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CONTROL OF ALDOSTERONE SECRETION IN ZONA GLOMERULOSA CELL SUSPENSIONS AND IN THE PERFUSED ADRENAL GLAND OF THE RAT

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Summary—The secretion of aldosterone and its responses to stimulation have been studied in rat adrenal zona glomerulosa tissue incubated as intact capsules or as collagenase-dispersed cell suspensions, and in intact perfused rat adrenal glands. Several differences are apparent in the functions of the various preparations. Aldosterone secretion rates are similar in incubated intact capsules and in the perfused gland. Relative to corticosterone, lower yields of aldosterone are obtained in dispersed glomerulosa cell *in vitro*. This may be related to the loss in the dispersed cells of a pool of tissue steroid (aldosterone or a precursor) which is revealed only in intact tissue incubations by trypsin stimulation of aldosterone secretion. Trypsin-released aldosterone is increased by prior dietary sodium restriction.

In addition, differences occur in the responses of dispersed cells and perfused glands to stimulation. Perfused glands from animals on a normal diet are less sensitive to stimulation by ACTH or α-MSH, but more sensitive than dispersed cells to angiotensin II amide. In the perfused gland, sensitivity of response (lowest effective concentration) to all three stimulants is increased by prior dietary sodium restriction, in contrast to dispersed cells in which increased sensitivity has been reported only to α-MSH. The perfused gland is particularly sensitive to angiotensin II amide, and a bolus administration of 1 amol gives significant stimulation in glands from animals on low sodium intake. Electrical (field) stimulation or dopamine administration at 10^{-6} mol/l (which is ineffective in dispersed cells) both depress aldosterone secretion by the perfused gland.

The data suggest that the sequestered pool of steroid is utilized in the perfused gland for aldosterone secretion. They furthermore suggest that in the intact gland there are mechanisms, which involve neural components, for intraglandular regulation of aldosterone secretion, which are lost in dispersed cells *in vitro*. Such mechanisms may be involved in sensitivity increases in sodium depletion.

INTRODUCTION

The study of the control of aldosterone secretion has been influenced by the available methodology. Early studies were based on the collection of the adrenal venous drainage [1–3], and later (following the advent of RIA techniques for aldosterone) peripheral plasma samples from animals exposed to different regimes of sodium intake or hormone treatment [4–6]. Over the past 15 yr, the emphasis has shifted to *in vitro* methods, following the introduction of the collagenase- (or trypsin)-dispersed cell systems [7–9]. There is little doubt that these techniques have contributed enormously to our understanding of the multiplicity of factors which may affect the zona glomerulosa and aldosterone secretion. However, there is relatively little information on how well the *in vitro* data reproduce the *in vivo* condition, or to what extent the function of the tissue is affected by the conditions of enzyme digestion and tissue dispersal. Some evidence suggests that this may be important: steroid secretion rates are considerably lower *in vitro* than *in vivo* and, in particular, aldosterone production *in vitro* appears to be sensitive to the degree of tissue disruption [10–12]. The relationship between the responsiveness of dispersed cells and the intact gland to stimulation by the physiological effectors is also difficult to determine with any certainty.

For these reasons it seemed to be essential to develop methods for studying the gland under conditions in which its architecture was undisturbed, in which stimulants were delivered to the gland through the vasculature in a manner approximating the physiological condition, and in which nevertheless the gland was completely isolated from the systemic circulation so that the content of the medium reaching the adrenocortical cells was closely defined and controlled. The *in situ* isolated perfused rat adrenal gland system was developed for this purpose [13].

Using known stimulants of the zona glomerulosa, this paper examines some of the responses of the *in situ* perfused rat adrenal in animals on a normal diet, or receiving limited sodium intake. *In vitro* studies are also described, which offer a comparison with the perfused gland, and suggest possible interpretations of some of its special characteristics.

MATERIALS AND METHODS

In vitro *methods*. Wistar or Sprague–Dawley rats maintained either at King's College London (KQC) or at St Bartholomew's Medical College were used

throughout. Males were used for whole capsule incubations and perfusions; both sexes were used for dispersed cell experiments. Animals were maintained on standard laboratory diets, except when the effects of dietary sodium restriction were studied. In this case, animals were maintained on a wholemeal flour diet supplemented with 1% $CaCO_3$, and control animals received 1% NaCl in addition. In these experiments animals were given only distilled drinking water [14].

Adrenal capsule preparations, containing mostly glomerulosa cells, but with 5–10% contamination by fasciculata cells, were incubated as whole tissue or as collagenase-dispersed cell preparations as previously described [10]. Incubations of whole tissue were in Krebs–Ringer bicarbonate solution with glucose (KRBG, 1 pair of glands/5 ml), and dispersed glomerulosa cells were incubated in KRBG with 1% bovine serum albumin (Sigma fraction V) (KRBGA, approximately 150,000 cells/5 ml). In intact tissue experiments, glands were first preincubated for 1 h, the medium was then changed and ACTH [1–24] (ACTH, Synacthen, Ciba-Geigy, 10^{-9} mol/l), or trypsin (Sigma, 2 mg/ml) were added to experimental flasks.

Intact adrenal perfusions in situ. The method used has been described in detail elsewhere [13, 15]. Briefly, medium is introduced, via the coeliac artery, into an isolated segment of aorta, from which the left adrenal arteries arise, and is collected from a pocket in the renal vein. The perfusion medium used in these studies was tissue culture Medium 199 (Difco Laboratories, Detroit, MI), modified by dilution (4:3) with KCl-free Krebs–Ringer bicarbonate to give a final potassium ion concentration of 3.9 mmol/l, with 0.5% BSA (fraction V, Sigma). Perfusate was delivered at a constant rate of between 0.4 and 0.8 ml/min throughout these experiments. Ten-min samples were collected. ACTH, α-MSH, or angiotensin II amide (Hypertensin, Ciba-Geigy) were administered in 0.1 ml bolus doses, but dopamine (Sigma, with 0.4% ascorbate) was infused over a 10-min period. The gland was subjected to electrical (field) stimulation following the method of Wakade[16] for 1 ms at 1 Hz and 60 V for 5 min using externally applied silver electrodes.

Steroids were extracted from incubation and perfusion media, and assayed by GLC and radioimmunossay, as previously described [17, 18].

RESULTS

The effects of trypsin and ACTH on aldosterone production by incubated capsule whole tissue and dispersed cells are shown in Fig. 1. Both preparations are stimulated by ACTH, but only intact tissue responds to trypsin with increased aldosterone secretion. The action of trypsin was greatly enhanced in tissue from animals maintained on a sodium-depleted diet (Fig. 2).

The perfused rat adrenal gland gave excellent dose-related responses to all of the stimulants used: ACTH, angiotensin II amide, and α-MSH. The sensitivity (lowest effective concentration) of these responses is shown in Table 1, and compared with the sensitivity of the responses of dispersed capsular cells. For both ACTH and α-MSH, the perfused gland is less sensitive then dispersed cells, but for angiotensin II amide the perfused gland is much more sensitive than the dispersed cell preparations.

Differences between the perfused gland and dispersed cells are more marked, however, when the data from the glands from sodium-restricted animals is considered. It will be clearly seen that sensitivity to α-MSH and ACTH as well as to angiotensin II amide is increased by this treatment in the perfused intact gland. The change in sensitivity to angiotensin II amide is particularly marked and in these experi-

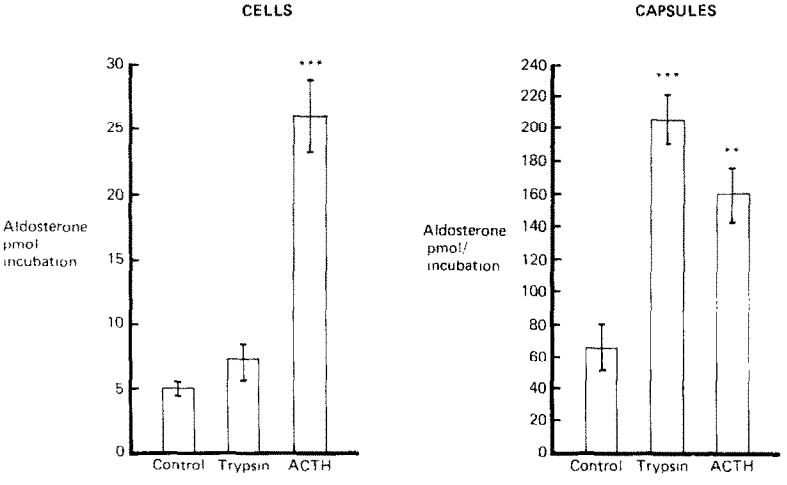

Fig. 1. Secretion of aldosterone by rat adrenal glomerulosa tissue incubated as intact capsules, and as collagenase-dispersed cell suspensions, and the response to trypsin and ACTH. $^{**}P < 0.01$, $^{***}P < 0.001$ relative to the appropriate controls, $n = 6$ throughout.

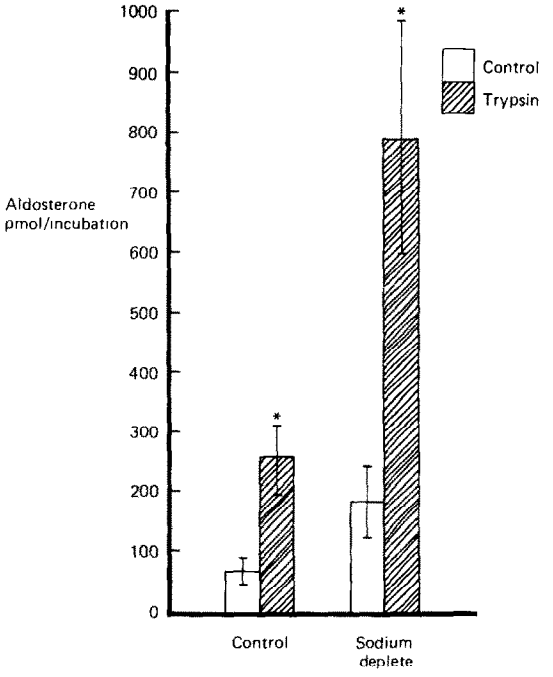

Fig. 2. Effect of prior dietary sodium restriction on the secretion of aldosterone in response to trypsin by rat adrenal zona glomerulosa tissue incubated as whole capsules. $*P < 0.05$, relative to the appropriate control.

ments 1 amol of the peptide elicited a significant increase in aldosterone secretion (Figs. 3–5, Table 1). The strong responses illustrated here in perfused glands from rats on low-sodium diets were to amounts of stimulant which were below the threshold dose in rats on a normal diet.

Dopamine (10^{-6} mol/l) or field stimulation decreased aldosterone production in the perfused gland (Fig. 6). Dopamine at this concentration and at 10^{-4} mol/l had no effect on dispersed cells (results not illustrated).

Table 1. Sensitivities (lowest effective doses, mol/l) of aldosterone responses to stimulants in incubated dispersed glomerulosa cells and in perfused adrenal glands from animals on normal diets, and subjected to dietary sodium restriction

	Normal	Na^+ restricted
A. Cells		
ACTH	5×10^{-13}	5×10^{-13}
α-MSH	10^{-7}	10^{-10}
Angiotensin II*	10^{-10}	10^{-10}
B. Perfused glands		
ACTH	1.5×10^{-10}	1.5×10^{-13}
α-MSH	3×10^{-7}	3×10^{-10}
Angiotensin II	5×10^{-13}	5×10^{-15}

*Data from Aguilera *et al.*[24]. Transient 4-fold increases in sensitivity were shown by these authors after 36 h sodium restriction, but not after 4 days.

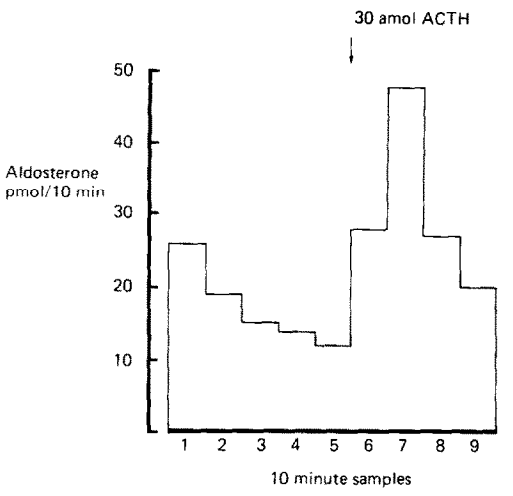

Fig. 3. An example of aldosterone secretion in response to ACTH stimulation by the perfused adrenal of a rat subjected to dietary sodium restriction. Significant stimulation ($P < 0.05$ or better) occurred with 30 fmol ACTH in normal animals ($n = 5$), and 300 amol in animals on low sodium intake ($n = 6$).

DISCUSSION

The results indicate that the *in situ* perfused intact adrenal gland of the rat offers a sensitive model for studying the control of corticosteroid secretion under conditions which approximate to the *in vivo* situation. There are several ways in which the functions of this preparation differ, however, from the

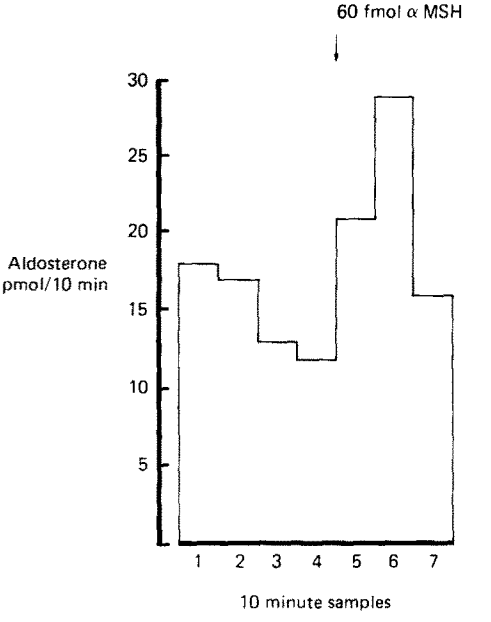

Fig. 4. Aldosterone secretion in response to α-MSH stimulation by the perfused adrenal of a rat subjected to dietary sodium restriction. Significant stimulation ($P < 0.05$ or better) occurred with 60 pmol α-MSH in normal animals ($n = 9$), and 60 fmol in animals on low sodium intake ($n = 5$).

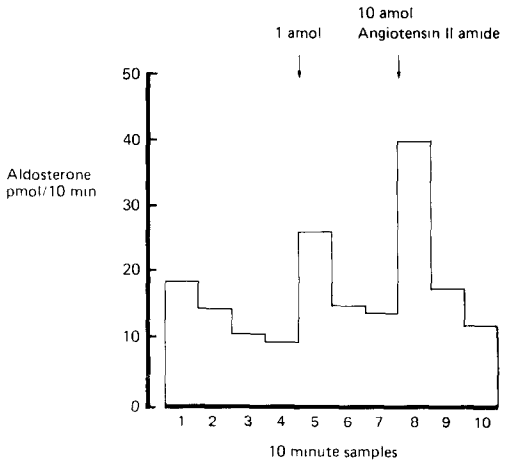

Fig. 5. Aldosterone secretion in response to angiotensin II amide stimulation by the perfused adrenal of a rat subjected to dietary sodium restriction. Significant stimulation ($P < 0.05$ or better) occurred with 100 amol angiotensin II amide in normal animals ($n = 4$), and 1 amol in animals on low sodium intake ($n = 7$).

widely applied methods of *in vitro* incubation of collagenase-dispersed cells. These, in turn, differ in their properties from incubated whole capsule tissue. In this paper, the experiments described were concerned with the mode of control of aldosterone secretion, but it should be noted that differences in function also occur in the mode of control of corticosterone secretion in the two preparations [19].

Nature of the secreted glomerulosa product. *In vitro*, different preparations of rat adrenal zona glomerulosa yield different steroid profiles. This is particularly exemplified by the relative amounts of corticosterone and aldosterone. If intact capsule tissue (consisting of glomerulosa cells, and some fasciculata cells, as well as the connective tissue capsule itself) is incubated, relatively large amounts of aldosterone are secreted under both basal or stimulated conditions. Corticosterone is always the major secreted product, however, and the corticosterone/aldosterone ratio is often about 3–4 [10,11]. In dispersed cell preparations of glomerulosa tissue from the same animals, kept under the same conditions, the ratio is higher, at about 10. It appears that as the tissue is disrupted, the capacity to form aldosterone may be reduced [12]. The results presented in Fig. 1 suggest a possible explanation for this discrepancy. It is clear that trypsin-releasable steroid occurs in intact tissue, but not in dispersed cells. This pool of releasable steroid may be aldosterone itself, or a precursor. In any event its possible physiological importance is indicated by the effect of dietary sodium depletion, which greatly enhances the yield of aldosterone on treatment with trypsin (Fig. 2). Rates of aldosterone production in the perfused intact gland, maximally about 30–35 pmol/10 min in glands from animals on a normal diet, are similar to those of intact glomerulosa tissue incubated *in vitro* [15]. This may indicate that the sequestered steroid pool is utilised as a source of aldosterone by the intact perfused gland.

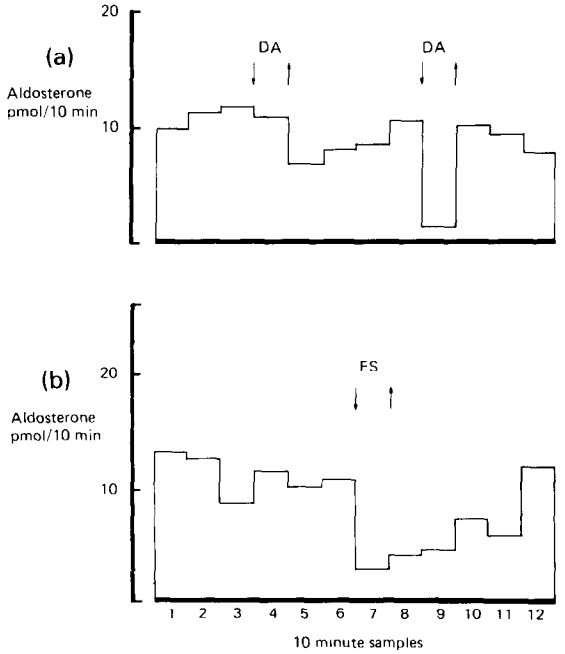

Fig. 6. Effects of (a) dopamine (10^{-6} mol/l) (DA) and (b) field stimulation (FS) on basal aldosterone secretion by perfused rat adrenal glands.

Control of aldosterone secretion. The data show strongly that the control of steroidogenesis also operates differently in the perfused gland, whole capsules and dispersed cells *in vitro*. In the first place the perfused gland is less sensitive to ACTH and to α-MSH stimulation than dispersed cells (Table 1), although the precise limits of sensitivity are difficult to determine. The slightly lower sensitivity of the perfused gland might be anticipated on the basis that before reaching the adrenocortical cells, the stimulants have first to cross the vascular endothelium. Remarkably, however, the system is much more sensitive than dispersed cells to angiotensin II amide.

Further differences between the perfused gland and dispersed cells become apparent when the effects of dietary sodium restriction are considered. Perfused glands from animals subjected to dietary sodium restriction respond significantly more sensitively to ACTH, α-MSH and to angiotensin II amide (Figs. 3–5, Table 1). Indeed, the sensitivity of the response to angiotensin II amide is such that as little as 1 amol of the peptide gives a significant increase in aldosterone secretion. Rough calculation suggests there are approximately 10^6 glomerulosa cells/gland, and this means that a bolus injection of the peptide giving about 1 molecule/10 glomerulosa cells is sufficient to give significant stimulation. This must rate as one of the most sensitive endocrine responses, and may imply that amplification of the signal occurs before it reaches the zona glomerulosa

cell. The data contrast sharply with the results with dispersed cells, in which prior sodium restriction for 4–14 days gives increased sensitivity only to α-MSH, and the sensitivity of responses to ACTH and angiotensin II are unaffected (Table 1). (It should be emphasized that "sensitivity" relates to the lower concentration of stimulant to give a significant effect: overall yields of aldosterone may be higher in cells from sodium-depleted animals, but the dose–response curves for ACTH and angiotensin II amide are not shifted to the left.)

Taken together, the data strongly suggest the existence in the perfused gland of mechanisms for the control of steroid secretion which are eliminated in dispersed cell preparations. In part, this is due to the loss of a tissue pool of aldosterone or its precursor(s) (Fig. 1, Refs. [10, 11]). However, further factors are implicated. It has been suggested that elements of the nervous or vascular systems may play a part in regulation of corticosterone secretion by the perfused gland [19]. The possibility that aldosterone is under the control of neurotransmitters, such as dopamine, has been widely reported, although the mechanisms involved have not been elucidated [20, 21]. In these experiments, both field stimulation and dopamine depressed aldosterone output (Fig. 6), although in common with other authors we have found no effect of dopamine (at a concentration of 10^{-6} mol/l) on basal aldosterone production by dispersed glomerulosa cells *in vitro* [22, 23]. The data suggest that neural mechanisms, which may affect the release of local regulating agents, may also play a part in the intraglandular control of aldosterone secretion. Such mechanisms could account for the changes in sensitivity to ACTH and angiotensin II amide with dietary sodium status which are found in the perfused intact gland, but not in dispersed glomerulosa cells *in vitro*. These possibilities would reward further study.

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