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Differential Effects of Warm- and Cold-Ambient Temperature on Blood Levels of β-Endorphin and Prolactin in the Rat (41288)

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Abstract. A comparison of the effects of warm- and cold-ambient temperature and physical immobilization on blood levels of immunoreactive β-endorphin (β-END) and prolactin (PRL) in rats was made. Both physical restraint (15–45 min) and warm-ambient temperature (36°, 20 to 360 min) evoked increases in circulating levels of β-END and PRL. By contrast, exposure to cold-ambient temperature (4°, 7½ to 360 min) increased blood levels of β-END but decreased circulating PRL. This divergence in plasma levels of β-END and PRL in cold-exposed animals suggests that: (i) separate hypothalamic mechanisms regulate the secretion of these two hormones, and (ii) bloodborne β-END does not normally stimulate PRL in cold-exposed rats. Therefore, under the present conditions the secretion of pituitary prolactin appears to be directly related to changes in body temperature and not plasma levels of immunoreactive β-END.

Recently β-endorphin (β-END) and several other endogenous opiate peptides have been isolated from mammalian brain and pituitary tissues (1). Demonstration of their abilities to produce analgesia via an interaction with brain opiate receptors has led to the hypothesis that opiate peptides have roles in the physiologic responses to painful stimuli (2, 3). In addition, recent studies indicate that endogenous opiate peptides may also act on the brain to influence neuroendocrine regulation and at present, most is known about their effects on the secretion of prolactin (PRL). Like morphine, β-END, methionine-enkephalin, and leucine-enkephalin have been reported to evoke PRL release when injected into rats, whereas opiates have no effect of PRL release *in vitro* (4–6). Opiate receptor-blocking drugs, naloxone and naltrexone, counteract the PRL response to administered opiates (4–6) and attenuate the physiologic release of PRL on proestrous afternoon and in response to stressful stimuli (6, 7). Furthermore, it has been observed that pituitary β-END and PRL are released together in response to electric footshock (8), immobilization and etherization (9, 10). Together these findings suggest that β-END released from the pituitary may in turn act on the brain to produce both analgesia and increase the secretion of PRL. To further investigate this possibility, we have studied the influence of warm- and cold-ambient temperature on blood levels of β-END and PRL in the rat. Previous reports in humans (11), rats (12), and cattle (13) indicate that the secretion of PRL varies in a direct relation to environmental temperature. Therefore, it was of interest to determine if changes in ambient temperature had similar effects on circulating levels of β-END. Because the plasma concentrations of β-END and PRL reported here were estimated by radioimmunoassays, the present data refer to amounts of β-END or PRL-like immunoreactivity.

Materials and Methods. Mature male Sprague–Dawley rats (Taconic Farms, Inc., Germantown, N.Y.) weighing 175 to 225 g were housed at 22°, lights on from 0600 to 1800 hr, and received food and water *ad libitum*. In addition, all animals were accustomed to daily handling for 5 days prior to each experiment. Immobilization was administered by taping the rats to test tube racks and placing them on their backs. For high-ambient temperature, animals were placed (four per cage, with water *ad libitum*) in a ventilated and lighted small animal surgical recovery unit (Kirschner Scientific, Aberdeen, Md.) heated to 36°. Cold-exposed animals were placed in individual cages in a ventilated

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and lighted chromatography chamber (Puffer-Hubbard, Grand Haven, Mich.) maintained at 4°. Control animals were housed at 22°. Following rapid decapitation at the times indicated under Results, trunk blood was collected in plastic tubes containing 600 μl of 10% ethylenediaminetetraacetic acid (EDTA) and chilled on ice. Plasma was separated by centrifugation and stored at −70° until assayed. Plasma levels of β-END were estimated by radioimmunoassay as previously described (Mueller, 1980) except using another antiserum (C-55) having the same specificity but higher titer (final dilution of 1:125,000). Briefly, the antiserum recognizes the C-terminal portion of camel β-END_{1-31} and cross-reacts with purified β-lipotropin (A. Parlow and D. Orth), acetylated β-END_{1-31}, β-END_{1-27} and acetylated β-END_{1-27} on an equimolar basis. *N*-Terminal fragments of β-END (methionine-enkephalin, leucine-enkephalin, and α-endorphin) and non-opiate hypothalamic and pituitary peptides are not detected by this assay. Since β-lipotropin and the β-END_{1-27} molecules are recognized, the β-END values reported here are probably overestimates of β-END_{1-31} levels. Plasma PRL was measured by the method described in the NIAMDD kit. Analysis of variance and Duncan's comparison test were used to test the significance of difference between control and experimental hormone values, and $P < 0.05$ was chosen as the level of significance.

Results. Physical immobilization evoked rapid increases in plasma levels of both β-END and PRL (Fig. 1). Circulating β-END was maximally increased from a control mean of 0.21 ± 0.06 to 2.91 ± 0.57 ng/ml by 30 min of restraint. The maximal PRL response (4.5-fold increase) was observed by 15 min of restraint. By 45 min of continuous immobilization, plasma levels of both hormones were significantly reduced from their respective maximal stress-induced values. As with physical immobilization, exposure of rats to high-ambient temperature was associated with increases in both β-END and PRL (Fig. 2). The maximal responses of the hormones were observed by 60 min for β-END and 20 min for PRL. By 360 min heat exposure,

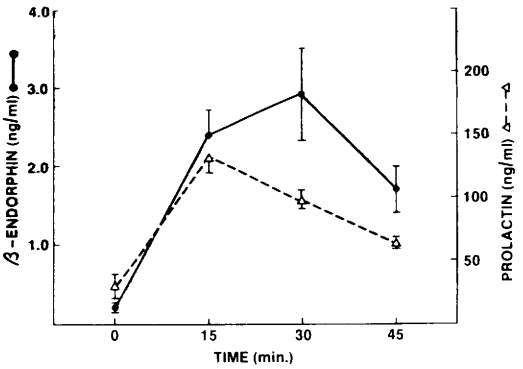

FIG. 1. Time course of effects of physical immobilization on plasma levels of β-END and PRL in rats. Each point represents the mean and SEM of eight determinations. As compared to nonstressed control values (0 min), plasma levels of both hormones were significantly increased ($P < 0.05$) by 15, 30, and 45 min restraint.

levels of β-END and PRL were still significantly increased above room temperature control values (4.3 and 3.3-fold, respectively). The time course of effects of cold-ambient temperature on blood levels of β-END and PRL are presented in Fig. 3. To account for the effects of animal handling alone on blood hormone levels, two groups of control animals (both maintained at 22°) were used. One group was sacrificed by 7½ min following transfer to a novel cage and the other by 120 min after transfer. As compared to the control animals sacrificed 120 min after cage transfer, control animals sacrificed by 7½ min after cage transfer had significantly higher plasma levels of both β-END (0.38 ± 0.02 ng/ml vs 0.31 ± 0.03 ng/ml) and PRL (38 ± 10 ng/ml vs 22 ± 2 ng/ml). For comparison of hormone values in animals acutely exposed to 4°, the mean β-END and PRL values of control animals terminated 7½ min after cage transfer has been plotted at the zero time point in Fig. 3. In cold-exposed animals plasma β-END values were maximally increased (3-fold) by 30 and 60 min and then returned to control values by 180 and 360 min. By contrast, circulating levels of PRL declined progressively throughout the cold exposure period. The first significant reduction from room temperature control values (38 ± 10 ng/ml) was observed by 30 min (17 ± 4 ng/ml) after

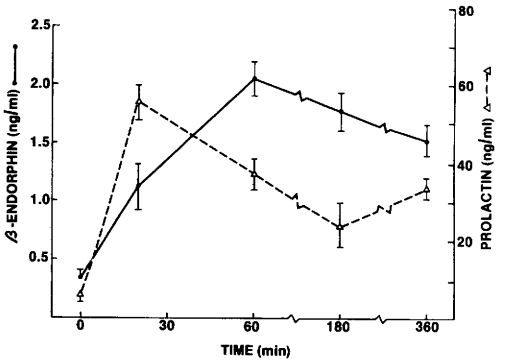

FIG. 2. Time course of effects at warm exposure 36° on plasma levels of β-END and PRL in rats. Each point represents the mean and SEM of eight determinations. The first significant increases ($P < 0.05$) in plasma β-END and PRL levels above room temperature control values (0 min) were observed at 20 min.

which plasma PRL values continued to decline to 26% of control values by 360 min exposure to 4°.

Discussion. The present findings demonstrate that in rats physical immobilization and exposure to warm-ambient temperature cause increases in circulating levels of β-END and PRL. By contrast, exposure to cold-ambient temperature increases blood levels of β-END but decreases circulating

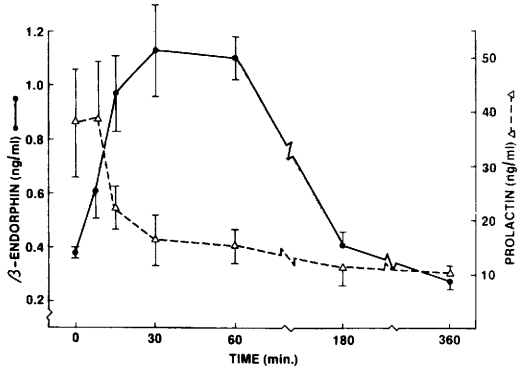

FIG. 3. Time course of effects of cold-ambient temperature (4°) on plasma levels of β-END and PRL in rats. Each point represents the mean and SEM of eight determinations. Plasma levels of β-END were significantly increased ($P < 0.05$) above room temperature control values (0 min) by 15, 30, and 60 min cold exposure. Plasma PRL levels were significantly ($P < 0.05$) reduced from control values by 30 min and at all times thereafter.

PRL. As compared to cold exposure, heat exposure and immobilization are two to three times more effective in stimulating β-END release. These differences may indicate that cold exposure is the least traumatic or stressful of the three treatments.

The present findings of a direct relationship between ambient temperature and circulating levels of PRL is consistent with the earlier reports of Mills and Robertsham in humans (11), Mueller *et al.* in rats (12), and Wetteman and Tucker in cattle (13). By contrast, Jobin *et al.* (14) and Harris *et al.* (15) noted acute (10–25 min) increases in blood levels of PRL following transfer of rats to a cold environment. In the present study no such rise in plasma PRL was observed after controlling for the effects of animal handling and cage transfer. Therefore, the brief elevation in plasma PRL values which may be noted following placement of rats in a 4° environment appears to be caused by the physical movement of the animals and is not a specific neuroendocrine response to cold exposure per se. Consistent with the view that cold ambient temperature inhibits PRL secretion, Harris and co-workers (15) also noted marked reductions (56 to 77%) in circulating PRL from room temperature control values in rats housed at 4° for longer than 25 min.

The divergence in plasma levels of β-END and PRL observed here in cold-exposed animals suggests that separate hypothalamic mechanisms regulate the secretion of these two hormones. In addition, there is much indirect evidence indicating that opiate peptides may be involved in the stimulatory control of PRL secretion in rats. Like morphine, exogenously administered opiate peptides stimulated PRL release and naloxone pretreatment inhibited this response (4, 5) and also attenuated the physiologic secretion of PRL rats (6, 7). Our findings indicate that although increased blood levels of β-END may facilitate PRL release in immobilized and heat-exposed rats, a similar situation does not exist in animals acutely exposed to cold-ambient temperature. These observations do not preclude a role for β-END or other opiate peptides in the stimulatory control of pituitary PRL but rather demonstrate that

blood-borne β-END does not stimulate PRL release in cold-exposed rats. Consistent with this view we have observed that the ability of morphine to evoke PRL release is diminished in cold-exposed rats (unpublished). Whether the failure of circulating opiates to stimulate PRL release in cold-exposed rats is a consequence of a decrease in body temperature cannot be determined from these experiments alone. Independent of blood-borne β-END, however, β-END (16) and methionine-enkephalin (17) have been shown to be concentrated in terminals of hypothalamic neurons. To what extent the release of these opiate peptides within the hypothalamus may be involved in the regulation of pituitary function remains to be determined.

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