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EFFECT OF THE ENDOTHELINS ON ALDOSTERONE SECRETION BY RAT ZONA GLOMERULOSA CELLS *IN VITRO*

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Summary—Endothelins are thought to be involved in the local regulation of blood flow and tissue function. These experiments were carried out to investigate the possible role of endothelins in the control of aldosterone secretion by the rat adrenal. Suspensions of zona glomerulosa cells were prepared by collagenase digestion of capsular tissue, and incubated in the presence of increasing concentrations of endothelin. Aldosterone was measured by RIA. All three peptides caused a dose-dependent increase in the secretion rate of aldosterone by zona glomerulosa cells. The minimum concentration of peptide required to give a significant response was 10^{-14} mol/l for endothelins 2 and 3 and 10^{-13} mol/l for endothelin 1. At a concentration of 10^{-7} mol/l endothelin 2 elicited a 20-fold increase over basal aldosterone secretion, while both endothelins 1 and 3 elicited a 30-fold increase ($P < 0.001$ in all cases). These results show that the endothelins are potent stimulators of aldosterone secretion, and suggest that these peptides may have a role in the control of zona glomerulosa function.

INTRODUCTION

The endothelins are a family of three recently identified vasoactive peptides which are structurally and pharmacologically distinct: endothelin 1 is made by endothelial cells; endothelin 3 is thought to be of neural origin; while the source of endothelin 2 is unknown (see Ref. [1] for a review). The endothelins have a wide range of biological properties [2], and receptors for these peptides have been identified in many tissues, including the adrenal gland [3]. The present studies are designed to investigate whether endothelins may have a role in the control of aldosterone secretion.

MATERIALS AND METHODS

Collagenase-dispersed rat zona glomerulosa cells were prepared and incubated as described previously [4]. Endothelin 1, 2 and 3 were purchased from Bachem U.K. (Saffron Walden, Essex) and ACTH 1-24 (Synacthen) was obtained from Ciba-Geigy (Horsham, Sussex). Aldosterone was measured by RIA [5].

RESULTS

Aldosterone was stimulated in a dose-dependent manner by each of the peptides tested (Figs 1–3). The minimum concentration of each peptide required for significant stimulation of aldosterone secretion was 10^{-13} mol/l for endothelin 1, and 10^{-14} mol/l for endothelins 2 and 3.

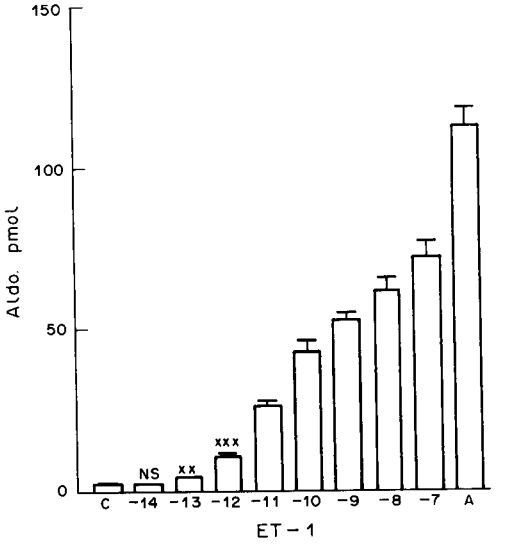

Fig. 1. Effect of endothelin 1 on aldosterone secretion by collagenase-dispersed rat zona glomerulosa cells (pmol/10^5 cells). C = control, A = ACTH (10^{-7} mol/l), numbers on the x-axis show the log concentration of endothelin 1 (mol/l). All bars show mean ± SEM. Significance compared with control: NS = not significant. $^{**}P < 0.01$, $^{***}P < 0.001$ (Student's t-test); $n = 4$ in each case.

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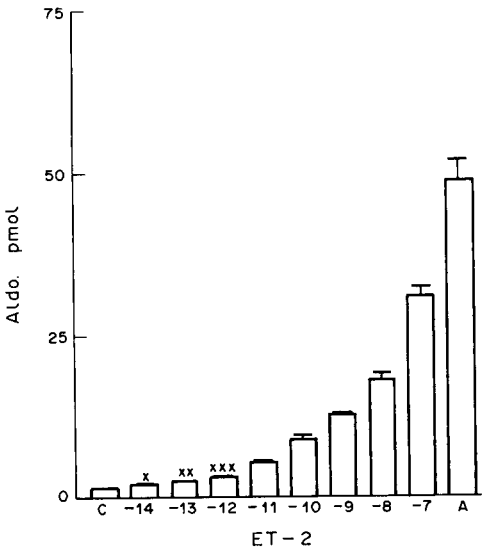

Fig. 2. Effect of endothelin 2 on aldosterone secretion by collagenase-dispersed rat zona glomerulosa cells (pmol/10^5 cells). C-control A = ACTH (10^{-7} mol/l), numbers on the x-axis show the log concentration of endothelin 2 (mol/l). All bars show means of 4 incubations, ±SEM. Significance compared with controls: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ (Student's t-test).

DISCUSSION

Previous studies from this laboratory have shown that there is a marked difference in responsiveness between the intact, perfused adrenal gland *in situ*, and collagenase-dispersed preparations of zona glomerulosa cells. There are several agents which stimulate aldosterone secretion by the intact perfused adrenal gland but which appear to be totally without effect on collagenase-dispersed adrenocortical cell preparations. These include calcitonin gene-related peptide and oxytocin [6, 7].

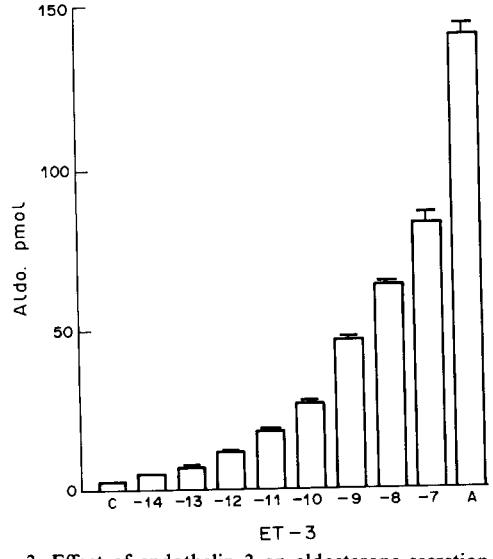

Fig. 3. Effect of endothelin 3 on aldosterone secretion by collagenase-dispersed rat zona glomerulosa cells (pmol/10^5 cells). C = control, A = ACTH (10^{-7} mol/l), numbers on the x-axis show the log concentration of endothelin 3 (mol/l). All bars show means of 4 incubations ± SEM. Significance compared with controls: $P < 0.001$ for all points (Student's t-test).

In experiments with other agents, such as angiotensin II, a much higher concentration of the peptide is required to stimulate steroidogenesis in incubations of collagenase-dispersed cells than is required to stimulate the intact adrenal [8]. The existence of novel intraglandular regulatory mechanisms has been postulated to account for these observations, although the nature of the mechanisms remains unclear. The present finding that the cells of the zona glomerulosa are sensitive to low concentrations of endothelin is particularly interesting in the light of reports of the high degree of vascularity of the adrenal gland [9], and thus the close proximity of adrenocortical cells to the cells of the vascular endothelium.

We propose that agents which stimulate aldosterone in the intact adrenal, but not in conventional *in vitro* adrenocortical preparations, may act by stimulating the vascular endothelium to release endothelin 1, which then acts locally to stimulate steroid secretion. We already have some evidence that this may be the case, since histamine, which has no effect on dispersed cells, stimulates steroidogenesis and endothelin secretion by the intact perfused adrenal gland [10]. It is also possible that the neurally derived peptide, endothelin 3, may be released locally and act on adrenocortical cells. Further work is clearly needed in this exciting area.

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