1976) and depletion of central catecholamines (Erinoff, Heller and Oparil, 1975) will all prevent development of hypertension.

The facts indicate that the nervous system is very important in the etiology of SHR hypertension.

1.3.6) Humoral Alterations in the SHR.

A large number of alterations in hormone levels have been observed in SH rats. Although considerable variation exists between the findings of various workers. Table 3.1 summarises the findings of a number of workers investigating plasma renin activity and illustrates the problems encountered with variability. A number of variations can be explained by differences in technique. For example anaesthesia (in the study of Sen et al., 1972) causes a 5-fold increase in PRA (Pettinger, Tanaka, Keeton, Cambell and Brooks, 1975). But not all the discrepancies can be explained in this way. Genetic divergence or slight differences in rearing conditions, such as diet, may help to explain the wide spectrum of results obtained.

No less confused are the studies of peripheral plasma levels of mineralocorticoids. Tan and Mulrow (1976) have shown increased plasma levels of DOC, 18-OH-DOC and aldosterone in pre-hypertensive SHR compared with age-matched wistar controls. In both SHR and controls the levels are lower in older animals and all differences between the two groups are abolished by 12 months of age. However, significantly increased secretion of mineralocorticoids in young SHR has only been shown for aldosterone (Freeman, Davis, Warshano-Aharon, Ulick and Weinberger, 1975), the evidence pointing to increased mineralocorticoid secretion with age (Moll et al., 1975). Alterations in the rate of clearance of mineralocorticoids may explain this discrepancy between plasma levels and rate of secretion.

Generally both plasma renin activity and mineralocorticoid levels (Melby, 1977) are thought not to be involved in the etiology of SHR

Table 3.1

Results of studies on plasma renin activity in the spontaneously hypertensive rat, representative of current literature (see text).

Plasma Renin Activity in SHR

Pre-hypertensive SHR c.f. Wistar	Hypertensive SHR c.f. Wistar	PRA with age Wistar	PRA with age SHR	Ref.
↑	-	No	↓	Sen et al., 1972.
Same	Same	Slight	Slight	Czyżewski et al., 1973.
-	-	No	↑	De Jong et al., 1972.
↓	↓	↓	↓	Tan et al., 1976.
Same	↑	No	↑	Bagby et al., 1979.

hypertension. However, studies investigating the wider interactions between the endocrine system have begun to demonstrate the importance of certain endocrine organs, such as the adrenals (Aoki, 1963; Louis and Spector, 1975). This suggests a disorder of the 'hypothalamic-pituitary—adrenal—gonadal' axis (Okamoto and Aoki, 1963; Iams and Wexler, 1977; 1979; Wexler, 1980; Wexler, 1981; McMurtry and Wexler, 1981).

1.3.7) Central Alterations in the SHR.

Some evidence exists for changes in the central nervous system (CNS) of the SHR. Both the depressor response to intravenously administered clonidine (Yamori, 1976b) and to pargyline (Fuentes, Ordaz and Neff, 1979) are greater in SHR than normotensive animals. This indication of a disorder in the central α-receptor mediated depressor response has been supported by findings of decreased noradrenaline levels and DBH activity in certain brain areas of SHR (Saavedra, Grobecker and Axelrod, 1978).

Intraventricular administration of both carbachol (Yamori, 1976b) and angiotensin (Hoffman, Phillips and Schmid, 1977) produced an increased blood pressure response in SHR animals. The response to angiotensin is particularly interesting as a number of changes in the brain renin-angiotensin system have been observed. Firstly, infusion of angiotensin antagonists lowers the blood pressure of SHRs to a greater extent than is seen for controls (Wickre, McDonald, Ban, Moffitt and Porter, 1978; Johnson, Simon, Schaz, Ganten, Ganten and Mann, 1978). Also some alterations in the number of angiotensin receptors found in various areas of the CNS (Mizuno, Shigetomi, Matsui, and Fukuchi, 1980), and a failure of receptor number to reduce on saline load (Ashida, Ohuchi, Yotsumoto and Yazaki, 1980) further implicate a

central renin-angiotensin disorder in these animals.

Despite the alterations in the pressor response, alterations in other central actions of angiotensin are disputed. Tabei, Maruyama, Kumada and Okamoto (1972) found an increase in ADH secretion resulting from increased central sympathetic activity, however Hoffman and coworkers (1977) observed no alterations in either the drinking response nor ADH release produced by intraventricular injection of angiotensin II.

1.3.8) Summary.

The SHR has proved to be an interesting animal for study, with a large range of both neural and humoral alterations compared with normotensive controls. Studies on both salt intake and excretion show marked changes in SHRs. However, little is known of the nature of ECF control in these animals. Further studies investigating salt and water balance in these animals may well produce insight into the the disordered mechanisms in the SHR. Such mechanisms may reflect changes occurring in human hypertension.

Chapter 2

METHODS

2.1) Measurement of Net Fluid Absorption from the Intestine.

2.1.1.) Weight Method.

Rats were anaesthetised with an i.p. injection of sodium pentobarbitone (75mg/Kg; Sagatal). A mid-line incision was made and a length of proximal jejunum (approximately 15cm), measured 1cm from the ligament of Trietz, was isolated, washed through with warmed physiological saline, gently emptied and made into a closed sac. Special care was taken during the preparation of the sac to avoid damaging the intestinal blood vessels. The sac was returned to the abdominal cavity for 15 minutes prior to the start of the experiment to allow recovery from surgical stress. At time Ø minutes the sac was filled with a known volume (W1) of Krebs' bicarbonate buffer*, pH 7.4 (Krebs and Henseleit, 1932), and the abdominal cavity again closed. After the experimental time period under study (between 15 minutes to 1 hour), the sac was removed from the animal and weighed (W2), then emptied and re-weighed (W3) to obtain the volume of the contents.

The tissue was stripped of all mesentery and blotted to obtain the wet weight of the sac (W4). In some experiments tissue dry weight was used in which case the tissue was dried in an oven to constant weight. Net fluid absorption from the sac can be calculated from the equation :

$$\text{Fluid absorption} = \frac{W1 - (W2-W3)}{W4}$$

The results were expressed as ml fluid absorbed/g wet weight tissue/hr .

* Table 4.5

2.1.2.) Radioactive Non-absorbable Marker Method.

Net fluid absorption was measured by the method of Bolton, Munday, Parsons and York (1975). Rats were anaesthetised and the sac prepared as previously described. The right femoral artery was cannulated and connected to a pressure transducer (Bell and Howell, Type 4) filled with heparinised saline. This was used for continous monitoring of mean arterial blood pressure on a Servoscribe RE541 potentiometric chart recorder. The right femoral vein was cannulated and connected to a constant rate infusion pump (Braun-Melsungen AG), through which isotonic saline or the drug solution under test was infused at a rate of 1.2 ml/hr. In those experiments requiring simultaneous infusion of another pharmacologic agent; the left femoral vein was also cannulated, and the infusion carried out as for the right femoral cannulation (See figure 4.1).

At t = 0 the sac was injected with buffer, containing [14C]-Polyethylene Glycol (PEG) 6000 (200,000 d.p.m./ml , specific activity of 15.8 μCi/mg) or [14C] Polyethylene Glycol 4000 (200,000 d.p.m./ml of specific activity 21.7 μCi/mg), as a non-absorbable marker. In the cases where 14C-PEG 4000 was used then the buffer also contained 5g/l of cold carrier PEG 4000.

The experiments were divided into two consecutive time periods; usually of 15 minutes duration. During the first, control, period isotonic saline was infused through the right femoral vein. This was followed by a second experimental period during which saline, drug or vehicle, was infused. Approximately 0.1 ml samples of the sac contents were removed by weighed 1ml syringes fitted with a 26G needle, at 0, 15 and 30 min and assayed for radioactivity by liquid scintillation counting in 10 ml of 50/50 toluene/methanol butyl-PBD.

The fluid transport rate for each period was calculated from the increase in the concentration of the marker.

Where:

V1 = Volume of fluid injected into sac.

V2 = Volume of fluid in first sample, removed at Ø minutes.

V3 = Volume of the second sample, removed at 15 min .

Then:

Volume of fluid transported in 1st. 15 minute period =

$$(V1-V2) - (V1-V2) \cdot \frac{\text{dpm Ø min}}{\text{dpm 15 min}}$$

Let this equal x.

Thus,

Volume of fluid transported in 2nd. 15 minute period =

$$(V1-V2) - (x+V3) - ((V1-V2)-(x+V3)) \cdot \frac{\text{dpm 15 min}}{\text{dpm 3Ø min}}$$

All dpm values were converted to dpm/Ø.1 ml The volume of fluid transported in each period was expressed as ml fluid absorbed/g wet weight tissue/hr .

The volume of fluid removed for each sample was measured, thus enabling the total transport to be calculated by the weight method. If the totals obtained by the two methods differed by more than 3Ø% the results were discarded.

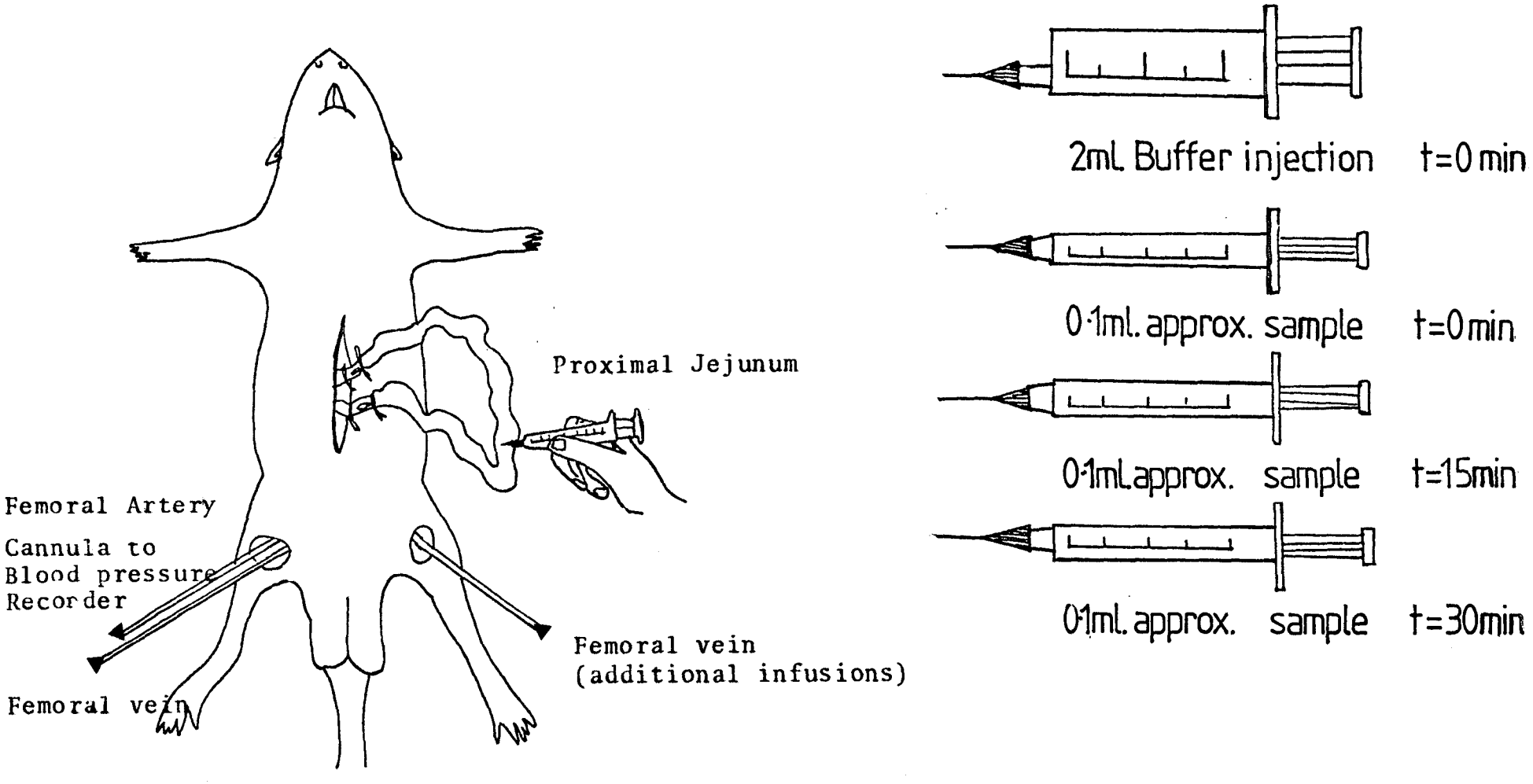


Figure 4.1 Rat intestinal sac preparation used in studies of intestinal fluid transport. Description of preparation in text.

2.1.3.) Measurements in Pithed and Nerve Stimulated Rats.

For pithed rats the time and length of the period of recovery from surgery, the order of preparation and the anaesthetic used were all varied as described under 'Results'. In some nerve stimulated animals three consecutive, 15 minute periods were used, an experimental period flanked by two control periods. The equation used to calculate absorption in this case was similar to that described above with the addition of a 3rd period.

2.1.4.) Measurements in Spontaneously Hypertensive Rats.

Because of the greatly increased rate of fluid absorption in these animals, the time of the absorption period was reduced to 10 minutes to ensure that absorption remained linear. In some experiments a single 15 minute absorption period was used as a comparison between groups of animals.

2.2) Validation of Methods.

As a closed sac is used in the intestinal transport studies (section 2.1) it is important to determine that the rate of fluid transport will not be influenced by the change in intra-luminal volume, and that absorption will remain linear over an acceptable length time period. The loss of fluid from the sac, as measured by the weight method, was determined for varying time periods, up to 90 minutes. Figure 4.2 shows the data from this study, demonstrating that absorption is linear with a low variability for experiments in Wistar animals lasting up to 60 minutes. It is important to note that this length of time will only hold true for typical Wistar 'basal' absorption, for when net absorption is increased a smaller 'true' linear period is likely. For this reason experiments of total duration

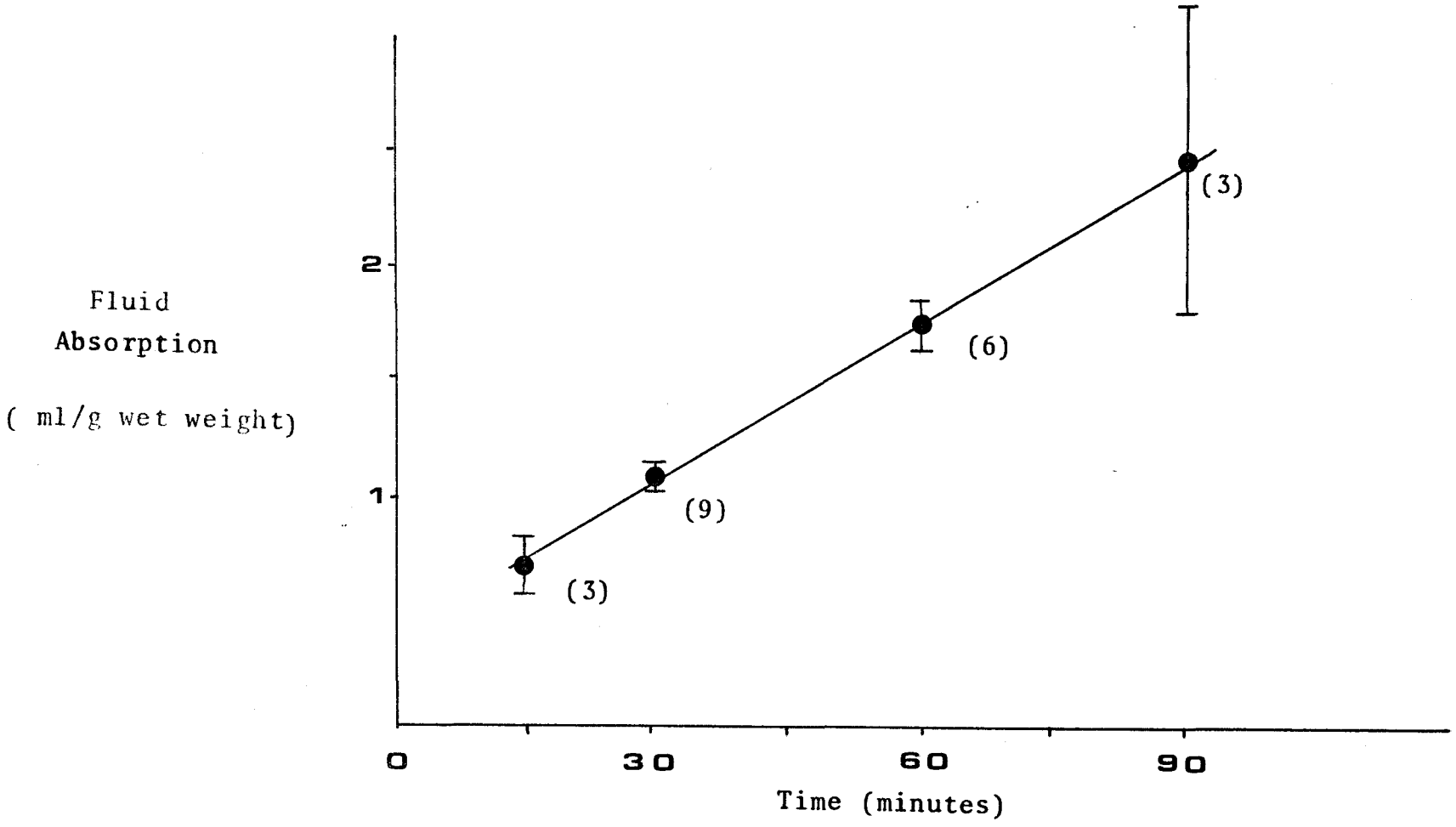


Figure 4.2 Intestinal fluid transport from jejunal sacs of anaesthetised wistar animals. Transport remains linear over 1 hour. Number of animals in parenthesis. Results expressed as mean ± S.E.M.

30 minutes (Wistar) and 20 minutes (SHR) were used.

The recovery of each radioactive marker used was determined for incubation periods of 30 minutes or 1 hour (table 4.1.) using the method described by Bolton (1975). The recovery of the smaller-sized PEG 4000 molecule was found to be lower, due to slight adsorption into the epithelial mucus layer. A cold carrier for a non-absorbable marker is commonly used in this type of study (for example Donowitz, Wicklern, Reynolds, Hynes, Charney and Zinner, 1979) and it was found that addition of 5g/ litre PEG 4000, greatly improved marker recovery as shown in the table.

Table 4.1. Results of recovery experiments, for 30 minute incubation. With and without carrier, at 5g /litre.

Radioactive marker	Recovery
[14-C] PEG 6000	90 ± 5% (7)
[14-C] PEG 4000	86 ± 2% (3)
[14-C] PEG 4000 + carrier	101 ± 5% (6)

2.3) The Rat Blood Pressure Preparation.

Systemic mean arterial blood pressure was measured according to the method of Noble (1973). Rats were anaesthetized with sodium pentobarbitone, as described previously. The right femoral artery and one or both femoral veins were cannulated. The arterial cannula was connected to a pressure transducer of the type previously described for continuous measurement of blood pressure (section 2.2). Dose response curves were constructed for single, intravenous injections in the presence and absence of blockers (where appropriate). In some cases drugs were infused intravenously at a rate of 1.2 mls / hour, rather

than being given as a single injection.

2.4) Pithed Rat Preparation.

300 g male wistar rats were either anaesthetized with sodium pentobarbitone (75mg/kg Sagatal) or with ether, and a tracheotomy performed. The animals were pithed with a 2mm diameter solid brass rod, or with a steel/teflon stimulating electrode (Gillespie, Maclaren and Pollock, 1970). Immediately after pithing the animals were placed on a heating box at 37 ^{o}C and connected to a respirator (60 breaths per minute at a depth of 5 ml). The jejunal sac preparation was carried out as described above. The sac was prepared after pithing in earlier experiments, and before pithing in the latter ones. Femoral artery and vein cannulations were carried out as for the standard anaesthetised preparation. Gallamine (27 mg/kg) was given intravenously prior to any stimulation, to prevent excessive neuromuscular activity.

2.5) Location of Segements in the Pithed Rat Preparation.

The animals were anaesthetised with ether and pithed with the stimulating electrode. A closed sac of jejunum was prepared, and a flexible polythene tube connected to a pressure transducer was tied into the distal end of the sac. With the sac filled with a sodium free isotonic tris-HCl solution (pH 7.4) relaxation and contraction of the intestine could be measured. Femoral vein and artery cannulations were carried out, for the injection of gallamine, and the measurement of mean arterial blood pressure. The output from the blood pressure transducer was also fed to a rate meter to give a measurement of heart rate.

2.6) Splanchnic Nerve Preparation.

The rats were prepared for measurement of fluid transport by the isotopic method (section 2.1.2). After all the surgery was complete the superior mesenteric artery was located, and the artery and nerves cleared of connective tissue. Twin hooked stimulating electrodes were then placed under the artery and nerves ready for stimulation.

In some experiments the nerves were dissected free from the artery. In other experiments both adrenals were tied off from the circulation to prevent leaked stimulation causing release of adrenaline and noradrenaline from the glands.

2.7) Measurement of Conscious Blood Pressure.

Conscious blood pressure was measured by the now standard procedure of tail-cuff occlusion (Byrom and Wilson, 1938; Maistrello, Spazzoli and Matscher, 1967; Newman and Looker, 1972). Figure 4.3 illustrates the apparatus used.

The animal under study was placed inside the restraining cage with as little disturbance as possible. The occlusion cuff was gently slid down to the base of the tail protruding from the cage, and the tail was then placed on the warm hot water bottle. The animal was left quietly for between 10 - 15 minutes both to allow it to settle down and to give time for the warmed tail to get a rich blood supply. After this time period a piezo-ceramic transducer (W. & W. Electronics A.G., Basel) was slid firmly onto the tail and pressure readings were then taken.

The tail-pulse measured by the transducer was fed to a times 100 amplifier (CFP preamplifier 8121) and then displayed on an oscillograph chart recorder (George Washington 400 MD/2). With a well warmed tail ,and the animal remaining still, a strong characteristically rhythmic pulse trace could be seen. After a stable base-line period of trace the

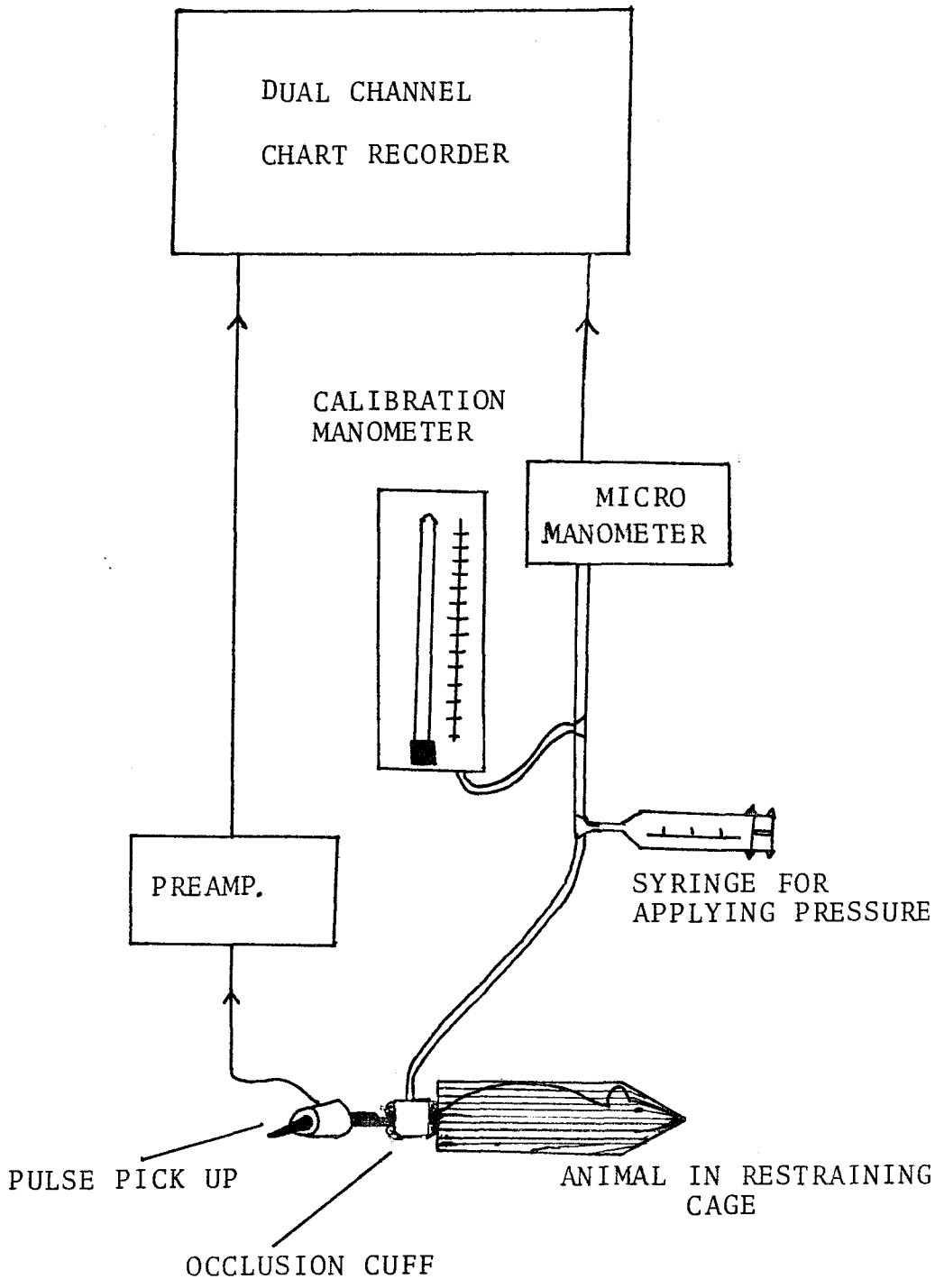


Figure 4.3 Apparatus for the measurement of conscious blood pressure by tail cuff. See text for description of use.

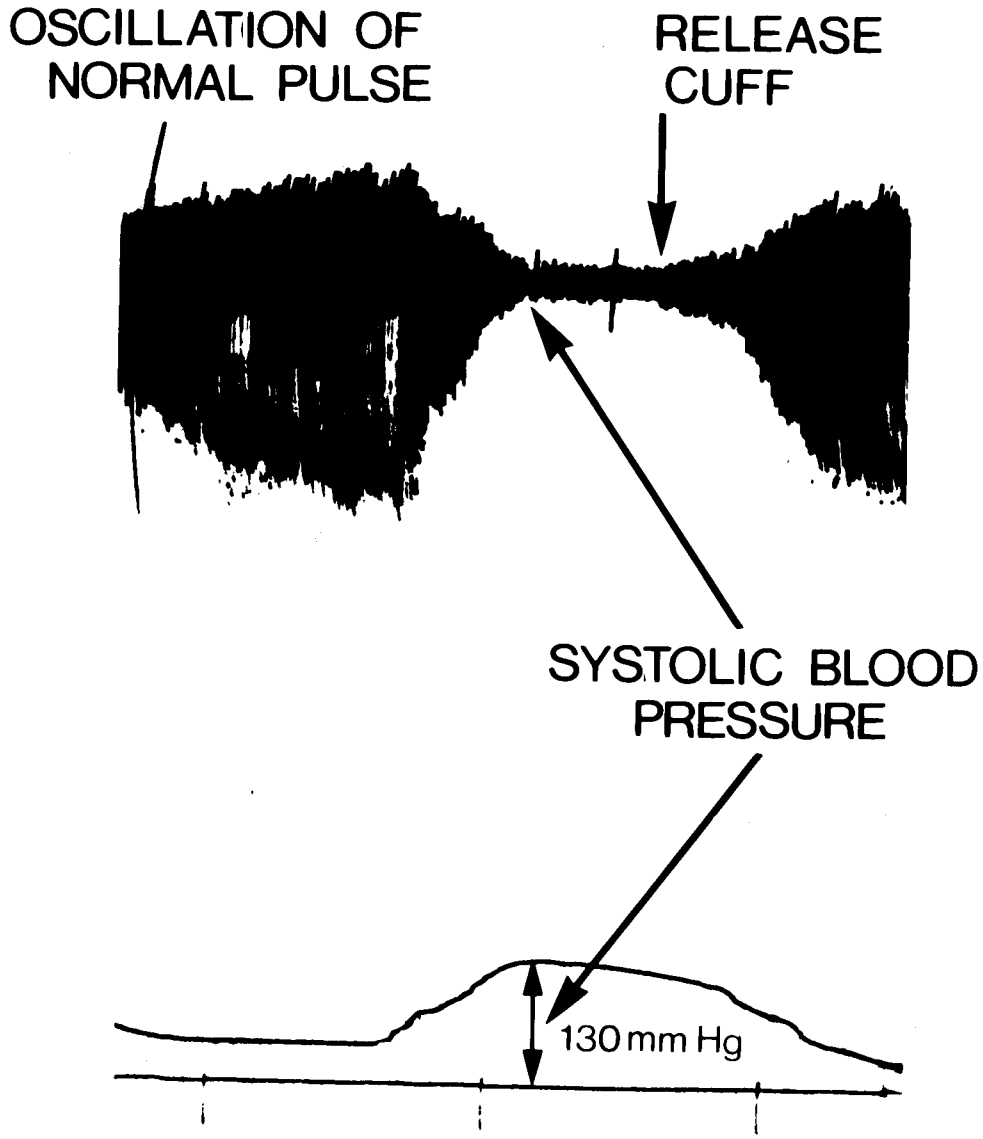


Figure 4.4 Typical chart recorder trace of pulse amplitude (top trace) and occlusion cuff pressure (lower trace) used to obtain values of conscious blood pressure from rats.

tail cuff was slowly occluded by increasing the pressure using the side-arm syringe. Systolic pressure was taken as the cuff pressure at the point of disappearance of the trace, and could be read off the chart (figure 4.4). Blood pressure values for animals were taken as the average value of six readings taken on two separate days.

This technique is both convenient and non-invasive and has been shown to have a close correlation with results obtained by direct methods (May, 1980). Stress produced by confinement in the restraining cage has been shown to have little effect on the blood pressure of normotensive animals, but may cause an increase in blood pressure in the SHR (Chiueh and Kopin, 1978). Thus the SHR values obtained may be slightly elevated in comparison with controls.

2.8) Treatment with 6-Hydroxydopamine.

Male wistar rats in the weight range 250-350g were anaesthetised with sodium pentobarbitone (75 mg/kg). The fur was shaved from the neck region and the neck was opened by a 2 cm incision in the ventral region. The left jugular vein was cleared and cannulated with polythene tubing (Portex PP25) by the method of Chapman, Chen, Munday, Philpot and Vasilescu (1980). Twenty-four hours after surgery the rats were injected through the indwelling cannula with drug or vehicle. The 6-hydroxydopamine hydrobromide (6-OHDA) was dissolved in 0.9% NaCl (w/v) solution containing 1% ascorbic acid, previously gassed for 1hr with nitrogen. Twice-daily injections (at 10 am and 4 pm) of a volume of 0.3 ml were carried out according to the scheme of Finch, Haeusler and Thoenen (1973) giving 2 X 50 mg 6-OHDA/kg on day 1 and 2 X 100mg/kg on day 7 after surgery. Control animals were injected under the same protocol with vehicle alone. Fluid transport by these animals was measured on day 8.

Several methods were used to assess the effectiveness of chemical sympathectomy. Firstly a group of treated and untreated animals were tested by the rat blood pressure preparation (section 2.3), using both exogenous noradrenaline and the endogenous noradrenaline releasing agent tyramine. Secondly the contractility of the anococcygeus muscle was studied (Gibson and Gillespie, 1973). This muscle is particularly suitable for testing the effectiveness of sympathectomy in individual animals as it is sensitive to 6-OHDA and is not disturbed during the transport studies.

Following transport studies the animals were killed and the right anococcygeus muscle was quickly excised, tied with cotton, and suspended in a 37^{o}C, oxygenated 30ml organ bath containing Krebs' bicarbonate saline (containing 11mM glucose). Tension was recorded isometrically with an Ether strain gauge tension transducer and displayed on a Servoscribe pen recorder. The muscle was mounted with a resting tension of 0.5g. Field stimulation was carried out using a pair of platinum loop electrodes embedded in epoxy resin (Araldite Ciba), and consisted of square wave pulses of 1.0 ms duration from a Grass model SD9 stimulator.

2.9)Assay of Noradrenaline Levels.

Rats to be assayed were killed by cervical dislocation and a 0.5 cm length of jejunum 15 cm from the ligament of Trietz was quickly excised. This tissue was then immediately dropped into liquid nitrogen. Following storage, the tissue was re-constituted and then homogenised. Noradrenaline was separated from other neurotransmitters on a Sephadex G-10 column and then read by fluorimetric assay according to the method of Earley and Leonard (1978). See figure 4.5 for standard curve.

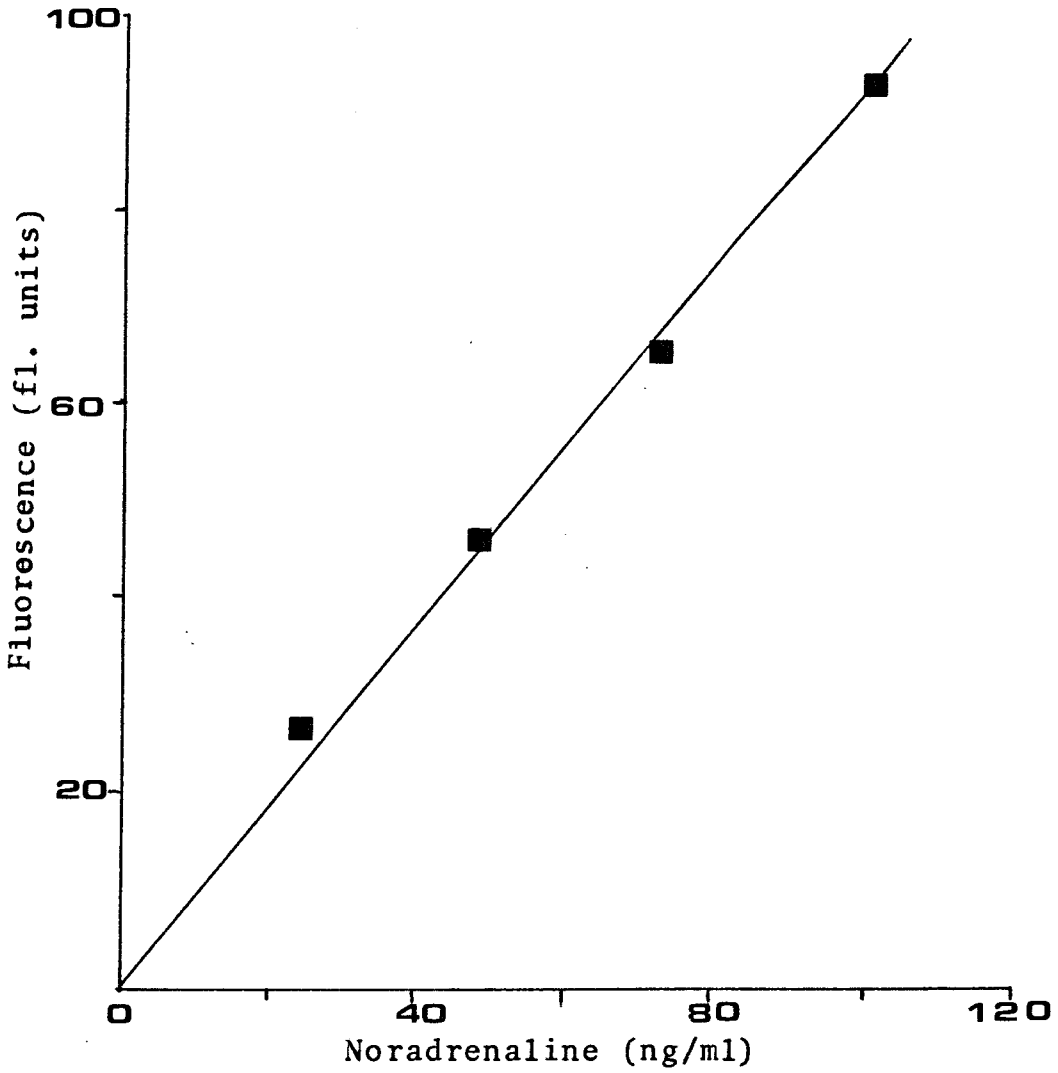


Figure 4.5 Standard curve for the determination of noradrenaline content by flurometric assay.

2.10)Adrenalectomy and Nephrectomy.

Rats were anaesthetised with ether, the fur just below the ribs and close to the spine on each side of the animal was shaved and an incision made. The kidney was exteriorised, and in the case of adrenalectomy, the adrenal located in the fat above the anterior pole, was removed together with surrounding fat. If a nephrectomy was to be performed then the renal artery and vein were tied off and the kidney excised. The body wall and skin were closed with sutures and the procedure repeated on the opposite side of the animal. The rats were recovered and maintained on a normal diet with 1% saline to drink.

2.11)Acute Nephrectomy.

Rats were anaesthetised with sodium pentobarbitone (75 mg/kg) and then nephrectomised as described in section 2.10. The anaesthetised animals were then left for 1.5 hours prior to any transport studies to allow the renin levels to be reduced. The half-life of renin in the rat is 20 minutes (Schaechtelin, Regoli and Gross, 1964).

2.12)Administration of Captopril.

Preliminary experiments demonstrated that administration of the converting enzyme inhibitor, Captopril, by the usual oral route did not produce adequate blockade (see section 3.3.6). Captopril was therefore administered intravenously 15 minutes prior to the transport studies. The extent of converting enzyme blockade was determined by bolus intravenous injections of angiotensin I in the rat blood pressure preparation.

2.13) Measurement of Sodium-Potassium-Sensitive ATPase.

Rats were killed by cervical dislocation and a 10cm length of jejunum, excised and placed on ice. The tissue was washed through with physiological saline and the intestine slit open by a longitudinal incision. Mucosal cells from the length of the intestine were scraped off using a glass slide. The cells were placed in an ice-cold homogenization tube and suspended in 10mls of cold sucrose buffer, containing 500mM sucrose, 10mM triethanolamine-hydrochloride and 0.1mM PMSF (pH 7.6). The suspension was homogenised manually with 30 strokes of the teflon homogenizer.

The Na—K—sensitive-ATPase activity was determined by the method of Jarnett and McKeel (1970). Suspensions of homogenate were incubated for 15 minutes in a 1ml volume of buffer containing 20mM tris HCl, 118mM NaCl, 5mM KCl, 3.5mM MgSO4, 20mM sodium azide and 3.5 mM ATP at pH 7.4. Half the tubes also contained 2mM ouabain to inhibit the Na—K-sensitive—ATPase. The reaction was started by the addition of homogenate and terminated by the addition of 0.2ml 25% TCA. The supernatant was assayed for inorganic phosphate (Sumner, 1944) and the precipitate for protein (Lowry, Rosebrough, Farr and Randall, 1951).

Preliminary experiments demonstrated that the reaction was independent of ATP concentration over the range used and linear at 15 minute incubation with protein concentration in the range 0.5 - 1.5 mg (as shown in figure 4.6). Values are expressed as μmoles phosphate produced/mg protein/hr. Values for Na—K—dependent—ATPase were obtained from the differences between tubes with total ATPase activity and those inhibited by ouabain.

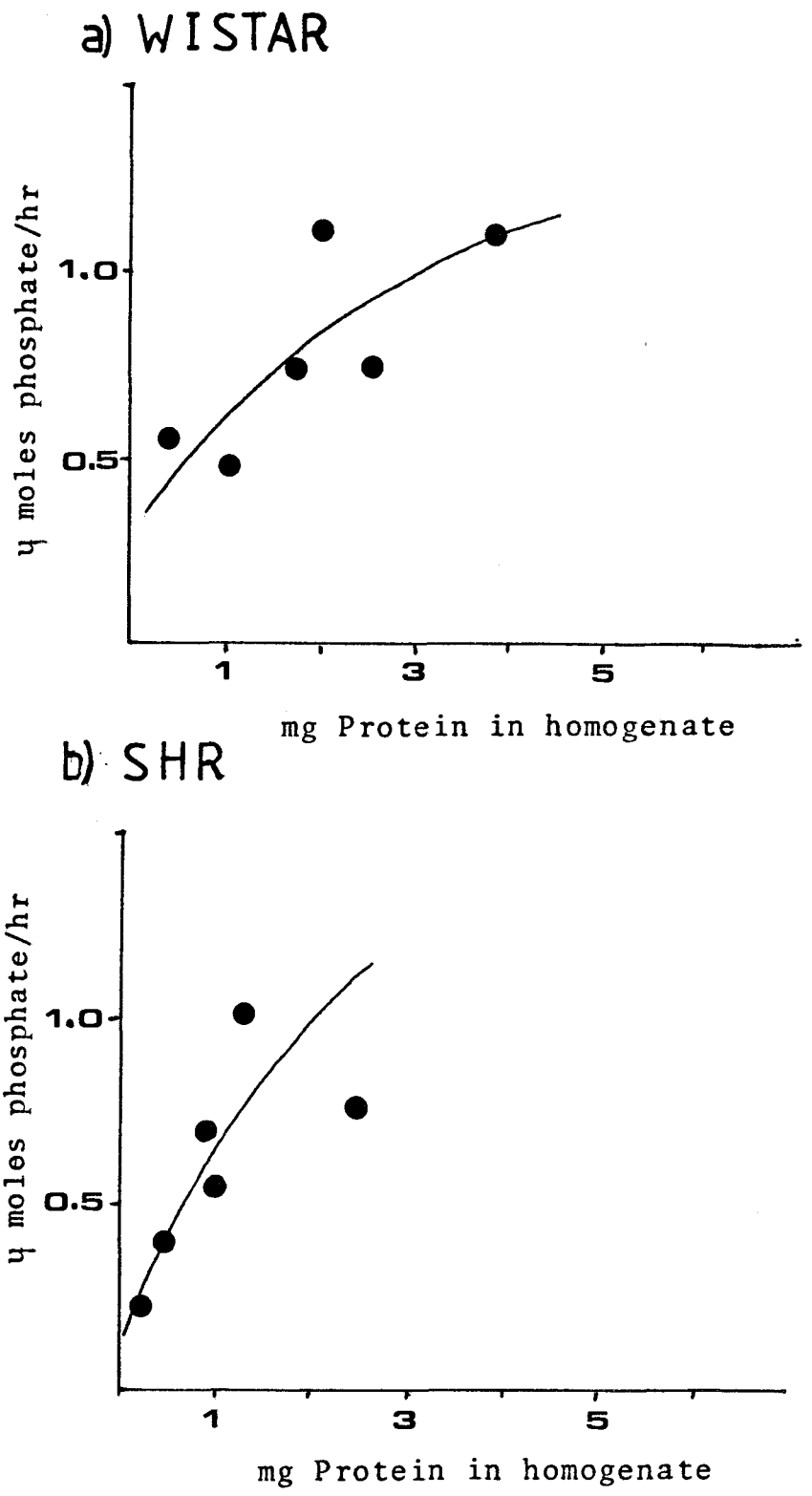


Figure 4.6 Phosphate production by sodium-potassium -sensitive ATPase in homogenate from jejunal mucosal scrapings for various protein concentrations. Experiment conducted for 15 minute incubation.

2.14)Chemical Dilutions and Administration of Drugs.

The majority of drugs used were made up in Ø.9% physiological saline and administered intravenously. The exceptions to this are given in table 4.2

Table 4.2 Drug Vehicle and Route.

Drug	Vehicle	Route
Apomorphine	4mM tartrate/saline	i.v.
Dopamine	4mM tartrate/saline	i.v.
Pargyline	saline	i.p.
Pentolinium tartrate	saline	i.p.
Prazosin	Ø.1% ascorbate/water	i.v.
Sulpiride	saline + a few crystals of Na dihydrogen orthophosphate	i.v.

In all cases the appropriate vehicle control was used. Radioactive markers were dissolved in Ø.9% saline to give a stock solution containing 1Øμ Ci/ml, and stored at -15 ^{o}C. Aliquots were removed as necessary and diluted in Krebs' bicarbonate buffer to the required concentrations before use.

2.15)Expression of Results.

Statistical significance was determined by Student's paired or un-paired t test, as appropriate. A P value of less than 5% was considered significant (Hodge and Seed, 1972).

2.16)Histology.

Histological examination where necessary was on a Ø.5 cm segment of jejunum immediately distal to that used for experiment. The tissue

was excised and placed in buffered formol saline (pH 7.4). After 48 hours it was removed, dehydrated, embedded in wax (table 4.3), and blocked. 20μ sections were cut from the blocks with a rotary microtome, the slides taken from the centre of the block being selected for staining. The slides were stained with haematoxylin and eosin (table 4.4) and mounted in DPX. The slides were examined under the microscope and categorised according to the scheme of Chiu et al. (1970).

Table 4.3 Dehydration and Blocking Protocol for Histological Studies.

Procedure	Time
Formol saline	2 - 7 days
Wash in water	15 minutes
50% alcohol	10 minutes
70% alcohol	10 minutes
90% alcohol	10 minutes
Absolute alcohol I	30 minutes
Absolute alcohol II	30 minutes
Xylene or inhibisol	30 minutes
Xylene or inhibisol	30 minutes
Wax embed in vacuum oven:-	
Wax I	1 hour
Wax II	1.5 hours
Wax III	overnight

Table 4.4 Staining Protocol for Histological Studies. Sections held onto slide with albumin.

Procedure	Time
Xylene	5 minutes
Xylene	5 minutes
Absolute alcohol I	2 minutes
Absolute alcohol II	2 minutes
90% alcohol	2 minutes
70% alcohol	2 minutes
Water	wash
Haematoxylin	20 minutes
Water	wash
Acid alcohol	quick dip
Water	wash
S.T.W.S.	until nuclei blue
Eosin	1 minute
water	wash
70% alcohol	1 minute approx.
90% alcohol	1 minute
Absolute alcohol II	1 minute
Absolute alcohol I	1 minute
Xylene	3 minutes
Xylene	5 minutes

Mount in DPX.

Table 4.5 Composition of Krebs' Bicarbonate Buffer, Used in Intestinal Absorption studies.

100 parts	0.9% NaCl
4 parts	1.15% KCl
3 parts	1.22% CaCl
1 part	2.11% KH_2PO_4
1 part	3.82% $MgSO_4 + 7 H_2O$
21 parts	1.30% $NaHCO_3$ (previously gassed with CO_2 for 1 hour).

Table 4.6 Commercial Origin of Drugs.

Compound	Source
6- hydroxydopamine	Aldrich
14-C PEG 4000	Radiochemical Centre, Amersham
14-C PEG 6000	Radiochemical Centre, Amersham
Angiotensin I	Sigma
Angiotensin II amide 5 valine	CIBA
Apomorphine hydrochloride	Macfarlan Smith, Edinburgh
ATP	Sigma
Atropine sulphate	Sigma
Captopril (SQ 14,225)	Squibb
Clonidine (Catapres)	Boehringer
Dopamine hydrochloride	Aldrich
Gallamine (Flaxedil)	May and Baker
Heparin BP, 5000 units/ml	Boots
Noradrenaline bitartrate	Koch-Light
Ouabain	Sigma
Pargyline (N-methyl-N-propargylbenzylamine HCl)	Aldrich
Pentolinium tartrate	May and Baker
Polyethylene glycol 4000	Hopkin and Williams

Phentolamine mesylate (Rogitine)	CIBA
Phenylephrine	Aldrich
Prazosin	Pfizer
Sodium pentobarbitone (Sagatal)(Nembutal)	May and Baker
Sulpiride	Ravizza
Triethanolamine- hydrochloride	Sigma
Yohimbine	Sigma

The gift of Captopril from E.R. Squibb Ltd., and Sulpiride from Ravizza are gratefully aknowledged.

Chapter 3

RESULTS

Section 3.1

CATECHOLAMINES AND INTESTINAL TRANSPORT

3.1.1) The Action of Dopamine on Intestinal Fluid Transport.

Much evidence has now been accumulated to suggest that noradrenaline is released to produce the observed stimulatory effects of angiotensin on intestinal fluid transport (Levens et al., 1979). However, studies with broad-spectrum α-blockers and sympathetic blocking agents have not yet conclusively demonstrated that the catecholamine neurotransmitter involved is actually noradrenaline. It is possible that the findings from these experiments might equally well be explained by implicating peripheral dopamine in the response.

Dopamine is an immediate pre-cursor of the adrenergic transmitter noradrenaline. However, much evidence now exists that dopamine acts as a neurotransmitter in its own right, both in the CNS (Woodruff, 1978) and in the periphery (Clark and Menninger, 1980). In the periphery dopamine acts as a modulator at prejunctional dopamine receptors (Stjarne and Brundin, 1975; Buylaert, Willems and Bogaert, 1979; Hope, McCulloch, Rand and Story, 1978; Takenaka, Sakanashi, Ishihera and Morishita, 1979; Rand, Majewski, Medgett, McCulloch and Story, 1980) and as an inhibitor of sympathetic transmission in ganglia such as the inferior mesenteric ganglia of the dog (Lins and Willems, 1974; Bogaert, de Schaepdryver and Willems, 1977). Furthermore postsynaptic dopamine receptors are also found, largely on vascular tissue. These vascular, vasodilator, receptors are particularly apparent in the vessels supplying organs with transporting epithelia such as the kidney (Goldberg, 1979; Imbs, Schmidt, Ehrhardt and Schwartz, 1979) and the dog ileum (Clark and Menninger, 1980).

As well as being found in transporting tissues, dopamine has also been shown to be likely to have a physiological role in several areas of salt and water homeostasis. It is suggested as modulating the release of renin (Sowers, Barrett and Sambhi, 1981), inhibiting

aldosterone production (Sowers, Golub, Tuck and Sowers, 1981) and possibly being involved in the mechanism of angiotensin-induced thirst (Fitzsimmons and Setler, 1975; Munday, Sumners and Woodruff, 1978).

Despite these indications that dopamine may indeed have a role in the control of intestinal fluid transport very little work has been conducted on the action of dopamine on transport. That such a study might yield results is suggested by the findings of Wiglusz and Korolkiewicz (1979) who have demonstrated that dopamine is capable of a dose-dependent stimulation of short circuit current in isolated frog skin. Upsher (1981) has also shown that the specific dopamine antagonist sulpiride can stereospecifically inhibit the transport stimulating actions of infused angiotensin on the in vivo rat jejunum.

With these results in mind a study was conducted on the possible role of dopamine in the control of intestinal fluid transport. To obtain an index of the potency of dopamine compared with noradrenaline initial studies on the pressor activity of infused dopamine were made. The results of this study, shown in figure 5.1 displays the typical multi-component curve expected from dopamine acting on both α-vasoconstrictor and dopamine- and β- vasodilator receptors (McNay and Goldberg, 1966; Chapman, Horn, Munday and Robertson, 1980). An infusion of the agonist noradrenaline at a rate of 7nmoles/kg/min will stimulate intestinal fluid transport (table 5.1) and produce a blood pressure increase of 29 ± 6 mmHg (4). Dopamine has a much weaker α-activity than noradrenaline (Lazner and de la Lande, 1974) and required an infusion of 50 X the amount of noradrenaline (351nmoles/kg/min) to obtain the same pressor response.

Using an in vivo sac preparation, the ability of exogenous dopamine to alter intestinal fluid transport was measured in the

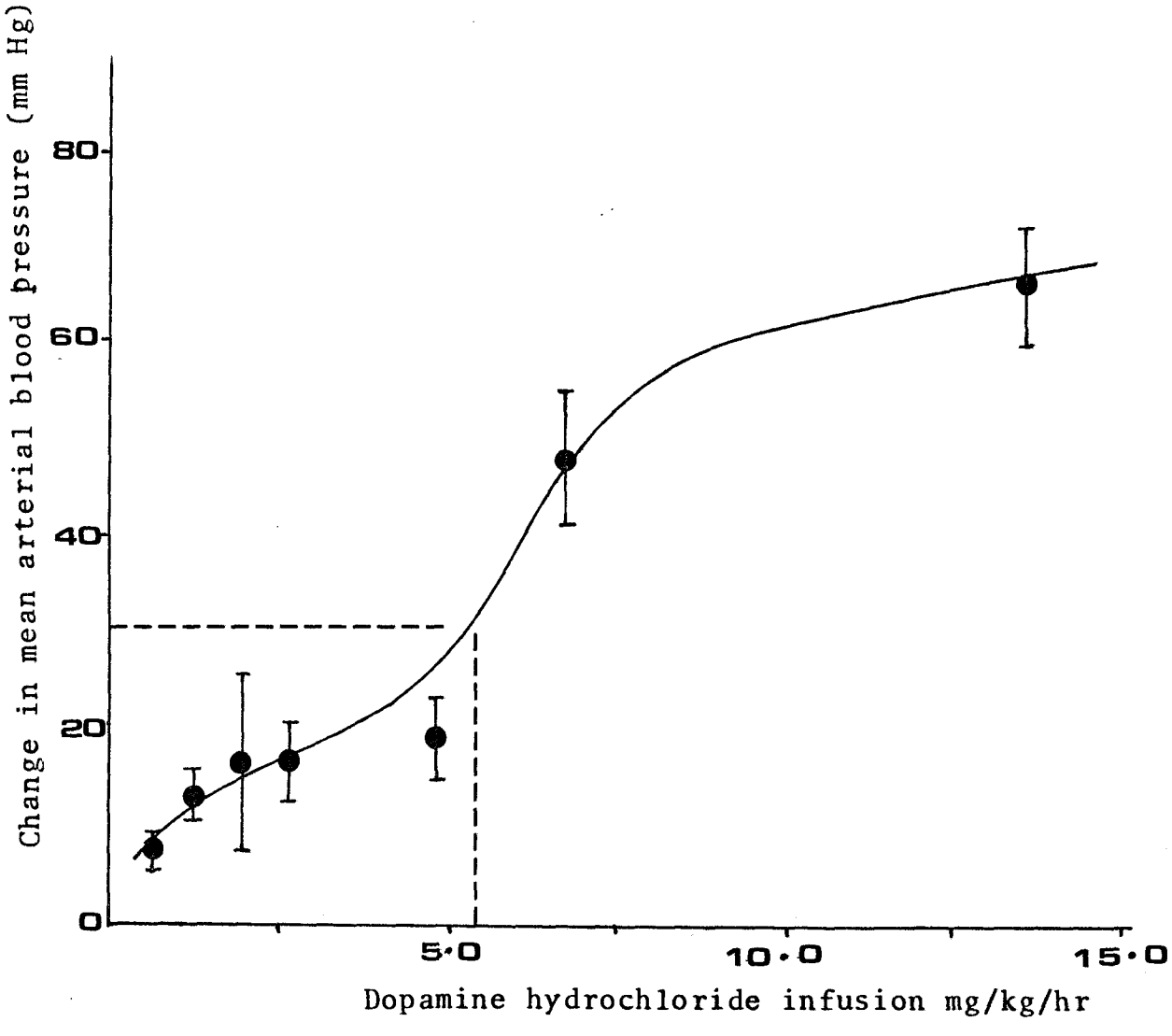


Figure 5.1 Dose response curve for the effect of infused dopamine on mean arterial blood pressure. Dashed-line indicates the pressor activity of the infusion of noradrenaline known to stimulate intestinal fluid transport, The equipotent pressor dose of dopamine can thus be found.

Table 5.1

The action of noradrenaline on intestinal fluid transport and mean arterial blood pressure. Absorption measured in two consecutive periods with saline infusion in first period. Experimental group had noradrenaline bitartrate dissolved in 0.9% saline infused at a rate of 7nmoles/kg/min in the second period. Results expressed as mean ± S.E.M. compared by Student's paired t test. Number of observations in parenthesis.

	Blood Pressure (mmHg)		Sig	Fluid Transport (ml/hr/g wet weight)		Sig
	Period 1	Period 2		Period 1	Period 2	
Control						
Saline/saline (4)	91 ± 8	95 ± 8	N.S.	0.88 ±.08	1.00 ±.20	N.S.
Experimental						
Saline/noradrenaline (5)	110 ± 6	139 ± 6	P<0.05	0.80 ±.20	2.04 ±.20	P<0.002

Table 5.2

The action of a range of doses of dopamine hydrochloride infusion on the rate of fluid absorption in rat jejunum. Dopamine hydrochloride dissolved in Ø.9% saline containing 4mM tartrate. Results expressed as mean ± S.E.M. compared by Student's paired t test. Number of observations in parenthesis.

	Blood Pressure (mmHg)		Sig	Fluid Transport (ml/hr/g wet weight)		Sig
Infusion in 2nd period	Period 1	Period 2		Period 1	Period 2	
4mM tartrate (6)	88 ± 6	9Ø ± 5	N.S.	1.2Ø ±.17	1.5Ø ±.21	N.S.
Dopamine-HCl 0.7nmoles/kg/min (1Ø)	97 ± 4	96 ± 4	N.S.	1.36 ±.12	1.56 ±.16	N.S.
Dopamine-HCl 7 nmoles/kg/min (6)	1ØØ ± 8	1Ø5 ± 8	N.S.	1.2Ø ±.16	1.44 ±.12	N.S.
Dopamine-HCl 351nmoles/kg/min (8)	94 ± 3	1Ø3 ± 3	P<Ø.Ø2	Ø.96 ±.16	1.85 ±.14	P<Ø.Ø1

jejunum of rats over two consecutive ,control and experimental, time periods. Table 5.2 shows the alterations in mean arterial blood pressure and intestinal transport on infusion of varying amounts of dopamine. Only the high, pressor, dose of dopamine has any action on transport.

The peripheral nervous system has a high level of catecholamine metabolising activity, this metabolism serving to inactivate neurotransmitters following release (section 1.2.6). It is therefore possible that dopamine is being metabolised more rapidly than infused noradrenaline and high concentrations of dopamine are therefore necessary to overcome this breakdown. Table 5.3 shows results for a series of experiments with prior administration of the monoamine oxidase inhibitor pargyline. Pargyline attaches covalently to this metabolising enzyme and prevents its action (Oreland, Kinemuchi and Yoo, 1973). Pretreatment with pargyline should therefore allow a more physiological dose of dopamine to have its effect. However, even with pargyline blockade, dopamine at the infusion rate of 7nmoles/kg/min has no effect on intestinal fluid transport.

Since dopamine acts on both α and β as well as dopamine receptors and high doses of dopamine have been shown to have predominantly α activity (McNay and Goldberg, 1966) it is possible that infusions of dopamine are not having their actions on intestinal fluid transport via specific dopamine receptors. This hypothesis was tested using both the specific dopamine antagonist sulpiride and the agonist apomorphine.

The substituted benzamide sulpiride has been shown to be a potent antagonist of peripheral dopamine receptors (Kohli, Volkman, Glock and Goldberg, 1978). Treatment with this drug would therefore be expected to abolish any true dopamine-receptor mediated effects. However, Table 5.4 shows that sulpiride infusion at a rate capable of blocking the

Table 5.3

The effect of exogenous infusions of dopamine on intestinal fluid transport of animals treated 3.5 hours prior to experiment with the monoamine oxidase inhibitor pargyline. Consecutive periods compared by Student's paired t test and expressed as mean ± S.E.M.. Number of observations in parenthesis.

Infusion in 2nd period	Fluid Transport (ml/hr/g wet weight)		Sig
	Period 1	Period 2	
Pargyline pre-treatment			
Saline (3)	1.68 ±.24	1.56 ±.36	N.S.
Dopamine-HCl 7nmoles/kg/min (6)	2.Ø4 ±.28	1.28 ±.28	N.S.
Control			
Saline (1Ø)	1.35 ±.14	1.42 ±.19	N.S.
Dopamine-HCl 7nmoles/kg/min (5)	1.96 ±.12	1.72 ±.2Ø	N.S.

Table 5.4

The action of sulpiride on the dopamine stimulation of fluid transport in the rat jejunum. Sulpiride infused simultaneously through the left cannula during dopamine infusions in second transport period. Consecutive periods compared by Student's paired t test and expressed as mean ± S.E.M.. Number of observations in parenthesis.

Infusion in 2nd period	Fluid Transport (ml/hr/g wet weight)		Sig
	Period 1	Period 2	
Dopamine HCl (4) Ø.7nmoles/kg/min +Ø.2mg/kg/min sulpiride	1.52 ±.12	1.28 ±.28	N.S.
Dopamine HCl (8) 351nmoles/kg/min +Ø.2mg/kg/min sulpiride	1.32 ±.16	2.Ø8 ±.24	P<Ø.Ø5
Dopamine HCl (4) 351nmoles/kg/min	1.76 ±.2Ø	2.36 ±.Ø8	P<Ø.Ø5
Dopamine HCl (1Ø) Ø.7nmoles/kg/min	1.36 ±.12	1.56 ±.16	N.S.
Saline (11) +Ø.2mg/kg/min sulpiride	1.51 ±.13	1.78 ±.Ø6	N.S.

dopamine vascular receptor (Woodruff and Sumners, 1979) has no effect on either dopamine—stimulated or basal transport. The agonist apomorphine has its predominant activity on β and dopamine receptors with almost no α activity (Bell, Conway and Lang, 1974), and would be expected to stimulate dopaminergic receptors, mimicking any true dopamine effect. However, apomorphine has no effect on jejunal fluid transport at infusion rates of 7nmoles/kg/min and 351nmoles/kg/min (table 5.5).

The results from experiments with sulpiride and apomorphine suggest that the observed stimulation of fluid transport obtained on infusing dopamine is unlikely to be mediated by either β or dopamine receptors. This possibility was further examined by testing the effectiveness of α-blockade on the dopamine response. Figure 5.2 gives the results for α-blockade of dopamine—stimulated jejunal fluid transport. The competitive α-blocker phentolamine produces a dose dependent inhibition of dopamine—stimulated transport, totally abolishing the effect at an infusion rate of 90µg/kg/min. Therefore, despite the ability of exogenous dopamine to stimulate intestinal fluid transport, dopamine is unlikely to be as important as noradrenaline in these effects, for exogenous dopamine both appears to be acting upon α receptors and is only active at a concentration 50 X the effective dose of noradrenaline.

3.1.2) The Effect of Raising Endogenous Catecholamine Levels.

Closer observation of the earlier results for pargyline pre-treatment reveals that the basal transport levels for pargyline treated animals are significantly above that of controls. Figure 5.3 gives the results for both pargyline pre-treated and un-treated animals in single 15 minute absorption experiments. Administration of the

Table 5.5

The action of apomorphine infusion on fluid absorption from rat jejunum *in vivo*. Apomorphine hydrochloride dissolved in Ø.9% saline containing 4mM tartrate and infused in the second consecutuve period. Consecutive periods compared by Student's paired t test and expressed as mean ± S.E.M.. Number of observations in parenthesis.

Infusion in 2nd period	Fluid Transport (ml/hr/g wet weight)		Sig
	Period 1	Period 2	
4mM tartrate in Ø.9% saline (6)	1.72 ±.24	1.84 ±.Ø8	N.S.
Apomorphine HCl 7nmoles/kg/min (9)	1.52 ±.2Ø	1.52 ±.12	N.S.
Apomorphine HCl 351nmoles/kg/min (4)	1.48 ±.12	1.76 ±.32	N.S.

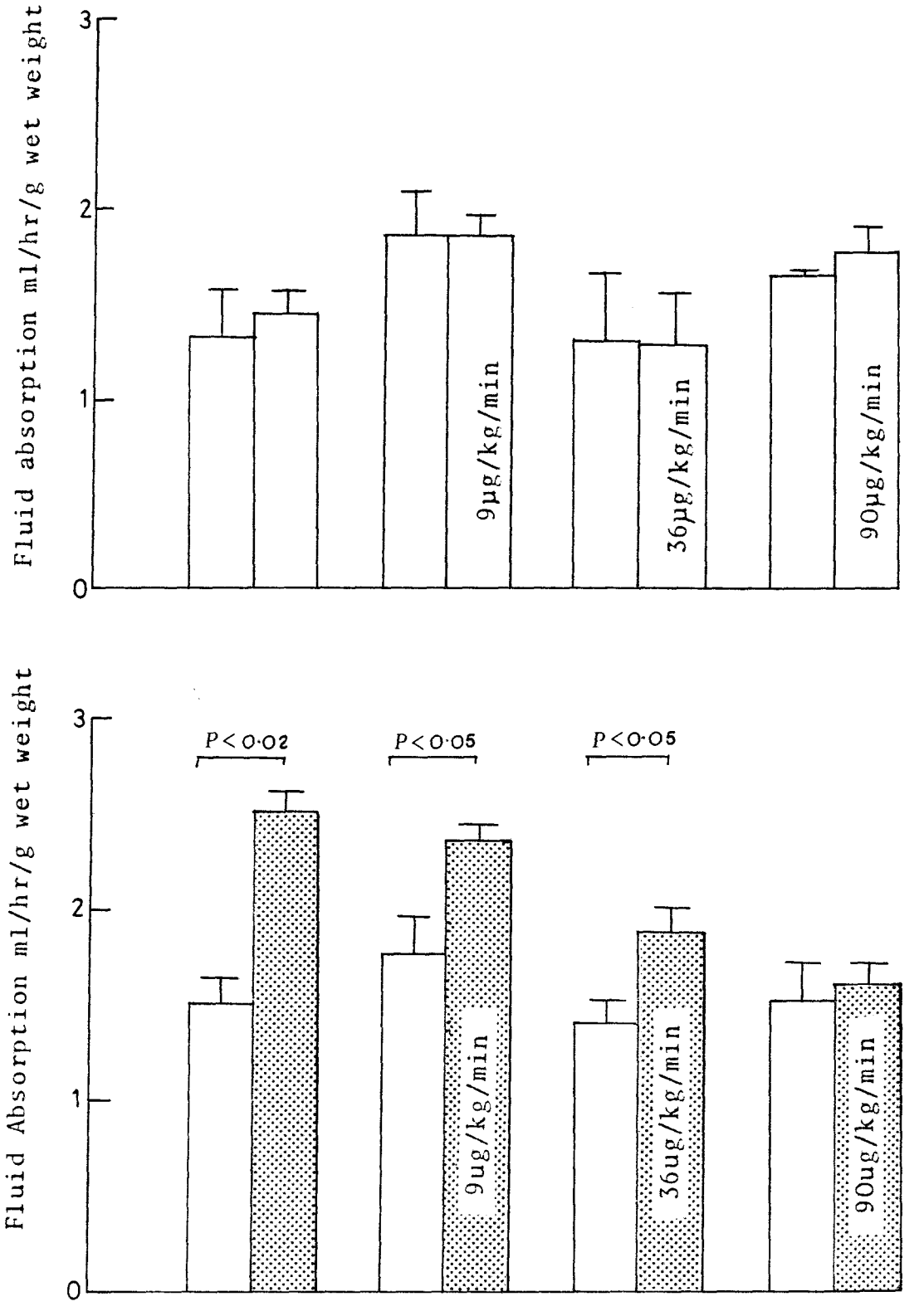


Figure 5.2 The effect of various doses of phentolamine on the stimulation of intestinal fluid transport by dopamine. Shaded bars have dopamine infused at a rate of 351 nmoles/kg/min. Paired bars represent control (1st) period of saline infusion and 2nd experimental period in with saline or dopamine with or without phentolamine are administered. Rate of infusion of phentolamine given as an inset within the bars, no value indicates no phentolamine infusion. Results given as mean ± S.E.M. 1st and 2nd periods compared by paired t test.

See table 5.6 for no. of observations per group.

Table 5.6

The effect of infusions of the α-blocker phentolamine mesylate (P) on dopamine-stimulated intestinal fluid absorption. Consecutive periods compared by Student's paired t test and expressed as mean ± S.E.M.. Number of observations in parenthesis. (Also see figure 5.2).

Infusion in 2nd period	Fluid Transport (ml/hr/g wet weight)		Sig
	Period 1	Period 2	
Saline (5)	1.32 ±.24	1.44 ±.12	N.S.
Phentolamine 9µg/kg/min (3)	1.84 ±.24	1.84 ±.12	N.S.
Phentolamine 36µg/kg/min (4)	1.44 ±.20	1.28 ±.28	N.S.
Phentolamine 90µg/kg/min (3)	1.64 ±.01	1.76 ±.12	N.S.
Dopamine 351nmoles/kg/min (4)	1.52 ±.12	2.52 ±.08	P<0.02
Dopamine 351nmoles/kg/min +P 9µg/kg/min (4)	1.76 ±.20	2.36 ±.08	P<0.05
Dopamine 351nmoles/kg/min +P 36µg/kg/min (8)	1.40 ±.12	1.88 ±.12	P<0.05
Dopamine 351nmoles/kg/min +P 90µg/kg/min	1.52 ±.20	1.60 ±.12	N.S.

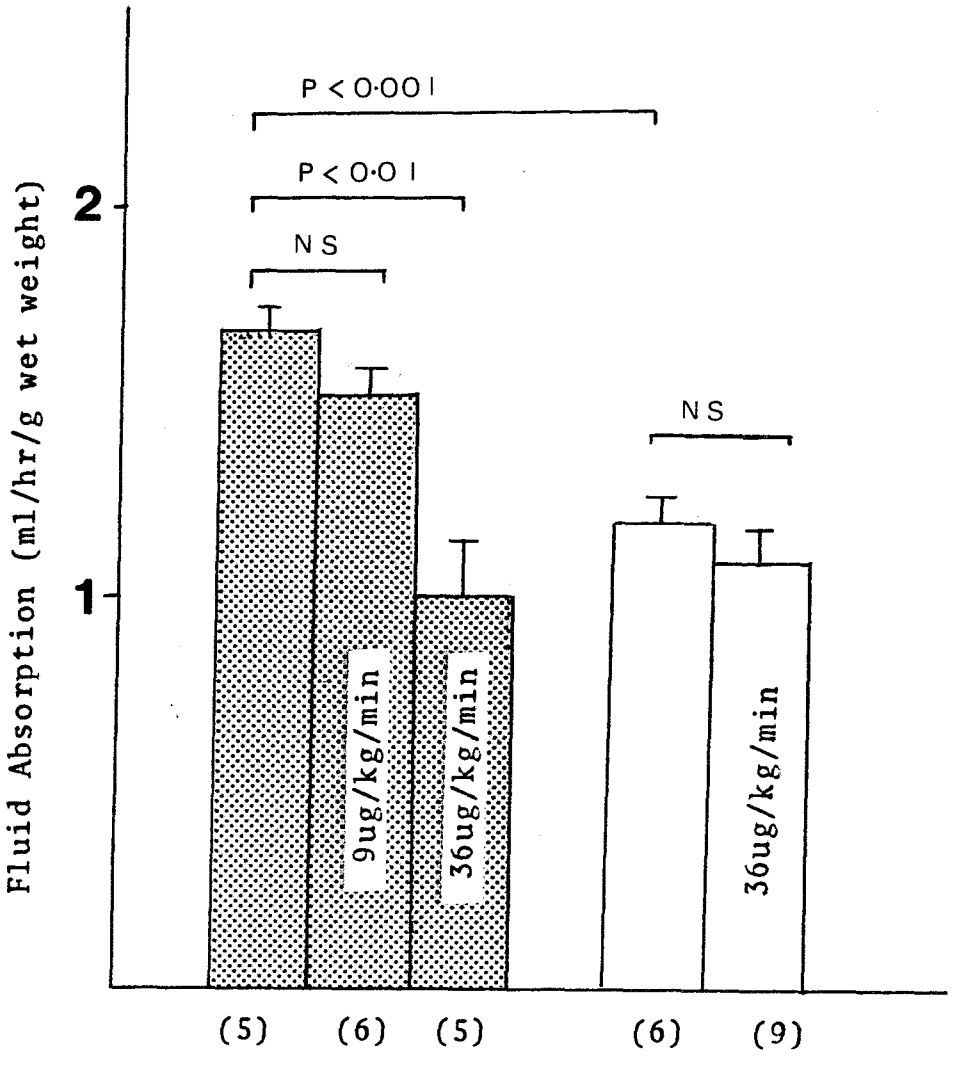


Figure 5.3 The effect of pargyline pre-treatment on intestinal fluid transport. Shaded bars represent animals pre-treated with pargyline 3½ hours prior to measurement of transport. Fluid transport measured as a single 15 minute period. Results expressed as mean ± S.E.M. and groups compared by Student's unpaired t test. Number of animals in parenthesis. In some groups phentolamine was infused during the transport measurements, in these cases the dose of blocker infused is given as an inset.

monoamine oxidase inhibitor pargyline under exactly these conditions has been shown to significantly increase endogenous levels of noradrenaline in the intestine (Munday, Poat and Upsher, 1980) and it may very well be these increased levels which stimulate transport. This was tested by use of the α-blocker phentolamine. Phentolamine at an infusion rate of 36µg/kg/min is able to return the increased transport to control levels, although it was without effect on transport in normal rats. This result supports the suggestion that pargyline is effecting an increase in transport via changes in endogenous noradrenaline levels.

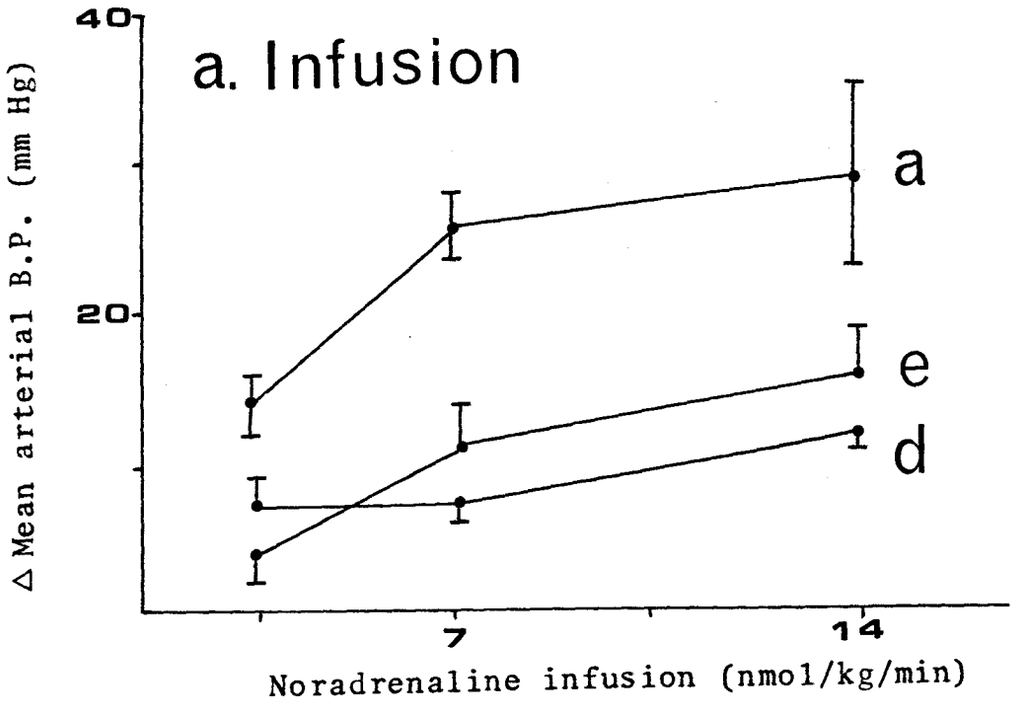
3.1.3) Characterisation of the Effector α Receptor.

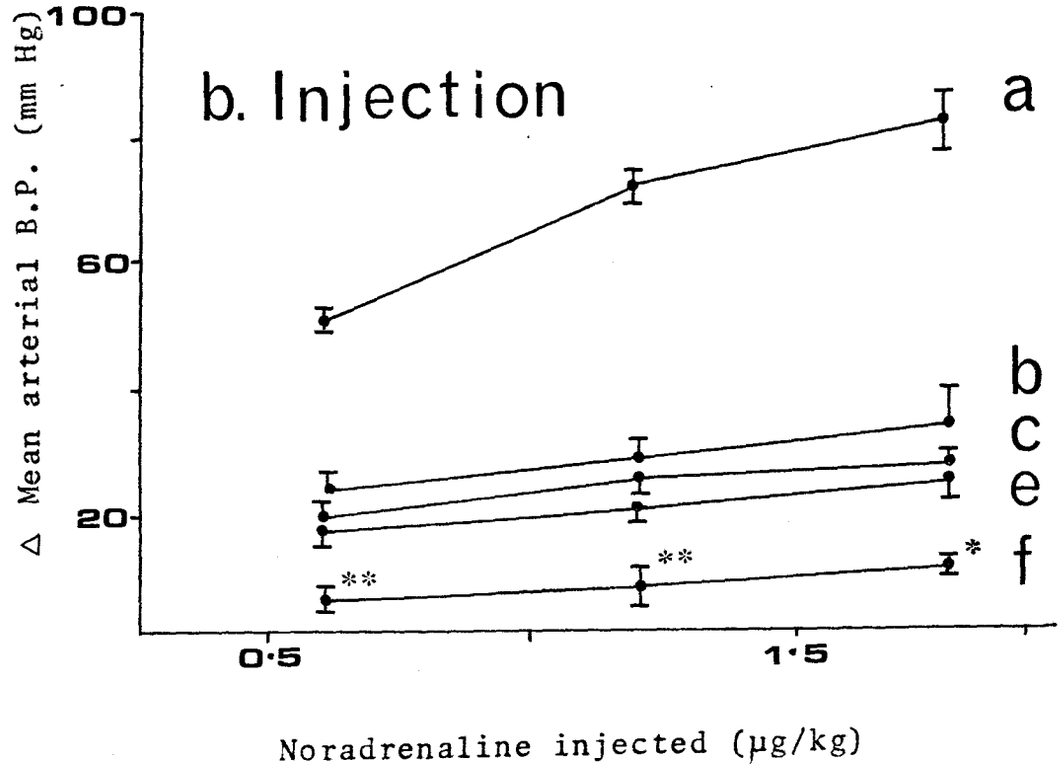
The evidence of the above investigations suggest that catecholamines, in particular noradrenaline, are able to stimulate intestinal fluid absorption. However, in these studies and those of other workers (Field and McColl, 1973; Levens et al., 1979), no attempt has been made at further characterisation of the α receptor involved. Little is known about either the location or the pharmacological specificity of the receptors. As discussed in the introduction (section 1.2.5) α receptors have been shown to have two pharmacological sub-types, designated α_1 (postsynaptic) and α_2 (pre- and post-synaptic). Broad-spectrum α-blockers such as phentolamine (U'prichard, Charness, Robertson and Snyder, 1978) do not distinguish between these receptors. More recently a large number of agonists and antagonists have become available which allow such studies to be carried out.

Prazosin is one such agent which has been shown to be a good specific postsynaptic α_1-antagonist (Davey, 1980), with insignificant α_2-receptor blocking activity. Figure 5.4 demonstrates the blocking

Figure 5.4 The action of the α blocker prazosin on mean arterial blood pressure increase produced by administration of noradrenaline, both by intravenous infusion and intravenous injection. a = no prazosin, b= 133µg/kg, c= 400µg/kg d= 600µg/kg, e= 1,200 µg/kg, f= 2,400µg/kg. In graph 'b' lines c,e and f are compared with line b by Student's unpaired t test. The points on lines c and e are not significantly different from those of line b. ** = $p < 0.02$ * = $p \leq 0.05$.

n = 3 in each group.


a. Infusion
40
20
Δ Mean arterial B.P. (mm Hg)
a
e
d
7
14
Noradrenaline infusion (nmol/kg/min)


b. Injection
100
60
20
Δ Mean arterial B.P. (mm Hg)
a
b
c
e
f
**
**
*
0·5
1·5
Noradrenaline injected (μg/kg)

action of a single injection of prazosin on the pressor response resulting from noradrenaline infusion, or noradrenaline injection. Mid to low doses of prazosin inhibit this noradrenaline pressor response, but only very high doses of 2,400µg/kg abolish it. This is similar to the observation of Drew and Whiting (1979). Table 5.7 gives the results for blockade of noradrenaline stimulated transport by prazosin. The stimulation of transport is remarkably resistant to prazosin blockade and noradrenaline will still stimulate fluid absorption in the presence of 133µg/kg and 300µg/kg prazosin, doses which significantly reduce the pressor effect. Prazosin will block the stimulation at a dose of 2,400 µg/kg and has no actions on control transport. Prazosin significantly reduces basal blood pressure at all doses apart from the high 2,400 µg/kg dose (an anomaly which is likely to be a reflection of different levels of anaesthesia).

The relatively specific α2-antagonist yohimbine (Doxey, Smith and Walker, 1977) has been shown to be able to abolish the 'prazosin resistant' pressor response to noradrenaline (Flavahan and McGrath, 1980). Table 5.8 shows the blocking action of this antagonist on both noradrenaline stimulated intestinal transport and the noradrenaline pressor effect. Low doses of 133µg/kg of yohimbine are able to abolish the noradrenaline stimulated transport but do not block the pressor effect. Yohimbine alone has no effect on basal transport despite its well documented pre-synaptic actions (Docherty and McGrath, 1979a).

Agonists for α1-and α2-receptors are generally not as specific as antagonists and are therefore less useful for receptor characterisation (Starke and Docherty, 1980), although they can provide useful additional information. Clonidine is well documented as being an agonist acting predominantly on α2-receptors (Docherty and McGrath,

Table 5.7

The blocking action of the α antagonist prazosin on noradrenaline—stimulated intestinal fluid absorption. Absorption measured in two consecutive periods, all groups had saline infusion in 1st period. Experiments have noradrenaline bitartrate infused in the 2nd period at a rate of 7nmoles/kg/min. Prazosin dissolved in 0.1% ascorbate in distilled water administered as a single i.v. injection just prior to the start of the 2nd period. Results expressed as mean ± S.E.M. compared by Student's paired t test. Number of observations in parenthesis.

	Blood Pressure (mmHg)		Sig	Fluid Transport (ml/hr/g wet weight)		Sig
	Period 1	Period 2		Period 1	Period 2	
Control						
Saline/saline (4)	91 ± 8	95 ± 8	N.S.	1.48 ±.12	1.72 ±.16	N.S.
Saline/prazosin 133µg/kg (3)	109 ± 6	93 ± 8	P<0.01	1.92 ±.36	1.20 ±.36	N.S.
Saline/prazosin 300µg/kg (3)	106 ± 2	78 ± 2	P<0.01	1.80 ±.28	1.76 ±.64	N.S.
Saline/prazosin 2400µg/kg (5)	95 ±10	79 ± 6	N.S.	1.56 ±.12	2.20 ±.08	N.S.
Saline/ascorbate (3)	78 ± 8	101 ± 1	N.S.	1.44 ±.40	2.12 ±.40	N.S.
Experimental						
Noradrenaline (NA)/saline (4)	100 ± 7	130 ± 6	P<0.02	1.12 ±.20	2.00 ±.28	P<0.05
NA/prazosin 133µg/kg (4)	102 ± 5	98 ± 2	N.S.	1.20 ±.08	1.84 ±.20	P<0.05
NA/prazosin 300µg/kg (4)	108 ± 6	114 ± 3	N.S.	1.52 ±.20	2.48 ±.04	P<0.05
NA/prazosin 2400µg/kg (6)	103 ± 2	107 ± 2	N.S.	1.64 ±.16	1.96 ±.12	N.S.

Table 5.8

The blocking action of the α-antagonist yohimbine on noradrenaline—stimulated intestinal fluid absorption. Absorption measured in two consecutive periods, all groups had saline infusion in 1st period. Experiments have noradrenaline bitartrate infused in the 2nd period at a rate of 7nmoles/kg/min. Yohimbine (YH) dissolved in Ø.9% saline administered as a single i.v. injection just prior to the start of the 2nd period. Results expressed as mean ± S.E.M. compared by Student's paired t test. Number of observations in parenthesis.

	Blood Pressure (mmHg)		Sig	Fluid Transport (ml/hr/g wet weight)		Sig
	Period 1	Period 2		Period 1	Period 2	
Control						
Saline/saline (1Ø)	91 ± 3	9Ø ± 3	N.S.	1.12 ±.Ø8	1.56 ±.12	N.S.
Saline/YH 13.3µg/kg (4)	1Ø2 ± 6	1Ø6 ±12	N.S.	1.36 ±.16	1.44 ±.24	N.S.
Saline/YH 133µg/kg (6)	97 ± 7	97 ±11	N.S.	1.ØØ ±.16	1.44 ±.28	N.S.
Saline/YH 3ØØµg/kg (4)	98 ± 3	88 ±1Ø	N.S.	1.28 ±.28	1.56 ±.16	N.S.
Saline/YH 1mg/kg (3)	113 ± 3	82 ± 7	P<Ø.Ø2	1.32 ±.Ø8	1.44 ±.Ø3	N.S.
Experimental						
Noradrenaline (NA)/saline (4)	11Ø ± 6	139 ± 6	P<Ø.Ø5	Ø.8Ø ±.12	1.6Ø ±.Ø8	P<Ø.Ø1
NA/yohimbine 13.3µg/kg (6)	98 ± 8	128 ± 6	P<Ø.Ø2	1.ØØ ±.16	1.72 ±.16	N.S.
NA/yohimbine 133µg/kg (8)	97 ± 9	127 ± 9	P<Ø.Ø5	1.24 +.Ø8	1.56 +.16	N.S.
NA/yohimbine 3ØØµg/kg (4)	99 ±11	1Ø6 ±14	N.S.	1.4Ø ±.Ø4	1.56 ±.12	N.S.
NA/yohimbine 1mg/kg (3)	95 ±12	126 ± 9	P<Ø.Ø5	1.44 ±.16	1.48 ±.24	N.S.

1979b). Table 5.9 summarises the action of this agonist on intestinal fluid transport, clonidine was found to stimulate transport when administered at a dose of 2.8nmoles/kg/min (cf. the stimulatory dose of noradrenaline of 7nmoles/kg/min). This stimulation was blocked by simultaneous infusion of the general α-antagonist phentolamine. Interestingly this stimulatory dose of clonidine was not pressor, the pressor response only being reached at an infusion rate of 56nmoles/kg/min.

α2- adrenoceptors are much less sensitive than α1- adrenoceptors to the α-agonist phenylephrine. Table 5.10 shows the stimulation of fluid transport obtained on infusion of phenylephrine at a rate of 7nmoles/kg/min. Indicating that the intestine will respond to both α1- and α2- agonists.

Table 5.9

The action of the α agonist clonidine (C) on intestinal fluid absorption. Absorption measured in two consecutive periods, all groups had saline infusion in 1st period. Various concentrations of clonidine dissolved in saline infused in 2nd period. Results expressed as mean ± S.E.M. compared by Student's paired t test. Number of observations in parenthesis.

	Blood Pressure (mmHg)		Sig	Fluid Transport (ml/hr/g wet weight)		Sig
	Period 1	Period 2		Period 1	Period 2	
Control						
Saline/saline (4)	80 ± 4	75 ± 5	N.S.	1.24 ±.20	1.20 ±.20	N.S.
Experimental						
Saline/C .28nmoles/kg/min (6)	90 ± 5	82 ± 8	N.S.	1.40 ±.16	1.52 ±.12	N.S.
Saline/C 2.8nmoles/kg/min (6)	83 ± 7	72 ± 6	N.S.	1.44 ±.07	1.84 ±.13	P<0.05
Saline/C 28nmoles/kg/min (4)	97 ± 4	111 ±11	N.S.	1.20 ±.16	1.88 ±.24	N.S.
Saline/C 56nmoles/kg/min (5)	85 ±10	144 ± 7	P<0.01	1.40 ±.20	1.68 ±.20	N.S.
Saline/C 2.8nmoles/kg/min(6) + 36µg/kg/min phentolamine	104 ±12	63 ± 4	P<0.05	1.44 ±.12	1.48 ±.20	N.S.

Table 5.10

The action of the α-agonist phenylephrine on intestinal fluid absorption. Absorption measured in two consecutive periods, all groups had saline infusion in 1st period. Phenylephrine dissolved in 0.9% saline containing 4mM tartrate infused in 2nd period. Results expressed as mean ± S.E.M. compared by Student's paired t test. Number of observations in parenthesis.

	Blood Pressure (mmHg)		Sig	Fluid Transport (ml/hr/g wet weight)		Sig
	Period 1	Period 2		Period 1	Period 2	
Control						
Saline/saline-tartrate (5)	95 ± 5	90 ± 3	N.S.	0.92 ±.16	1.28 ±.20	N.S.
Experimental						
Saline/Phenylephrine (6) + 7nmoles/kg/min	98 ± 4	123 ± 7	P<0.01	1.00 ±.12	1.80 ±.16	P<0.01

Section 3.2

ANGIOTENSIN AND THE NERVOUS SYSTEM

3.2) Angiotensin and the Nervous System.

Both noradrenaline and angiotensin have been shown to have actions on intestinal fluid transport both *in vivo* (Hubel, 1976; Bolton et al., 1975) and *in vitro* (Brasitus, Field and Kimberg, 1976; Davies et al., 1970). These findings, together with the knowledge that angiotensin has been shown to release noradrenaline from both sympathetic nerve endings (Starke, 1977) and the adrenal medulla (Peach, 1971), led Levens and coworkers (1979) to investigate the possibility that angiotensin may be having its stimulatory actions on intestinal fluid transport through the release of noradrenaline. These workers found support for this idea as the action of both agents could be blocked by blockade, and depletion of endogenous noradrenaline by reserpine prevented the angiotensin response (Levens, Munday, Parsons, Poat and Stewart, 1978).

3.2.1) Mechanism of Action of Angiotensin.

Although further work using the noradrenaline releasing agent tyramine (Munday, Parsons, Poat and Upsher, 1980) and the monoamine oxidase inhibitor pargyline (section 3.1.2) has confirmed that endogenous noradrenaline can stimulate intestinal fluid transport little is known of either the site or mechanism by which angiotensin may act on the sympathetic nervous system. Starke (1977) suggests a number of mechanisms of angiotensin enhanced noradrenaline release that may occur in various tissues, including an increase in stimulus evoked release, an increase in the basal overflow or an inhibition of neuronal uptake of noradrenaline.

If sympathetic nerve firing is necessary for the action of angiotensin on the intestine then prevention of nerve firing by a

procedure such as ganglion blockade would be expected to prevent angiotensin's action. In this study the effect of ganglion blockade was investigated. The ganglion blocker pentolinium at a dose of 30mg/kg i.p. produces a good sympathetic blockade demonstrated by a drop in systemic blood pressure from 90 ± 3 to 53 ± 3 (9) mm Hg. This blood pressure fall remains steady over the 30 minutes of the two consecutive time periods indicating a reliable ganglion blockade. Fluid transport was measured in two consecutive periods in both blocked and control animals. The second experimental period of angiotensin or noradrenaline infusion is expressed as a percentage of the initial control period (see table 5.11). Angiotensin is unable to stimulate intestinal fluid transport in ganglion blocked animals, although the stimulatory response of noradrenaline is unimpaired. Pentolinium itself has no effect on basal transport rate, transport being 1.38 ± 0.12 (9) in control and 1.44 ± 0.11 (7) in pentolinium-treated. These results are consistent with the hypothesis that angiotensin stimulates fluid transport by increasing stimulus evoked release from firing intestinal sympathetic neurones.

3.2.2) Site of Action of Angiotensin.

The fluid transport stimulating actions of angiotensin have been shown to be likely to occur through the release of noradrenaline. However, angiotensin has been shown to be capable of stimulating release from both sympathetic nerves and the adrenal medulla (Peach, 1977). Angiotensin will still stimulate transport in isolated *in vitro* everted sacs (Davies et al., 1970) and is capable of stimulating transport *in vivo* following adrenalectomy (Mariscotti, 1980) but the evidence still remains largely indirect that angiotensin is acting on the sympathetic nerves.

Table 5.11 The effect of 30mg/kg pentolinium tartrate given i.p. 2 minutes before the start of the first absorption period on the responses of rat jejunum *in vivo* to i.v. infusions of angiotensin II and noradrenaline. Saline was infused during the first control absorption period and saline or saline containing angiotensin II (7pmol/kg/min) or noradrenaline (7nmol/kg/min) during the second absorption period. Values are given as the means ± S.E.M. and expressed as a percentage of the 2nd period compared with the 1st. Results compared by Student's unpaired t test. Number of observations in parenthesis.

Infusion during 2nd period	Fluid transport 2nd period as % of 1st	Sig
0.9% saline	119 ± 8.2 (29)	N.S.
0.9% saline + Pentolinium	127 ± 29 (7)	P<0.01
Angiotensin II	178 ± 17 (32)	N.S.
Angiotensin II + Pentolinium	109 ± 7.5 (11)	
Noradrenaline	172 ± 24 (8)	N.S.
Noradrenaline + Pentolinium	180 ± 25 (8)	

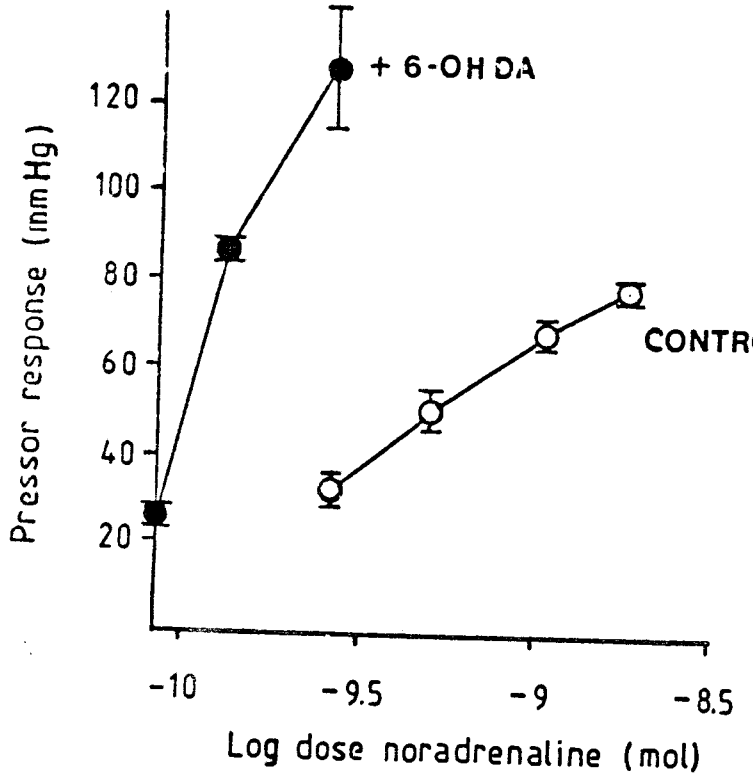
In this study the effect of destruction of the sympathetic nerves by pretreatment with 6-hydroxydopamine on angiotensin stimulated fluid transport was investigated. 6-hydroxydopamine causes selective destruction of adrenergic nerve terminals (Tranzer and Thoenen, 1968; Bennett, Burnstock, Cobb and Malmfors, 1970). The protocol of Finch, Haeusler and Thoenen (1973) (2 x 50mg/kg on day 1; 2 x 100mg/kg on day 7, administered intravenously through chronic cannulae) was used as these workers had shown a complete impairement of adrenergic nerve function 24 hours following the final dose of 6-hydroxydopamine.

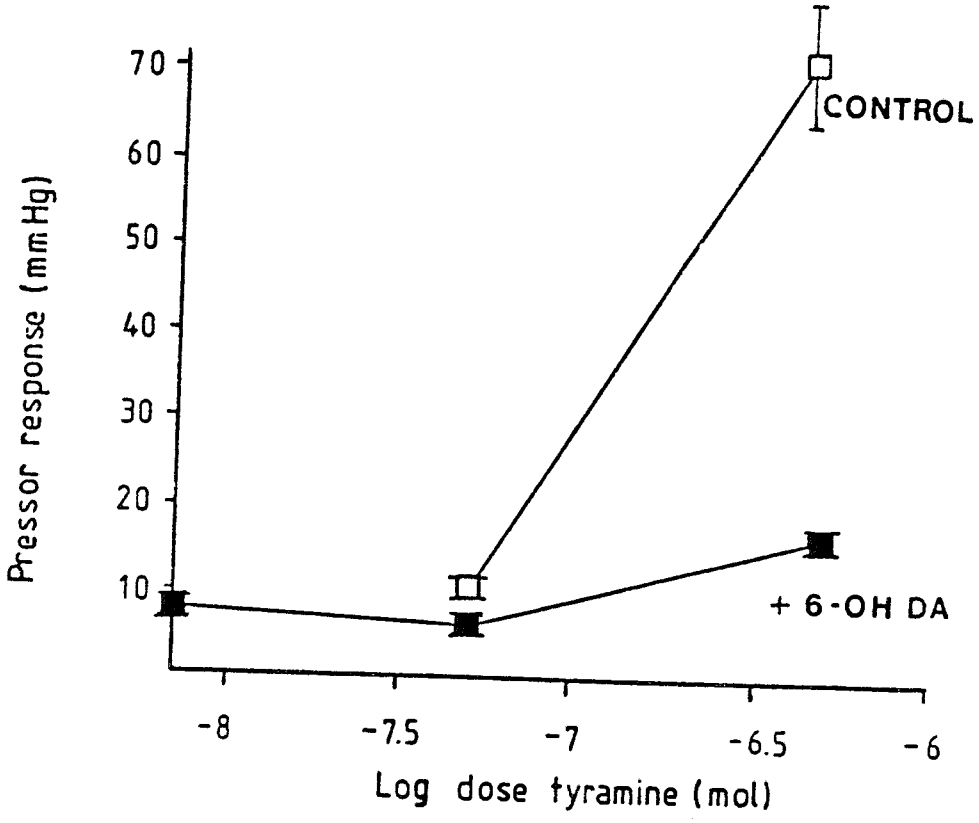
Prior to the transport studies the effectiveness of the sympathectomy was tested using the rat blood pressure preparation. Figure 5.5 shows the results obtained for the pressor effects of both noradrenaline and tyramine. Noradrenaline shows the expected supersensitivity resulting from receptor proliferation following good denervation (Langer 1975). The noradrenaline releasing agent tyramine has a much reduced pressor response due to depleted noradrenaline stores.

As a second test, following each transport study the anococcygeus muscle was removed from the animal and tested for response to field stimulation (Gibson and Gillespie, 1973). The anococcygeus has a dense motor adrenergic innervation but is particularly resistant to 6-hydroxydopamine. The greatly attenuated response of denervated animals shown in figure 5.6 further confirms the good denervation occuring on 6-hydroxydopamine pre-treatment. Curiously the anococcygeus preparation did not show supersensitivity to added noradrenaline, although this may be a result of its resistance to complete adrenergic destruction.

Figure 5.5 The effect of chemical sympathectomy by 6-hydroxydopamine on the pressor response obtained from exogenous injections of tyramine and noradrenaline. Sympathectomised animals have an increased pressor response to noradrenaline, and a reduced response to the noradrenaline releasing agent tyramine.

Results are expressed as mean ± S.E.M. of groups of between 2 - 4 animals.


+ 6-OH DA
CONTROL
Pressor response (mm Hg)
120
100
80
60
40
20
-10
-9.5
-9
-8.5
Log dose noradrenaline (mol)


CONTROL
+ 6-OH DA
Pressor response (mm Hg)
70
60
50
40
30
20
10
-8
-7.5
-7
-6.5
-6
Log dose tyramine (mol)

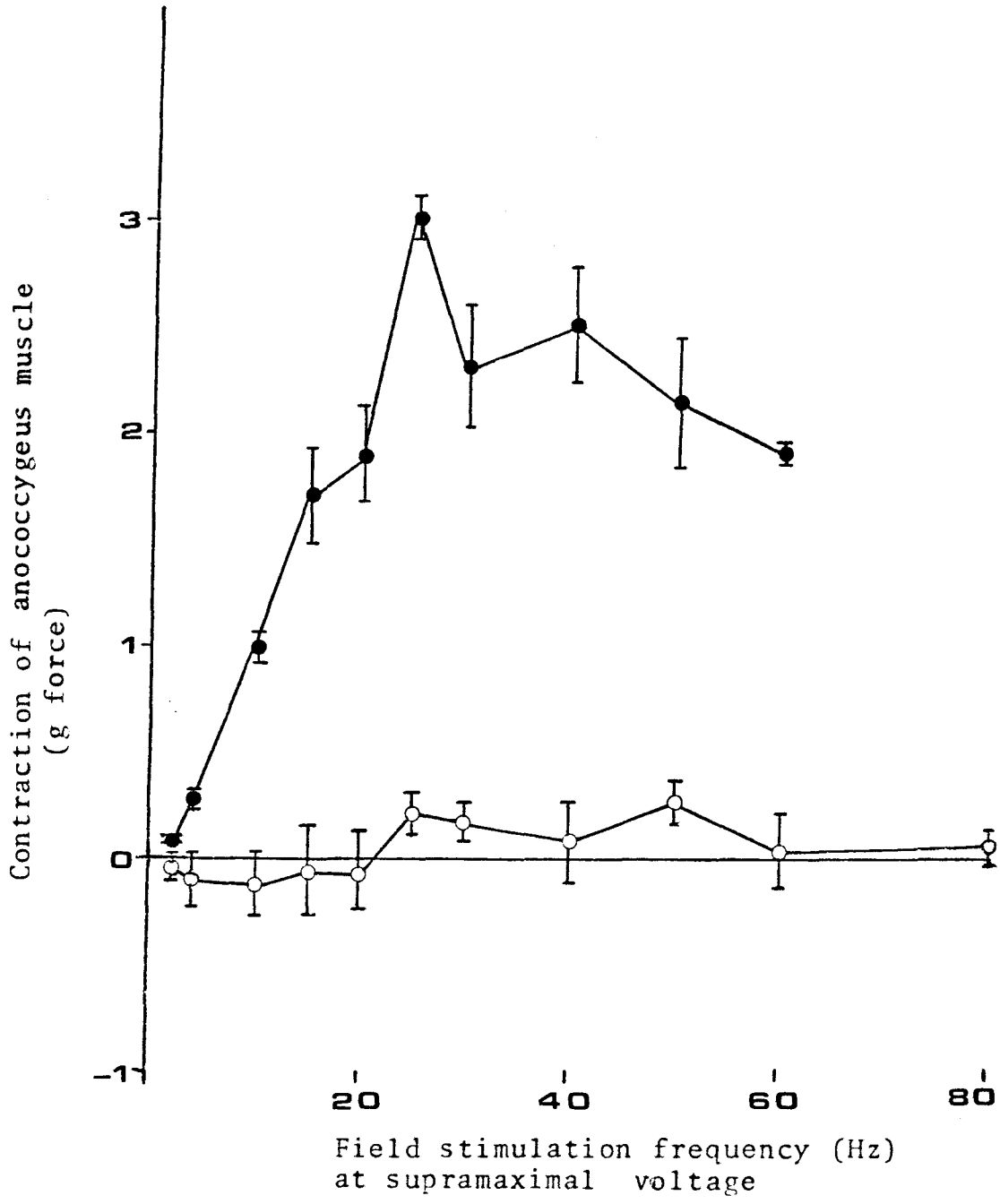


Figure 5.6 The effect of chemical sympathectomy with 6-hydroxydopamine on the contractility of the anococcygeus muscle in rats. Untreated animals (●) Treated animals (○). Results expressed as mean ± S.E.M. for results pooled from two groups of 8 animals.

Transport studies were carried out as two consecutive transport periods comparing an initial control period with a following experimental period of angiotensin or noradrenaline infusion (see table 5.12). Pre-treatment with 6-hydroxydopamine abolished the response to angiotensin, whilst the response to exogenous noradrenaline remained unaffected. A comparison of the transport rates of saline-treated controls (1.45 ± Ø.18 ml/hr/g wet wt) with those of 6-hydroxydopamine pre-treated saline controls (1.42 ± Ø.23 ml/hr/g wet wt) showed that there were no significant changes.

These findings are consistent with the view that angiotensin has its stimulatory actions on intestinal fluid transport through effects on the sympathetic nervous system. Chemical sympathectomy destroys the adrenergic nerves whilst leaving the postsynaptic α-receptors intact (Kostrezewa and Jacobowitz, 1974), therefore removing the site of action of angiotensin, but allowing exogenous noradrenaline to still have its effect.

3.2.3) The Role of the Sympathetic Nervous System.

The above results illustrate the role of the sympathetic nervous system in angiotensin-stimulated transport. Although, as previously discussed, little is known about the exact site of action of angiotensin upon the sympathetic nervous system. Still less is known about the interaction of the sympathetic nerve supply itself with intestinal transport processes (see section 1.2.1Ø). The pithed rat preparation of Gillespie, Maclaren and Pollock (197Ø) has been used for a number of nerve studies and is particularly useful as it allows stimulation of discrete segments of the spinal cord with minimal disturbance of the nerve supply. Preliminary studies with this preparation demonstrated that spinal segments could indeed be easily

Table 5.12

The effects of treatment by 6-hydroxydopamine (6-OHDA) on the responses of rat jejunum *in vivo* to i.v. infusions of angiotensin II and noradrenaline. Saline (Ø.9%) was infused i.v. during the first control absorption period and saline or saline containing angiotensin II or noradrenaline during the second absorption period. Consecutive periods compared by Student's paired t test and expressed as mean ± S.E.M.. Number of observations in parenthesis.

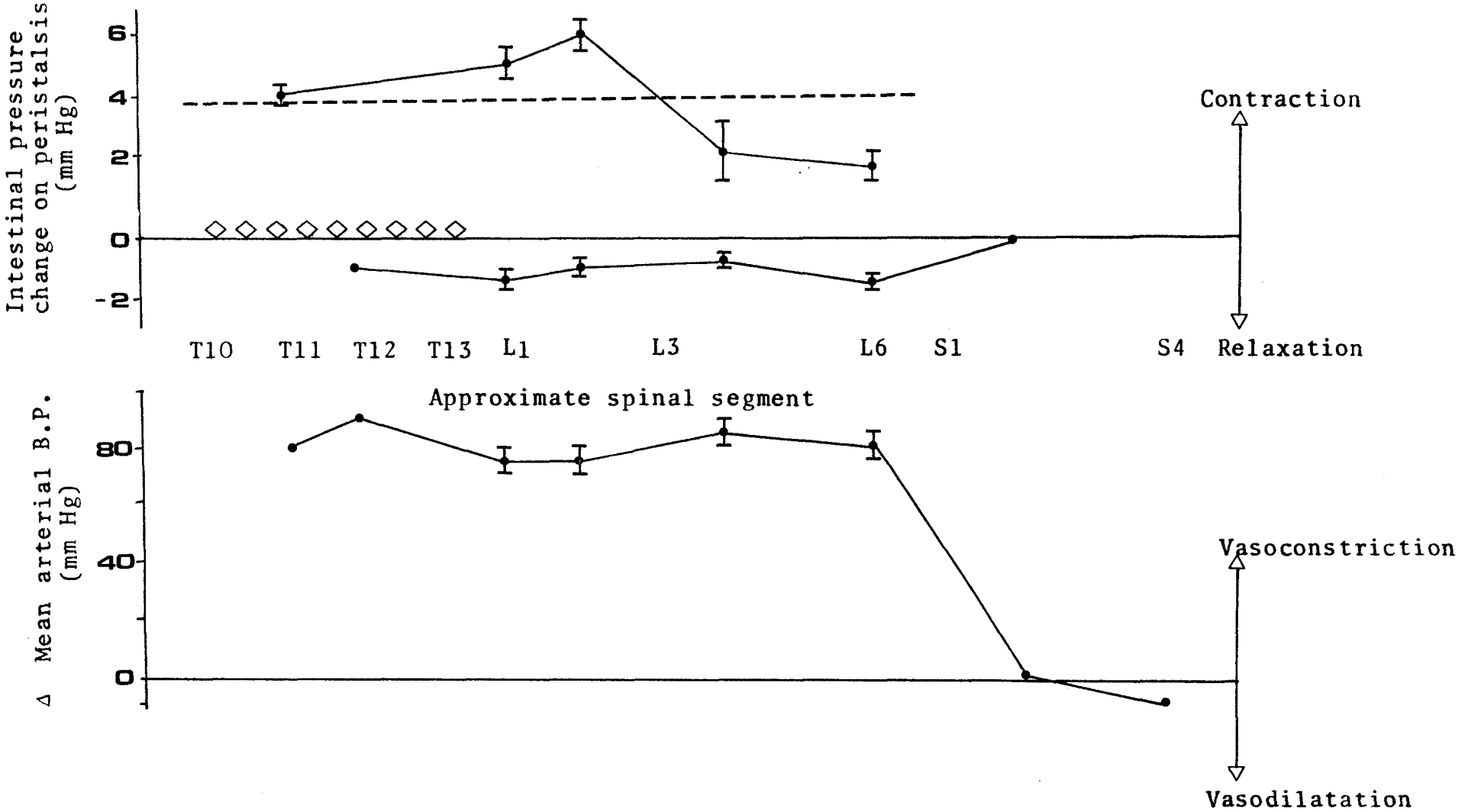
Infusion in 2nd period	Fluid Transport (ml/hr/g wet weight)		Sig
	Period 1	Period 2	
Control			
Ø.9% saline (4)	1.55 ±.12	1.35 ±.14	N.S.
Angiotensin II 7pmol/kg/min (5)	1.49 ±.14	2.16 ±.15	P<Ø.Ø1
Noradrenaline 7nmol/kg/min (4)	1.17 ±.21	2.Ø3 ±.22	P<Ø.Ø5
6-OHDA Treated			
Ø.9% saline (4)	1.37 ±.Ø4	1.47 ±.23	N.S.
Angiotensin II 7pmol/kg/min (5)	1.65 ±.11	1.57 ±.15	N.S.
Noradrenaline 7nmol/kg/min (6)	1.16 ±.19	2.32 ±.44	P<Ø.Ø5

located, and a profile showing alterations in intestinal motility and mean arterial blood pressure was produced (figure 5.7).

The results of the initial studies on intestinal absorption in this pithed preparation are shown in figure 5.8. Absorption was both low and erratic in the pithed animals compared with normal anaesthetised controls. Pithed animals have lost all sympathetic tone and have a concomitantly lower blood pressure (reduced from 87 ± 8 (5) to 38 ± 1 (9) mm Hg.). It is possible that this reduced blood pressure may reduce the intestinal perfusion pressure with a resultant reduction in the ability of the intestine to clear metabolites. However, neither increasing the duration of the recovery period following surgery (table 5.13) nor carrying out surgery prior to pithing (table 5.14) produced a stable linear transport from the pithed preparations. Administration of Ø.5mg/kg atropine produced a stable, although reduced intestinal transport indicating that to some extent a cholinergic imbalance was occuring.

Many anaesthetics are known to produce artefacts in animal preparations and the ether anaesthetic used prior to pithing is known to stimulate cholinergic effects. The possibility that the anaesthetic ether might be having some action producing poor absorption in this preparation was investigated using sodium pentobarbitone (7Ømg/kg i.p.). Table 5.15 shows the results for this study and demonstrates that nembutal—anaesthetised animals have greatly improved transport than their ether anaesthetised counterparts. The results also show that intestinal transport is beginning to deteriorate in pithed animals if a 1 hour surgical recovery period is used, this recovery period was therefore reduced to 15 minutes.

Figure 5.7 The influence of stimulating discrete spinal segments by the method of Gillespie et al., (1970) on mean arterial blood pressure and depth of peristaltic relaxation and contraction in proximal jejunum. Basal pressure prior to stimulation is denoted by the dotted line. Regions at which spinal stimulation produces an increase in heart rate are shown by: ◇◇◇◇◇◇. Results are expressed as mean ± S.E.M. and are pooled from observations in three animals.


Intestinal pressure change on peristalsis (mm Hg)
6
4
2
0
-2
Contraction
Relaxation
T10
T11
T12
T13
L1
L3
L6
S1
S4
Approximate spinal segment
Δ Mean arterial B.P. (mm Hg)
80
40
0
Vasoconstriction
Vasodilatation

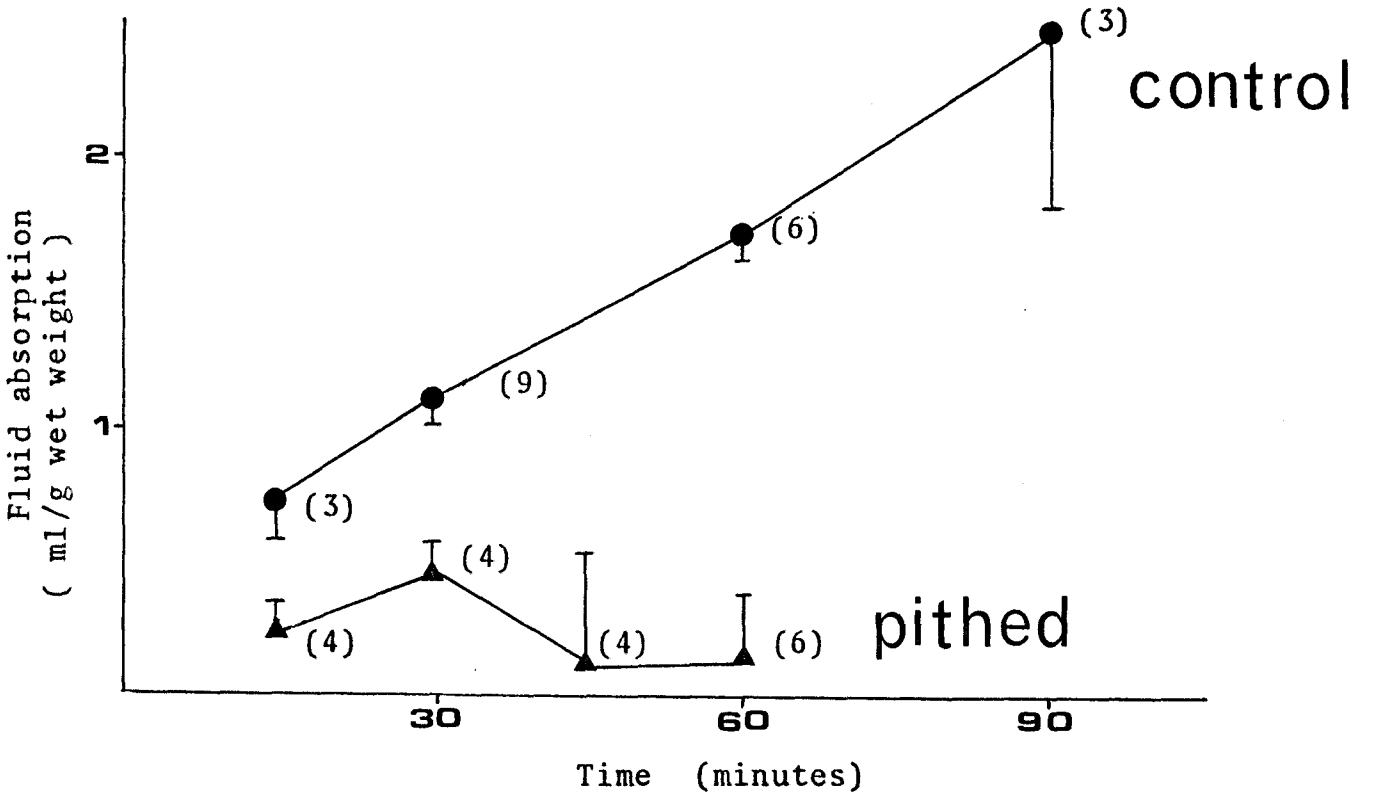


Figure 5.8 Transport rate from jejunal sacs over different time periods. Transport for ether anaesthetised pithed rats compared with nembutal anaesthetised un-pithed. Results expressed as mean ± S.E.M. Number of animals in parenthesis.

Table 5.13

The effect of increasing the recovery period after preparation of pithed, ether anaesthetised, animals to allow clearance of metabolites. Results expressed as mean ± S.E.M., negative values indicate net secretion. Number of observations in parenthesis.

Surgical recovery period	Fluid Transport (ml/hr/g wet weight)	
	Period 1	Period 2
15 minutes (3)	Ø.88 ±.ØØ	Ø.2Ø ±.44
3Ø minutes (5)	2.36±1.48	Ø.48 ±.32
6Ø minutes (4)	1.64 ±.52	-Ø.24 ±.12
6Ø minutes (4) + Ø.5mg/kg atropine i.p.	Ø.68 ±.28	Ø.6Ø ±.12
Control (unpithed) (6) 15 minutes recovery	1.36 ±.44	1.6Ø ±.4Ø

Table 5.14

The effect of preparing the gut sac prior to pithing in ether anaesthetised pithed rats. One hour recovery period from surgery. Results expressed as mean ± S.E.M., negative values indicate net secretion. Number of observations in parenthesis.

Treatment	Fluid Transport (ml/hr/g wet weight)	
	Period 1	Period 2
Control (un-pithed) (9)	1.76 ±.4Ø	1.28 ±.32
Pithed prior gut preparation (3)	Ø.28 ±.32	1.68 ±.56
Pithed later gut preparation (4)	1.64 ±.52	-Ø.24 ±.12

Table 5.15 Results showing the effects of anaesthesia with ether and sodium pentobarbitone on the intestinal fluid transport in pithed rats. Results expressed as absorption rate in ml/hr/g wet weight tissue. Absorption measured as a single 15 minute absorption period in proximal jejunum. Values given as mean ± S.E.M. and compared with non-pithed control animals by Student's unpaired t test.

Treatment	Absorption rate		Sig
1 hour recovery			
Pithed + ether	0.53 ± 0.16	(12)	P < 0.001
Control	1.97 ± 0.11	(10)	
Pithed + pentobarbitone	1.13 ± 0.21	(7)	P < 0.01
15 minute recovery			
Pithed + ether	-0.16 ± 0.48	(3)	P < 0.01
Control	1.58 ± 0.16	(4)	
Pithed + pentobarbitone	1.76 ± 0.16	(3)	N.S.

Despite the improvements produced on the change of anaesthesia to sodium pentobarbitone and the reduction of the surgical recovery period to 15 minutes, the pithed preparation still had to be tested for linearity of transport in consecutive periods. Table 5.16 and table 5.17 show the results for 2 and 3 consecutive 15 minute periods respectively. Transport was found to be roughly linear but was still lower than in unpithed animals. Furthermore, the variation between different experiments was found to be high, producing standard errors as much as 50% of the mean. It is possible that the low perfusion pressure in this preparation is producing an impairment of intestinal function (Winne, 1979; Varro, Blaho, Csernay, Jung and Szarvas, 1965; Shepherd, 1979). Samples of intestine from both pithed and control animals were therefore compared histologically, according to the scheme of Chiu, McArdle, Brown, Scott and Gurd (1970). No obvious lesion damage was seen in either group, although a large number of goblet cells were present in the villi of the pithed animals. Owing to the high variability still experienced with the transport studies from this preparation, its use was discontinued.

In a number of preparations, for example in the cat (Brunsson et al., 1979), the sympathetic innervation can be stimulated by the splanchnic outflow feeding the viscera. The large splanchnic nerve bundle can be dissected off the mesenteric artery about 1cm distal to its origin in the aorta. This preparation was attempted in the rat with a number of dissection techniques and a variety of electrodes. The results in table 5.18 demonstrate that the nerve preparation disturbs the normal absorptive physiology, affecting linear transport. Even when great care was taken to prevent any restriction of the superior mesenteric artery the results for control studies still proved to be

Table 5.16 Comparison between sodium pentobarbitone anaesthetised pithed rats and sodium pentobarbitone anaesthetised non-pithed rats. Experiments were conducted with 15 minutes recovery period from surgery. Results expressed as absorption rate in ml/hr/g wet weight tissue. Values presented as mean ± S.E.M. Number of observations in parenthesis. Period 1 and period 2 are two consecutive periods of fluid absorption which are compared by Student's paired t test.

Treatment	Fluid absorption		Sig
	Period 1	Period 2	
Pithed (MABP = 38 ± 1)	1.52 ± 0.20	1.00 ± 0.20	N.S.
Control (MABP = 87 ± 8)	1.32 ± 0.24	1.44 ± 0.12	N.S.

Table 5.17

Test of linearity of transport in anesthetised pithed rats compared with un-pithed controls. Results expressed as mean ± S.E.M.. Number of observations in parenthesis.

Group	Fluid Transport (ml/hr/g wet weight)		
	Period 1	Period 2	Period 3
Control (4)	1.12 ±.36	1.28 ±.12	1.00 ±.16
Pithed (7)	0.72 ±.03	0.56 ±.28	0.60 ±.16

Table 5.18

Results showing splanchnic nerve stimulation and effects of splanchnic nerve preparation on jejunal fluid absorption. Stimulation given as '30-seconds-on 30-seconds-off' bursts for the whole 2nd 15 minute period. Results expressed as mean ± S.E.M.. Number of observations in parenthesis.

Procedure	Fluid Transport (ml/hr/g wet weight)			
	Period 1	Period 2	Period 3	
No electrode	2.00 ±.52	2.60 ±.56	2.00 ±.24	(4)
Electrode no stimulation	1.36 ±.16	2.20 ±.32	2.16 ±.28	(6)
5Hz 20V stim.	1.00 ±.20	1.80 ±.16	1.60 ±.28	(5)
8Hz 20V stim.	1.32 ±.20	1.44 ±.04	1.44 ±.08	(3)
10Hz 20V stim.	0.92 ±.24	2.48 ±.32	1.96 ±.24	(4)

highly variable. In the light of this erratic baseline this preparation was considered unsuitable for the required studies.

Section 3.3

INTESTINAL TRANSPORT IN THE SPONTANEOUSLY HYPERTENSIVE RAT

3.3) Intestinal Transport in the Spontaneously Hypertensive Rat.

The Okamoto strain of spontaneously hypertensive rat (SHR) have been shown to have a number of alterations in physiology that are of interest in the study of salt and water homeostasis. SHRs have been reported as having a greater salt appetite and fluid intake than normotensive controls (Catalanotto et al., 1972; Fregly, 1975) as well as alterations in the renal handling of sodium (Farman and Bonvalet, 1975; Willis et al., 1976). Furthermore, these animals are known to have an increased level of sympathetic activity (Okamoto et al., 1967) and altered renin-angiotensin system activity (Czyzewski and Pettinger, 1973), two factors known to influence the transport of salt and water by the intestine. No literature exists on the properties of intestinal salt and water transport in these animals so a study was undertaken.

3.3.1) SHR Transport Compared with Wistar.

An initial absorption study was carried out comparing the fluid absorption in male SHRs with male wistar normotensive controls. Table 5.19 shows the results of this study, demonstrating that SHRs have linear transport over two consecutive periods and that fluid absorption rate is significantly higher than that of controls. This transport rate was so much increased that the absorption time period used for SHRs was reduced from 15 to 10 minutes in order to ensure that transport remained linear. This study was continued with age-matched hypertensive (BP = 163 ± 6 (29) mm Hg.) and normotensive (BP = 114 ± 5 (9) mm Hg.) animals greater than 13 weeks of age to ensure that the SHR were on the hypertensive plateau (see figure 3.1). Table 5.20 shows the absorption rate for both groups of animals expressed both per g wet weight and per g dry weight of tissue. The tissue water content is significantly reduced in SHR although transport is still significantly increased when

Table 5.19 Investigation into the rate and linearity of intestinal fluid absorption in spontaneously hypertensive rats compared with normotensive wistar animals. Results show two consecutive absorption periods, each of 10 minute duration for SHR and of 15 minute duration for wistar. Values presented as mean ± S.E.M. Number of observations in parenthesis. Consecutive periods compared by Student's paired t test.

Group	Blood Pressure (anaesthetised)	Fluid transport (ml/hr/g wet weight)		Sig
		Period 1	Period 2	
Wistar(4)	95 ± 4	1.12 ± 0.12	1.32 ± 0.20	N.S.
SHR(6)	131 ± 7	2.04 ± 0.16	2.00 ± 0.12	N.S.

Table 5.20 Comparison of basal transport rate between wistar and spontaneously hypertensive rats. Absorption from proximal jejunum measured as a single 15 minute period by isotope Animals in both groups were over 13 weeks of age. Results expressed as mean ± S.E.M. and compared by Student's unpaired t test. Number of observations in parenthesis.

	Wistar	SHR	Sig
Conscious blood pressure (mm Hg)	114 ± 5 (9)	163 ± 6 (29)	P<0.001
Fluid transport (ml/hr/g wet weight)	1.32±.04(26)	1.96±.08(33)	P<0.001
Fluid transport (ml/hr/g dry weight)	5.68±.28(16)	8.12±.44(25)	P<0.001
Tissue water content (ml/g dry weight)	3.82±.04(46)	3.29±.07(25)	P<0.001

Table 5.21 Comparison of basal transport between young (less than 7 week old) male wistar and spontaneously hypertensive animals. Absorption measured as a single 15 minute period by isotope. Results expressed as mean ± S.E.M. and compared by Student's unpaired t test. Number of observations in parenthesis.

	Wistar (6)	SHR (4)	Sig
Fluid transport (ml/hr/g wet weight)	1.24±0.12	2.72±0.20	P<0.001
Fluid transport (ml/hr/g dry weight)	6.72±0.80	14.80±1.40	P<0.01
Tissue water content (ml/g dry weight)	4.47±0.12	4.38±0.11	N.S.

expressed per g dry weight. Tissue dry weights were not signficantly different between groups of animals (table 5.20).

It is possible that the increased rate of intestinal transport could be the direct result of the increased blood pressure in the SHR group of rats. This was tested by investigating intestinal transport in young male pre-hypertensive animals (<7 weeks old). The results shown in table 5.21 demonstrate that fluid transport is still higher in SHR, although interestingly, in these young animals, there is no significant difference in tissue water content. Figure 5.9 shows a further comparison between fluid transport rate and age in SHRs. The fluid absorption rate is greater in the young pre-hypertensive animals and actually decreases with age and increasing blood pressure. This profile with age is reminiscent of similar figures illustrating the relationship between water intake and age (see figure 5.10).

Table 5.22 gives the results of similar experiments conducted with female animals. Curiously, although conscious blood pressure is increased in >13 week female SHR compared with age-matched controls, and the tissue water content of SHR is significantly less, the fluid transport of the normotensive control animals is at the same high level as the SHR.

3.3.2) Is Transport Related to Blood Pressure ?

If the increased blood pressure does lead to an increase in transport in SHR then these two factors should be related. Figures, 5.11, 5.12, and 5.13 show the results of plotting conscious blood pressure against fluid transport for male SHR and female SHR, and anaesthetised blood pressure against fluid transport for male SHR. In no case was any correlation observed, indicating that either the fluid

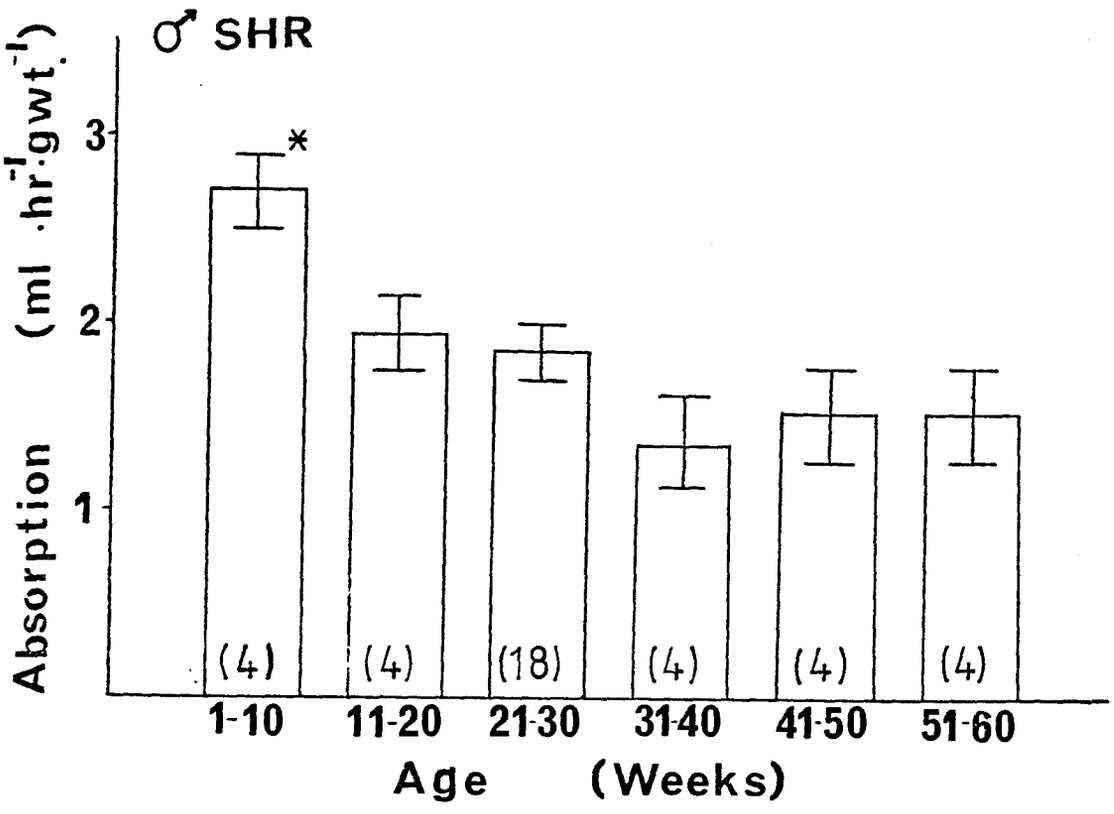
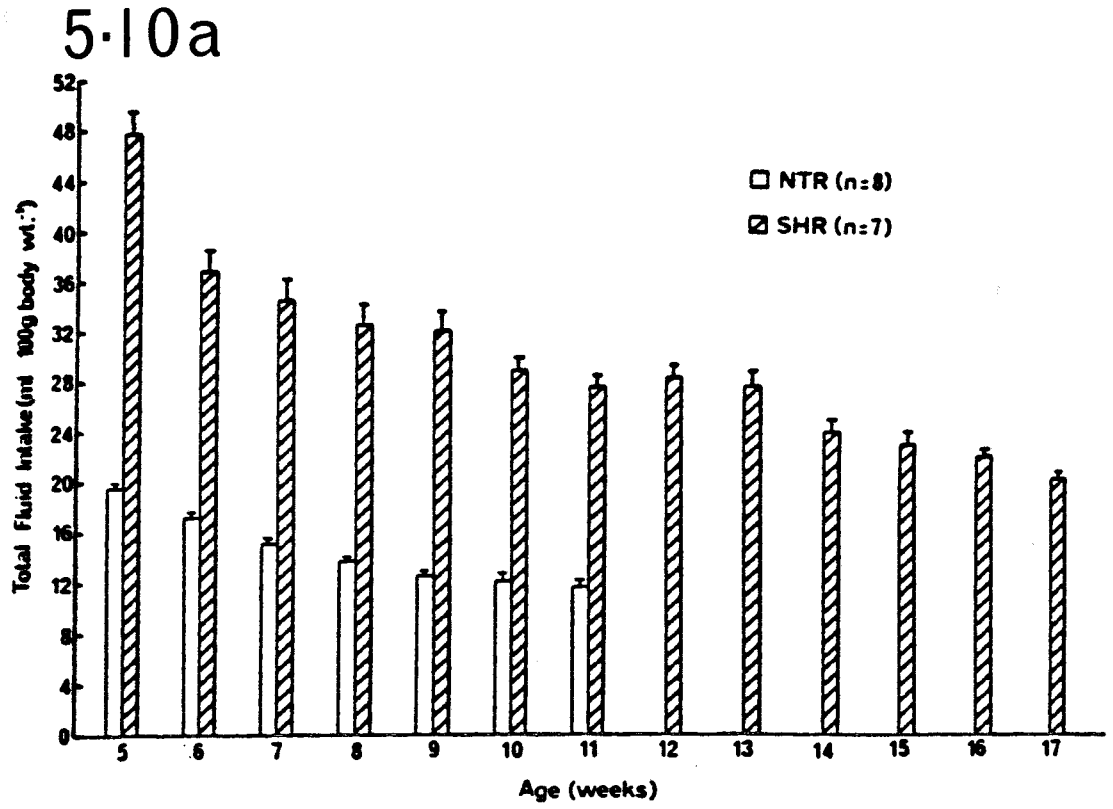


Figure 5.9 The alteration of intestinal fluid transport in the spontaneously hypertensive rat with age. Results expressed as mean ± S.E.M. Number of observations in parenthesis.
* denotes that first group is significantly different from all other groups when compared by Student's unpaired t test ($P < 0.05$).



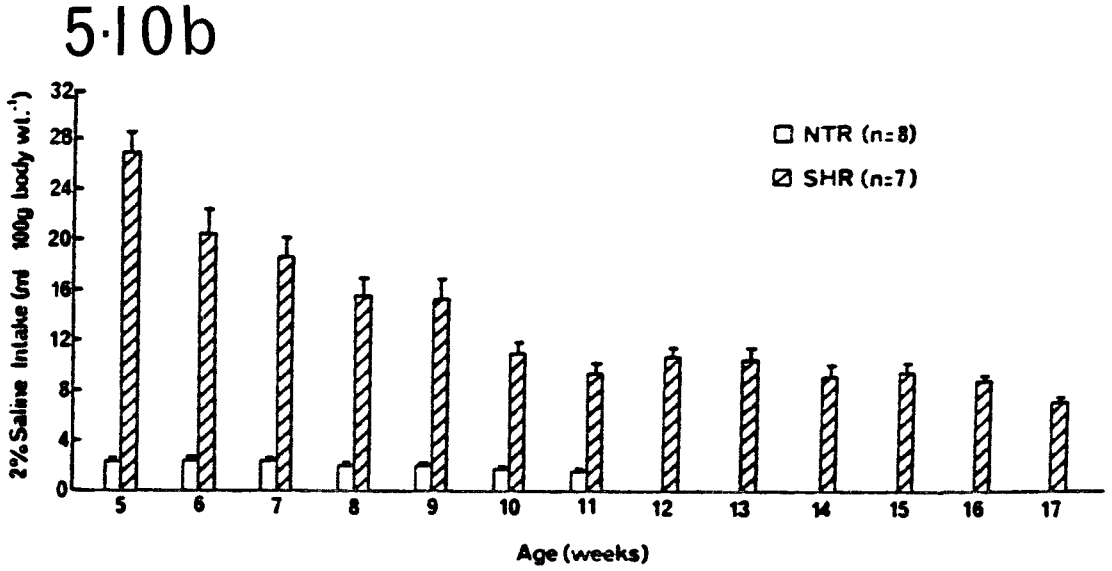


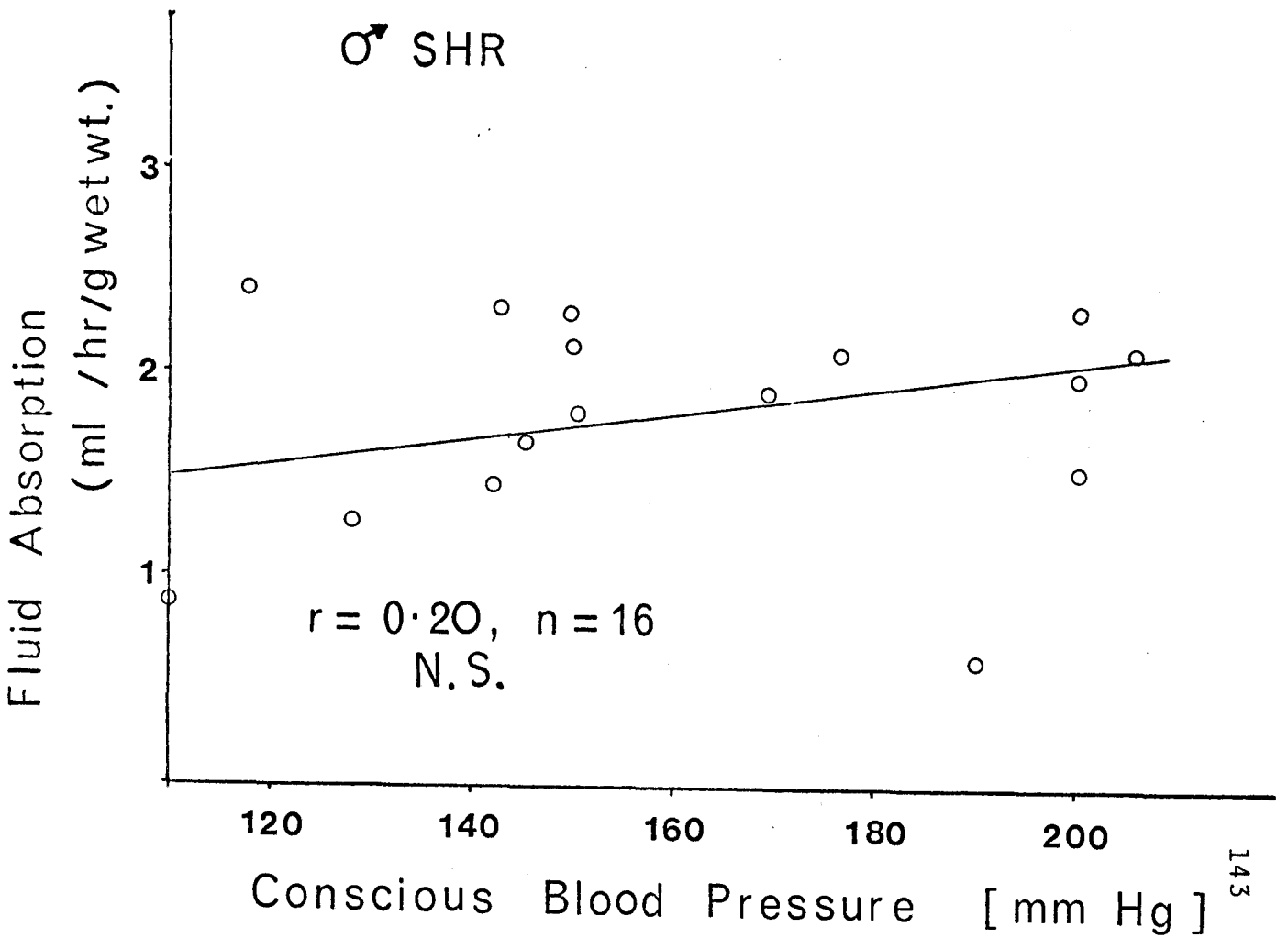
Figure 5.10 The 24 hour total fluid and 2% saline intakes of SHR and NTR at different ages. Values are the mean ± S.E.M. of the 24 hour intakes for each week.

Results reproduced from C.N. May (1980) with permission.

Table 5.22 Comparison of the basal transport between female wistar and spontaneously hypertensive rats. Absorption measured as a single 15 minute period by isotope. All animals were older than 13 weeks of age. Results expressed as mean ± S.E.M. and compared by Student's unpaired t test. Number of observations in parenthesis.

	Wistar (4)	SHR (14)	Sig
Conscious blood pressure (mm Hg)	118 ± 8	169 ± 5	P<0.001
Fluid transport (ml/hr/g wet weight)	2.04±0.32	2.16±0.12	N.S.
Fluid transport (ml/hr/g dry weight)	8.58±1.36	8.64±0.68	N.S.
Tissue water content	3.96±0.34	3.00±0.15	P<0.02

Figure 5.11 Fluid absorption in male spontaneously hypertensive rats plotted against conscious blood pressure. r is the correlation coefficient.



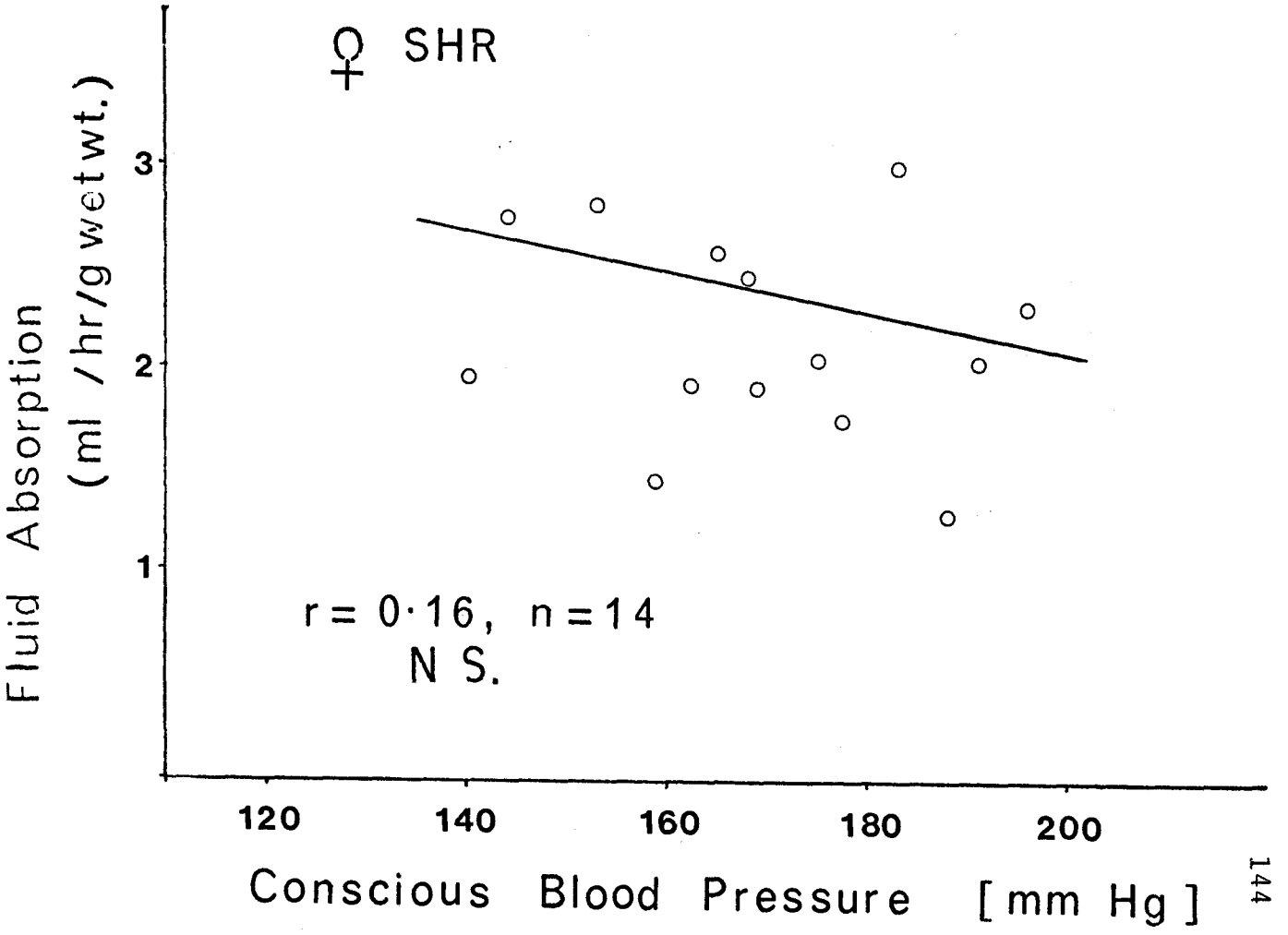


Figure 5.12 Fluid absorption in female spontaneously hypertensive rats plotted against conscious blood pressure. r is the correlation coefficient.

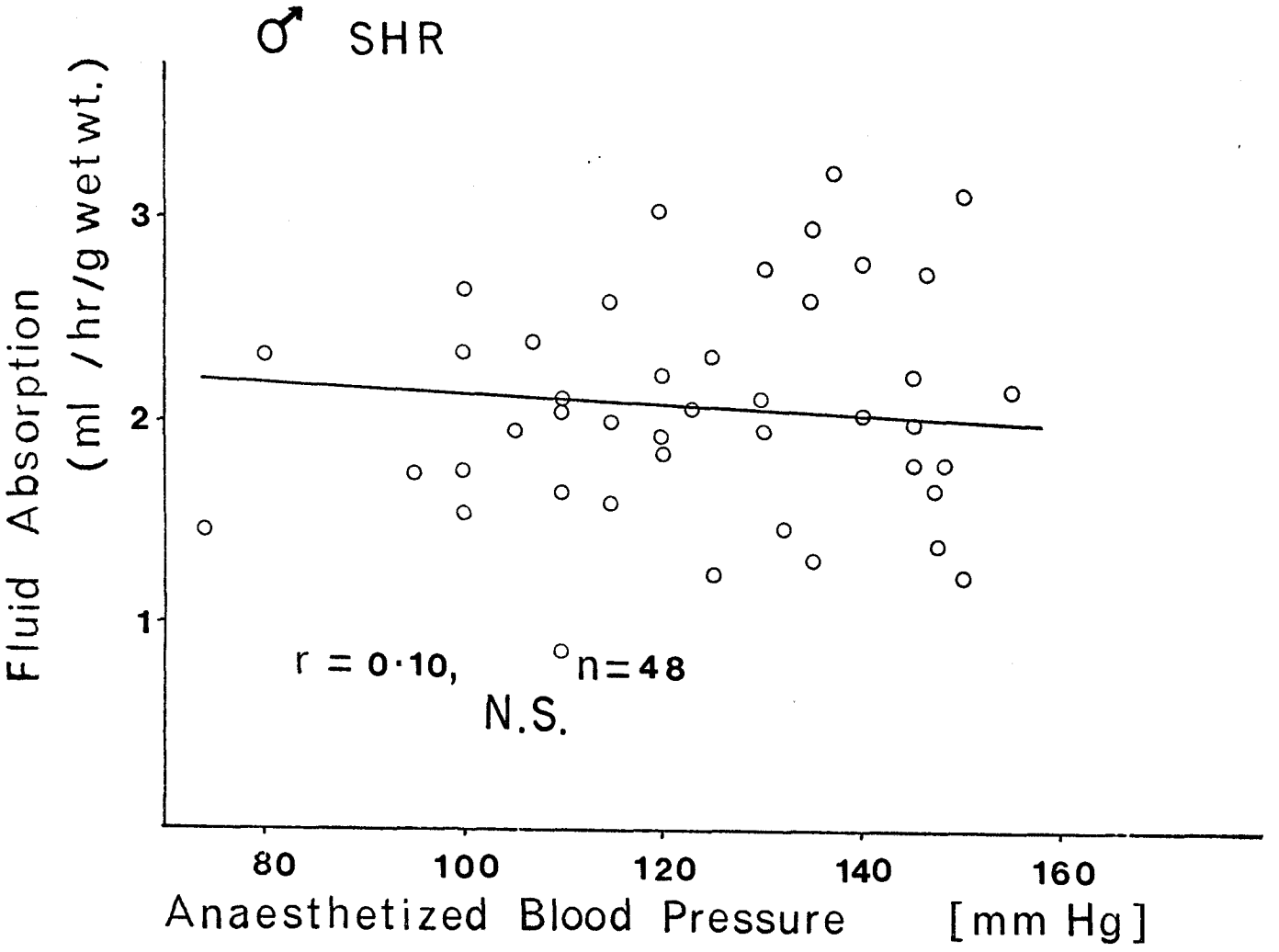


Figure 5.13
Results of fluid absorption studies plotted against the anaesthetised blood pressure for male spontaneously hypertensive rats. r is the correlation coefficient.

transport and blood pressure are unrelated or that some additional factor is masking any relationship.

3.3.4) The Action of Noradrenaline on SHR Fluid Transport.

This increase in intestinal transport could be a result of an increase in sympathetic activity leading to an increase in noradrenaline levels. Noradrenaline is well known as being able to stimulate intestinal transport in a dose dependent fashion. If noradrenaline was responsible for increased transport then a change in sensitivity to infused noradrenaline might be anticipated. In a series of standard two consecutive absorption period experiments the effect of infusions of various doses of noradrenaline on transport in SHR and wistar animals was studied. Figure 5.14 shows the result of this study. The results of which are shown as transport rate during noradrenaline infusion expressed as a percentage of the first control period during saline infusion. SHR show a decreased sensitivity to noradrenaline and only an infusion of noradrenaline at the rate of 70nmoles/kg/min will stimulate intestinal fluid transport. The slight inhibition of transport seen at the very high infusion dose of 700nmoles/kg/min is probably due to vascular effects.

SHR have been reported as having increased circulating levels of endogenous noradrenaline (Grobecker, et al., 1975). If this is the case then a reduced sensitivity to exogenous noradrenaline may very well be expected. In order to investigate the possibility that greater tissue noradrenaline levels may be present, sections of jejunum from 20 week old SHR were assayed for noradrenaline content and compared with wistar controls. However, table 5.23 presents the results of this assay, and show no significant difference between SHR and control tissue levels of noradrenaline. This appears to be inconsistent with the observed

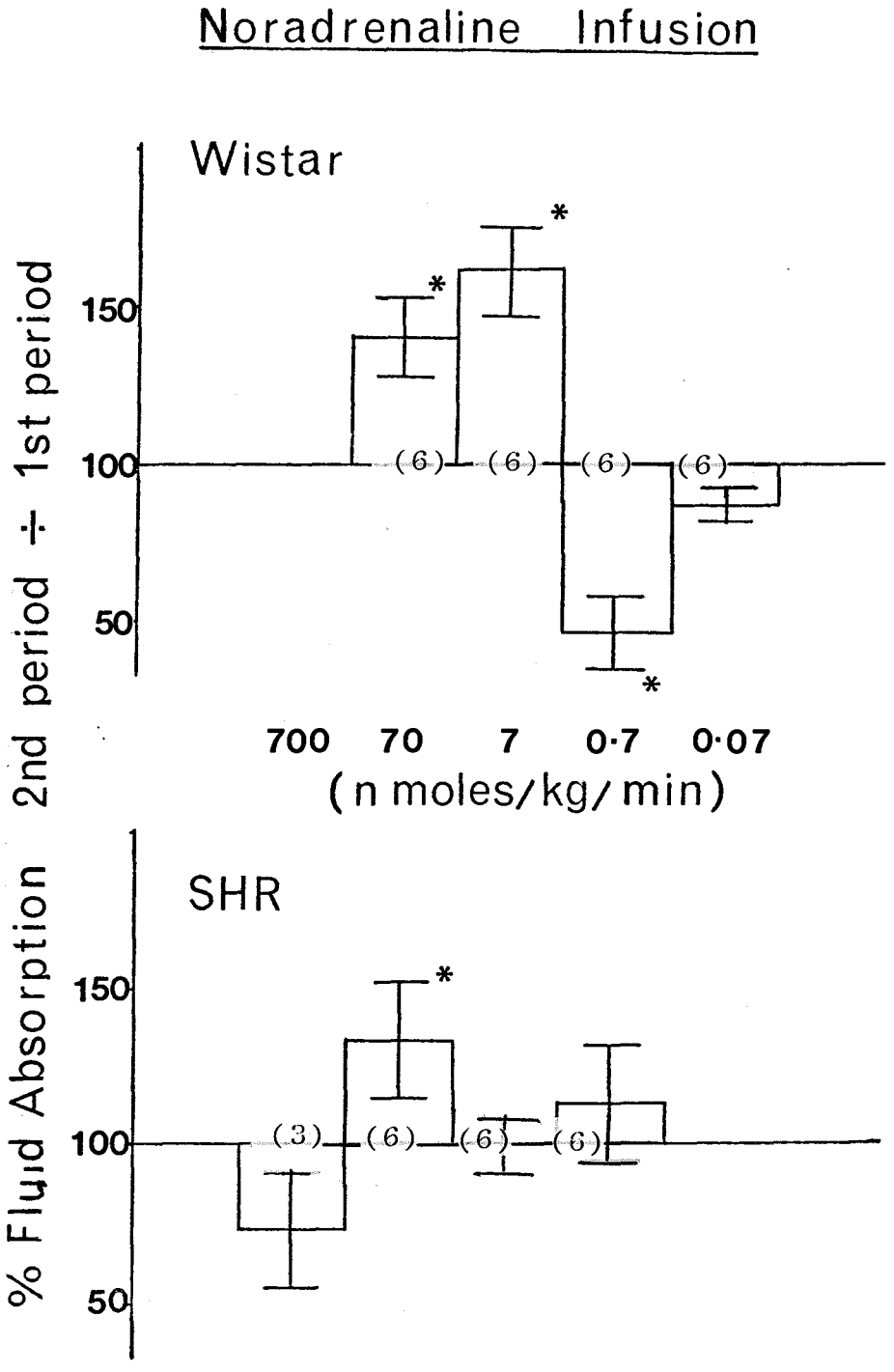


Figure 5.14 The action of different concentrations of noradrenaline bitartrate infusion on intestinal fluid transport in the in vivo jejunum of wistar and spontaneously hypertensive rats, Results expressed as mean ± S.E.M. First and second periods compared by Student's paired t test. * = $P < 0.05$

Table 5.23 The results of the assay for tissue noradrenaline in jejunal tissue from 20 week old wistar and spontaneously hypertensive male rats. Values are expressed as mean ± S.E.M. and compared by Student's unpaired t test. Number of animals in parenthesis.

	Wistar (3)	SHR (3)	Sig
Conscious blood pressure (mm Hg)	122 ± 4	203 ± 7	P<0.001
Noradrenaline content (µg/g wet weight)	0.81 ± 0.33	0.77 ± 0.16	N.S.

alteration in sensitivity.

3.3.5)Are the Alterations Seen in SHR Transport Due to Neural or Catecholamine Influences ?

Although changes were observed in the response of SH rats to exogenous noradrenaline, this result alone does not conclusively suggest that an increase in the levels of circulating noradrenaline are responsible for the observed increase in transport. A more conclusive result might be anticipated from the use of ganglion blockers and α-blockade in the SHR. Pentolinium tartrate given as an i.p injection prior to the second consecutive absorption period produced a significant fall in mean arterial blood pressure in both wistar and SHR. However, there was no significant reduction in the rate of intestinal transport for either group of animals (see figure 5.15). Although this result makes it unlikely that increased sympathetic activity is producing alterations in basal transport increased circulating levels of noradrenaline may have a similar effect.

Figure 5.16 gives the result of experiments conducted infusing the α-blocker phentolamine mesylate during the second period of fluid absorption. As with ganglion blockade phentolamine produces a significant fall in mean arterial blood pressure in both wistar and SHR animals, with no significant change in rate of fluid absorption. It can be argued that the dose of 36µg/kg/min for phentolamine infusion may not be sufficient to block the levels of noradrenaline present. However, this dose has been shown to satisfactorily block raised endogenous levels of noradrenaline produced by pargyline (section 3.1.2), and no real changes have been observed in actual tissue levels of noradrenaline in the SHR (section 3.3.4). From both these results it would seem unlikely that either the increased circulating levels of noradrenaline or the increased sympathetic activity reported for these

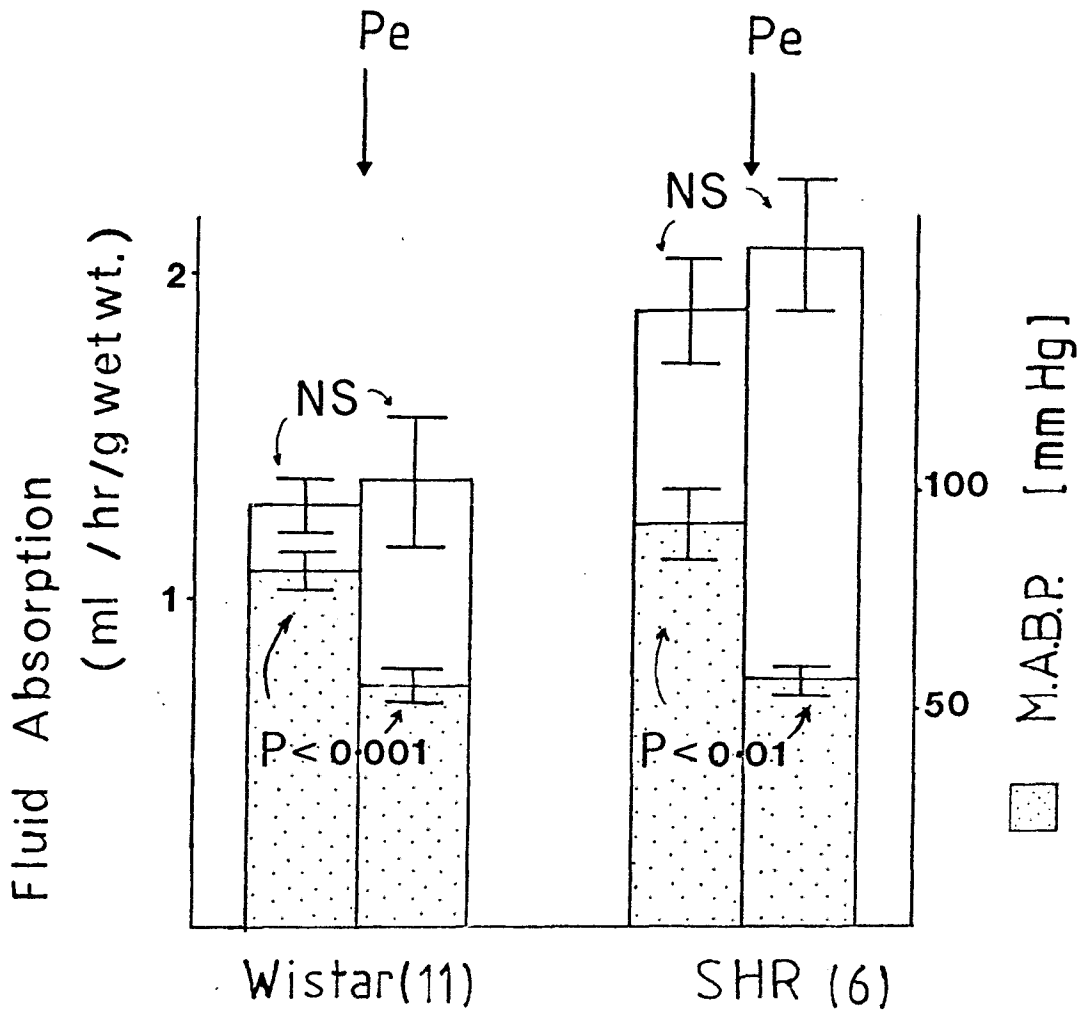


Figure 5.15 The action of the ganglion blocking agent pentolinium tartrate on intestinal fluid transport and mean arterial blood pressure in wistar and spontaneously hypertensive rats. Results expressed as mean ± S.E.M.. Number of observations in parenthesis. Paired histograms are data for 1st and second consecutive absorption periods. Pentolinium (Pe) injected at a dose of 30mg/kg i.p. 2 minutes prior to the start of the second absorption period.

Consecutive periods compared by Student's paired t test.

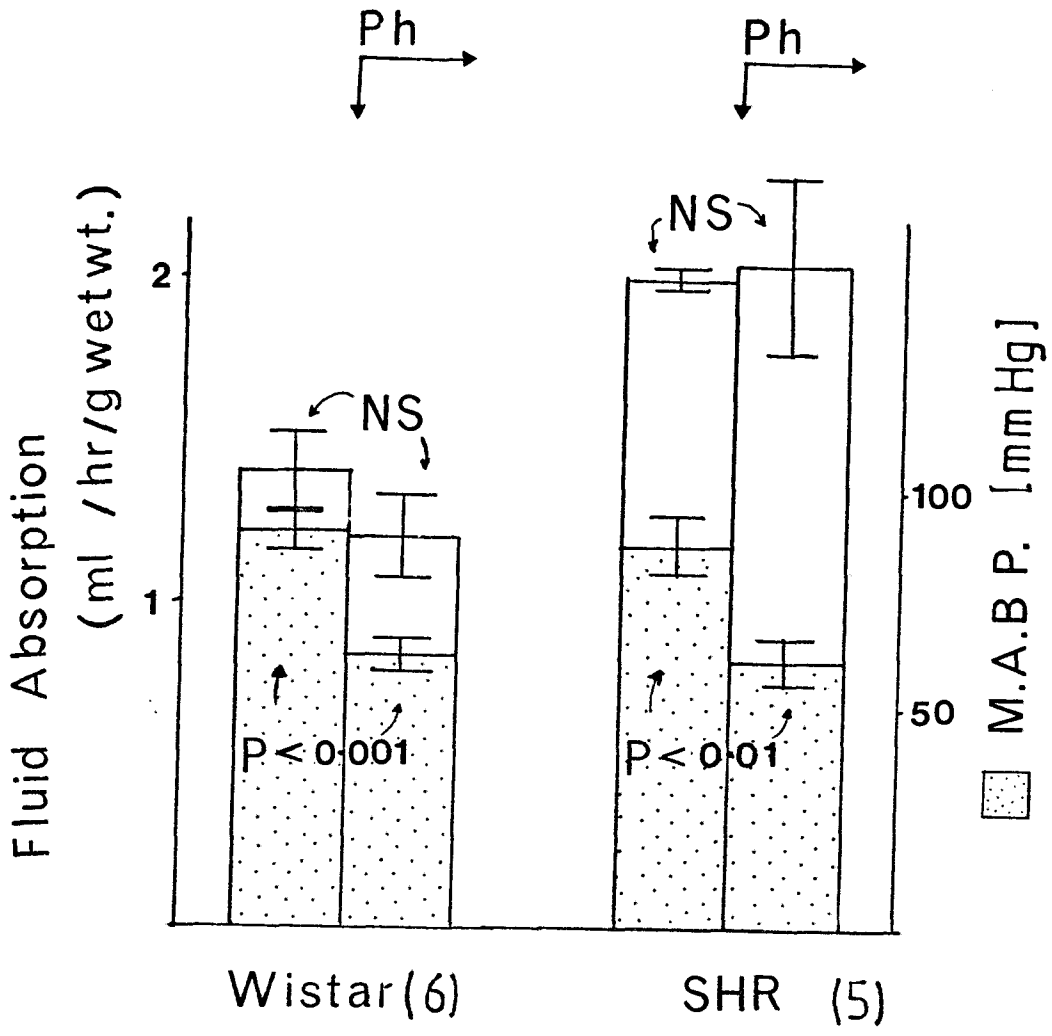


Figure 5.16 The action of the α blocker phentolamine mesylate on intestinal fluid transport and mean arterial blood pressure in wistar and spontaneously hypertensive rats. Results expressed as mean $\pm$ S.E.M. Number of observations in parenthesis. Paired histograms are data for 1st and 2nd consecutive periods. Phentolamine (Ph) infused at a rate of 36µg/kg/min i.v. throughout 2nd period. Consecutive periods compared by Student's paired t test.

animals is producing the observed increase in transport rate.

3.3.6)Are the Alterations Seen in SHR Intestinal Transport Due to Alterations in the Renin-Angiotensin System ?

As discussed earlier there are a large number of reports that the SHR has alterations in the activity of the renin-angiotensin system(RAS) (section 1.3.6). As angiotensin is known to stimulate intestinal fluid transport the observed increase in SHR transport may be due to this alteration in the RAS. Nephrectomy has been shown to greatly reduce endogenous renin (Blaquier, 1965). A study was therefore conducted on the effect of nephrectomy on the intestinal transport rate of the SHR. In initial experiments 24 hour nephrectomy and/or adrenalectomy was performed. However, these rats seemed unable to survive the stress of such an acute operation, the survival rate was 1 in 10. Experiments were therefore conducted acutely ligating both kidneys in Nembutal-anaesthetised animals 1.5 hours prior to measurement of intestinal fluid transport. Renin in the rat has a half-life of 20 minutes and so levels are greatly reduced after 1.5 hours (Schaechtelin, Regoli and Gross, 1964; Lee, 1969).

Table 5.24 summarises the results of this study and shows that there are no significant differences between sham operated or nephrectomised animals. Transport remains at a high, typically SHR, level in both groups. It would be wrong to assume that the kidney is the only source of renin, as a large number of tissues are thought to have their own isorenin system (Ganten, Hutchinson, Haebara, Schelling, Fischer and Ganten, 1976) and indeed nephrectomy alone does not always reduce basal intestinal transport levels. Another approach is to inhibit angiotensin converting enzyme. The antihypertensive agent SQ14,225 (Captopril) is well known for its ability to block angiotensin

Table 5.24a The effect of acute bilateral nephrectomy by ligation 1½ hours prior to experiment on basal intestinal fluid transport in spontaneously hypertensive rats. Results are expressed as mean ± S.E.M. and compared by Student's unpaired t test. Number of animals in parenthesis.

	Sham (4)	Nephrectomy(5)	Sig
Conscious blood pressure (mm Hg)	156 ± 16	162 ± 6	N.S.
Fluid transport (ml/hr/g wet weight)	2.20 ± 0.16	2.12 ± 0.20	N.S.
Fluid transport (ml/hr/g dry weight)	11.32 ± 1.32	11.02 ± 1.20	N.S.
Tissue water content (ml/g dry weight)	4.13 ± 0.41	4.15 ± 0.32	N.S.
Tissue dry weight (g)	0.18 ± 0.02	0.17 ± 0.01	N.S.

Table 5.24b The effect of acutely administered captopril (0.24mg/kg) given i.v. 15 minutes prior to absorption studies on basal intestinal transport in spontaneously hypertensive rats. Results expressed as mean ± S.E.M. and compared by unpaired Student's t test. Number of animals in parenthesis.

	Saline (4)	Captopril(5)	N.S.
Conscious blood pressure (mm Hg)	168 ± 3	163 ± 7	N.S.
Fluid transport (ml/hr/g wet weight)	2.28 ± 0.24	2.20 ± 0.28	N.S.
Fluid transport (ml/hr/g dry weight)	15.40 ± 5.91	12.13 ± 1.54	N.S.
Tissue water content (ml/g dry weight)	4.99 ± 0.75	5.87 ± 1.33	N.S.
Tissue dry weight (g)	0.14 ± 0.004	0.1 5 ± 0.02	N.S.

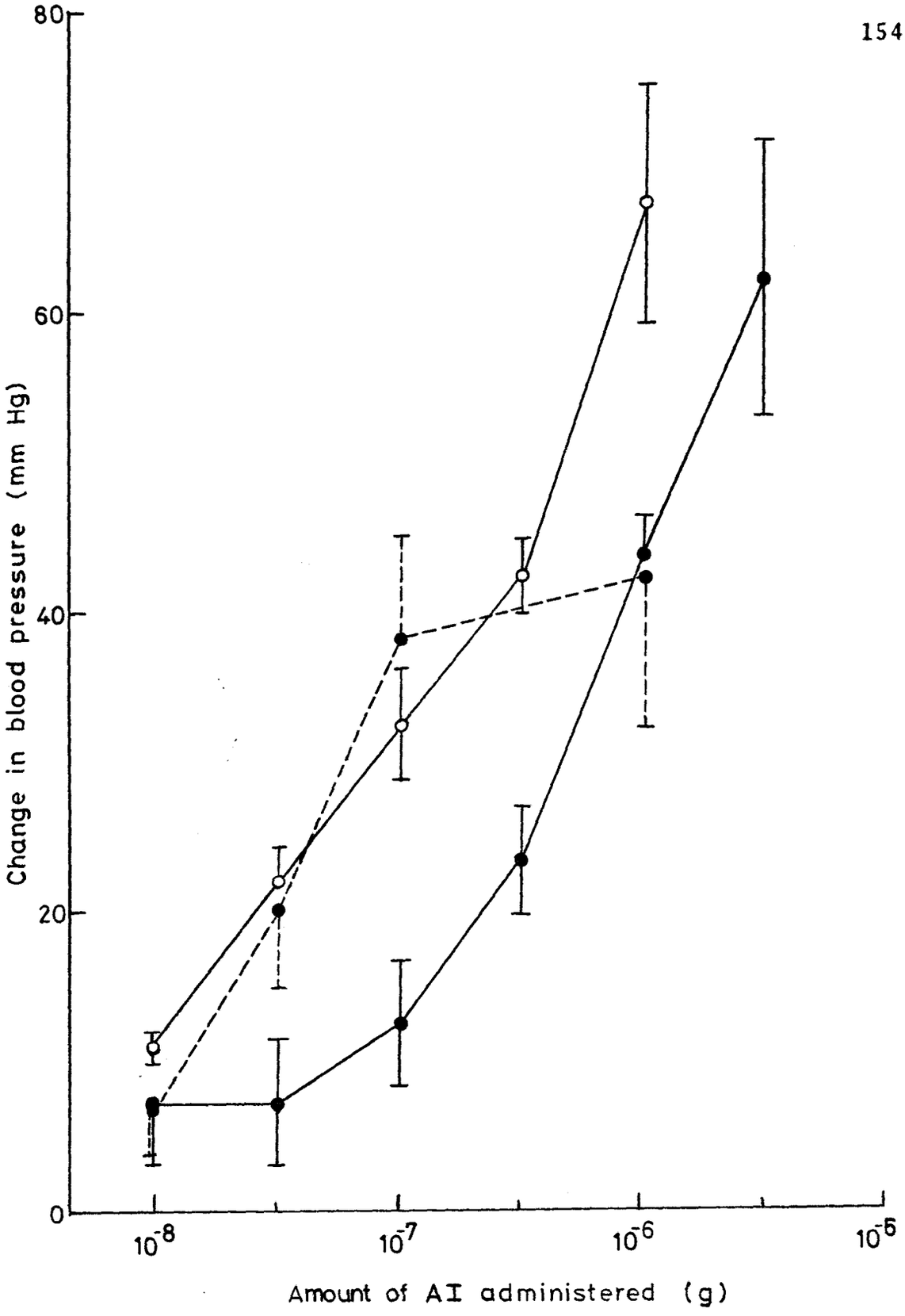


Figure 5.17 The blocking action of Captopril administered by oral and intravenous routes on the pressor response produced by single injections of angiotensin I.
○——○ = no blockade, ●——● i.v. Captopril 0.24mg/kg , ●- - - - -● = oral Captopril 5mg/kg.

converting enzyme(ACE) (Kokubu, Ueda, Ono, Kawabe, Hayashi and Kan, 1980; Levens, Peach, Vaughan, Weed and Carey, 1981). Before any transport studies were carried out the best route of administration was tested. Figure 5.17 shows the inhibition of ACE produced by both oral and acute intravenous Captopril, demonstrated as a reduction in the pressor response to injected angiotensin I. The results show that oral Captopril was not found to be as effective as the intravenous route. Thus acute intravenous doses of 4 X the ID50 value, for the rat (Patchett et al., 1980), were used.

Table 5.24 presents the results of this study and shows that acutely administered Captopril has no effect on SHR transport. It therefore seems unlikely that the renin-angiotensin system is the cause of the increased transport seen in the SHR.

3.3.7) In Vitro Electrical Measurements in the SHR.

As it is unlikely that either noradrenaline or angiotensin are involved in the observed increase in transport, more information about the mechanism of altered transport is required. Although a useful physiological preparation, the *in vivo* bulk fluid absorption studies give little insight into the mechanism and ion movements that may be occuring in the intestine of the SHR. A useful *in vitro* preparation sensitive to noradrenaline which is used to study intestinal ion transport is the stripped epithelial sheet across which electrical measurements can be taken (Field, Fromm and McColl, 1971). Sheets of jejunum taken from 13 week old age-matched male SHR and wistar animals were compared in such a study (see table 5.25).

A number of significant changes were seen in SHR when compared with wistar animals. Firstly transepithelial resistance is reduced in

Table 5.25 Electrical parameters obtained from in vitro ussing chamber studies on proximal jejunum from both wistar and spontaneously hypertensive rats. Both groups of animals were 13 weeks of age and male. Results expressed as mean ± S.E.M. and compared by Student's unpaired t test. Number of observations in parenthesis

	Wistar (8)	SHR (10)	Sig
Resistance (Ω)	57.7 ± 5.4	43.2 ± 3.5	P<0.05
Conductance (mmho/cm^2)	16 ± 1	21 ± 2	P<0.05
Potential difference (mV)	1.2 ± 0.3	2.0 ± 0.4	N.S.
Short circuit current (μA/cm^2)	23 ± 2	42 ± 7	P<0.05

the SHR, indicating a greater tissue conductance in these animals. Secondly there was a greater short circuit current (current measured with no electrochemical gradient present across the tissue) in the SHR indicative of increased active transport processes. Values for transepithelial potential differences may also have been altered in the SHR, but as tissue resistance has changed no real conclusions can be drawn. Interestingly these results indicate that SHR have alterations in both passive permeability (conductance) and active transport (short circuit current). Increased active transport normally suggests the increase in activity of an ion pump.

3.3.8) Sodium—Potassium-Sensitive-ATPase Activty in the Intestine of SHR.

The alterations in short circuit current in the electrical parameter study above suggest an increase in active transport. This increase could be due to an alteration in activity in one or a number of a variety of ion pumps, preferably those involving sodium transport which would then help explain the increase in fluid absorption seen in the SHR. The most prominent ion pump found in the mammalian intestine is the sodium—potassium—sensitive-ATPase. Furthermore Na^{+}—K^{+} sensitive-ATPase changes have been implicated in both human hypertension (Blaustein, 1977; Garay and Meyer, 1979) and in the SHR (Jones, 1973; Friedman, 1979). In order to investigate the possibility that transport changes were due to an increase in this ion pump, ATPase activity was measured in this study.

Initial observations from mucosal scrapings of both wistar and SHR animals produced the results for ATPase activity shown in figure 4.6

Table 5.26 summarises the sodium—potassium—sensitive-ATPase activity found in these two groups of animals, taken from the initial

Table 5.26 Results of sodium—potassium—sensitive—ATPase assay in mucosal scrapings from jejunum of 20 weeks old wistar and spontaneously hypertensive male rats. Results taken from the linear portion of activity and expressed as mean ± S.E.M. Groups compared by Student's unpaired t test. Number of animals in parenthesis.

	Wistar (3)	SHR (3)	Sig
Conscious blood pressure (mm Hg)	122 ± 4	203 ± 7	P<0.001
ATPase activity (phosphate production μmol/hr/mg protein)	0.78± 0.24	0.78± 0.16	N.S.

linear portion of the activity curve. From these data it is apparent that SHR show no alteration in ATPase activity under the conditions in which the assay was carried out.

Chapter 4

DISCUSSION

Section 4.1

THE ACTION OF CATECHOLAMINES AND THE NATURE OF THEIR CONTROL OF INTESTINAL FLUID TRANSPORT

4.1) The Action of Catecholamines and The Nature of Their Control of Intestinal Fluid Transport.

4.1.1) The Action of Dopamine on Intestinal Fluid Transport.

In contrast to the large number of drugs and hormones used in the study of secretion very few pharmacological agents have been used in the study of intestinal fluid absorption. Noradrenaline and adrenaline have almost exclusively been the subject of all the major studies (Field and McColl, 1973; Hubel, 1976) and very little attention has been paid to other putative transmitters that may be involved in the control of intestinal fluid absorption rather than secretion. The catecholamine dopamine is found in adrenergic neurons as a precursor for noradrenaline. Although it has more recently become apparent that dopamine is in fact a neurotransmitter in its own right, both in the CNS (Woodruff, 1978) and in the periphery (Clark and Menninger, 1980). In peripheral systems dopamine has been shown to have two major actions. Firstly it acts as an inhibitory modulator both on pre-synaptic receptors of nerves (such as sympathetic adrenergic) and on sympathetic ganglia such as the inferior mesenteric ganglia of the dog (Libet, 1970; Lins and Willems, 1974; Hope, McCulloch, Rand and Story, 1978; Hope et al., 1980; Rand, Majewski, Medgett, McCulloch and Story, 1980). Secondly it acts on specific dopaminergic postsynaptic receptors such as those in the vascular tissue of both the kidney (Goldberg, 1979; Imbs, Schmidt, Erhardt and Schwartz, 1979) and the ileum of the dog (Clark and Menninger, 1980).

Physiologically dopamine may also be of interest in the study of ECF control due to its suspected role in the modulation of renal renin release (Sowers Barrett and Sambhi, 1981), and its ability to inhibit aldosterone production (Sowers, Golub, Tuck and Sowers, 1981).

Furthermore, sulpiride an atypical neuroleptic drug known for its specific blocking actions on peripheral dopamine receptors (reviewed by Jenner and Marsden, 1979) has been shown to increase levels of serum prolactin (Mancini, Guitelman, Vargas, Debeljuk and Apericio, 1976: See Ensor, 1978 for a review of prolactin's actions.) as well as having many actions on the gastrointestinal tract.

Recently Wiglusz and Korolkiewicz (1979) have demonstrated that dopamine causes a dose-dependent stimulation of short circuit current across frog skin epithelia. However, very little is known about the actions of dopamine on transporting epithelia in general. In this study the actions of dopamine on in vivo jejunal fluid transport was studied. A high infusion rate of 351nmoles/kg/min of dopamine stimulated intestinal fluid absorption although lower rates of infusion were without effect. This was true even following pretreatment with a monoamine oxidase inhibitor suggesting that the low sensitivity of the preparation is not due to the rapid metabolism of dopamine. This observed stimulation of transport is interesting but far from a conclusive demonstration that dopamine receptors are involved in the response. Furthermore the dopamine agonist apomorphine which has less activity on α and β adrenoceptors was ineffective in stimulating intestinal fluid transport. Again the specific antagonist sulpiride, when infused simultaneously with the stimulatory dose of dopamine, did not block the response. The fact that these experiments produced negative results is very suggestive of the response being due to dopamine acting on α or β adrenoceptors rather than specific dopamine receptors. In fact dopamine is known to have affinity for both α and β as well as dopamine receptors (McNay and Goldberg, 1966).

Earlier studies on the effects of both adrenaline and noradrenaline on intestinal fluid transport have shown the actions of these agents to be blocked by α blockers such as phentolamine and dihydroergotamine, but unaffected by β blockers such as propranolol (Field and McColl, 1973; Levens et al., 1979). These findings led workers to believe that the effects on intestinal fluid transport were mediated by an α receptor. Dopamine has been shown to have an α agonist activity 10 - 100 X less potent than noradrenaline (Lazner and de la Lande, 1974). At the 50 X greater dose used in this study, dopamine is likely to have an α activity equal to that of the usual stimulatory dose of noradrenaline. The possibility that infusions of dopamine in this study may be acting on α receptors was tested by α blockade with the competitive antagonist phentolamine. Phentolamine produced a dose-dependent reduction in dopamine stimulation of intestinal fluid absorption with a complete blockade at an infusion rate of 90µg/kg/min. Upsher (1981) has reported the ability of dopamine in vitro to reduce both transepithelial p.d. and short circuit current in stripped rat ileum. This effect is also able to be blocked by α blockade. Therefore both of these findings support the idea that exogenous dopamine stimulates intestinal transport by acting on α receptors.

Physiologically a peripheral stimulatory action produced through dopamine receptors is unlikely, especially as dopamine is seen to have predominantly inhibitory actions on the elements of homeostatic control described above. Postsynaptic dopaminergic vasodilatation might possibly promote fluid absorption, however, a postsynaptic α response is a far more likely explanation. A complete dismisal of a role for dopamine would, however, be unwise. Any possible presynaptic effects of dopamine are likely to be difficult to detect by studying "end

responses" and dopamine may indeed still have a role in this system.

4.1.2) Nature of the α Receptor Mediating the Control of Intestinal Fluid Transport.

The findings discussed above suggest that α receptors play a role in the control of intestinal fluid transport. Much of this evidence was obtained from the use of blocking drugs such as phentolamine and dihydroergotamine. These drugs have good specificity for α receptors but do not descriminate between α receptor sub-types (Steer, Khorana and Galgoi, 1979; U'Prichard, Charness, Robertson and Snyder, 1978). These α receptor sub-types (designated $^{\alpha}1$ and $^{\alpha}2$) have a different pharmacological specificity. $^{\alpha}1$ receptors are found post-synaptically whilst $^{\alpha}2$ receptors are present pre-synaptically as well, as has been found more recently, on post-synaptic sites (for example Timmermans and Van Zwieten, 1980). In this study the effects of the more specific $^{\alpha}1$ receptor antagonist prazosin and the $^{\alpha}2$ receptor antagonist yohimbine on the ability of noradrenaline to stimulate intestinal fluid transport were investigated.

Prazosin at high, non specific, doses was able to inhibit both noradrenaline stimulation of intestinal fluid absorption and alterations in blood pressure. However, at moderate pharmacological doses prazosin was found to be able to block the pressor response produced on noradrenaline infusion but was unable to block noradrenaline stimulated intestinal fluid absorption produced by the same infusion. In contrast to this the $^{\alpha}2$ antagonist yohimbine blocks noradrenaline stimulated transport at doses well below those which block the pressor response. The pressor response to noradrenaline is known to be predominantly (of the order of 70%, Drew and Whiting, 1979)

due to activity on α1 post-synaptic receptors. The remaining 30% of the pressor effect due to actions on postsynaptic α2 receptors is blocked by yohimbine (Drew and Whiting, 1979; Flavahan and McGrath, 1980). Interestingly it appears to be α blockade by the α2 receptor blocking agent that is more effective at abolishing the fluid transport effects of noradrenaline, indicating that α2-type receptors may be important in producing the effect. Further results using the α1 agonist phenylephrine and the predominantly α2 agonist clonidine demonstrated that both of these agents were able to stimulate intestinal fluid transport when infused at a similar rate to the stimulatory dose of noradrenaline. The dose of clonidine having no effect on blood pressure even although transport was stimulated. Thus the picture appears to be confused. The use of antagonists suggests that noradrenaline stimulation of intestinal fluid transport is via an α2 receptor, yet agonists failed to confirm this.

Before conclusions can be drawn from these studies a number of important points should be considered. Firstly the specificities of each drug for their proposed site of action are open to question. Agonists generally are far less specific than antagonists for α1 or α2 receptor sub-types (Starke, 1981). Clonidine, in particular, has been shown to have some α1 receptor affinity (Ruffolo, Waddell and Yaden, 1980). Secondly, systemic infusions of drugs into an *in vivo* preparation introduce problems in differential metabolism between drugs, disparient distribution, as well as widespread systemic actions such as the central depressor actions of clonidine (Dollery and Reid, 1973) and the pre-synaptic blocking actions of yohimbine (Starke, Borowski and Endo, 1975). Thirdly, the noradrenaline stimulation of

intestinal fluid transport is an 'all or nothing' effect (Upsher, 1981) so that inhibitors tend to either completely block the response or leave it unaffected. Dose response curves are not possible and therefore the standard pharmacological comparison of ID50 cannot be made between antagonists.

The independence of the fluid transport effects from blood pressure is well illustrated in these studies. Transport is able to be stimulated even if blood pressure does not alter. Agents such as angiotensin have similarly been shown to have their transport-stimulating actions without altering intestinal haemodynamics (Mandel and Saperstein, 1962).

Despite the reservations mentioned above, these results, in particular the relative effectiveness of the two antagonists on the noradrenaline stimulation of fluid transport and blood pressure, are compelling. The evidence suggests that receptors of α_2-type alone or a mixed population of α_1 and α_2 (as found in vascular smooth muscle) may mediate the stimulatory response of noradrenaline on intestinal fluid transport. Recently, in a study on whole homogenate from guinea pig ileum, Tanaka and Starke (1979) have detected α_2-type receptors not located on sympathetic nerve terminals and preliminary results using [3-H] clonidine and [3-H] prazosin as ligands show that isolated epithelia from rat jejunum contains specific sites for both of the ligands (Cotterell, personal communication). Further research into the pharmacology of this system using a wider spectrum of agonists and antagonists in an *in vitro* preparation such as the everted sac should help clarify the picture.

4.1.3) The Action of Endogenous Noradrenaline and the Nervous System on Intestinal Fluid Transport.

Studies on the action of exogenous infusions of agents such as catecholamines give indications, but little confirmation of the physiological role of such agents in the control of intestinal fluid transport. The fact that noradrenaline has an action upon intestinal fluid transport is given further perspective by the recent findings of Brunsson and coworkers (1979) that sympathetic nerve stimulation in the cat will enhance fluid absorption. The role of endogenous noradrenaline is supported by the findings in this study that pre-treatment with the monoamine oxidase inhibitor pargyline, known to increase endogenous noradrenaline in the intestine 10 fold (Munday, Poat and Upsher, 1980), will stimulate intestinal fluid transport. Similarly, Munday, Parsons, Poat and Upsher (1980) showed that the pharmacological agent tyramine, which releases endogenous noradrenaline from nerve vesicles (Vanhoutte, 1978), was able to stimulate intestinal fluid transport both *in vivo* and *in vitro*.

Curiously neither α blockade, ganglion blockade, nor denervation with 6-OHDA produce any reduction in basal fluid transport. This suggests that the nerves are either not firing during basal transport or that they are firing at a low rate below the threshold at which noradrenaline has any action. This could be due to rapid removal of released noradrenaline by metabolic or uptake processes. Both of these possible mechanisms for the sympathetic control of intestinal fluid transport provide an interesting contrast with the sympathetic neural control of the kidney. Here the nerves are continually active, promoting reabsorption of water and electrolytes, and physiological control can actually be exerted upon the kidney by a reduction in

firing rate. Such reductions occur following factors such as an increase in intravascular volume (Ricksten, Noresson and Thoren, 1979; Ricksten, Yao, Dibona and Thoren, 1981). Unlike the kidney, the intestine can play no serious role in excreting fluid and electrolytes and it is probably for this reason that a high spontaneous sympathetic tone is not present.

The lack of receptor tone in this system is similar to that proposed by Powell and Tapper (Tapper et al., 1978; Powell and Tapper, 1979) in their hypothesis of cholinergic—adrenergic interaction. Figure 6.1 illustrates the proposed models. In model A the postganglionic adrenergic fibres of the sympathetic nervous system terminate on the epithelial cells. They release catecholamines which effect receptors on the basolateral cell membranes and promote fluid absorption. Postganglionic parasympathetic innervation also terminate on the epithelia and promote secretion. Model B requires that the postganglionic nerves terminate on the ganglia of the intramural plexus rather than directly on the epithelial cells, and for proper control neccesitates the sympathetic and parasympathetic systems to be in constant tone. Both the findings of Tapper and Powell and the results from this study indicate that it is unlikely that the receptors controlling intestinal fluid transport are under tone. Furthermore, Browning, Hardcastle, Hardcastle and Redfern (1978) have produced evidence that absorption and secretion may occur at different locations in the villus. They suggest that absorption occurs at the villus tip whilst secretion occurs in the crypts. A modified version of the Tapper and Powell model A, with adrenergic and cholinergic nerves leading to different cells may best fit the available data. (Intestinal nerve morphology and mechanism is further discussed in section 4.2).

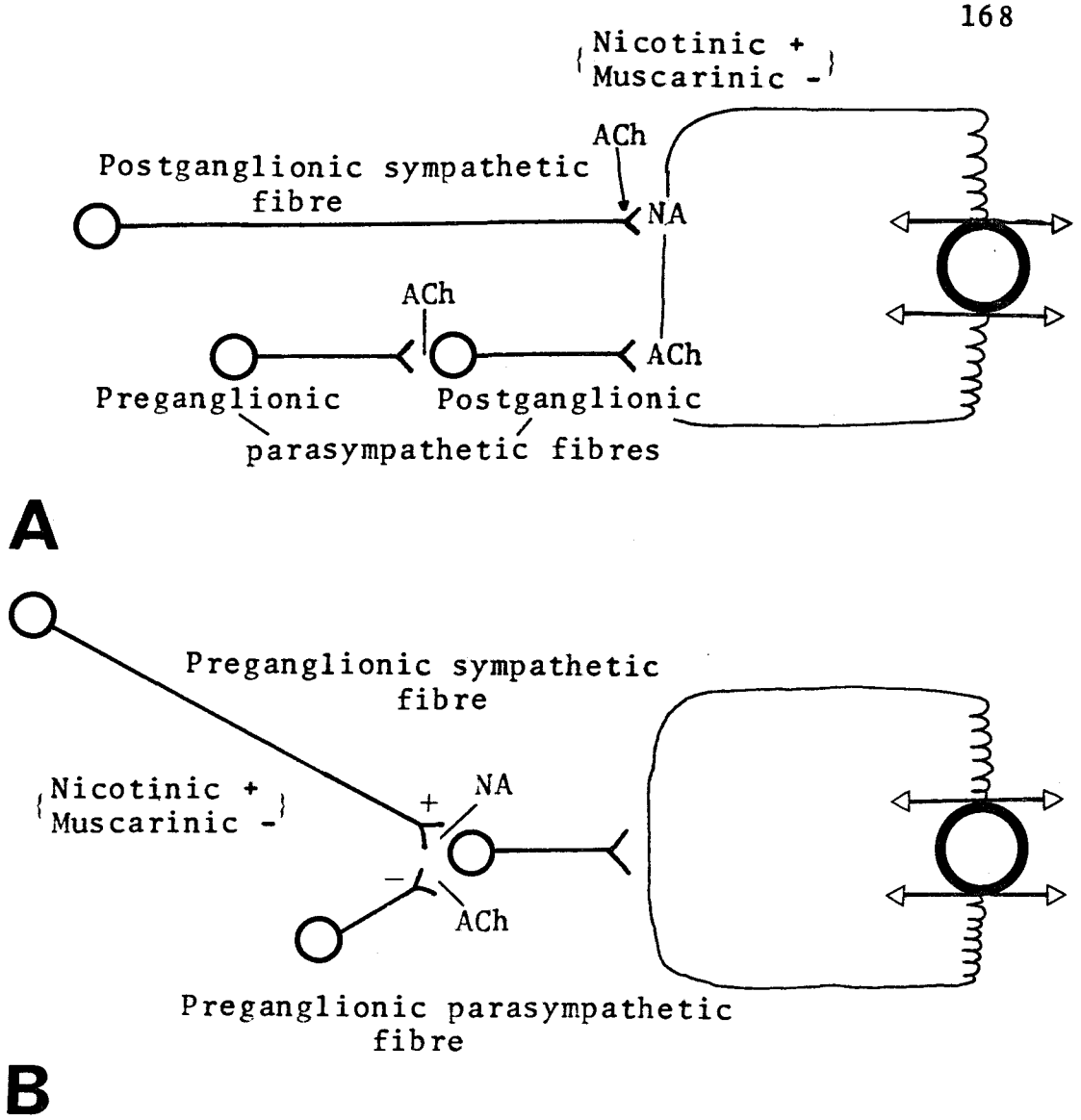


Figure 6.1 Diagrams of the two models proposed by Powell and Tapper for the cholinergic-adrenergic interaction in the control of intestinal fluid transport. In model 'A' both sympathetic (stimulating absorption) and parasympathetic (stimulating secretion) systems directly innervate the epithelial cells. In model 'B' both parts of the autonomic nervous system are proposed to modulate nerves which keep the epithelia in constant 'tone'. These models are further described in the text.

Section 4.2

MECHANISMS OF ACTION OF ANGIOTENSIN ON INTESTINAL FLUID TRANSPORT

4.2) Mechanisms of Action of Angiotensin on Intestinal Fluid Transport.

Earlier studies on the intestine have shown that the polypeptide hormone angiotensin II is able to stimulate intestinal fluid transport in a number of regions and preparations including the *in vitro* everted sac (Crocker and Munday, 1970; Davies, Munday and Parsons, 1970) and the *in vivo* jejunum (Bolton et al., 1975). Furthermore angiotensin is able to stimulate electroneutral extrusion of sodium and chloride from kidney cortex slices (Munday, Parsons and Poat, 1971).

However, noradrenaline has been shown to have similar actions to angiotensin on fluid and ion transport. As noradrenaline stimulates transport in *in vitro* ileal preparations of rabbit (Field and McColl, 1973) and rat (Upsher, 1981) and stimulates fluid absorption from *in vivo* rat intestine (Hubel, 1976). Levens and coworkers (1977) noticed striking similarities between the actions of these two agents. Furthermore they were able to demonstrate that both angiotensin and noradrenaline effects in the intestine were blocked by α but not β blockers (Levens et al., 1979). These findings were confirmed by further studies on angiotensin stimulated extrusion from kidney cortex slices (Brunton et al., 1978). It was therefore suggested that angiotensin may very well be having its effects on intestinal fluid transport through the release of noradrenaline. In support of this idea is the ability of angiotensin to release noradrenaline from both sympathetic nerves and the adrenal medulla (Peach, 1977).

Further evidence that angiotensin is likely to be having its actions through the release of endogenous noradrenaline can be observed by considering the effectiveness of the α blocker phentolamine in blocking the stimulation of transport produced by a number of agents.

For example, exogenous noradrenaline infused at a rate of 7 nmoles/kg/min will stimulate intestinal fluid transport, and this stimulation is blocked by a simultaneous infusion of phentolamine at a rate of 9μg/kg/min (Upsher, 1981). In the study reported in this thesis it was observed that the stimulation of fluid transport produced by raising endogenous noradrenaline levels with pargyline, was only blocked by infusion of a higher concentration of phentolamine (36μg/kg/min). Interestingly this higher concentration of phentolamine is also necessary to block the effects of angiotensin upon intestinal fluid transport (Levens et al., 1979; Upsher, 1981). The exact concentration of noradrenaline released at the proposed effector site is not known, although some workers have estimated the synaptic concentration of noradrenaline to be of the order of 10^{-5} M during maximum nerve firing (Ljung and Wennergren, 1971; Bevan, 1976). It is therefore possible that endogenous noradrenaline has a higher effective concentration than an exogenous infusion, therefore requiring a higher blocking dose of phentolamine.

If angiotensin does have its actions by the release of noradrenaline then both the adrenal medulla (Peach, 1971) and adrenergic nerves (Starke, 1977) are likely sources. Noradrenaline depletion by the pharmacological agent reserpine has been shown to be more effective on post-ganglionic adrenergic nerves than on the adrenal medulla (Carlsson, Rosengren, Bertler and Nilsson, 1957). The findings of Levens and coworkers (1978) that reserpine will prevent the action of angiotensin on intestinal fluid transport may therefore suggest that angiotensin's action on the nervous system may be the important action. In this study the possibility that angiotensin is releasing noradrenaline from sympathetic nerves was tested by sympathetic

denervation using the pharmacological agent 6-hydroxydopamine (6-OHDA). After administration of 6-OHDA sympathetic nerves are damaged and become incapable of releasing neurotransmitters. In this study 6-OHDA pre-treatment was shown to abolish the response to infusions of angiotensin II whilst infusions of noradrenaline, acting on the still intact postsynaptic receptors (Kostrezewa and Jacobowitz, 1974) still had its effect. This data is therefore consistent with the view that angiotensin is acting by the release of noradrenaline from adrenergic sympathetic nerves.

The 6-OHDA experiments are subject to several criticisms. Firstly, Porlier, Nadeau, de Champlain and Bichet (1977) demonstrated increased plasma renin activity and circulating catecholamine levels in dogs treated with 6-OHDA. It is therefore possible that the inability of the intestine of 6-OHDA treated rats to respond to low infusion rates of angiotensin could be due to the high endogenous levels of the hormone. However, similar studies using rats treated with 6-OHDA in a similar experimental procedure to the one employed here did not show any increase in plasma renin activity (Bennett and Gardiner, 1978). Also if plasma angiotensin levels were raised in the 6-OHDA experiments in this study it would be expected that an increase in fluid transport would be observed in the 6-OHDA treated rats compared with controls. In the same way, any increase in plasma catecholamine concentrations secondary to 6-OHDA administration would also be expected to be seen as an increase in the rate of fluid transport during the first absorption period.

As well as destroying noradrenaline containing nerves, 6-OHDA has been shown to deplete dopamine to a degree that suggests that dopaminergic nerves can also be damaged. However, as discussed earlier,

dopamine is unlikely to be important in the stimulation of intestinal fluid transport as it is 5Ø X less effective than noradrenaline. A further series of 6-OHDA treatment experiments using a prior pre-treatment with desmethylimipramine would help clarify this situation. Desmethylimipramine is able to prevent the uptake of 6-OHDA into noradrenergic neurons (Tessel, Kennedky, Burgess and Borchardt, 1978) and would thus allow specific destruction of dopaminergic nerves.

Recently Levens, Peach and Carey (1981) have conducted a series of experiments examining the effects of both chronic and acute administration of guanethidine on the ability of infusions of angiotensin II to stimulate intestinal fluid transport. They found that both treatments prevented the action of angiotensin on fluid transport. When given acutely guanethidine prevents the release of noradrenaline from adrenergic nerve endings and therefore blocks adrenergic neural transmission. When given chronically over a period of several weeks guanethidine produces a selective peripheral sympathectomy with destruction of adrenergic fibres whilst leaving the adrenal medulla intact. Furthermore, Mariscotti (198Ø) has demonstrated that angiotensin is still able to effect intestinal fluid transport following bilateral adrenalectomy. The evidence is therefore strong in support of the idea that angiotensin is having its actions through the release of noradrenaline from peripheral sympathetic nerves.

The action of angiotensin promoting noradrenergic effects has been proposed to occur by a number of possible mechanisms, which may be divided into two major categories. Firstly angiotensin may increase the noradrenaline-elicited response by a spontaneous mechanism not requiring the nerves to be active, such as by increasing basal overflow

of noradrenaline (Khairallah, 1972; Roth, 1972) or by increasing the sensitivity of the effector cells to noradrenaline (Day and Moore, 1976). Alternatively angiotensin may increase the amount of noradrenaline released per nerve impulse (McCubbin and Page, 1963; Zimmerman and Whitmore, 1967) and therefore require sympathetic nerves to be active in order for angiotensin to have an effect.

The latter mechanism is supported by the greatest weight of evidence (Starke, 1977) so the dependence of the effect of angiotensin on sympathetic firing was investigated. Ganglion blockade with pentolinium tartrate prevents peripheral sympathetic nerve activity and was also found to abolish the stimulation of intestinal fluid transport produced by infusions of angiotensin. Exogenous infusions of noradrenaline, acting postsynaptically, were unaffected by the blockade and were still able to stimulate fluid transport. These findings therefore lend support to the idea that angiotensin requires spontaneously firing nerves for these actions.

In contrast, angiotensin is also capable of stimulating intestinal absorption in isolated stripped everted sacs of rat intestine, a preparation which could be considered to have little or no intrinsic nerve activity. Furthermore, preliminary studies not reported in this thesis have shown that under certain conditions angiotensin may be able to promote the spontaneous overflow of noradrenaline from intestinal tissue. This may be similar to the ability of angiotensin to spontaneously release dopamine from striatal slices (Simonnet and Giorguieff-Chesselet, 1979).

Figure 6.2 illustrates three proposed models for the action of angiotensin and noradrenaline on intestinal fluid transport. In model A

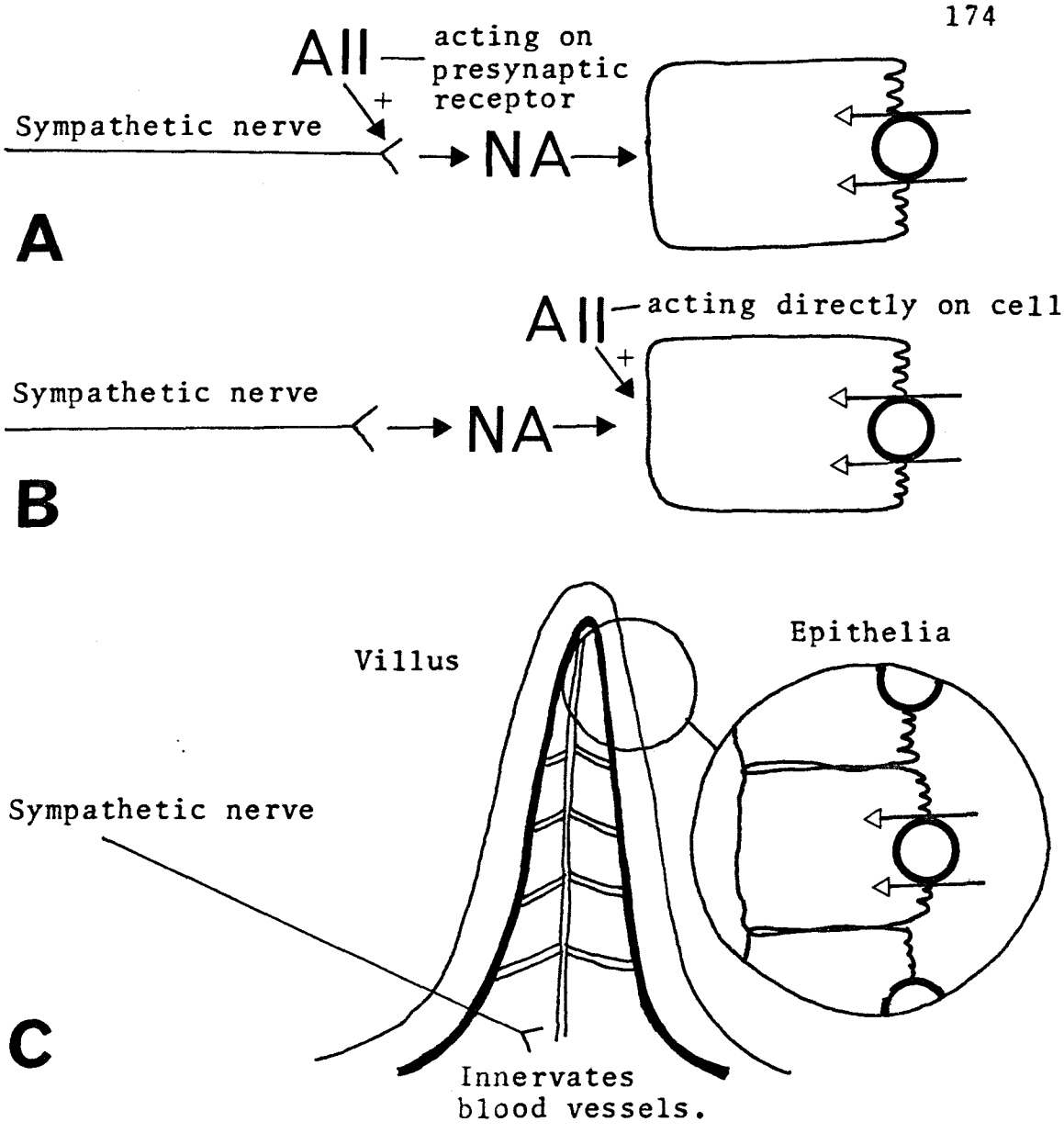


Figure 6.2 Three proposed models for the mechanism of action of angiotensin II, noradrenaline and the sympathetic nervous system in the control of intestinal fluid transport. Model 'C' involves no direct epithelial innervation. The illustrated brushed-border pump-site location is purely diagramatic and simply represents absorption of sodium, chloride and water from the lumen.

a noradrenergic postganglionic nerve fibre synapses on or close to the transporting epithelia. Nerve impulses cause noradrenaline to be released from the nerve ending which then acts on α receptors located on the epithelial cell. Stimulation of these receptors activate an ion pump mechanism which produces an electroneutral transport of sodium and chloride ions. This movement of ions results in a parallel absorption of water from the intestinal lumen (section 1.1.17 - 1.1.2Ø). It is proposed that under stable physiological conditions these nerves are firing only at a low rate, releasing noradrenaline at a level below the threshold of the system. Increased release of noradrenaline, produced for example by an increase in sympathetic nerve activity will raise the levels of noradrenaline above this threshold and intestinal fluid absorption will be stimulated. Circulating angiotensin II is able to act on the sympathetic nervous system at one or a number of sites including presynaptic receptors on sympathetic nerve terminals. This action on the nerve then enhances the release of noradrenaline and again stimulates fluid absorption.

Model B suggests that an alternative site for the action of angiotensin may be on the epithelial cell itself. The action of such a receptor could be to directly stimulate fluid transport without the mediation of noradrenaline (Diez de los Rios, Labajos, Manteca, Morell and Souviron, 198Ø) or to alter the threshold of the epithelia to respond to noradrenaline (Day and Moore, 1976). The experimental evidence which supports the first model could as readily fit model B. Experiments with ganglion blockade and sympathectomy are all likely to reduce the amount of basal release of endogenous noradrenaline and this could be below the threshold that any increase in tissue sensitivity produced by angiotensin can achieve. Providing evidence contrary to

this idea are the experiments by Munday, Poat and Upsher (1980) using the stripped epithelial sheet. This intestinal preparation has been found to be unable to respond to angiotensin, probably due to insufficient availability of tissue noradrenaline. However, even prior administration of sub-threshold doses of noradrenaline were unable to produce a response to angiotensin as would indeed be expected if angiotensin was altering the sensitivity of the epithelia to noradrenaline.

Several authors have produced no evidence for noradrenergic fibres entering the villi (Jacobowitz, 1965; Ahlman, Enerbak, Kewenter and Storm, 1973; Brunsson et al., 1979). Such a villus innervation is naturally essential for the sympathetic nerve based models discussed above. Brunsson and coworkers (1979) in their studies on cats have proposed an alternative model, model C. In this model the sympathetic nervous system innervates vascular tissue supplying the villus and controls fluid absorption by altering the villus blood flow, an important factor in the proposed counter-current mechanism of absorption (Hallback, Jodal, Sjoquist and Lundgren, 1979). More recent studies in the rat (Thomas and Templeton, 1981) have demonstrated adrenergic nerve fibres extending towards the tip of the villus. These nerves are seen to remain close to blood vessels and, although they have not yet been shown to innervate epithelia directly, may be in close enough proximity to allow noradrenaline release to directly effect ion transport (as described in model A ;Field, Brasitus, Sheerin and Kimberg, 1976; Hubel, 1976).

Certainly none of these models are either definitive or exclusive. And models such as the counter-current hypothesis require active ion

transport processes as well as blood flow control for efficient operation. This may be especially true in species such as the rat where a more 'squat' villus vascular anatomy appears to make blood flow factors less important than is seen to be the case in the cat (Winne, 1975; personal communication). Furthermore, additional complications including the possible involvement of intrinsic, possibly peptidergic, nerves (Powell and Tapper, 1979b) make any proposed model far from straightforward.

Further studies investigating the distribution and localisation of both angiotensin-and α-adrenergic-receptors are presently being conducted by a number of workers. The information from these studies will therefore provide us with an important further insight into the mechanisms of action and interaction between angiotensin and the sympathetic nervous system in their role in the control of intestinal fluid absorption.

Section 4.3

CONTROL OF INTESTINAL FLUID TRANSPORT IN THE SPONTANEOUSLY HYPERTENSIVE RAT

4.3)Control of Intestinal Fluid Transport in the Spontaneously Hypertensive Rat.

The strain of spontaneously hypertensive rat (SHR) developed by Okamoto and Aoki (1963) has produced much interest as a true genetic hypertensive model (Trippodo and Frohlich, 1981). In spite of some criticism that these animals may not be a good model of human hypertension (McGiff and Quilley, 1981) there has been a wealth of studies showing many interesting differences between SHR and their normotensive counterparts. Amongst these investigations a number of workers have shown that SHR have alterations in salt and water balance, however, little work has been conducted to investigate the control of extracellular fluid balance in these animals.

Not only have SHR been found to have a greatly increased thirst and salt appetite when compared with normotensive controls (Catalanotto et al., 1972; Fregly, 1975; May, 1980), but they have been shown to have alterations in their renal handling of both sodium and water (Farman and Bonvalet, 1975; Willis et al., 1976; Mullins and Banks, 1976). However, reports of alterations in total body water and ECF composition and volume that may be a product of changes in intake and excretion of water and electrolytes, are less clear and still remain disputed (Trippodo, Walsh and Frohlich, 1978; Willis and Baeur, 1978).

Over a number of years, studies on the nature of intestinal fluid transport have produced evidence linking the control of intestinal fluid transport with ECF homeostasis (for example Mariscotti et al., 1977). Therefore, in order to obtain further information on any alterations in extracellular fluid status and water balance in the SHR, intestinal transport was studied. Male SHR rats of all ages were found to have a higher rate of intestinal fluid absorption than male wistar

controls, and this increase in intestinal transport had no correlation with conscious blood pressure. In fact young SHR in the pre-hypertensive phase of their growth curve were found to have an even higher fluid absorption rate than older hypertensive SHR. A profile of decreasing intestinal fluid absorption with age and development of hypertension, similar to that observed for salt appetite and water intake (May, 1980), was found.

In addition to these observed changes in fluid transport the water content of SHR intestinal tissue (obtained by drying the tissue) was found to be lower in mature SHR compared with age-matched normotensive controls. In contrast to these findings, the tissue water content of young SHR was found to be the same as that of controls. A reduction in intestinal tissue water content therefore appears to occur in the SHR, either with age or with both age and the development of hypertension. This idea is supported by the fact that neither tissue water content nor intestinal fluid transport were found to alter with age in the normotensive wistar males.

Curiously, although mature hypertensive female SHR still have a lower intestinal tissue water content than their age-matched female wistar controls, female SHR did not show any significant alterations in intestinal fluid transport. This was not due to female SHR having fluid transport at normal 'wistar' levels but rather that the fluid transport in female wistar was elevated above that usually observed (Mariscotti, 1980). The exact reason for this increase is unclear. Previous observations on the intestinal transport of female wistar rats has shown no significant changes during the oestrus cycle (Munday, personal communication) although intestinal fluid transport has been shown to increase and decrease with seasonal changes (Evans, Bolton, Munday and Parsons, 1975). Very many endocrine hormones have actions on intestinal

fluid transport and alterations in any one of a number of factors could cause these observations. Because of the difficulty in having stable control female animals, males were used exclusively in all further studies.

The markedly increased rate of transport seen in the SHR may be involved with the development of hypertension. Further information on the mechanisms thought to be involved in the etiology of hypertension in the SHR may therefore provide further insight into the possible mechanisms producing rapid fluid transport. The sympathetic nervous system is now known to be involved in the control of intestinal fluid transport (section 4.1) and the action of catecholamines on transport processes are also well documented. Interestingly, SHR have been reported as having increased sympathetic nerve activity when compared with controls (Okamoto et al., 1977; Judy et al., 1979). And some workers have shown that SHR have increased catecholamine synthesis and turnover in tissues such as the heart (Louis, Spector, Tabei and Sjoerdsma, 1969) and the kidney (Yamori, 1972) as well as increased circulating levels of noradrenaline (Grobecker et al., 1975). Indeed the alterations seen in the sensitivity of intestinal fluid transport to infusions of noradrenaline in this study, although in contrast to the increased adrenoreceptor sensitivity reported in SHR vascular tissue (Webb and Vanhoutte, 1979), are typical of the pharmacological receptor sub-sensitivity that high endogenous levels of noradrenaline are likely to produce. In contrast to these findings no difference was found in tissue noradrenaline content when noradrenaline was assayed in samples of small intestine from mature SHR and wistar animals. In fact the SHR tissue noradrenaline levels obtained were similar to those found by other workers in normal wistar rats (Upsher, 1981).

In fairness, an assay of tissue noradrenaline levels may not give a true indication of the amount of catecholamines that may be present in the circulation. Furthermore, mature SHR have been found to have decreased sympathetic activity from that seen in young animals of the strain (discussion following Yamori, 1979) and some workers have not been able to see any significant difference between sympathetic activity in mature SHR and normotensive controls (Lais and Brody, 1975).

The observed sub-sensitivity of intestinal fluid transport to respond to exogenous noradrenaline suggests that either endogenous noradrenaline or the sympathetic nervous system are involved in the observed increases in transport. However, further investigation provided contrary evidence. Neither α blockade with phentolamine nor prevention of sympathetic nerve firing by ganglion blockade with pentolinium tartrate produced any alteration in the observed high rate of fluid transport. The fact that these blocking procedures are normally effective on normotensive Wistar is well demonstrated. Both phentolamine and pentolinium have been shown to abolish the stimulatory-action of infusions of angiotensin on intestinal fluid transport (section 4.2). Furthermore, infusions of phentolamine will also prevent the stimulatory-action of both endogenous and exogenous noradrenaline on intestinal fluid transport (section 4.1). It is therefore unlikely that either noradrenaline or the sympathetic nervous system are directly involved in producing the high rate of intestinal fluid transport observed in the SHR.

As discussed in the introduction (section 1.3.6) a number of contrasting observations have been made on the activity of the

renin-angiotensin system in the SHR. In the normal rat angiotensin is known to be able to both stimulate and inhibit intestinal transport dependent on the dose. It has been suggested that this stimulation may be via the mediation of noradrenaline (Levens et al., 1979) or by a direct action on the intestine (Diez de los Rios et al., 1980; Levens et al., 1980). Therefore altered levels of angiotensin II may be important in the mechanism of elevated transport observed in the SHR. This possibility was tested by inhibiting the production of angiotensin II by the renin-angiotensin system by two methods. Firstly, bilateral nephrectomy was carried out to remove renal renin (Blaquier, 1965) and secondly, as non-renal sources of renin have been found in tissues such as the mesenteric artery and spleen (Ganten, Hayduck, Brecht, Boucher and Genest, 1970), SHR were treated with captopril (SQ 14,225) an inhibitor of the converting enzyme which converts angiotensin I to angiotensin II. Both these procedures would be expected to reduce circulating levels of angiotensin II and may therefore produce a reduction in the rate of fliud absorption. However, neither of these procedures produced any alterations in the high rate of intestinal fluid transport in the SHR. The results therefore suggest that endogenous levels of angiotensin II are unlikely to be involved in the observed high rate of intestinal fluid transport.

The *in vivo* loop (*in vivo* sac) preparation provides useful information on the physiology of bulk fluid movements but can give little insight into the nature of any ion movements that may be occuring. The study of the mechanism of transport in the SHR was therfore extended by measuring *in vitro* electrical parameters in a Ussing chamber stripped epithelial sheet of jejunum (Ussing and Zerhan, 1951). Thirteen week old SHR were compared with age-matched wistar

animals. The results from these studies show possible alterations in intestinal ion transport for both passive and active processes. Firstly, passive permeability changes are indicated by the decreased tissue resistance which demonstrates that ions are able to move more freely. A number of reports exist describing tissue permeability changes in hypertensive states (reviewed by Trippodo and Frohlich, 1981) including changes in capillary permeability in the SHR (Rippe, Lundin and Folkow, 1978). However these alterations may very well be developing as a result of hypertension rather than being causative. A series of experiments conducted with young pre-hypertensive SHR would help clarify the situation.

Alterations in an active transport process are indicated by the observed increase in short circuit current in intestinal tissue from SHR. Short circuit current is a measure of the ion movements occuring with no net electrochemical gradient. Both this change in active transport and the alteration in passive permeability may very well be involved in the observed increase in rate of intestinal fluid absorption. Little can be said from simple electrical parameter studies as to which active transport processes may be altered. However, the sodium-potassium-ATPase is the most predominant active transport mechanism for sodium in the intestine (section 1.1.13) and disorders in this particular pump have been implicated both in human hypertension (Blaustein, 1977; Garay and Meyer, 1979) and in the SHR (Jones, 1973; Friedman, 1979; reviewed by Trippodo and Frohlich, 1981). The possibility of an increase in activity of this pump was therefore tested. No alteration was found in the Na^+-K^+-dependent-ATPase activity between SHR and normotensive controls. This finding reduces, but does not eliminate, the possibility that Na^+-K^+-dependent-ATPase or a similar

ion pump is involved in the observed transport changes. In fact the conditions of the assay may not be representative of the environment found in each group of animals and thus the observed rates of ATPase activity may not be applicable. Also an alteration in ion pump stoichiometry that may possibly occur in the SHR would not be recognised by examining the production of inorganic phosphate. Mineralocorticoids such as aldosterone (section 1.1.7) as well as glucocorticoids (Donowitz et al., 1979) are known to increase sodium pump activity, and in fact current thoughts on the etiology of hypertension in the SHR consider that a disorder of the endocrine interaction between the elements of the 'hypothalamic-pituitary-adrenal-gonadal' system may exist (Wexler et al, 1980; 1981). Certainly such a hormone imbalance might very well explain both the transport changes and the unusual susceptability of the SHR to stress (producing death following surgery) found both in this study and by other workers (Wexler, 1981). The increased levels of transport in young SHR may very well be due to elevated levels of plasma aldosterone as have been suggested to explain the increased thirst and salt appetite in young animals (May, 1980), these ideas could be simply tested by treatment with aldosterone antagonists such as spironolactone. Furthermore, a large number of other active transport systems exist in the intestine, transporting both anions and electrolytes (for example, section 1.1.15). Alterations in any one of a number of these pump systems could produce the observed effects. Experiments measuring ion fluxes would greatly clarify the situation.

In conclusion many questions still remain to be answered on the nature of the observed high rate of fluid transport in the SHR. In particular the mechanism behind the alteration in ion transport

requires further study, as do the possible role of disorders in the 'hypothalamic-pituitary-adrenal-gonadal' axis. Such further investigation may very well provide useful information on the nature and etiology of processes similar to human essential hypertension.

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