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10. Iprindole hydrochloride (10 mg/kg) was injected intraperitoneally at various times before the injection of (+)amphetamine sulfate (18.4 mg/kg, intraperitoneally). Brain levels of amphetamine measured 2 hours after injection of amphetamine were 55 ± 6 ng/g in rats not receiving iprindole. When amphetamine was injected 1, 4, 16, 24, or 48 hours after administration of iprindole, amphetamine concentrations in brain tissue were 157 ± 8, 148 ± 10, 167 ± 5, 160 ± 10, and 80 ± 4 ng/g, respectively. All of these values differed significantly from those in the group not first treated with iprindole ($P < .05$). Blank values for untreated rats or rats treated with iprindole alone were not significantly different from those at zero time.
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28 January 1980; revised 18 March 1980

β-Lipotropin: A New Aldosterone-Stimulating Factor

Abstract. *β-Lipotropin stimulated the production of aldosterone in collagenase-dispersed rat adrenal capsular cells. The maximum response obtained with β-lipotropin was the same as the response obtained with corticotropin and was greater than that obtained with angiotensin II. These data suggest that β-lipotropin may play a role in aldosterone regulation.*

Although it is well known that angiotensin II (AII), potassium ion, and adrenocorticotropic hormone (ACTH) stimulate aldosterone secretion, it has become evident that all aspects of the regulation of aldosterone secretion cannot be fully explained by the known stimulators. Previous reports (*1*, *2*) suggest that non-ACTH pituitary factors may play a role in regulation of aldosterone secretion. In 1965, β-lipotropin (β-LPH) was isolated from the ovine pituitary gland (*3*). Both ACTH and β-LPH are contained in a common precursor molecule present in the pituitary gland (*4*). Recently β-LPH became the focus of interest as a prohormone of opioid peptides, but there is no information available regarding the effect of β-LPH on the adrenal gland. In this report, we discuss the effect of sheep and human β-LPH on adrenal steroidogenesis in rat adrenal collagenase-dispersed capsular and decapsular cells. Results indicate that β-LPH has a potent effect on aldosterone production.

For each experiment, 18 to 20 female Sprague-Dawley rats (180 to 220 g), fed a regular sodium diet for at least 2 weeks, were used (*5*). After the animals were decapitated, the adrenal glands were removed and separated into capsular (mainly glomerulosa cells) and decapsular (fasciculata, reticularis, and medullary cells) portions. Each portion was minced with scissors, washed with medium 199 (*6*), and incubated with collagenase (2 mg per milliliter of medium 199) for 20 minutes at 37°C under 95 percent O_2 plus 5 percent CO_2. To disperse capsular and decapsular cells, medium 199 containing bovine serum albumin (BSA; 2 mg/ml) was added to the collagenase-treated tissues, which were then agitated with a Pasteur pipette. The cell suspensions were filtered through gauze into a 50-ml polyethylene centrifugation tube. The dispersed cells were pooled and centrifuged at 100*g* for 15 minutes, and the sedimented cells were resuspended in fresh medium 199 containing BSA (2 mg/ml). After the capsular and decapsular cells were harvested, these cell suspensions (average cell count, 100,000 per tube) were incubated in 1 ml of medium 199 containing BSA (2 mg/ml), with various amounts (10^{-11} to $10^{-5}M$) of sheep and human β-LPH, for 2 hours at 37°C under 95 percent O_2 and 5 percent CO_2. The β-LPH was isolated from sheep or human pituitary glands as described (*7*). Amino acid sequences of sheep and human β-LPH have been elucidated (*3*, *8*). The purity of the β-LPH preparations was verified; a single band was noted when disc electrophoresis on 7 percent polyacrylamide gel at *p*H 4.5 was used, and glutamic acid was the only amino terminal residue noted by the dansyl method (*9*). The β-LPH concentration was determined by ultraviolet absorption. In suspensions of capsular cells, the production of aldosterone in response to synthetic [Asp^1-Ile^5]AII ($10^{-4}M$) (Asp, aspartic acid; Ile, isoleucine) and porcine ACTH (86 I.U./mg; $4 \times 10^{-6}M$) were compared in each experiment. The maximum steroidogenic response to synthetic human ACTH was the same as it was to porcine ACTH. These concentrations are well above the maximum stimulating doses (*5*). In suspensions of decapsular cells, response to the same dose of porcine ACTH was compared in each experiment. Aldosterone and corticosterone were measured directly by radioimmunoassay (*5*, *10*).

At 10^{-8} to $10^{-7}M$, sheep β-LPH stimulated production of aldosterone by the capsular cells, but not that of corticosterone by the decapsular cells (Fig. 1). Human β-LPH produced a similar effect. In a single experiment with synthetic sheep β-LPH (*11*), aldosterone stimulation was similar to that caused by the natural product. A significant increase in aldosterone production was obtained with $10^{-9}M$ β-LPH [from the control value of 32.9 ± 1.9 (standard error) ng per 100,000 capsular cells to 38.6 ± 2.0 ng per 100,000 capsular cells; $P < .02$]; the half-maximum increase in aldosterone production was obtained at a β-LPH concentration between $3 \times 10^{-8}M$ and $10^{-7}M$ (Fig. 2). The maximum response

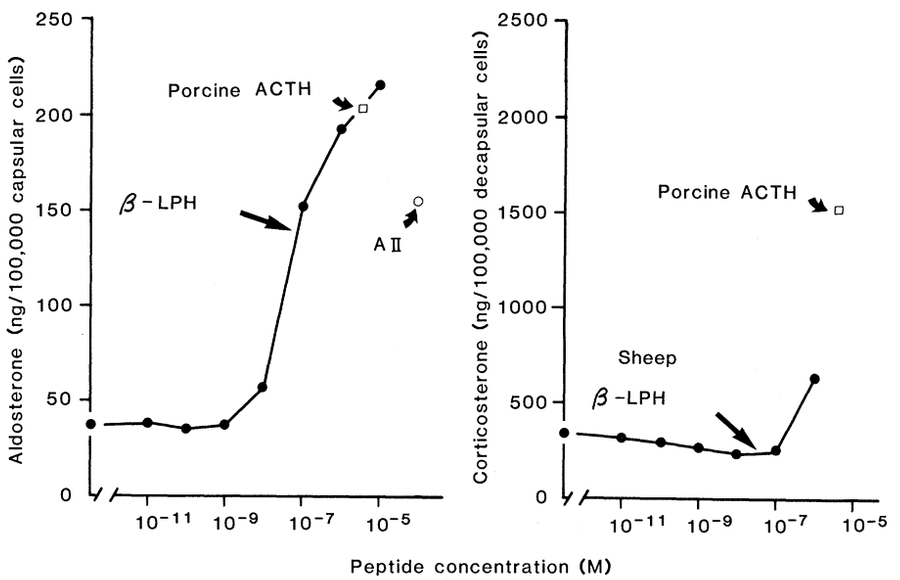

Fig. 1. Aldosterone and corticosterone production by sheep β-LPH in capsular and decapsular cells in a representative experiment. Each point represents the mean of duplicate incubations. Peptide concentrations are expressed as moles per liter of incubation medium.

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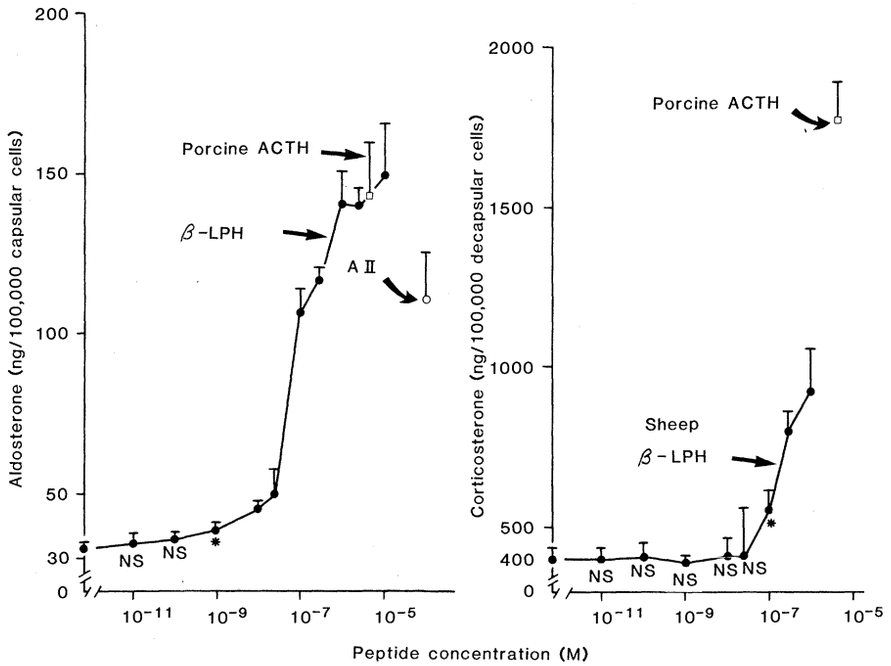

Fig. 2. Aldosterone and corticosterone production by β-LPH. Each point represents the mean ± standard error of eight experiments (four experiments with human β-LPH and four experiments with sheep β-LPH); *NS*, not significantly different from the control value; asterisk, significantly different from the control value ($P < .02$). All other points are significantly different from the control value ($P < .01$).

obtained with β-LPH (144.9 ± 15.5 ng per 100,000 capsular cells) is greater than that obtained with AII (110.8 ± 16.1 ng per 100,000 capsular cells; $P < .01$) and the same as that obtained with ACTH (142.7 ± 17.3 ng per 100,000 capsular cells). However, the concentration of β-LPH (about $5 \times 10^{-8} M$) required to induce half-maximum aldosterone production is approximately 100 times higher than that required for AII (*5*). The corticosterone response to β-LPH is less sensitive than the aldosterone response, with no significant increases at concentrations less than $10^{-7} M$. In contrast, the sensitivities of the aldosterone and corticosterone responses to ACTH are approximately equal (*5, 12*). This difference in the sensitivities of the aldosterone and corticosterone responses suggests that β-LPH and ACTH have different modes of action. Furthermore, there was no detectable ACTH in the β-LPH preparations when millimolar concentrations were assayed by an ACTH radioimmunoassay that could detect 1 pmole of ACTH. Also, we have found that doses of sheep β-LPH that maximally stimulated production of aldosterone did not significantly increase production of adenosine 3′,5′-monophosphate (cyclic AMP) by the adrenal cells. In contrast, maximally stimulating doses of synthetic human ACTH caused a 4.5-fold increase in cyclic AMP production. In other experiments, various amounts (10^{-8} to $10^{-4} M$) of Met^5-enkephalin (Met, methionine) and of α- and β-endorphin did not stimulate aldosterone and corticosterone production. Several lines of evidence suggest that unidentified factor or factors may contribute to aldosterone regulation under some conditions. In the rat, a non-ACTH pituitary factor is essential for the aldosterone response during sodium restriction (*1*). The clinical syndrome of primary aldosteronism associated with bilateral adrenal hyperplasia suggests the presence of an unknown stimulator. Recently, a glycoprotein extracted from human urine has been found to stimulate aldosterone production by capsular cells in vitro and to produce hypertension and hyperaldosteronism when it is administered to rats (*13*). Our results show that β-LPH has a potent aldosterone-stimulating effect. These data raise the question whether β-LPH and possibly other pituitary factor is essential for the aldosterone response during sodium restriction in some types of primary aldosteronism.

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14. This work was supported by NIH grants H1-19644-01 and GM-2907.

3 January 1980; revised 13 March 1980

Ethanol: Novel End Product of Vertebrate Anaerobic Metabolism

Abstract. *During periods of short-term experimental anoxia, the goldfish produces metabolic carbon dioxide and does not accumulate lactate to the extent expected. The goldfish has evolved a novel pathway of vertebrate anaerobic metabolism in which glucose carbon is metabolized to ethanol and excreted.*

Most vertebrates can tolerate only very short excursions into anoxic environments. The common goldfish, however, is remarkably resistant to total anoxia. At low temperatures, during winter (when resistance is maximum), the goldfish can survive several days in the complete absence of oxygen (*1, 2*). Although the metabolic basis for this capability is unknown, it does not seem to reside in classical vertebrate glycolysis for two reasons. First, metabolic CO_2 is known to be an anaerobic end product resulting, at least in part, from the catabolism of glucose (*3, 4*). Second, lactate does not accumulate to the extent predicted either on the basis of glycogen depletion or on the duration of anoxia (*1, 4*). In fact, less than half the glycogen utilized during anoxia can be accounted for in terms of lactate accumulation (*4*). Nor does lactate appear to be excreted. Prosser *et al.* (*5*) looked for but were unable to detect lactate in the water of hypoxic fish. They did however find an excretory product which they were unable to identify but which they postulated was the result of glucose fermentation.

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