

Searching for an inhibitory action of blood-borne β -endorphin on LH release

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Summary. Concentrations of β -endorphin were quantified in peripheral blood plasma of sheep by a radioimmunoassay that cross-reacted with β -lipotrophin. Plasma concentrations of β -endorphin increased abruptly after physical confinement, bacteraemia, and electroacupuncture treatment for induction of analgesia. In these experimental situations in which plasma concentrations of β -endorphin increased, plasma concentrations of LH often decreased. To test the hypothesis that increases in blood-borne β -endorphin actually caused the decrease in LH release, naloxone was administered to antagonize the opioid receptors at which blood-borne β -endorphin might act. In no case did administration of naloxone disrupt the temporal correlation between experimentally induced increases in plasma β -endorphin and decreases in plasma LH. It was concluded that the increases in blood-borne β -endorphin did not cause the decrease in LH release. Other research investigated whether β -endorphin might be delivered via blood from pituitary to hypothalamus in locally enriched concentrations. Even when pituitary release of β -endorphin was acutely stimulated, it was not possible to demonstrate retrograde delivery of β -endorphin to the hypothalamus without dilution in the systemic circulation. In conclusion, it is unlikely that blood-borne β -endorphin inhibits the release of LH, and β -endorphin should not be classified as a hormone until blood concentrations of the peptide can be shown to exert some effect at a location distant from its site of secretion.

Introduction

It has been 10 years since Li & Chung (1976) isolated and identified a unique peptide from an extract of pituitary glands from camels and suggested the name β -endorphin for this peptide. This same laboratory had reported earlier (Li *et al.*, 1975) that this extract from 500 camel pituitaries lacked β -lipotrophin which had been abundantly present in pituitary extracts from other species. Li *et al.* (1975) suggested that the camel may be unique with regard to β -lipotrophin or that β -lipotrophin was destroyed before they received the glands from the slaughterhouse in Iraq. Li & Chung (1976) observed that the amount of β -endorphin which they found in their extract of camel pituitaries approximated the amount of β -lipotrophin which one would expect to be present using calculations based on pituitaries from other domestic species. Within the same year, there appeared three reports showing that several identified, as well as unidentified, fragments of β -lipotrophin from camels, pigs and sheep possessed morphine-like activities in bioassays and in radioreceptor assays (Bradbury *et al.*, 1976; Cox *et al.*, 1976; Lazarus *et al.*, 1976). In these studies the peptide corresponding to residues 61 to 91 at the C-terminus of β -lipotrophin produced the greatest effects and, in agreement with the earlier suggestion of Li & Chung (1976), it has come to be universally called β -endorphin. Many advances have been made in our understanding of β -endorphin in the decade since its discovery. These include recognition that (1) β -endorphin is only one of many proteolytic cleavage products of β -lipotrophin and its precursor pro-opiomelanocortin (POMC), (2) β -endorphin is synthesized from POMC in the pars anterior and pars intermedia of the pituitary

gland as well as in brain tissue, and (3) β -endorphin is secreted into blood concomitantly with adrenocorticotrophic hormone (ACTH), another product of POMC (Imura *et al.*, 1983; Akil *et al.*, 1984).

Blood-borne β -endorphin

Shortly after the discovery of β -endorphin, several laboratories developed radioimmunoassays to demonstrate its presence in blood (Guillemin *et al.*, 1977; Holtt *et al.*, 1978; Akil *et al.*, 1979). Concentrations of blood-borne β -endorphin have now been measured in many species, and in general they parallel changes in ACTH and/or adrenocortical steroids in blood (Vuolteenaho *et al.*, 1982; Orth *et al.*, 1982; DeSouza & Van Loon, 1983). We have validated methods for quantifying β -endorphin immunoreactivity in blood plasma of rats (Davis *et al.*, 1983), horses (Bossut *et al.*, 1983) and sheep (Leshin & Malven, 1984b). A major problem of β -endorphin quantification is that most antisera to β -endorphin also recognize the β -endorphin sequence when it is still part of the β -lipotrophin molecule. Therefore, a portion of the estimated β -endorphin in plasma represents β -lipotrophin unless a separation step precedes the immunoassay. Although the fluctuations of β -endorphin and β -lipotrophin appear parallel in most cases, the relative contribution of each molecule to the measured concentration is not always known for every plasma sample.

The source of most blood-borne β -endorphin is thought to be the pars anterior of the pituitary gland. Selective removal of the pars anterior leaving the pars intermedia and pars nervosa intact in rats markedly reduced basal concentrations of β -endorphin in plasma (Przewlocki *et al.*, 1982), but these authors also concluded that during stress the pars intermedia may secrete some β -endorphin into blood. However, the cells of the pars anterior and pars intermedia process POMC differently, and the production of β -endorphin appears to be regulated differently in the two tissues (Smyth & Zakarian, 1980; Shiomi & Akil, 1982; Millan *et al.*, 1982; Lim & Funder, 1983). Other possible sources of blood-borne β -endorphin include the brain (Zakarian & Smyth, 1982) and peripheral organs such as the gonads and other tissues (see Autelitano *et al.*, 1986, for review) because β -endorphin is synthesized in a variety of tissues, but their contribution to plasma β -endorphin relative to that of the pituitary gland is probably minor.

Is β -endorphin a hormone?

β -Endorphin produced in neural tissue can act in a neurocrine or paracrine manner to influence opioid receptors in adjacent neural elements. This paper will not address such actions of β -endorphin but will focus on the question of whether β -endorphin is a hormone. By definition, a hormone travels in blood to a site some distance from its site of secretion and modulates a physiological process. β -Endorphin is secreted into blood by the pituitary gland and concentrations of blood-borne β -endorphin fluctuate widely, both acutely and chronically. The only hormonal criterion which has been difficult to satisfy is modulation of a physiological process by blood-borne β -endorphin (Cox & Baizman, 1982). I propose to restrict the remainder of my remarks to this subject using data primarily from our laboratory and focussing mainly on the inhibition of release of pituitary LH. There is considerable evidence that in specific physiological states unidentified endogenous opioid peptides inhibit LH release in ewes (Malven *et al.*, 1984; Brooks *et al.*, 1986a, b; Gregg *et al.*, 1986) and other species (see Malven, 1986, for review). A large part of this evidence has been obtained through use of naloxone, an antagonist of opioid receptors. When administration of naloxone abruptly increases plasma concentrations of LH, it can only be concluded that naloxone antagonized the LH inhibitory action of some unidentified opioid. However, such an effect of naloxone, which readily passes the blood-brain barrier, does not establish whether a blood-borne opioid was antagonized or whether naloxone antagonized a locally produced opioid

acting on the pituitary LH cells or on neurones which release LH-releasing hormone (LHRH) or which modulate LHRH neurones (Malven, 1986). To demonstrate an LH-inhibitory action of blood-borne β -endorphin, we have examined several situations in which there is an association between elevated plasma β -endorphin concentrations and depressed plasma LH values (i.e. a temporal correlation). To test whether this temporal correlation was in fact a cause-effect relationship, we have attempted to disrupt the association by administering naloxone. Our rationale was that systemic administration of naloxone would reach all the sites reached by blood-borne β -endorphin and should be able to block the interaction of this β -endorphin with those opioid receptors which mediate the suppression of LH release. If naloxone attenuates the suppression of LH release, we can conclude that blood-borne β -endorphin is a possible mediator of the effect. If naloxone has no effect on the suppressed levels of LH, it seems unlikely that the elevated blood concentrations of β -endorphin are responsible for the inhibition of LH release.

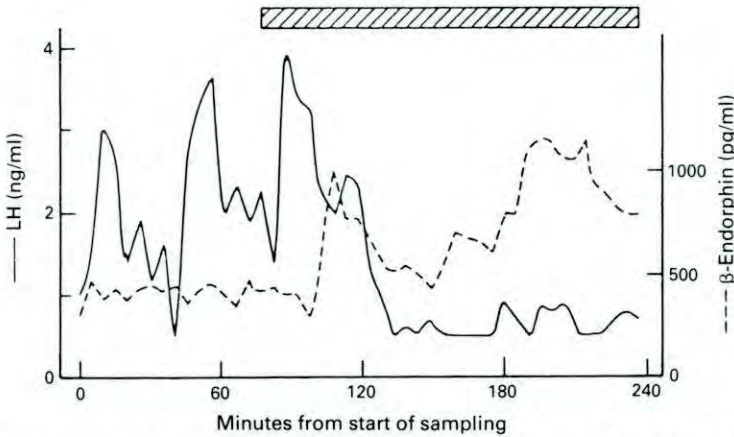


Fig. 1. Plasma profiles of LH and β -endorphin in one ovariectomized ewe. During the period of physical confinement (hatched bar), the ewe was maintained in an enclosed chamber ($1.8 \times 0.7 \times 1.6$ m) which moderately restricted movement and produced visual isolation identical to that described by Rasmussen & Malven (1983).

Inhibition of LH during confinement stress and bacteraemia

Episodic secretion of LH in ovariectomized ewes decreased after imposition of physical confinement of the ewes. As ewes habituated to confinement, the frequency and amplitude of LH peaks increased (Rasmussen & Malven, 1983). Although plasma concentrations of β -endorphin were not reported by Rasmussen & Malven (1983), one example, of an ovariectomized ewe exposed to a similar environment is presented in Fig. 1. The profile of plasma LH showed two distinct peaks before the onset of confinement and a third peak soon after confinement. At that point (about 105 min after start of sampling), the plasma concentration of β -endorphin increased abruptly, and there were no more peaks of LH for the remainder of the experiment. In this ewe, a temporal association clearly existed between the confinement-induced increase in β -endorphin and suppressed release of LH.

Another method of increasing plasma concentrations of β -endorphin is by induced bacteraemia. Intravenous administration of bacteria or their endotoxins provokes substantial increases in plasma β -endorphin (Carr *et al.*, 1982; Leshin & Malven, 1984a). Figure 2 shows 4 examples of ovariectomized ewes in which such a bacteria-induced increase of β -endorphin occurred. Plasma LH was fluctuating episodically during each sampling period as expected of ovariectomized ewes.

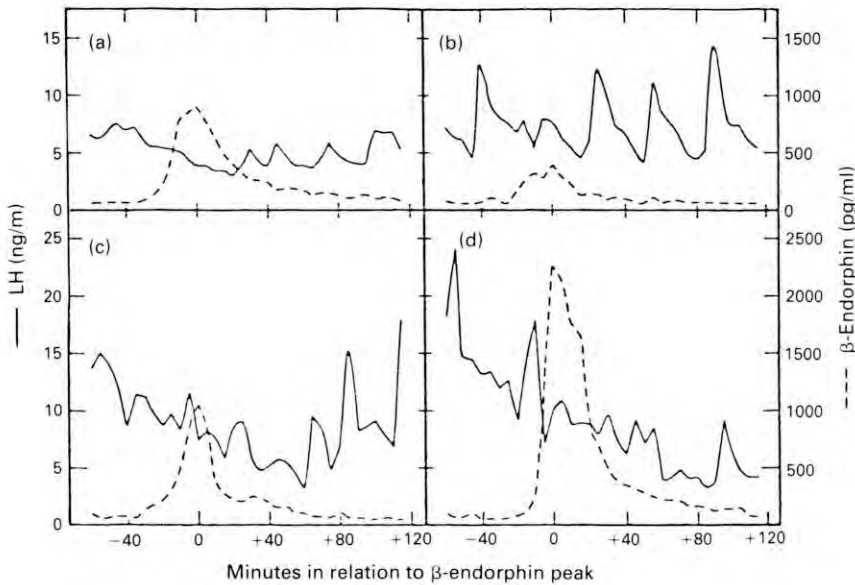


Fig. 2. Plasma profiles of LH and β -endorphin in 4 ovariectomized ewes plotted relative to the peak of β -endorphin in plasma. Each panel presents a sampling sequence wherein a transient increase in plasma β -endorphin occurred probably as a result of bacteraemia (taken from Exp. 1 of Leshin & Malven, 1984a)

The increases in plasma β -endorphin in Figs 2(a) and 2(b) occurred concurrently with lower than usual peaks of plasma LH. Profiles of LH in Figs 2(c) and 2(d) were less regular, but there appeared to be some slight inhibition of LH release during the periods of transiently elevated β -endorphin concentrations and plasma LH values continued to decline steadily during the period of the β -endorphin discharge into blood. These examples in Fig. 2 did not involve a standardized induction of bacteraemia and in some cases the bacteraemia was probably inadvertent (Leshin & Malven, 1984a).

To investigate more thoroughly the temporal association between decreased LH and increased β -endorphin during bacteraemia, Leshin & Malven (1984a) administered a standardized bolus of cultured *E. coli* to ovariectomized ewes. A variety of cardiovascular, thermoregulatory and hormonal effects were noted after *E. coli* infusion, and plasma LH decreased gradually while appearing to become less episodic. Mean plasma LH values during the 180-min period after *E. coli* infusion were lower ($P < 0.05$) than during the 180-min period before infusion (Fig. 3). To determine whether opioid receptors were required for this temporal association, naloxone was infused at a dosage of 2.5 mg/kg/h for the entire 6-h sampling period. Naloxone infusion had no significant effect on the mean LH profile either before or after *E. coli* infusion, and the profile in Fig. 3 represents an average of both saline and naloxone infusions. If our dosage of naloxone was adequate, these results indicate that the bacteria-induced increase in blood-borne β -endorphin was not acting via naloxone-sensitive opioid receptors to produce the concurrent suppression of plasma LH.

Effects of electroacupuncture on opioid-LH interactions

Electroacupuncture treatment of specific loci in male and female sheep confirmed the Chinese veterinary literature by quantifying the resulting analgesia (Bossut *et al.*, 1986). Application of this electroacupuncture treatment to castrated male sheep inhibited plasma LH (Malven *et al.*, 1984)

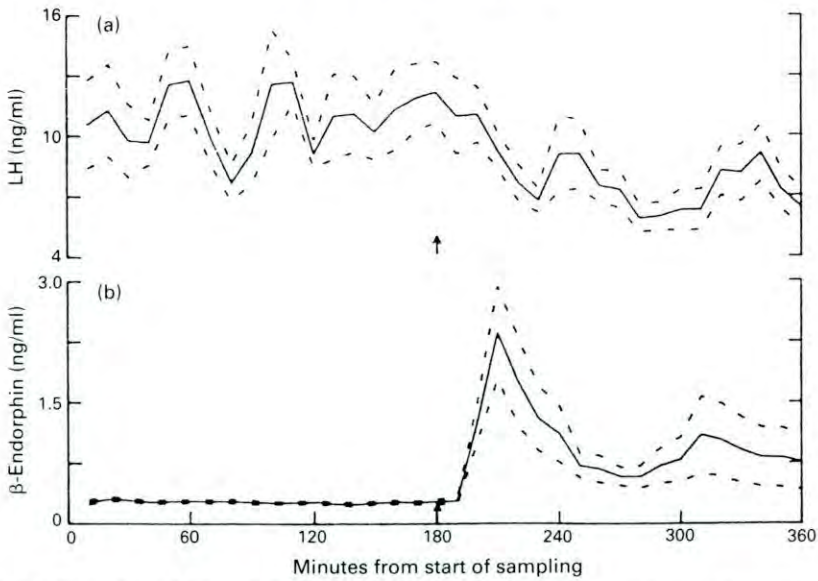


Fig. 3. Profiles of (a) LH and (b) β -endorphin in plasma during 6-h sampling. At 180 min (denoted by arrows), cultured *E. coli* were injected into one jugular vein. Trials in which saline or naloxone (2.5 mg/kg/h) were infused continuously were combined because there were no statistical differences between them. Solid lines depict means ($n = 10$) and broken lines depict the s.e.m. (Adapted from Leshin & Malven, 1984a.)

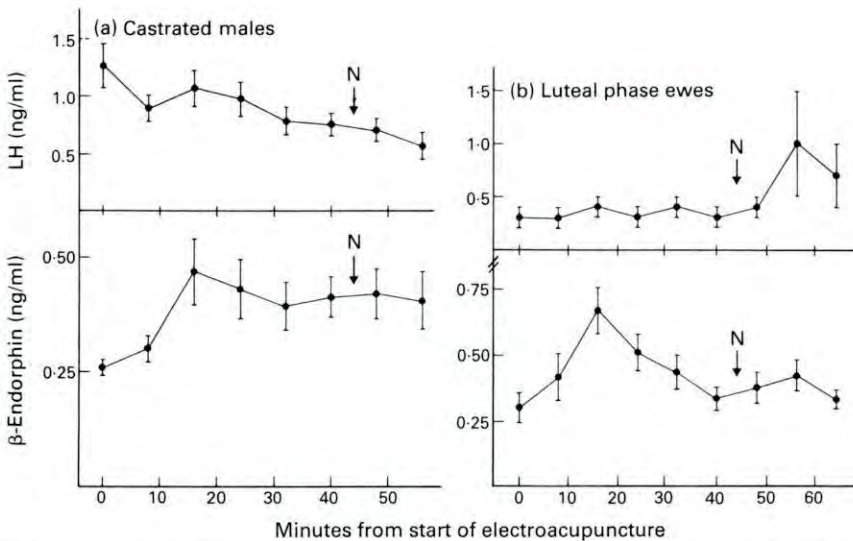


Fig. 4. Plasma profiles of LH and β -endorphin in (a) castrated male sheep and (b) cyclic luteal-phase ewes subjected to electroacupuncture for induction of cutaneous analgesia (redrawn from the data of Malven *et al.* (1984) and of Bossut *et al.* (1986)). Means are presented \pm s.e.m. with 16–19 (a) and 8–12 (b) observations per mean. Time of naloxone injection is denoted by the arrow (N). LH values of luteal-phase ewes used as controls for the electroacupuncture treatment and which received the same dosage of naloxone have been presented elsewhere (Malven *et al.*, 1984).

and increased plasma concentrations of β -endorphin (Fig. 4a). This temporal correlation between depressed LH and elevated β -endorphin concentrations in plasma was similar to those observed during confinement and bacteraemia. Injection of naloxone (1.1 mg/kg) during the period of depressed plasma LH values failed to stimulate release of LH (Fig. 4a) suggesting that blood-borne β -endorphin was probably not responsible for the electroacupuncture-induced suppression of LH in those castrated males.

Malven *et al.* (1984) reported that naloxone administration (1.1 mg/kg) to cyclic female sheep abruptly stimulated the release of LH provided that the ewes were in the luteal phase of the oestrous cycle. This observation, as well as that of Trout & Malven (1984), indicates that during progesterone-induced suppression of LH release an unidentified endogenous opioid was inhibitory to LH release and that a moderate dosage of naloxone was able to antagonize this opioid thereby stimulating LH release. When luteal-phase ewes were treated with electroacupuncture for induction of cutaneous analgesia, injection of naloxone at the effective dosage for control ewes (Malven *et al.*, 1984) did not increase plasma LH significantly (Fig. 4b). Electroacupuncture treatment of these ewes also increased plasma β -endorphin (Fig. 4b), similar to but not as sustained as its effect in castrated males (Fig. 4a). Therefore, electroacupuncture treatment of luteal-phase ewes increased plasma β -endorphin and blocked the ability of naloxone to stimulate the release of LH. It seems unlikely that blood-borne β -endorphin could be responsible for antagonizing the LH-releasing effect of naloxone during electroacupuncture treatment of luteal-phase ewes, but it remains a possibility that the dosage of naloxone, which was adequate to stimulate LH release during sham treatment, subsequently became inadequate during acupuncture-induced elevations of plasma β -endorphin.

Retrograde delivery of pituitary β -endorphin to the brain

The vascular anatomy of the pituitary gland is consistent with retrograde delivery of secreted pituitary hormones to the hypothalamus and brain without dilution in the systemic circulation (Page, 1982). Since opioid receptors are present in large numbers in the brain, there is a strong physiological rationale for delivery to the brain of enriched concentrations of pituitary-derived β -endorphin. Moreover, surgical removal of all or parts of the pituitary gland reduced the concentrations of β -endorphin in hypothalamic tissue (Ogawa *et al.*, 1979; Przewlocki *et al.*, 1982). To investigate the possibility of retrograde delivery of β -endorphin in ewes, Leshin & Malven (1985) implanted catheters for blood sampling into the dorsal longitudinal sagittal sinus very near the place where diencephalic venous effluent entered the sinus. Plasma concentrations of β -endorphin were measured in pairs of samples withdrawn simultaneously from the sagittal sinus and from the carotid artery at regular 20-sec intervals. Our rationale was that retrograde delivery of pituitary-derived β -endorphin to the brain should be reflected in more β -endorphin in the sagittal sinus samples than in the paired carotid artery sample.

Release of β -endorphin into blood was provoked by induced bacteraemia and less frequently by injection of naloxone and pentobarbitone sodium. There were very few series of blood samples over the entire experiment in which concentrations of β -endorphin were consistently greater in the sagittal sinus than in the carotid artery. We concluded that these few series were insufficient to establish retrograde delivery of pituitary-derived β -endorphin to the diencephalon. Rather, they may represent β -endorphin being secreted into blood from the diencephalon. In summary, our failure to demonstrate enriched concentrations of β -endorphin in diencephalic effluent blood of ewes did not support the hypothesis of retrograde delivery, but negative results such as these cannot exclude such a possibility. Since this experiment did not demonstrate locally enriched concentrations of β -endorphin in the vicinity of its CNS receptors, we cannot counteract the contention (Cox & Baizman, 1982) that blood concentrations of β -endorphin are generally much lower (picomolar range) than the levels needed to affect in-vitro opioid binding sites (nanomolar range).

Conclusions

Blood-borne β -endorphin satisfies several of the criteria for being a hormone, but demonstrating an action of β -endorphin on distant sites regulating LH release has not yet been achieved. Temporal correlations between elevated plasma concentrations of β -endorphin and suppressed release of LH were repeatedly demonstrated, but administration of naloxone at dosages which should antagonize all the opioid effects of β -endorphin failed to relieve the suppression of LH release. These results suggest that the temporal correlations were not reflecting a cause and effect relationship. Moreover, retrograde blood-borne delivery of enriched concentrations of β -endorphin to the brain could not be demonstrated. In summary, β -endorphin cannot yet be classified as a hormone on the basis of its effects on LH release because it fails to satisfy the criterion of acting at a location distant from its site of secretion.

This manuscript is published as Journal Paper No. 10,792 of the Indiana Agricultural Experiment Station. I thank L. S. Leshin and D. F. B. Bossut who conducted many of the experiments from which the present data were taken; and S. A. Haglof for technical assistance with all of the experiments.

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