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FACTORS AFFECTING THE TRYPSIN INDUCED RELEASE OF ALDOSTERONE IN RAT ADRENAL ZONA GLOMERULOSA TISSUE

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Summary—The yields of aldosterone obtained during incubation of whole adrenal capsule tissue from the rat (consisting of the connective tissue capsule itself, all of the glomerulosa tissue, and some fasciculata) cannot apparently be accounted for by the gland's capacity for *de novo* synthesis of this steroid. Recent studies with proteolytic enzymes and inhibitors suggest that in part aldosterone output may result from the activation of proteolytic events which release aldosterone from a sequestered intraglandular pool. These proteolytic events are mimicked by the addition of trypsin to whole tissue incubations *in vitro*. Experiments were carried out to determine what factors may govern the size of such intraglandular steroid pools. The most remarkable effect was that prior sodium depletion greatly enhanced the yield (2–3-fold) of aldosterone on subsequent incubation of adrenal capsules with trypsin, to an extent far greater than the increase in basal (non trypsin induced) aldosterone output in this tissue. Although betamethasone (20 μg/ml in drinking water) and the converting enzyme inhibitor captopril (7.2 mg/day) eliminated trypsin releasable steroid in control animals, they had no effect on the enhanced levels of trypsin releasable steroid seen with sodium depletion. The data suggest that trypsin releasable steroid pools are variable in accordance with the physiological requirements of the animal, particularly in sodium depletion.

INTRODUCTION

The existence of sequestered pools of steroid in the intact rat adrenal zona glomerulosa which are utilised as secretory reserves has been suggested by indirect experimental evidence [1, 2]. More recently, the specific release of aldosterone and 18-hydroxycorticosterone on incubation with trypsin was shown to occur [3], even when *de novo* synthesis of steroids was inhibited [4].

Further studies with protease inhibitors suggested that trypsin may be replicating the effects of endogenous proteases [5]. This paper describes the preliminary results of experiments designed to investigate the factors which affect the scale and extent of the trypsin induced release of aldosterone.

EXPERIMENTAL

Rats of the Wistar strain were taken from the colony maintained at St Bartholomew's Medical College. Animals received the following pretreatments *in vivo*: (1) Control group, normal diet (laboratory diet no. 1, Spratt's, Reading, Berkshire) and water *ad libitum*. (2) Captopril (Capoten, Squibb; 7.2 mg/kg per day, i.p., for 5 days). (3) Dietary sodium restriction [6] for 2 weeks. (4) Dietary sodium restriction for 2 weeks with captopril treatment as above for the last 5 days. (5) Betamethasone (Betnesol, Glaxo; 20 μg/ml with 1% glucose in drinking water for 2 weeks). (6) Dietary sodium restriction and betamethasone as above for 2 weeks.

Blood was collected following decapitation of the animals, and plasma stored frozen until required for extraction. Capsular tissue was incubated and aldosterone extracted and measured as previously described [4, 7]. Half of the adrenal pairs from each *in vivo* group were incubated with no additions, half with the addition of trypsin (2 mg ml^{-1}, Sigma, 11,250 BAEE units per mg protein).

RESULTS

The most marked effect of the various treatments was that capsules from sodium depleted animals invariably yielded much greater amounts of aldosterone on incubation with trypsin compared with tissue from control animals (Figs 1, 2 and 3), and as expected, plasma from sodium restricted animals contained more aldosterone than controls.

The converting enzyme inhibitor, captopril (Fig. 1), reduced plasma aldosterone in control animals: its effects in sodium deplete animals are less marked. In the control (non-trypsin) incubations of adrenal tissue aldosterone output was decreased following captopril pretreatment in the sodium deplete (but not in the control) group. Most notably however, although captopril reduced trypsin releasable aldosterone in control animal tissue it had no significant effect on the amount of trypsin-released aldosterone in the

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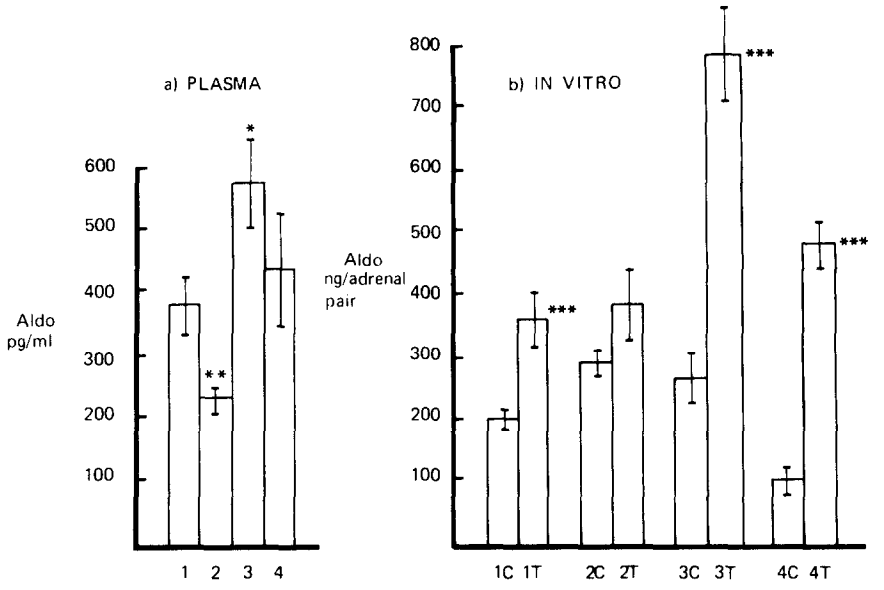

Fig. 1. Effects of sodium depletion and captopril pretreatment *in vivo* on: (a) plasma aldosterone (aldo) and (b) trypsin-releasable aldosterone *in vitro*. In all of the figures *in vivo* treatments are ($n = 12$) referred to by number (see Experimental), letters refer to *in vitro* treatments ($n = 6$). 1 = control diet; 2 = captopril-treated; 3 = sodium depleted; 4 = captopril treated + sodium depleted; C = control incubation; T = incubation with trypsin (2 mg ml^{-1}). In all Figures, * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$ compared with relevant controls by Student's t-test or variance ratio, as appropriate.

adrenal tissue from sodium deplete animals (Figs 1 and 3).

Betamethasone pretreatment also reduced circulating aldosterone in both control and sodium depleted animals (Fig. 2) and in addition it reduced aldosterone released in control (non-trypsin) incubations of tissue from sodium depleted animals. However, although trypsin-releasable aldosterone was eliminated from control tissue it was not affected by betamethasone pretreatment in tissue from sodium deplete animals.

DISCUSSION

In contrast to *de novo* synthesis the effect of trypsin on intact glomerulosa tissue is taken to indicate the release of relatively large amounts of aldosterone from steroid stores sequestered within the tissue, although this remains a hypothesis since more direct evidence is still lacking [3, 4].

The control of aldosterone secretion is known to be multifactorial [8–10]. Physiologically the two primary stimulants of increased aldosterone secretion are sodium restriction and decreases in extracellular fluid

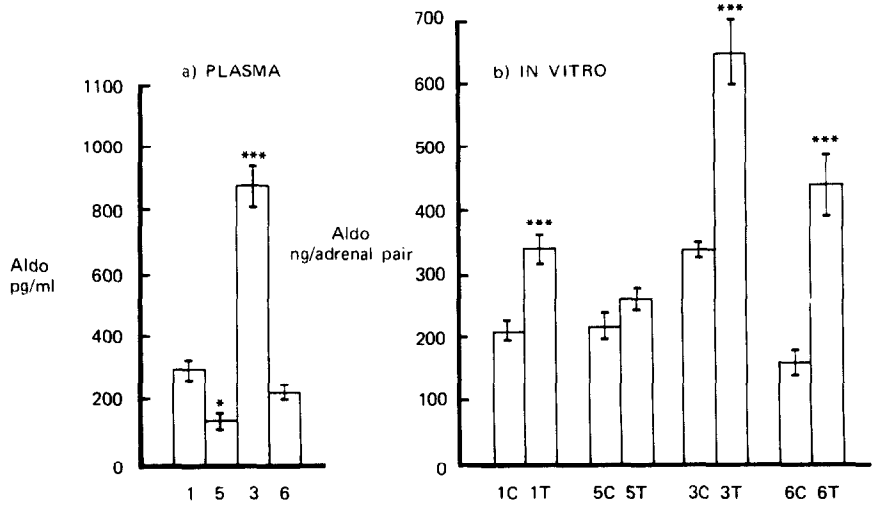

Fig. 2. Effects of sodium depletion and betamethasone pretreatment *in vivo* on: (a) plasma aldosterone ($n = 12$) and (b) trypsin-releasable aldosterone *in vitro* ($n = 6$). 1 = control; 5 = betamethasone-treated; 3 = sodium-depleted; 6 = betamethasone treated + sodium depleted; C = control incubation; T = incubation with trypsin (2 mg ml^{-1}). Other symbols as for Fig. 1.

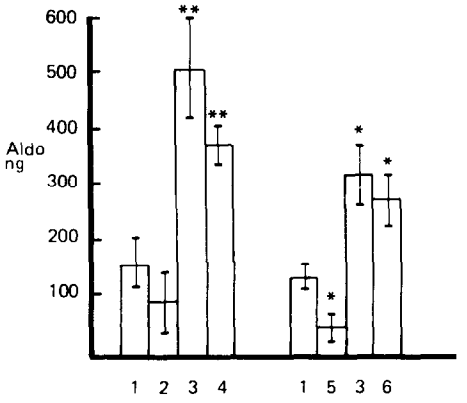

Fig. 3. Summary of effects of the *in vivo* treatments ($n = 12$) on the magnitude of the *in vitro* release of aldosterone by trypsin, derived by subtracting the control (non-trypsin) mean value for aldosterone production per pair of incubated glands from each of the trypsin-treated values ($n = 6$) for each pretreatment group. 1 = control; 2 = captopril-treated; 3 = sodium-depleted; 4 = captopril-treated + sodium depleted; 5 = betamethasone-treated; 6 = betamethasone-treated + sodium depleted. Significant differences are from control means, symbols as for Fig. 1. (NB: There was no significant release of aldosterone by trypsin compared with control incubations in groups 2 and 5).

and blood volume. Of the multiplicity of factors which are known to affect the gland, however, it has proved difficult to unravel the precise means by which these effects are elicited. At different times over the last few years various factors, notably plasma potassium and angiotensin II, have been favoured by different groups [8–13], and in addition the pituitary must be involved, since the response to sodium depletion is diminished by both hypophysectomy in experimental animals [14] and hypopituitarism in man [15].

The most remarkable effect in these preliminary experiments is the great increase in trypsin releasable aldosterone seen after a period of dietary sodium restriction (Figs 1, 2, 3). This is particularly noteworthy since adrenal tissue *in vitro* rapidly "forgets" changes induced by stimuli applied *in vivo*: the preincubation step was deliberately included so as to reduce the high "background" of *de novo* synthesis. The effects of sodium depletion on adrenal zona glomerulosa function *in vitro* have been widely studied and characterised. For example, basal aldosterone output is increased, and the magnitude of the responses to stimulants such as angiotensin II or potassium is enhanced [12].

Although both betamethasone and captopril pretreatments reduced the response to trypsin in tissue from sodium replete animals (Fig. 3), the present results give no indication of the involvement of these effectors in increasing the magnitude of the trypsin releasable aldosterone pool in sodium depletion. They suggest, for example, that angiotensin II is not involved, since treatment with the converting enzyme inhibitor captopril decreased circulating aldosterone concentrations, as previously reported [17], and also reduced basal levels of aldosterone released *in vitro*, but had no effect on the increment of aldosterone released in response to trypsin in tissue from sodium deplete rats (Figs 1 and 3). However, to define the role of angiotensin II more precisely, further experiments with varying doses of captopril would be required.

The importance of pituitary ACTH secretion in controlling circulating aldosterone concentrations is seen in the effects of betamethasone (Fig. 2) pretreatment, which greatly reduced circulating aldosterone levels, although its effect on amounts produced during *in vitro* incubation varied. In particular, however, it had no effect on the amount of aldosterone released by trypsin in sodium depleted tissue (Fig. 3). Trypsin releasable aldosterone in rat adrenal glomerulosa tissue responds to sodium depletion in a manner which suggests that it could be utilised by the animal as a secretory reserve according to physiological demand. Mechanisms should be sought for this response but the preliminary data reported here do not suggest a major role for angiotensin II or the pituitary. One possibility, not studied in these experiments, is that potassium may be directly involved, since it is difficult to differentiate between the actions of sodium depletion and elevated potassium under the conditions used.

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