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Induction of hypovolaemia by thirst-inducing doses of renin or angiotensin II

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Summary

1. Intravenous injection of five Goldblatt units (Gbl.U) of renin, or intravenous infusion of 2·5 μg of angiotensin into nephrectomized rats decreased plasma volume by $22 \pm 2\%$ (calculated from the increase of the haematocrit) or by $14 \pm 2\%$ (calculated from the increase in protein concentration) within 2 minutes. Fifteen minutes and 60 min after injection or infusion, the plasma volume (calculated from both parameters) was still 10% lower than initially.

2. The initial discrepancy between haematocrit values and plasma protein concentration was neither due to mobilization of red blood cells (r.b.c.) from the spleen, nor to an increase in r.b.c. volume.

3. The observed decrease in plasma volume was large enough to explain the drinking response to renin and angiotensin. Renin may thus induce or aggravate hypovolaemia rather than signalling its occurrence to hypothalamic receptors.

Introduction

Intravenous injection of renin (Fitzsimons, 1969; Haefeli & Peters, 1970), or intravenous infusion of angiotensin (Fitzsimons & Simons, 1969) causes nephrectomized rats which are in fluid balance to drink water. In rats, nephrectomy decreases the drinking response to depletion of their extracellular fluid volume by subcutaneous injection of hypertonic colloids (Blass & Fitzsimons, 1970), or to partial hypovolaemia by ligation of the abdominal vena cava (Fitzsimons, 1969). In normal rats both these situations elicit an acute increase of renin secretion. Renin could be the mediator of this type of extracellular thirst. This hypothesis was supported by the observation that angiotensin, albeit in large doses, induced drinking when injected directly into the hypothalamus (Epstein, Fitzsimons & Simons, 1968).

On the other hand, Nairn, Masson & Corcoran (1956), Giese (1963) and Cuthbert & Peart (1970) have shown that high doses of renal extracts or partially purified renin induce, in nephrectomized rats, effusions of fluid into serous cavities, swelling of the pancreas and an increase of the haematocrit with an unchanged or decreased plasma protein concentration (Cuthbert & Peart, 1970). All these changes were recorded 24 h–6 days after injection of renin, and occurred only in nephrectomized or anuric rats. This loss of plasma volume has been assumed to be due to an increased permeability of the vascular walls to proteins.

Our study was undertaken to find out if any loss of plasma volume is induced by high doses of renin at the time when renin increases water intake, that is within the

first hour following the injection, and whether such a loss is large enough to account for the drinking. If this were so, renin could induce thirst by causing hypovolaemia rather than by informing the hypothalamus of a decrease of the blood volume.

Methods

Male Wistar rats weighing 350–400 g, on food and water *ad lib.*, were nephrectomized under light ether anaesthesia. Ninety minutes later they were anaesthetized again with pentobarbitone (40 mg/kg intraperitoneally). The carotid artery was exposed and cannulated. Heparin (0·2 ml ; 0·5% in saline) was injected through the cannula. After another 10 min, blood was taken from the carotid directly into three microhaematocrit tubes (Capilets, Dade, Miami, Fla., USA) and five Goldblatt units (Gbl.U) of purified hog renin (Nutritional Biochemical Corporation, Cleveland, Ohio, USA), in 0·5 ml saline, or 0·5 ml saline were injected through the cannula. Blood samples for measuring the haematocrit were then obtained at various intervals, that is 2, 5, 8, 15, 30 or 60 min after injecting renin. Not more than one postrenin sample was taken from any one individual rat, in order to avoid haemorrhagic hypovolaemia. Five to twelve experiments were performed for each of the time intervals stated.

In similar experiments, 0·5 ml of blood was withdrawn before and 2, 8, 15 or 60 min after injecting renin for measuring the plasma protein concentration by a Kjeldahl procedure. Each experimental group comprised five animals.

The same experiment was performed in a group of five rats which had been splenectomized, under light ether anaesthesia, 48 h before, in order to prevent a possible increase of haematocrit by red blood cells originating from the spleen. In some experiments, 0·5 ml of an angiotensin solution containing 2·5 μg of val_5 angiotensin II—amide (Hypertensin, Ciba) were infused within 5 min into the nephrectomized rats. The control animals received 0·5 ml of 0·9% NaCl.

A possible influence of angiotensin on the volume of red blood cells (r.b.c.) was searched for *in vitro*, using the following technique. Three millilitres of blood from the carotid of a rat, nephrectomized 90 min earlier, were collected in tubes kept at 37° C. One microgramme of angiotensin dissolved in 0·1 ml of saline was added and the mixture was incubated for 10 minutes. The haematocrit was measured immediately before adding angiotensin and at the end of the incubation period. Five such experiments were performed with angiotensin, while 0·1 ml of saline was added to the blood in five simultaneous experiments.

The plasma volume for 100 ml of r.b.c. was calculated as: $(100-\text{Hct}) \times 100/\text{Hct}$ (Hct=haematocrit); the plasma volume per gramme of plasma protein as: $(1{,}000-\text{P}) \times 1{,}000/\text{P}$ (P=plasma protein concentration in grammes per litre). Plasma volume after injecting renin or infusing angiotensin was expressed as a fraction of the preinjection control value for each individual animal. Numerical values are expressed as means $\pm$ standard error.

The haematocrit value and the plasma protein concentration decreased slowly and progressively in the nephrectomized control rats as described previously by Cuthbert & Peart (1970). The increases caused by the injection of renin or infusion of angiotensin were therefore calculated for each treated animal as the actual increase from the preinjection value plus the mean decrease in the control group.

Results

Intravenous injection of 5 Gbl.U of renin into nephrectomized rats induced a rapid rise of the haematocrit which reached a peak after 2 min, decreased slightly up to the fifteenth min and then remained approximately constant, though higher than before the injection, up to 60 minutes (Fig. 1). In control rats, the haematocrit decreased progressively and fell to $-3{\cdot}8 \pm 1{\cdot}1$ within 1 hour.

The increase in the plasma protein concentration was significantly smaller than the increase of the haematocrit 2 min after injecting renin, but not later. Figure 2

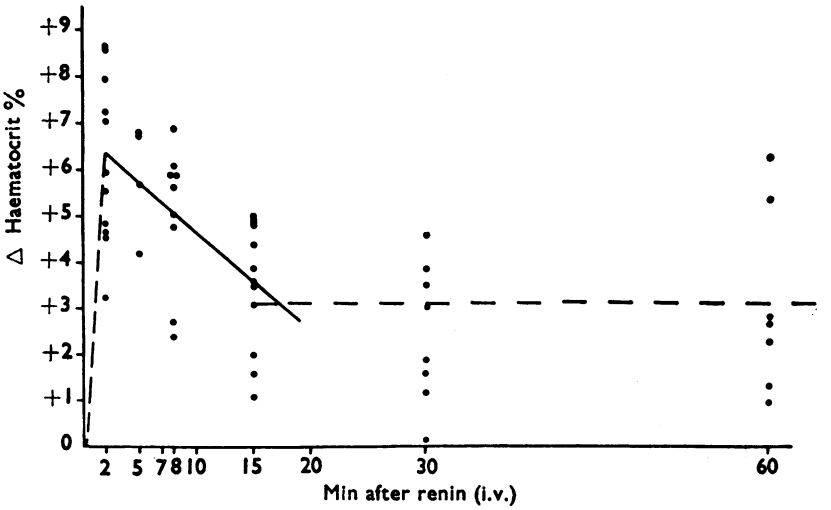

FIG. 1. Change in haematocrit values at various times after intravenous injection of renin (5 Gbl.U) in rats. Dots are individual points. The full line is a regression line calculated from the increases of haematocrit values 2–15 min after injection. The horizontal interrupted line shows the mean increase in haematocrit between 15 and 60 min after injection. For the calculation of individual values see **Methods.**

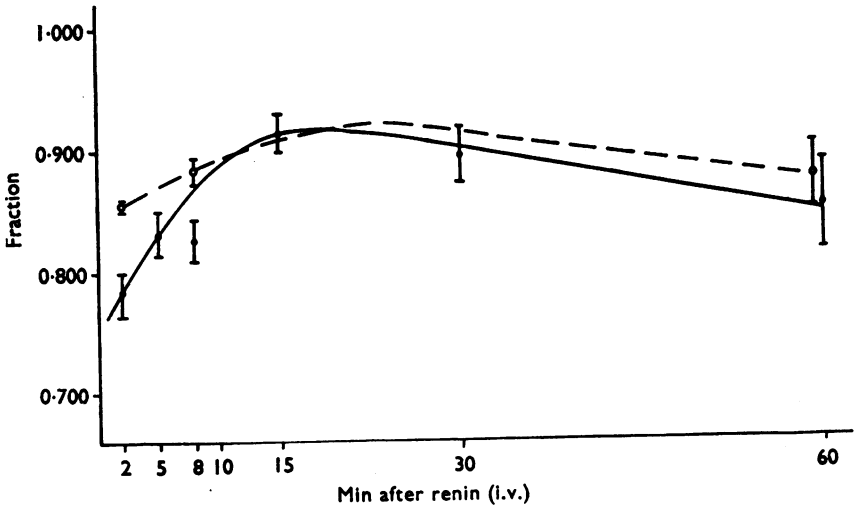

FIG. 2. Total plasma volume after intravenous injection of renin (5 Gbl.U) as a fraction of preinjection plasma volume, calculated either from changes in haematocrit values (lower curve) or from changes in plasma protein concentration (broken line). The curves shown were adapted visually.

shows the changes of plasma volume per 100 ml of r.b.c. or per gramme of plasma protein. Two minutes after injecting renin, the loss of plasma volume calculated from the increase of the haematocrit was greater than that calculated from the increase in plasma protein concentration, indicating a small loss of plasma protein occurring simultaneously with a greater loss of protein-free fluid. From 15 min onwards, the loss of plasma volume per 100 ml r.b.c. and per gramme of protein became equal, indicating that only protein-free fluid had left blood vessels.

Infusion of angiotensin had the same effect as injecting renin. The dose of angiotensin used induced approximately the same rise in blood pressure as the dose of renin injected (+40–50 mmHg (1 mmHg≡1·333 mbar)). Five minutes after the end of the infusion of angiotensin, the haematocrit value was increased by $5{\cdot}2 \pm 0{\cdot}6$ vol. % and 10 min after the end of the infusion by $5{\cdot}7 \pm 0{\cdot}9$ vol. %.

Adding angiotensin to blood *in vitro* did not significantly influence the haematocrit which was $48{\cdot}5 \pm 2{\cdot}0$ vol. % before and $47{\cdot}7 \pm 3{\cdot}4$ vol. % 10 min later. No change occurred in the haematocrit values of the control experiment.

In splenectomized nephrectomized rats, the haematocrit rose to the same extent as in nephrectomized rats. It increased by $+4{\cdot}8 \pm 2{\cdot}0$ vol. % within 5 minutes.

Discussion

A maximal increase of the haematocrit was measured as soon as 2 min after injecting renin. At this time, the increase in protein concentration was less pronounced. Therefore the loss of plasma volume was smaller when calculated from the change of the protein concentration than from the increase of the haematocrit. This may indicate that the fluid escaping from the vascular bed contained less protein than the plasma. It could also mean that there was a small loss of protein-free fluid from the plasma and a simultaneous passage of sequestered r.b.c. into the circulating blood. The mobilization of r.b.c. could be a direct effect of angiotensin or a consequence of drug induced hypovolaemia. Stricker (1968) has suggested a similar explanation for a similar discrepancy observed after subcutaneous injection of polyethylene-glycol. Removal of the spleen, which is the main r.b.c. reservoir did not, however, depress the increase of the haematocrit after renin. This explanation therefore appears unlikely. Finally, an increase in the size of the r.b.c., due to an inhibition of the sodium pumps of the r.b.c. by angiotensin, could account for the greater increase of the haematocrit value as compared with the protein concentration. If this were so, angiotensin should also cause r.b.c. to swell *in vitro*. No such swelling was observed in our experiments.

Between 2 and 15 min after injection, we observed a fall in the haematocrit values and a smaller decrease in the plasma protein concentration. Both phenomena could be due to reentry of fluid from the extracellular space into the circulating blood, when the blood pressure begins to fall after the initial peak. The fluid reentering the vessels must be assumed to contain less protein than plasma, though possibly slightly more than the fluid originally extravasated. Protein could be adsorbed to constituents of the vascular walls during the first 2 min after injecting renin, and could subsequently be carried back into the plasma with the reentering fluid.

Between 15 and 60 min there was a very small, though statistically not significant increase of the haematocrit and the plasma protein concentration. If this

increase were real, it might be the first expression of the simultaneous loss of protein and water from blood which becomes detectable 22 h later (Cuthbert & Peart, 1970). In our experiments, the loss of plasma volume 2 min after renin amounted to $-22 \pm 2\%$ as calculated from changes in the haematocrit value and to $-14 \pm 2\%$ as calculated from the increase of protein concentration; at 15 min the values were $-9 \pm 2\%$ (haematocrit) and $-12 \pm 1\%$ (plasma protein) (Fig. 2). The loss of plasma volume appears large enough to explain the drinking response observed. Stricker (1968) has shown that a drinking response after subcutaneous injection of polyethylene-glycol occurs when the plasma volume falls by 9% (calculated from the haematocrit value) or by 6% (calculated from the plasma protein concentration). Renin or angiotensin thus appear to induce drinking by causing hypovolaemia rather than by informing cerebral receptors on hypovolaemia.

The observation that hypovolaemia induced by other means causes more drinking in the presence than in the absence of the kidneys (Blass & Fitzsimons, 1970) could mean that endogenous renin aggravated the hypovolaemia. It appears more difficult to explain the drinking response to angiotensin injected into the hypothalamus (Epstein *et al.*, 1968). Angiotensin could, however, cause a topical vasoconstriction and a loss of fluid from hypothalamic vessels. Decrease of the blood volume in a circumscribed area of the hypothalamus could be a signal for hypovolaemic drinking.

A loss of plasma fluid into the extravascular space cannot easily be demonstrated in non-nephrectomized rats, because of the well known diuretic effect of renin and angiotensin (Peters, 1963). Fitzsimons (1969) as well as ourselves (unpublished) observed a smaller drinking response to intravenous renin in normal than in nephrectomized rats. This difference may be due to the smaller increase of blood pressure in normal animals. It is, however, difficult to explain why renin diuresis does not enhance the drinking response. If injection of renin, or an acute liberation of endogenous renin induces drinking by raising the blood pressure, and forcing fluid out of the blood vessels, the absence of drinking responses to chronic increases of plasma renin activity (Haefeli & Peters, 1970) becomes readily understandable. An increase in blood pressure does not, however, appear to be the only factor responsible for the loss of plasma volume and the drinking response to renin or angiotensin. Thus, Nairn *et al.* (1956) did not observe any effusions into serous cavities after noradrenaline, while Fitzsimons & Simons (1969) did not observe any drinking response to pressor doses of vasopressin.

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