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EFFECTS OF ANGIOTENSIN II ON FLUID TRANSPORT, TRANSMURAL POTENTIAL DIFFERENCE AND BLOOD FLOW BY RAT JEJUNUM *IN VIVO*

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SUMMARY

1. A method has been described for the measurement of fluid transport by rat jejunum *in vivo* over two consecutive 30 min periods.

2. Subpressor infusion rates of angiotensin (0·59 ng/kg per minute) stimulate fluid transport, while high (pressor) infusion rates (590 ng/kg per minute) inhibit fluid absorption.

3. Both the inhibitory and stimulatory effects of angiotensin on fluid transport are not accompanied by any change in the transmural p.d., total blood flow to the jejunum or distribution of blood flow within the wall of the jejunum.

4. These results are discussed in relation to the mechanism of action of angiotensin on fluid transport and its role in sodium and water homoeostasis.

INTRODUCTION

It is generally assumed that aldosterone is the major hormone involved in the control of sodium homoeostasis, although it is becoming increasingly evident that angiotensin must also be considered as a physiologically important sodium retaining hormone apart from its proposed role (Kaplan & Bartter, 1962) in the control of aldosterone secretion.

The rate of renin secretion, and consequently the circulating angiotensin concentration, is dependent on the sodium status of the animal; sodium depletion consistently elevates plasma renin levels whereas sodium loading is usually associated with a fall in the plasma renin concentration (Vander, 1967). Furthermore, physiological concentrations of angiotensin have been shown to stimulate sodium transport by a range of mammalian transporting epithelia. For example angiotensin, at low concentrations,

enhances sodium transport by *in vitro* preparations of rat jejunum (Crocker & Munday, 1970), rat colon (Davies, Munday & Parsons, 1970) and rat kidney (Munday, Parsons & Poat, 1971). Similarly, the kidney *in vivo* responds to low infusion rates of the hormone with an anti-natriuresis, but it is controversial whether this is due to direct stimulation of tubular sodium re-absorption mechanisms (Barraclough, Jones & Marsden, 1967) or is secondary to changes in intrarenal blood flow (Bonjour & Malvin, 1969).

Much of the evidence implicating angiotensin as a salt retaining hormone has been obtained from studies carried out on *in vitro* intestinal preparations and, as such, may be subject to criticism. Consequently, the following experiments were undertaken to investigate the effects of intravenous infusions of angiotensin on fluid transport, and thus indirectly sodium transport, by rat jejunum *in vivo*.

METHODS

Animals

Male albino Wistar rats, weighing approx. 300 g, were starved overnight before use.

Experimental procedure

The rats were anaesthetized with pentobarbitone sodium (100 mg/100 g body wt.) I.P. after which the trachea, left common carotid artery and both femoral veins were cannulated. A pressure transducer was connected to the carotid cannula in order to continuously monitor arterial blood pressure. The femoral vein cannulae were then independently attached to separate syringes in a Palmer constant-rate infusion pump so that either 0·9% NaCl (containing 50 u. heparin/ml.) could be infused into one femoral vein or, alternatively, angiotensin in isotonic heparinized saline could be infused into the second femoral vein. The rate of infusion was always 1·0 ml./hr apart from the first min of any infusion when it was increased to 0·1 ml./min in order to establish the patency of the cannula.

A mid line abdominal incision was made through the skin and muscle layers in order to expose the viscera. The proximal end of the jejunum was found at the ligament of Treitz and ligatured, after which a second ligature was tied approx. 20 cm distal to the first. The resulting isolated segment of proximal jejunum was then thoroughly washed with isotonic saline and gently emptied. Finally, two new ligatures were tied around the central region of the isolated segment of proximal jejunum to give a closed sac approx. 15 cm in length. Great care was taken with the placing of all ligatures so that the blood supply to the sac of jejunum was not impaired.

Measurement of fluid transport

Approximately 5 ml. Krebs bicarbonate buffer (Krebs & Henseleit, 1932) containing [^{3}H]inulin (200,000 d.p.m./ml.), as a non-absorbable marker, was injected into the sac through a small diameter (26 S.W.G.) hypodermic needle. The jejunum was gently massaged to mix the sac contents, and at 0 min a 0·1 ml. sample was removed through a hypodermic needle and assayed for tritium. Immediately after

this, the sac of jejunum was returned to the abdominal cavity and saline infused into one of the femoral veins at a rate of 1 ml./hr.

After 30 min, the jejunum sac was again exposed and massaged to mix the contents. A second 0·1 ml. sample of the sac contents was removed and assayed for tritium. The sac was returned to the abdomen, and at the same time the saline infusion was stopped and replaced by an infusion of angiotensin in saline, at the same rate, through the other femoral vein for a second 30 min period.

Finally, at the end of 60 min the contents of the sac were mixed and a third sample collected and assayed for radioactivity. The sac of jejunum was removed from the animal by cutting the intestine immediately beyond each ligature and weighed. It was then emptied, blotted by a standardized procedure on filter paper (Whatman No. 50) and re-weighed to obtain the volume of the contents and the weight of the sac. The precise volume of buffer injected into the sac at the beginning of the experiment and the volume of subsequent samples were obtained by weighing.

Inulin is a non-absorbable marker so that, following the absorption of fluid from the jejunum sac, there is an increase in the inulin concentration. From the volume of fluid in the sac at 0 min and the concentrations of inulin in the sac contents at 0, 30 and 60 min and the volume of all samples removed, fluid absorption from the sac during two consecutive 30 min periods was calculated.

A second procedure for assessing fluid transport was routinely carried out as a check on the measurement of fluid transport by the inulin method. In this, fluid transport over the whole 60 min period was calculated from the volume of the contents of the jejunum sac at 0 and 60 min and the total volume of all samples removed from the sac during the experiment.

Measurement of transmural potential difference

In some experiments the transmural potential difference, p.d., of the jejunum sac was measured. A length of 30 pp Portex tubing containing 3 M-KCl in 4% agar gel was used as the mucosal electrode and ligatured into the distal end of the jejunum sac. An I.P. agar–saline bridge was used as the serosal electrode. Both electrodes were connected, via calomel half-cell electrodes, to a Vibron electrometer which was used to measure the p.d. across the mucosal and serosal electrodes. The transmural p.d. was continuously monitored on a recorder. The asymmetry between the intraluminal and serosal electrodes was always checked before experiments by placing their tips together in isotonic saline and did not exceed 0·3 mV. The mean transmural p.d. was obtained from the mid-point of the best straight line through the p.d. recording.

Measurement of blood flow to the jejunum

Blood flow to the jejunum was obtained from the product of cardiac output and the percentage of cardiac output passing to the jejunum. Animals were prepared as described previously for measurements of fluid absorption and jejunum blood flow determined during the infusion of saline or angiotensin in saline.

(*a*) *Cardiac output.* The thermal dilution method of Fegler (1954) was used. The left common carotid artery was cannulated and a thermistor inserted so that the tip lay just inside the aortic arch. Precisely 0·1 ml. of 0·85 g/100 ml. saline, at room temperature, was injected every 5 min through a cannula placed in the left jugular vein. Cardiac output was calculated from the arterial temperature–dilution curves obtained at 5 min intervals over a 30 min period during the infusion of saline and during a second 30 min period whilst saline or angiotensin in saline was infused through the femoral veins at a rate of 1 ml./hr.

(*b*) *Percentage of cardiac output distributed to the jejunum.* Two independent methods were used to measure this parameter.

The first method was essentially that described by Sasaki & Wagner (1971) with the exception that macro-aggregated iodinated (^{131}I) human serum albumin was used as an alternative to microspheres. The majority of these particles had a diameter between 20 and 50 μm and consequently were trapped by intestinal capillaries so that the percentage of intracardiac administered macro-aggregated albumin collected in the jejunum could be taken as a measure of the percentage of cardiac output delivered to the jejunum. Nylon 00 tubing, filled with heparanized saline and attached to a blood pressure transducer, was used to cannulate the right carotid artery. The cannula was inserted into the left ventricle and its position verified by the large increase in the pulse pressure as the cannula passed the aortic valves. Saline was infused I.V. for 15 or 45 min after which 0·2 ml. (1 μc) macro-aggregated albumin was injected into the left ventricle. When the effects of angiotensin were studied, saline was infused for 30 min followed by angiotensin for 15 min after which the macro-aggregated albumin was injected. The sac of jejunum was removed from the animal 10 min after the injection of macro-aggregated albumin, opened, damp blotted, weighed and assayed for ^{131}I in a γ-well scintillation counter.

The second method was that of Sapirstein (1958) which depends on the observation that ^{86}Rb is almost completely cleared from the plasma during its passage through an organ. In these experiments, 0·2 ml. (1 μc) ^{86}Rb Cl was injected into a femoral vein and 45 sec later the intestine removed, blotted, weighed and assayed for ^{86}Rb in a γ-well scintillation counter.

Radioactivity assay

Each 0·1 ml. sample of intestinal contents, containing [H^3]inulin, was added to a counting vial containing 10 ml. scintillation fluid (0·8% butyl PBD in 1:1, v/v methanol:toluene) and counted in a Phillips automatic scintillation counter. Intestines, containing ^{86}Rb or ^{131}I, were dissolved in conc. HNO^4, made up to 5 ml. with distilled water and counted in a γ-well scintillation counter. All counts were corrected for background and quenching to give d.p.m.

Expression of results

Fluid absorption is defined as the loss of fluid from the jejunum sac during the experiment and is recorded as ml./30 min per gram wet weight of sac. There is no significant change in the water content of the jejunum following the infusion of angiotensin, the wet/dry wt. ratio being $5·9 \pm 0·4$ (5) following the infusion of saline and $6·1 \pm 0·3$ (4) and $6·2 \pm 0·7$ after the infusion of 0·59 and 590 ng angiotensin/kg per minute respectively. Transmural p.d. is taken as the mean p.d. recorded over a 30 min period. Student's *t* test was used to determine the significance of differences between means.

Materials

Angiotensin (Hypertensin, Ciba) was diluted with sterile saline to a concentration of 10 μg/ml. and stored in sealed siliconized ampoules. Immediately before use, the angiotensin was diluted to a suitable concentration in isotonic saline.

[^{3}H]inulin ^{86}RbCl and ^{131}I-labelled macro-aggregated albumin were obtained from the Radiochemical Centre, Amersham.

RESULTS

The use of non-absorbable markers to measure fluid transport has been well documented. In a comprehensive study, Miller & Schedl (1970) showed that less than 2% of inulin was lost from an intestinal perfusate during a 3 hr test and concluded that this material is suitable for measuring fluid transport in preparations of small intestine. The effectiveness of inulin as a non-absorbable marker was confirmed in the modified preparation of jejunum used in these experiments. Fluid transport was

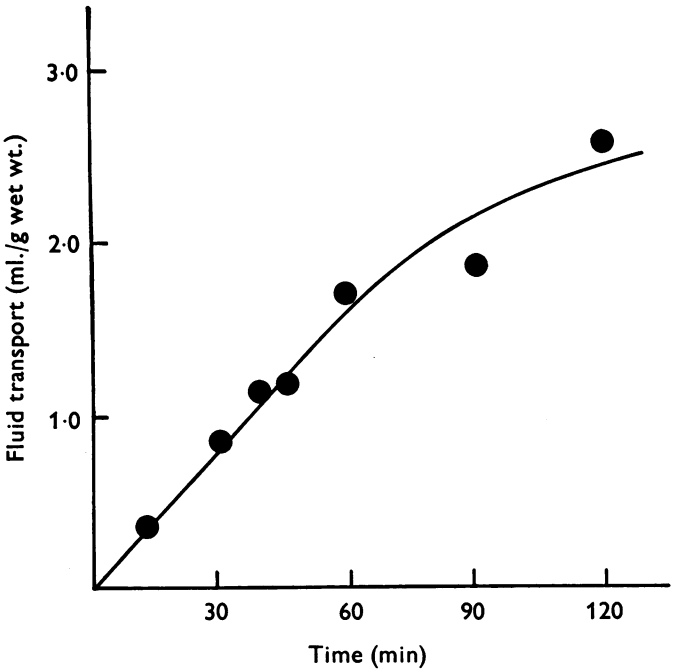

Fig. 1. The rate of fluid absorption, as a function of time, from sacs of rat jejunum *in vivo*. Each point represents the mean of two observations.

determined in a series of animals by both the inulin method and by the direct technique of measuring the loss of weight of the sacs. Over a period of 60 min, in eight preparations, fluid transport measured by the inulin method ($1{\cdot}48 \pm 0{\cdot}10$ ml./g wet wt. jejunum per hour) was not significantly different from that obtained by direct weighing ($1{\cdot}59 \pm 0{\cdot}13$ ml./g wet wt. jejunum per hour). Furthermore, in the presence of 30 mg/100 ml. carrier inulin, $93{\cdot}6 \pm 4{\cdot}1$ % of the inulin injected into the sac was recovered at the end of the experiment.

The suitability of the preparation was further tested by measuring

TABLE 1. The effects of angiotensin on fluid transport by rat jejunum

I.V. infusion (1·0 ml./hr)		Fluid absorption (ml./30 min per gram wet weight jejunum)		Significance
First 30 min	Second 30 min	First 30 min	Second 30 min	(P)
Saline (0·9 %)	Saline (0·9 %)	0·60 ± 0·047 (5)	0·65 ± 0·10 (5)	n.s.
Saline (0·9 %)	Angiotensin (0·59 ng/kg per min)	0·69 ± 0·040 (5)	1·31 ± 0·079 (5)	< 0·001
Saline (0·9 %)	Angiotensin (590 ng/kg per min)	0·70 ± 0·026 (5)	0·23 ± 0·058 (5)	< 0·001

Fluid absorption was measured over two consecutive 30 min periods. Saline was infused I.V. at a rate of 1·0 ml./hr during the first 30 min and saline or angiotensin in saline, at the same rate, for the second 30 min. Results expressed as mean ± S.E. of mean. Number of observations in parentheses.

the rate of fluid transport for varying periods to a maximum of 2 hr. The results of these experiments are given in Fig. 1 and show that the rate of fluid transport is constant for the first 60 min. After 60 min the rate of fluid absorption is reduced, presumably as a consequence of the small volume of fluid remaining in the sac and available for transport from this time.

Having established the validity of using inulin as a non-absorbable marker to measure fluid transport from isolated sacs of rat jejunum and having shown that absorption is constant over a 60 min period, the following experiments were carried out to study the effects of angiotensin

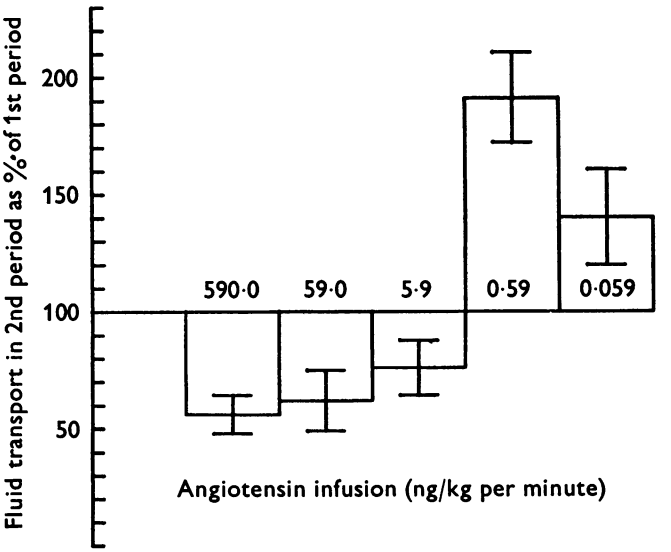

Fig. 2. Dose dependent actions of angiotensin on fluid transport by rat jejunum *in vivo*. Angiotensin was infused during the second period and fluid transport is expressed as rate of transport in the second period as a percentage of that in the first (control) period. Bars represent S.E. of mean.

on fluid transport by this preparation. Fluid transport was measured over two consecutive 30 min periods. Isotonic saline was infused I.V. during the first 30 min period while saline or angiotensin in saline was infused during the second period. In this way the first 30 min absorption period was used as a control for the second period. The results of these experiments are presented in Table 1.

There was no significant difference between fluid absorption in the first and second 30 min periods in those experiments where saline was infused continuously, confirming the results presented in Fig. 1 that

isolated sacs of jejunum transport water at a constant rate over a period of 60 min. The infusion of low doses of angiotensin (0·59 ng/kg body wt per minute) during the second period resulted in a highly significant increase in the rate of fluid absorption from the sacs compared with that in the first (control) period. In contrast, transport of fluid from the jejunum was markedly reduced during the infusion of high doses of angiotensin (590 ng/kg body wt. per minute) throughout the second period.

The dose-dependent, reversible nature of the response was investigated further by studying the effects of infusing a range of doses of angiotensin on fluid transport. In Fig. 2, fluid transport during the second period has been expressed as a percentage of the rate of transport during the first period. The infusion of 5·9 ng angiotensin/kg body wt. per minute during the second period was without significant effect on fluid transport, whereas lower doses stimulated and higher doses inhibited fluid absorption.

Carotid blood pressure was measured in all of these experiments and sample traces are presented in Fig. 3. The top trace gives the blood pressure during the infusion of saline throughout the first and second periods. Under these conditions, blood pressure remained constant apart from a transient fall at 30 min while the intestine was handled in order to obtain a sample of luminal fluid. Similarly, the infusion of 0·59 ng angiotensin/kg body wt. per minute during the second period, an infusion rate which significantly increases the rate of fluid absorption, had no effect on carotid blood pressure. In contrast, the infusion of 590 ng angiotensin/kg body wt. per minute, a dose which inhibits fluid transport, gave a sustained pressor response of about 20 mmHg. The transient increase in blood pressure following the infusion of both doses of angiotensin is probably due to the initial 1 min infusion at a rate of 0·1 ml./min.

The transport of sodium across epithelial tissues is often associated with a change in transmural p.d. Indeed, the transmural p.d. appears to be generated, under many circumstances, as a consequence of the active transport of sodium (Keynes, 1969). However, in rat jejunum, there is evidence for the existence of both electrogenic and non-electrogenic sodium transport mechanisms (Barry, Smyth & Wright, 1965).

Experiments were carried out to determine whether the changes in sodium transport, following the administration of angiotensin, are associated with changes in jejunum transmural p.d. The results of these experiments are given in Table 2. The transmural p.d. remained steady at approximately 3 mV when saline was infused during both the first and second periods. Similarly, the infusion of 0·59 ng angiotensin/kg per minute, which significantly increased fluid transport, or 590 ng angiotensin/kg per minute, which markedly reduced fluid transport, were unaccom-

panied by any significant change in the p.d. developed across the tissue indicating that the hormone may exert its effects through a non-electrogenic sodium transport mechanism.

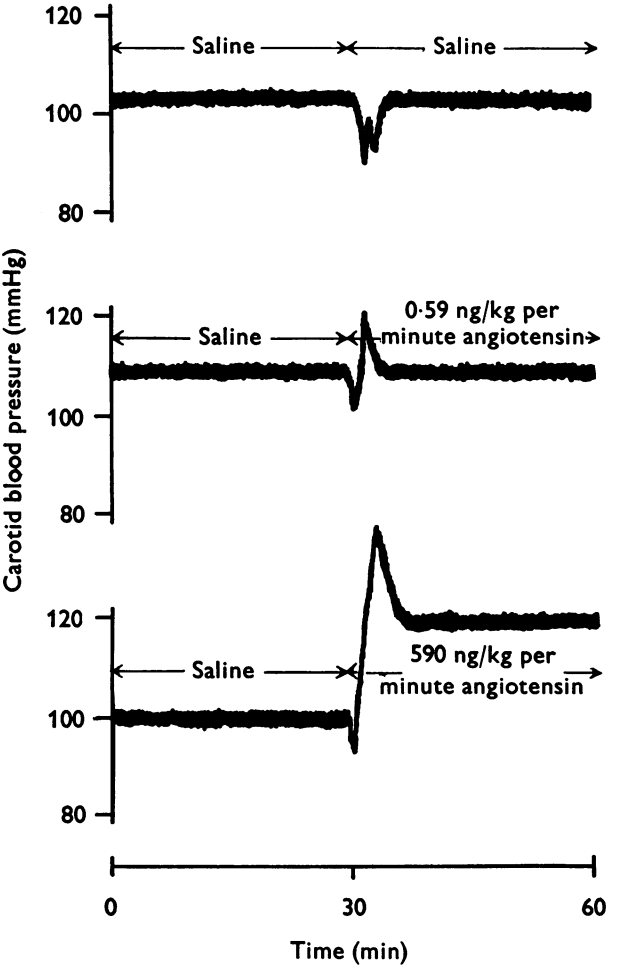

Fig. 3. Rat blood pressure recorded during experimental infusions of saline and angiotensin.

Fig. 4 gives typical recordings of the transmural p.d. of rat jejunum *in vivo*. The recordings presented in Fig. 4*A* are typical examples of transmural p.d. obtained during 30 min saline infusions following infusions of 0·59 ng/kg per minute angiotensin. Transmural p.d. remained essentially constant over the first 30 min period of the experiments and showed no consistent change during the second period when sodium, and

TABLE 2. The effects of angiotensin on fluid transport and transmural p.d. of rat jejunum *in vivo*

I.V. infusion (1·0 ml./hr)		Fluid absorption (ml./30 min per gram wet weight)			Transmural p.d. (mV)		
First 30 min	Second 30 min	First 30 min	Second 30 min	Significance (*P*)	First 30 min	Second 30 min	Significance (*P*)
Saline (0·9 %)	Saline (0·9 %)	0·74 ± 0·05 (5)	0·59 ± 0·08 (5)	n.s.	3·2 ± 0·3 (5)	3·1 ± 0·3 (5)	n.s.
Saline (0·9 %)	Angiotensin (0·59 ng/kg per minute)	0·81 ± 0·05 (4)	1·20 ± 0·09 (4)	< 0·01	3·5 ± 0·6 (4)	3·2 ± 0·6 (4)	n.s.
Saline (0·9 %)	Angiotensin (590 ng/kg per minute)	0·70 ± 0·03 (5)	0·22 ± 0·03 (5)	< 0·001	3·3 ± 0·5 (5)	3·1 ± 0·3 (5)	n.s.

Fluid absorption and transmural p.d. were measured over two consecutive 30 min periods. Saline was infused I.V. at a rate of 1·0 ml./hr during the first 30 min and saline or angiotensin in saline, at the same rate, for the second 30 min. Results expressed as mean ± S.E. of mean. Number of observations in parentheses.

consequently fluid, transport was increased. The ability of the equipment to detect electrogenic ion movement is also demonstrated in Fig. 4. The addition of glucose to the luminal fluid (recording *B*) caused a large and repeatable increase in transmural p.d.

Bonjour & Malvin (1969) concluded, from their experiments, that the antinatriuretic effect following low infusion rates of angiotensin is secondary

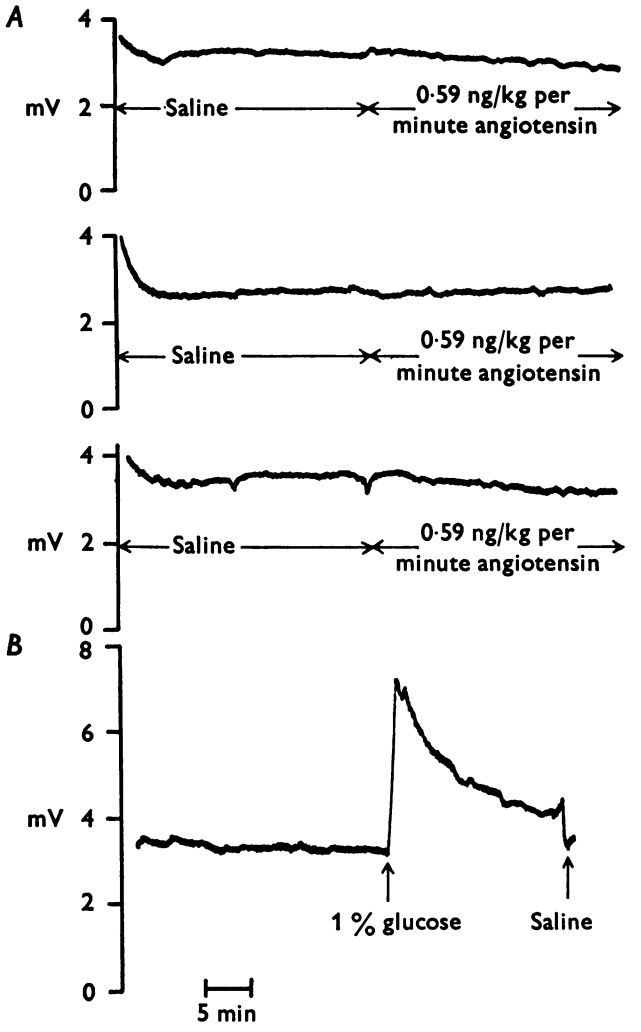

Fig. 4. P.d. recordings across rat jejunum *in vivo*. *A*, gives three examples of transmural p.d. over two consecutive 30 min periods. Saline was infused for the first 30 min and angiotensin (0·59 ng/kg per minute) in saline during the second 30 min period. *B*, shows the effect of adding glucose to the luminal fluid on transmural p.d.

to a redistribution of intrarenal blood flow. A similar effect may be responsible for one or both of the actions of angiotensin on intestinal transport function and consequently a study was carried out on the effects of high and low infusion rates of the hormone on intestinal blood flow.

Blood flow to the jejunum was calculated from cardiac output and the percentage of cardiac output passing to the jejunum during the infusion of saline, 0·59 ng and 590 ng angiotensin/kg per minute. Fractional distribution of cardiac output to the jejunum was determined by two methods, namely ^{86}Rb and macro-aggregated albumin. The results of these experiments are presented in Table 3 and show that angiotensin

TABLE 3. The effects of angiotensin on jejunum blood flow

	Jejunum blood flow (ml./min per gram wet weight)	
Infusion (1 ml./hr)	^{86}Rb method	MAA method
Saline	1·46 ± 0·32 (11)	1·35 ± 0·34 (11)
Angiotensin (0·59 ng/kg per minute)	1·25 ± 0·15 (11)	1·26 ± 0·22 (11)
Angiotensin (590 ng/kg per minute)	1·15 ± 0·15 (11)	1·54 ± 0·29 (11)

Blood flow was calculated from the product of cardiac output and fractional distribution of either ^{86}Rb or macro-aggregated albumin, *MAA*, to the jejunum.

Results expressed as mean ± S.E. of mean. Number of observations in parentheses. There was no significant change in jejunum blood flow under the conditions studied.

has no statistically significant effect on jejunum blood flow when infused at rates which significantly stimulate or inhibit fluid transport by the tissue.

Blood flow distribution within the wall of the small intestine is comprised of parallel circulations through the mucosa, submucosa and muscle layers. The possibility that angiotensin, although not producing changes in total blood flow to the intestine, may cause a redistribution of blood flow within the intestinal wall, was tested by measuring the flow to the three regions in the presence and absence of angiotensin. This was achieved by dissecting out the regions following the labelling of the vasculature with intracardiac administered macro-aggregated albumin. Assuming that the macro-aggregated albumin was firmly trapped in the capillary beds, and not redistributed during the separation procedure, the percentage of cardiac output passing to each region was calculated from the amount of macro-aggregated albumin in each fraction. It is apparent from the results given in Table 4 that angiotensin, at infusion rates which give

TABLE 4. The effects of angiotensin on blood flow to the mucosa, submucosa and muscle of jejunum

Infusion (1·0 ml./hr)	Blood flow (% cardiac output/g wet wt.) Mucosa	Submucosa/ mucosa	Muscle
Saline	0·25 ± 0·03 (8)	14·3 ± 2·0 (8)	0·26 ± 0·06 (8)
Angiotensin (0·59 ng/kg per minute)	0·34 ± 0·07 (8)	12·4 ± 1·5 (8)	0·34 ± 0·08 (8)
Angiotensin (590 ng/kg per minute)	0·31 ± 0·06 (8)	13·4 ± 0·8 (8)	0·35 ± 0·06 (8)

Percentage cardiac output distributed to regions of the intestine was obtained from the fraction of intracardiac administered macro-aggregated albumin present in the fractions which were separated by dissection. The submucosa/mucosa fraction was that remaining after removal of the muscle and a portion of the mucosa. Results expressed as mean ± S.E. of mean. Number of observations in parentheses. There was no significant change in blood flow to any region following the infusion of angiotensin.

both a stimulation and inhibition of fluid transport, does not significantly alter the distribution of blood flow within the wall of the intestine.

DISCUSSION

Several indirect observations have indicated that extra-adrenal factors exist which regulate sodium homoeostasis. Clarke, Miller & Shields (1967) demonstrated that spironolactone, a competitive inhibitor of aldosterone, does not block the increase in the rate of sodium absorption following sodium depletion in dogs, while Crocker & Munday (1970) showed that adrenalectomy depresses fluid transport by rat jejunum *in vitro* to a lesser extent than feeding the animals high sodium diets. These observations, together with the findings that circulating levels of angiotensin are dependent on the sodium status of the animal (Vander, 1967) and that the hormone has profound effects on both intestinal (Crocker & Munday, 1970) and kidney (Barraclough *et al.* 1967) sodium transport capacities, strongly support the view that angiotensin may be one of the postulated extra-adrenal sodium-retaining hormones.

Previous evidence that angiotensin alters the handling of salt and water by the intestine was obtained using *in vitro* preparations. The present studies show that angiotensin modifies intestinal absorption *in vivo*, thus eliminating the remote possibility that the effects of the hormone on transport function might be artifacts of the *in vitro* condition.

It has been demonstrated that low infusion rates of angiotensin increase

the rate of fluid transport across rat jejunum *in vivo* whereas high infusion rates result in an inhibition of jejunum fluid absorption. These observations are qualitatively similar to the dose-dependent, biphasic actions of the hormone on rat colon *in vitro* (Davies, Munday & Parsons, 1970) and rat kidney *in vivo* (Barraclough *et al.* 1967) and thus appears to be a consistent feature of the responses of transporting epithelia to angiotensin. The infusion rates of angiotensin which produce a stimulation of fluid transfer are subpressor and are probably within the physiological range of hormone concentration (Catt, Cain & Coghlan, 1967). In contrast, the inhibitory effects of pressor doses of angiotensin, though interesting, are of doubtful physiological significance. Nevertheless, the high circulating levels of the hormone, in certain pathological conditions, warrants further investigation of the inhibitory response.

There is some controversy regarding the mechanism of action of angiotensin on epithelial transport function. In the rat kidney *in vivo*, Barraclough *et al.* (1967) have attributed both the antinatriuretic and natriuretic effects to direct actions of the hormone on tubular transport mechanisms while Bonjour & Malvin (1969) concluded that only the natriuresis, caused by high infusion rates of angiotensin, is due to direct tubular effects and the antinatriuresis, produced by low doses of the hormone, is secondary to changes in intrarenal blood flow. The observations by Davies *et al.* (1970) that angiotensin has a dose dependent, biphasic action on sodium transport by rat colon mucosa *in vitro* demonstrates that both actions of the hormone in this tissue are, at least in part, direct actions on epithelial transport mechanisms.

Apart from direct actions, it is possible that angiotensin may have secondary effects on intestinal transport function under conditions *in vivo*. The hormone is known to induce the secretion of aldosterone from the adrenals and this would be expected to stimulate sodium absorption. Such an effect is unlikely to contribute to the responses observed in the present series of experiments since changes in fluid transport are observed within 30 min, whereas Crocker & Munday (1969) noted a long latent period between the administration of aldosterone and the subsequent increase in jejunum fluid transport.

As in the kidney, angiotensin may alter blood flow distribution to the intestine and, as a consequence of this, modify fluid absorption. The effects of angiotensin on blood flow to the alimentary canal are contradictory. In anaesthetized rats, hepatic blood flow is either increased (Gomori, Tackacs & Kallay, 1962) or unchanged (Mandel & Sapirstein, 1962). The results reported here show that infusions of angiotensin, at rates which produce both a marked stimulation and inhibition of intestinal fluid transport, have no significant effect on total blood flow to the

jejunum or on its distribution within the tissue. The relationship between intestinal blood flow and absorption is poorly defined although Love, Matthews & Veall (1972) demonstrated that very large flow changes have to be induced before transport is altered. Such large changes in intestinal blood flow did not occur in the experiment reported here and consequently, it would seem likely that the transport effects we have observed following both high and low infusion rates of angiotensin are due to direct actions of the hormone on the jejunum mucosa.

In recent years, evidence has accumulated to indicate that there are at least two modes of active sodium transport in the kidney, these being a sodium–potassium exchange pump, which is dependent on the presence of potassium and inhibited by ouabain, and a potassium-independent pump which is inhibited by ethacrynic acid (Whittembury, 1968; Whittembury & Proverbio, 1970). Similarly, Barry *et al.* (1965) demonstrated the existence of two pumps in rat jejunum; one electrogenic and associated with active sugar absorption and the other non-electrogenic and responsible for bulk sodium and fluid movement.

Munday *et al.* (1971) studied the two modes of sodium extrusion from rat kidney cortex slices and showed that angiotensin-stimulated sodium transfer is mediated through increased activity of the potassium-independent, ouabain-insensitive pump. Similarly, Shaikh (1972) demonstrated that angiotensin will enhance sodium transport in rat colon *in vitro* in the presence of high concentrations of ouabain. Shaikh (1972) also found that the stimulation of sodium transport by angiotensin in colon *in vitro* is not associated with any change in transmural p.d. The latter observation is similar to that obtained in the present series of experiments using rat jejunum *in vivo* where there was no apparent change in the magnitude of the transmural p.d. during the infusion of angiotensin at rates which both increased and decreased fluid absorption. However, it should be emphasized that there is much evidence to support the existence of low resistance shunts across the rat upper intestine. These shunts could attenuate potentials, generated across either the mucosal or serosal cell membranes, resulting in an insignificant change in transmural p.d.

Nevertheless, the above observations are consistent with the view that two modes of sodium transport are present in the intestinal mucosa; an electrogenic and a non-electrogenic mechanism. Angiotensin appears to alter the activity of the non-electrogenic mode of sodium transfer with no effect on the electrogenic mechanism. It would seem likely that this non-electrogenic pump may be equated to the potassium-independent, ouabain-insensitive and ethacrynic acid-sensitive sodium pump which has been described in the kidney. Why this pump is non-electrogenic is a matter for speculation, but may be explained by the work of D. J. Smith

(personal communication) who, using kidney slices, found that the potassium-independent sodium pump has an absolute requirement for chloride ions. It may be that this pump transfers sodium and chloride ions in the same direction in an equimolar ratio. The apparent non-electrogenic nature of angiotensin-stimulated sodium transport may explain the inability of some (Barbour, Gill & Bartter, 1964) to obtain an effect of angiotensin on transport function of epithelial tissues when measuring sodium transport by electrical methods.

There are many similarities between the effects of angiotensin on kidney *in vivo*, intestine *in vitro* and intestine *in vivo*. All tissues show dose-dependent, biphasic responses at similar concentrations of the hormone. The responses are rapid in all tissues and in both the *in vivo* and *in vitro* intestine have been shown to be associated with non-electrogenic processes. However, one discrepancy exists; that of pre-treatment of the animals. In order to obtain a response to angiotensin using rat intestine *in vitro*, the endogenous sources of sodium-retaining hormones must first be suppressed by prior adrenalectomy and nephrectomy or by salt loading the animals (Crocker & Munday, 1970; Davies *et al.* 1970). Similarly, Barraclough *et al.* (1967) obtained an antinatriuretic response to angiotensin in rat kidney *in vivo*, only when the animals had been salt loaded. In contrast, no prior treatment of the rats is necessary in order to obtain responses to angiotensin in both jejunum *in vivo* as shown in the experiments described in this paper, and kidney cortex slices *in vitro* (Munday *et al.* 1971). Why different preparations require varying preliminary treatments in order to exhibit responses to added angiotensin is by no means clear, but may relate to the constraints imposed by the inherent characteristics of the alternative preparations.

It is now becoming increasingly evident that angiotensin has an important and central role in the maintenance of the extracellular fluid volume. Under extreme conditions, the pressor actions of the hormone (Pickering & Prinzmetal, 1938) will limit the consequences to the cardiovascular system of a large fall in the extracellular fluid volume. Furthermore, on a moment to moment basis, angiotensin regulates extracellular fluid volume by contributing to the control of drinking behaviour (Fitzsimons & Simons, 1969), the secretion of antidiuretic hormone (Mouw, Bonjour, Malvin & Vander, 1971) and sodium retention by both the alimentary canal and the kidneys. Increased sodium retention is achieved by stimulating aldosterone secretion from the adrenal cortex (Kaplan & Bartter, 1962) and by the direct actions of angiotensin on kidney and intestine transport function.

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