Beta Endorphin Selectively Stimulates Aldosterone Secretion in Hypophysectomized, Nephrectomized Dogs

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ABSTRACT We examined the effects of synthetic human β-endorphin (βEND) and a stable methionine (Met)-enkephalin analogue on aldosterone and cortisol secretion rates in anesthetized, hypophysectomized, and nephrectomized dogs and compared them to those of (1–39) ACTH. The circulation of the adrenal glands was completely isolated on the arterial and venous sides (Hilton Pouch). The peptides were infused to deliver 3 pmol/min into the aortic "pouch." Blood was collected from the vena caval pouch, which received blood only from the adrenal gland. Secretion rates of aldosterone and cortisol were calculated as the product of adrenal blood flow and venous steroid concentration. Duplicate steroid measurements were obtained during a control period, at 10, 30, and 50 min of peptide infusion and during a postcontrol period. βEND increased aldosterone secretion rate from 2.4±0.5 ng/min (mean±SEM) to 3.2±0.9 ng/min at 10 min (N.S.), 8.2±2.5 ng/min ($P < 0.05$) at 30 min and 11.0±3.7 ng/min ($P < 0.05$) at 50 min of infusion. Cortisol secretion rate was not affected by infusion of βEND. Infusion of the stable Met-enkephalin analogue D-alanine[2]; Metphenylalanine[4], Met(*O*)-enkephalin-ol or saline alone had no effect on aldosterone or cortisol secretion rates. ACTH infusion increased mean aldosterone secretion rate by ~215% and significantly stimulated cortisol secretion rate. These results indicate that βEND selectively stimulates aldosterone secretion with a potency similar to that of an equimolar dose of ACTH.

INTRODUCTION

There is considerable indirect evidence suggesting that an unidentified pituitary factor other than ACTH plays a role in the regulation of aldosterone production by the adrenal glands of rat, dog, and man (1–7). The nature of this postulated factor is unknown.

Several structurally-related peptides with opioid activity have been isolated from the pituitary and brain of several animal species, including man (8–10). The role of opiate peptides synthesized in the pituitary gland and released into the blood stream is not clear. In the pituitary gland, β-endorphin (βEND),[1] the pharmacologically most potent endorphin with regard to opioid activity, is synthesized as part of a much larger precursor hormone (11) that has been given several names, including pro-opiolipomelanocortin (pro-OLMC), to indicate that it contains the amino acid sequences for ACTH, β-lipotropin (βLPH), γLPH [(1–58) βLPH], β-melanocyte-stimulating hormone [βMSH, (41–58) βLPH], βEND [(61–91) βLPH], methionine (Met)-enkephalin [(61–65) βLPH], and an amino-terminal portion (16,000 fragment). Immunoreactive βEND has been detected in human plasma (12) and has been shown to be secreted from the pituitary gland concomitantly with ACTH and other peptide fragments of pro-OLMC in response to a variety of stimuli, including hypoglycemic stress (12–14).

Intravenous injection of a synthetic peptide representing a portion of the amino terminal or 16,000 fragment of pro-OLMC, termed γ_3-MSH, potentiated the stimulation of corticosterone and aldosterone by (1–24) ACTH in hypophysectomized rats (15). Furthermore, the amino-terminal region of pro-OLMC has been reported to potentiate slightly but significantly the action of (1–24) ACTH in isolated rat adrenocortical cells (16). In this study synthetic αMSH, synthetic porcine βMSH, synthetic human βEND and highly purified ovine βLPH were found to have no effect on corticosterone secretion.

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[1] *Abbreviations used in this paper*: βEND, β-endorphin; βLPH, β-lipotropin; βMSH, β-melanocyte-stimulating hormone; pro-OLMC, proopiolipomelanotropin.

The naturally occurring human γMSH precursor glycopeptide, (1–77) pro-γMSH, has been found to potentiate ACTH stimulation of corticosterone and aldosterone secretion by perfused rat and human adrenal cells (17). Synthetic γ_3-MSH and the N-terminal fragment of pro-OLMC (16,000 fragment), purified from porcine anterior pituitaries, stimulated aldosterone release by human adrenal adenoma cells in vitro (18). Cortisol was not measured in this study. Similarly, synthetic human lysine γ_3-MSH (19) had a direct stimulatory effect on aldosterone and cortisol secretion from human adenomatous and nonadenomatous cells in vitro.

Recently, βLPH and βMSH were observed to stimulate aldosterone production in collagenase-dispersed rat capsular adrenal cells (zona glomerulosa cells) without affecting corticosterone production in zona fasciculata and reticularis cells (20, 21). Beta-endorphin and Met- or leucine(Leu)-enkephalin had no effect on steroid production by adrenal cells in this system. The above findings prompted us to examine the effects of βEND or Met-enkephalin on steroidogenesis of adrenal glands in hypophysectomized nephrectomized dogs.

METHODS

Female foxhounds (Veterinary Resources Branch, National Institutes of Health), weighing 20–25 kg, were used for the experiments. The animals were fed a constant diet (Prescription Diet h/d, Hill's Division Riviana Foods, Inc., Topeka, KS), containing 0.01% sodium for 6 d. This diet was supplemented by 10 g/d sodium chloride yielding a total sodium intake of 263 meq/d. In the morning and evening of the day before the experiment each dog received dexamethasone sodium phosphate (Dexasone, Legere & Company, Inc., Scottsdale, AZ), 0.5 mg intramuscularly, and an additional 1.0 mg was injected on the morning of the experiment. The animals were anesthetized with sodium pentobarbital (30 mg/kg) intravenously and mechanically ventilated with a Harvard respirator (Harvard Apparatus Co., S. Natick, MA). Blood pressure was measured by a pressure transducer (Statham Instruments, Inc., Hato Rey, Puerto Rico) and recorded on a Brush 440 recorder (Gould Inc., Cleveland, OH).

The pituitary gland was removed by a dental drill through a transsphenoidal approach at the beginning of the operation. The adrenal glands were then prepared for perfusion by a technique previously described by Hilton et al. (22). In this procedure the circulation of the glands is completely isolated on the arterial and venous sides. This technique allows perfusion of the adrenal glands by the animal's own heart without interruption of blood supply and without trauma to the glands. Blood from a donor dog that had not been hypophysectomized was transfused at a constant rate to replace the blood withdrawn and to maintain the blood pressure of the animal. Both kidneys were removed. The animal was then given 5 mg/kg heparin i.v. The final preparation consisted of an aortic pouch at the arterial side from which blood flows only to the adrenal glands, and a vena caval pouch that receives blood only from the adrenal glands and from which blood for determination of flow rate and hormone concentration can be collected. For collection of venous blood samples a small polyethylene catheter was threaded into the inferior vena cava via the remaining segment of the left renal vein so that its tip was situated at the level of the entrance of the adrenal veins into the vena cava. The portion of blood received by the vena caval pouch that was not withdrawn for sampling, was routed back to the general circulation.

Normal saline was infused at a rate of 0.247 ml/min into the aortic pouch by a Harvard pump (model 901, Harvard Apparatus Co., Inc.) during control (30 min) and postcontrol (60 min) periods. The peptides were dissolved in normal saline and equimolar doses (3 pmol/min) were infused into the arterial pouch for 50 min. Beginning 30 min after completion of the preparation of the animal, duplicate blood samples for measurement of plasma aldosterone and cortisol were collected from the venous pouch at 0, 20 min (control), 30, 50, 70 min (experimental), and 90, 110, and 130 min (postcontrol). Adrenal blood flow was calculated by timing the adrenal venous outflow of a given volume of blood into a graduated tube.

Synthetic human βEND was purchased from Beckman Instruments, Inc., Palo Alto, CA. Porcine (1–39) ACTH (grade II, product A 6002) was purchased from Sigma Chemical Co., St. Louis, MO. The stable Met-enkephalin analogue D-alanine^2, Metphenylalanine^4, Met (*O*)-enkephalin-ol (FK 33-824) was a gift from Sandoz Ltd., Basel, Switzerland. This analogue has been shown to have the same biological activity as Met-enkephalin but is not rapidly metabolized in plasma (23, 24).

Plasma concentrations of aldosterone and plasma cortisol were measured by specific radioimmunoassays (25–27) by Hazelton Laboratories, Inc., Vienna, VA, under a special contract. The interassay coefficient of variation for cortisol was 8.5% (n = 10) and the intraassay coefficient of variation was 3.1% (n = 10). The interassay coefficient of variation for aldosterone was 15.7% (n = 15) and the intraassay coefficient of variation was 5.8% (n = 15). Secretion rates of cortisol and aldosterone were calculated from adrenal blood flow rate and steroid concentrations. Animals in whom a high basal cortisol secretion suggested incomplete removal of the pituitary gland were not included in the study. Potassium concentration in adrenal venous plasma was measured on a flame photometer. Statistical analysis was performed by analysis of variance with repeated measures and by the Student's t test for paired observations. All values reported represent the mean±SEM.

RESULTS

βEND had virtually no effect on cortisol secretion (Fig. 1, lower panel). Mean cortisol secretion rate was 0.11±0.03 μg/min during the control period, 0.09±0.01 μg/min at 10 min of infusion, 0.11±0.02 μg/min at 30 min, and 0.11±0.03 μg/min at 50 min. In contrast, βEND (3 pmol/min) increased aldosterone secretion rate [repeated measures ANOVA F (d.f. = 5.7) = 2.49, $P < 0.05$] from a control value of 2.43±0.54 ng/min to 3.18±0.87 ng/min at 10 min, 8.23±2.45 ng/min at 30 min, and 10.96±3.69 ng/min at 50 min (Fig. 1, upper panel). 20 min after discontinuation of βEND infusion, aldosterone secretion rate was still significantly elevated (9.6±3.4 ng/min, $P < 0.05$) and returned only partially to control levels. The smallest

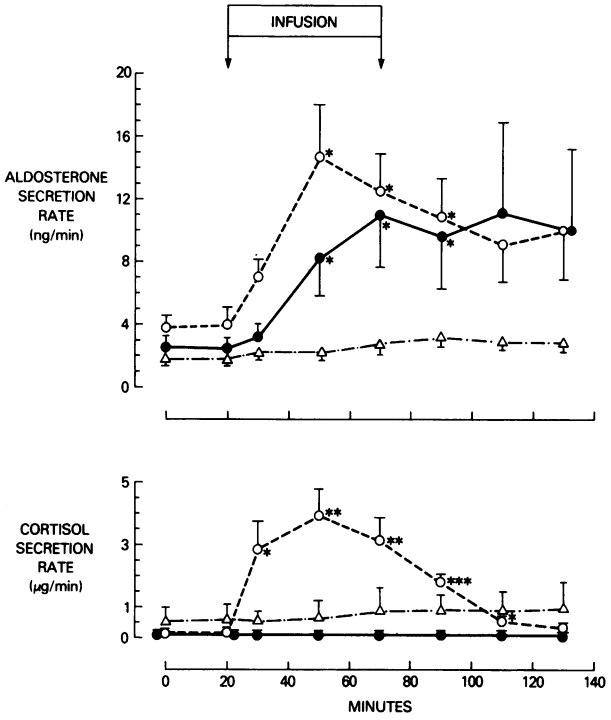

FIGURE 1 Effect of infusion of βEND (n = 6), (1–39) ACTH (n = 6), or normal saline (n = 5) on aldosterone and cortisol secretion rates. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$, compared with control. ●, βEND, 3 pmol/min; ○, ACTH, 3 pmol/min; △, normal saline.

dose of βEND examined was 0.3 pmol/min (two animals), which caused a maximum increase in aldosterone secretion rate of 185 and 331%, respectively. Saline infusion alone had no effect on either aldosterone or cortisol secretion rates (Fig. 1). Similarly, infusion of FK 33-824 (3 pmol/min) did not alter either aldosterone or cortisol secretion rate (Table I).

Infusion of (1–39) ACTH increased aldosterone secretion rate [$F(5, 7) = 3.63$, $P < 0.01$] from 3.98±1.12 ng/min to 7.04±1.05 ng/min at 10 min, 14.72±3.38 ng/min at 30 min, and 12.56±2.34 ng/min at 50 min. Even though there was a gradual decline after discontinuation of ACTH infusion, aldosterone secretion had returned only partially to control level 60 min after the end of the infusion (Fig. 1, upper panel). A different pattern was observed for cortisol secretion. Mean cortisol secretion rate increased [$F(5, 7) = 8.40$, $P < 0.001$] from 0.13±0.03 μg/min to 2.89±0.88 μg/min at 10 min, 3.94±0.87 μg/min at 30 min and 3.15±0.69 μg/min at 50 min. 60 min after discontinuation of ACTH infusion, cortisol secretion rate had returned to control levels.

Adrenal venous plasma potassium concentration did not change during the course of βEND infusion (3.6±0.2 meq/liter at the end of the control period, 3.4±0.3 meq/liter at the end of βEND infusion). Adrenal blood flow was not affected by infusion of any of the peptides or of saline.

DISCUSSION

Considerable indirect evidence indicates that in addition to ACTH an unknown pituitary factor may regulate aldosterone secretion (1–7). It has been speculated that one of the pro-OLMC-derived pituitary peptides could be that factor. It has been shown that βLPH, injected intraperitoneally, increases plasma aldosterone concentration in dexamethasone-treated rats in vivo (28). However, a relatively high peptide dose (10 μg/100 g body wt) was needed to increase aldosterone secretion. Recently, it has been reported that βLPH and βMSH selectively stimulated aldosterone production in collagenase-dispersed capsular cells from rat adrenal glands (20, 21). The C-terminal βLPH fragments, βEND and Met-enkephalin, had no effect on steroidogenesis in this in vitro system. In these studies, the smallest βLPH concentration to cause a small but nonsignificant increase in aldosterone production was 10^{-9} M, a concentration that is at least 100 times higher than that present in normal human peripheral plasma (20). The authors concluded that βMSH was the aldosterone-stimulating core of βLPH. It should be noted, however, that βMSH does not normally exist in human plasma, but that it may be an artefact produced by degradation of βLPH during extraction (29, 30).

In other studies, high concentrations (more than 10^{-8} M) of purified βLPH from human and ovine pituitaries were required to stimulate both aldosterone and corticosterone production by rat adrenocortical cells in vitro (31).

TABLE I
Effect of the Stable Met-Enkephalin Analogue FK 33-824 (3 Pmol/Min) on Secretion Rates of Aldosterone and Cortisol (n = 3)

	Control	Exp. (50 min)	Postcontrol
Aldosterone secretion rate, *ng/min*	4.51±3.06	5.03±3.50	5.04±3.33
Cortisol secretion rate, *μg/min*	0.59±0.12	0.70±0.16	0.73±0.17

A preferential effect on aldosterone production was not observed. In this study sufficient immunoreactive ACTH concentrations were found in the βLPH preparations to account, at least partially, for their effect on aldosterone and corticosterone production. It was suggested that the remainder of the steroidogenic activity was due to the heptapeptide core sequence common to ACTH, αMSH, βMSH, βLPH, and γLPH, rather than due to a specific action of βLPH.

The synthetic peptide γ_3-MSH, which represents a portion of the amino terminal or 16,000 fragment of pro-OLMC, has been shown to potentiate the stimulation of aldosterone and corticosterone production by ACTH in hypophysectomized rats (15) and isolated rat adrenocortical cells (16) but γ_3-MSH itself had no steroidogenic activity. In a subsequent study, γ_3-MSH has been reported to stimulate aldosterone release from human adrenal adenoma cells in vitro (18). Similarly, synthetic Lys-γ_3-MSH caused direct stimulation of cortisol and aldosterone in isolated human adrenal adenoma cells (19). Addition of the morphine antagonist naloxone to superfused rat adrenocortical quarters or slices significantly potentiated the effect of ACTH on corticosterone production, whereas pharmacological dosages of naloxone alone (10^{-3} and 10^{-4} M) produced a transient insignificant decrease in corticosterone formation, followed by a small increase (32). Although Met-enkephalin did not have an effect in this system, administration of ACTH 2 h after Met-enkephalin caused a 34% greater increase in corticosterone release than the same dose of ACTH added before Met-enkephalin. In another study, intravenous injection of βEND or intraperitoneal injection of either morphine or naloxone in hypophysectomized rats had no effect on corticosterone content of the adrenal gland (33). However, morphine potentiated and naloxone inhibited the response of corticosterone to ACTH (33). Aldosterone was not measured in these experiments.

In the present study, infusion of βEND into the adrenal gland of hypophysectomized, nephrectomized dogs caused a significant increase in aldosterone secretion but not cortisol secretion. Infusion of the stable Met-enkephalin analogue D-Ala^2, MePhe^4, Met(*O*)-enkephalin-ol had no effect on either aldosterone or cortisol secretion rates. An equimolar dose of (1–39) ACTH, which elicited significant increases both in aldosterone and cortisol secretion, produced an absolute increase in aldosterone secretion similar to that of βEND.

The reason that βEND stimulates aldosterone secretion in the *in situ* perfused dog adrenal gland but not in rat adrenal cortical cells in vitro (20, 21, 31) is not clear. Additional studies are needed to clarify this question. It is possible that species differences or differences in the experimental system used are responsible for the different results. Species differences have, for instance, been shown for the lipolytic effect of βLPH (34, 35). Therefore, it is conceivable that species differences exist also for the effect of βEND on aldosterone production. This possibility is intriguing in view of recently published data suggesting that the βLPH/βEND ratio in the dog anterior pituitary lobe is much lower than that in the rat (36). Furthermore, it remains to be seen if acetylated βEND that accounts for a large portion of βEND-like material released from the posterior-intermediate lobe of the pituitary gland, at least in rat, beef, and monkey, is equally or more potent than βEND with regard to stimulation of aldosterone.

Since a portion of the blood received by the vena caval pouch, after transiting the adrenal gland, was routed back to the general circulation, the effect of βEND on aldosterone secretion in the autoperfused dog adrenal gland may be indirectly mediated by release of a secondary hormone. It is also possible that βEND may have caused the adrenal medulla to release a substance capable of stimulating aldosterone production. Experiments in which the effect of βEND on steroid production by isolated canine zona glomerulosa cells is examined are necessary to answer these questions.

The smallest dose of βEND examined in our experiments that significantly stimulated aldosterone secretion was ~0.3 pmol/min or 1 ng/min. Adrenal venous plasma immunoreactive βEND, which comprises both β-endorphin and β-lipotropin, has been found to be >333 pg/ml in an anesthetized dog (Güllner, H.-G., and D. N. Orth, unpublished observations). Since adrenal blood flow is ~8 ml/min, an infusion of 1 ng/min may increase the concentration in adrenal blood by 125 pg/ml, or ~30%, resulting in a 200–300% increase in aldosterone secretion. Thus, under certain conditions, circulating concentrations of βEND may be high enough to stimulate aldosterone secretion suggesting that βEND may play a physiological role in the regulation of aldosterone secretion.

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