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Effects of Endorphins on Prolactin and Growth Hormone Secretion in Rats (40219)

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Morphine has been reported to stimulate both prolactin (PRL) and growth hormone (GH) release in the rat *in vivo* (1, 2) but not *in vitro* (3). The GH response to morphine was partially blunted in animals with hypothalamic ventromedial lesions (4). PRL and GH release induced by morphine were shown to be reversed by concurrent administration of naloxone, a specific opiate antagonist (1). These observations suggest that morphine stimulates PRL and GH release possibly acting through an opiate receptor in the central nervous system.

The presence of endogenous opiate activity was recently demonstrated in the brain. Hughes *et al.* (5) first isolated Met^5-enkephalin and Leu^5-enkephalin from porcine brain as peptides which bind opiate receptors. Guillemin *et al.* (6) isolated α-endorphin from porcine hypothalamus and posterior pituitary. Met^5-enkephalin and α-endorphin were identical to the sequence of 61–65 and 61–76 amino acids of β-Lipotropin (β-LPH) first isolated from sheep pituitary (7), respectively. The C-terminal fragment, $\beta\text{-LPH}^{61\text{–}91}$ (β-endorphin) was isolated from camel pituitary gland (8). These peptides have potent opiate activity and bind opiate receptors (5, 8–10).

Since these results suggest a possible role of opioid peptides in regulating PRL and GH secretion, and β-endorphin and Met^5-enkephalin were recently reported to enhance PRL and GH release (3, 11, 12), we designed this experiment to compare the effect of these opioid peptides on PRL and GH secretion in the rat, with special reference to the minimal effective dose and time course of plasma hormone responses.

Materials and methods. Male Wistar rats weighing 180–220 g were used throughout the experiments. They were maintained in a light and temperature controlled room (14 hr light, 10 hr dark, 25 ± 1°), and were fed Oriental Laboratory Chow (Oriental Yeast Co., Tokyo) and water *ad lib.*

After an overnight fast, the animals were anesthetized with urethane (150 mg/100 g body wt ip). Thirty min later, test materials were injected into the lateral ventricle of the rat through a stainless steel cannula (23 guage), which was stereotaxically inserted at a point 1.2–1.5 mm lateral to the sagital suture, 1 mm posterior to the bregma, and 4.2–4.6 mm lower than the top of the skull. The test material was dissolved in physiological saline containing 0.24% Fast Green FCF (Chroma Co., Stuttgart), a dye marker, and injected in a volume of 10 μl per rat. The completeness of intraventricular injection was proved by macroscopic examination after the experiments.

The following drugs were employed: β-endorphin (Peninsula Labs., Calif.), α-endorphin (Peninsula Labs., Calif.), Met^5-enkephalin (Protein Research Foundation, Osaka), Leu^5-enkephalin (Protein Research Foundation, Osaka) and naloxone hydrochloride (Endo Labs., NY).

Blood samples of 0.6 ml were withdrawn from the jugular vein immediately before and 10, 20 and 40 min after the injection of test materials or control saline solution, according to the method described previously (13). Blood samples were promptly centrifuged, and plasma was kept at −20° until assayed.

Plasma PRL and GH levels were measured by specific radioimmunoassay (14, 15) using the kit kindly supplied by the NIAMDD, the Rat Pituitary Hormone Distribution Program. The minimum detectable quantity of plasma PRL and GH was 1.0 ng/ml and the coefficient of variation between assays averaged 12% and 9%, respectively. Student's t test was used for the statistical evaluation.

Results. Intraventricular injection of β-endorphin (10 ng, 100 ng, 350 ng and 1 μg per rat) resulted in a significant and dose-related increase in plasma PRL levels in the rat (Fig.

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1). Plasma GH was also elevated by the higher doses of β-endorphin (350 ng and 1 μg) but not by the smaller doses of β-endorphin (10 ng and 100 ng) (Fig. 1). Control saline solution did not significantly change either plasma PRL nor GH.

Concomitant intravenous administration of naloxone (250 μg) significantly blunted the increases of plasma PRL and GH induced by β-endorphin (1 μg), as shown in Fig. 2.

Intraventricular injection of α-endorphin (10 ng, 100 ng, 1 μg and 10 μg per rat) caused

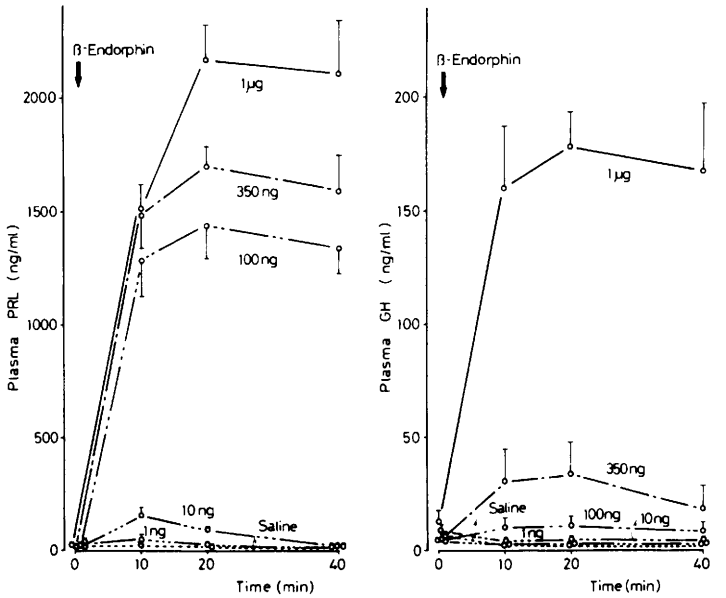

FIG. 1. Plasma rat PRL and GH levels following the intraventricular injection of various doses of β-endorphin (10 ng, 100 ng, 350 ng and 1 μg per rat). All values are the mean ± SE of the determinations in six to seven animals.

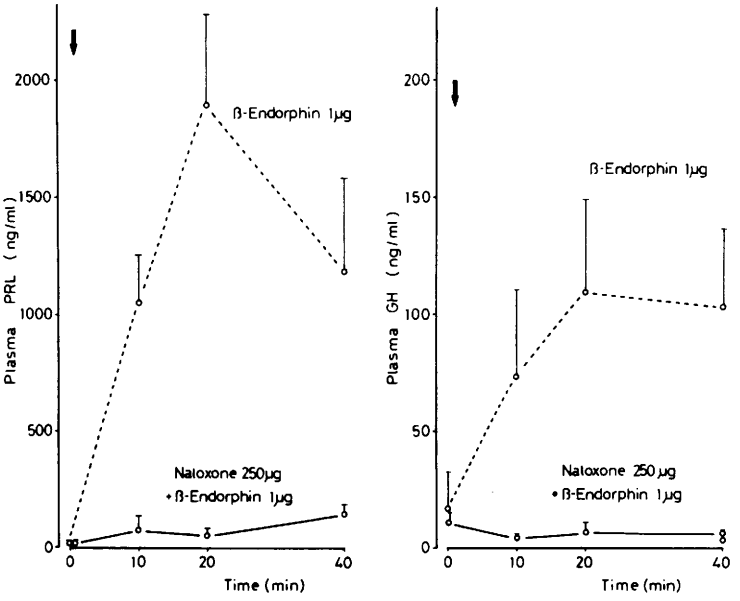

FIG. 2. Effect of naloxone (250 μg) administered intravenously on rat PRL and GH responses to β-endorphin (1 μg) injected intraventricularly. All values are the mean ± SE of five to six animals.

a significant and dose-related increase in plasma PRL levels in the rat (Fig. 3). Plasma GH was significantly elevated by α-endorphin only at the dose of 10 μg and not affected by smaller doses of α-endorphin (Fig. 3). Naloxone (250 μg iv) injection significantly suppressed plasma PRL and GH responses to α-endorphin (10 μg) as shown in Fig. 4.

Intraventricular injection of Met^5-enkephalin (10 ng, 1 μg and 10 μg) and Leu^5-enkephalin (1 μg and 10 μg) raised plasma PRL levels dose-dependently, respectively (Fig. 5).

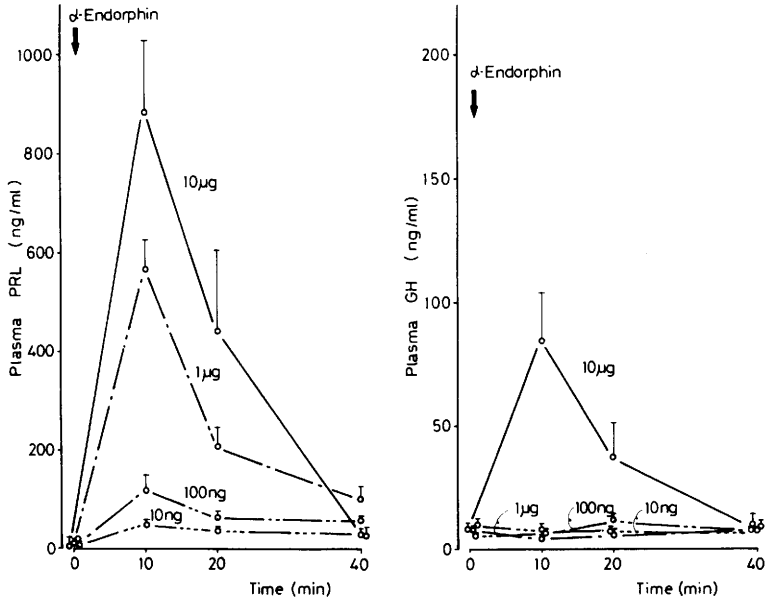

FIG. 3. Plasma rat PRL and GH levels following the intraventricular injection of α-endorphin (10 ng, 100 ng, 1 μg and 10 μg per rat). All values are the mean ± SE of five to six animals.

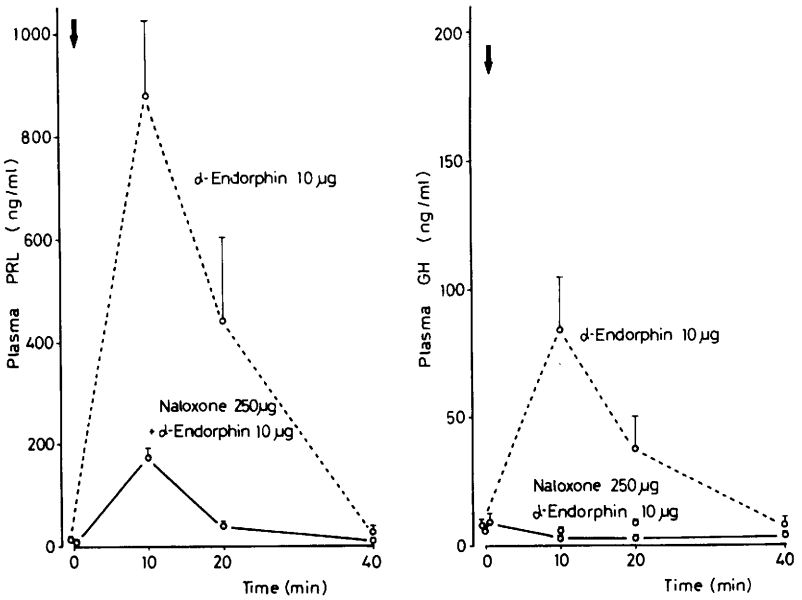

FIG. 4. Effect of naloxone (250 μg) administered intravenously on rat PRL and GH responses to α-endorphin (10 μg) injected intraventricularly. All values are the mean ± SE of six animals.

However, neither Met[5]-enkephalin nor Leu[5]-enkephalin changed plasma GH at the doses examined.

Figure 6 shows a dose-response curve, constructed by plotting the mean of the maximum plasma PRL and GH increments above the basal level after each dose of β-endorphin, α-endorphin and Met[5]-enkephalin against the logarithm of molar concentration. The dose-response curves were not parallel, but β-en-

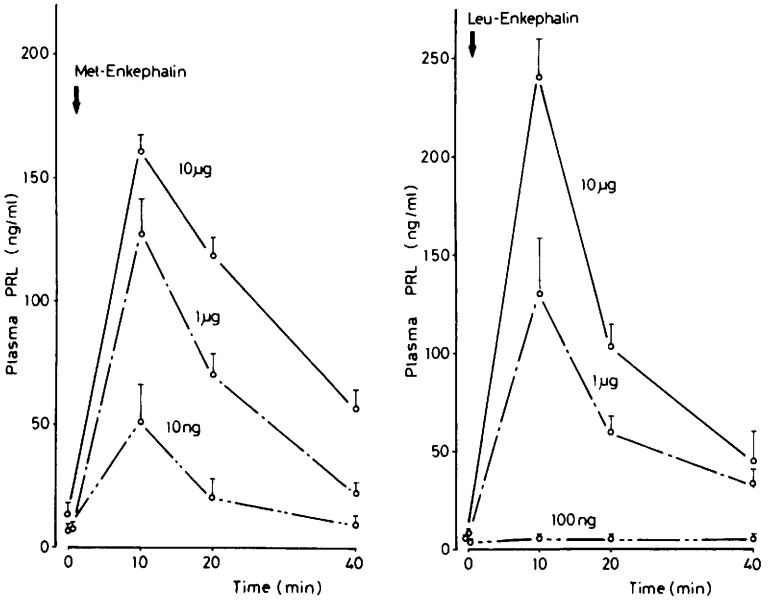

FIG. 5. Effects of intraventricular injection of Met[5]-enkephalin (10 ng, 1 μg and 10 μg) and Leu[5]-enkephalin (100 ng, 1 μg and 10 μg) on plasma PRL levels in rats. All values are the mean ± SE of five to seven animals.

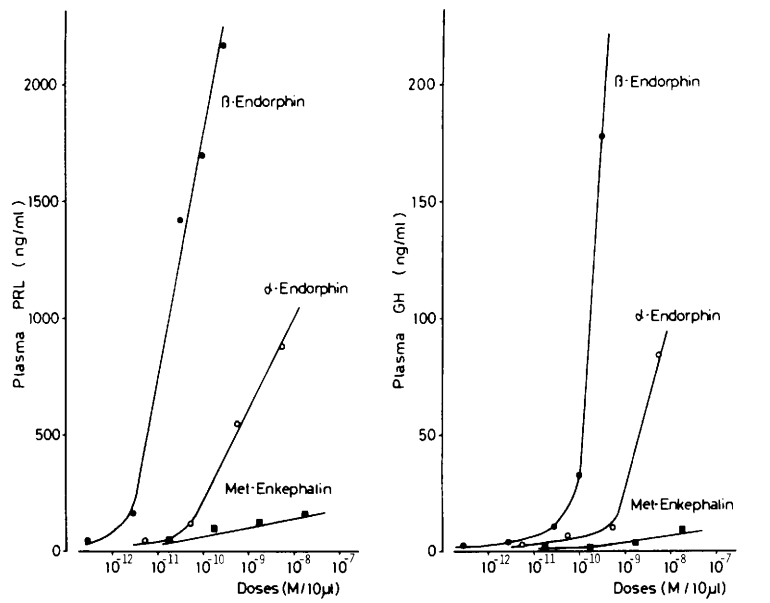

FIG. 6. Plasma PRL and GH responses to log dose of β-endorphin, α-endorphin and Met[5]-enkephalin on a molar basis. Each point represents the mean ± SE of the maximum plasma PRL and GH increments in each test group after the administration of the indicated dose of peptide into the lateral ventricle in the rat.

dorphin is apparently the most potent, followed by α-endorphin and then Met[5]-enkephalin in stimulating PRL and GH release. When the increment of these hormones above the basal level was compared after the injection, PRL was found to be more increased than GH by these opiate peptides. Smaller doses of α- and β-endorphins raised plasma PRL but not GH.

Discussion. PRL and GH secretion is influenced by a variety of naturally occurring brain compounds, which include biogenic amines, TRH, substance P and neurotensin (13, 16–18). The recent identification of brain peptides with opiate like activity (5, 6, 8–10) raised the possibility that these peptides, beside their role as modulators of pain (19), could also be involved in the control of hypothalamo-pituitary functions.

Bruni *et al.* (20) reported that naloxone, an opiate antagonist, reduced serum PRL and GH in rats decapitated after the intraperitoneal injection. It suggests that the endogenous opiates may help to maintain basal serum PRL and GH levels.

We observed in the present experiment that intraventricular injection of β-endorphin and α-endorphin caused a significant increase in plasma PRL and GH in urethane-anesthetized rats. Plasma PRL was also elevated by Met[5]-enkephalin and Leu[5]-enkephalin, but GH release was not affected by these peptides at the doses examined. The stimulating effects of higher doses of β-endorphin, Met[5]-enkephalin and Leu[5]-enkephalin on PRL and GH secretion were previously demonstrated in unanesthetized rats (12, 21) and in steroid-primed and anesthetized rats (3). In agreement with these reports, we observed that β-endorphin, the longest peptide, was the most potent to stimulate PRL and GH release among these opioid peptides. PRL and GH responses to these opioid peptides were significantly inhibited by naloxone. Met[5]-enkephalin, the shortest peptide, was reported to be rapidly inactivated in rat brain (22). These results suggest that the stimulating activity of these peptides involves an opiate receptor dependent step and the potency may depend on resistance to enzymic inactivation.

The dose-response curves obtained with these peptides in PRL and GH release were not parallel. If the effective dose is considered on a molar basis, these peptides may play a more important physiological role in the regulation of PRL than of GH secretion in the rat.

The exact mechanism by which the opiate agonists stimulate PRL and GH release needs further investigation. *In vitro* studies, direct action of these peptides on the pituitary was not observed (3). Morphine is reported to affect biogenic amines in the brain (23–25). It is possible, therefore, that opioid peptides may modulate the release of hypothalamic hormones, acting somewhere in the central nervous system. However, the endogenous somatostatin may not play a role in the mechanism by which the opioid peptide stimulates PRL and GH release, since PRL and GH responses to opioid peptides were also obtained in rats treated with antiserum against somatostatin (12, 21).

Summary. Intraventricular injection of β-endorphin and α-endorphin, in doses of 10 ng to 10 μg per rat, resulted in a significant and dose-related increase in plasma prolactin (PRL) levels in urethane-anesthetized male rats. β-endorphin (350 ng and 1 μg) and α-endorphin (10 μg) also caused a significant increase in plasma growth hormone (GH), although smaller doses of these peptides had no significant effect. The increases in plasma PRL and GH induced by β-endorphin were larger than those induced by α-endorphin when compared on a molar basis. Plasma PRL and GH responses to β-endorphin (1 μg) and α-endorphin (10 μg) were both significantly blunted by naloxone (250 μg), an opiate receptor blocking agent, when it was simultaneously injected intravenously. Met[5]-enkephalin (10 ng, 1 μg and 10 μg) and Leu[5]-enkephalin (1 μg and 10 μg) also significantly elevated plasma PRL levels, but less so than did β- and α-endorphins, whereas plasma GH concentrations were not changed at the doses examined. These results suggest that endorphins stimulate PRL and GH secretion in a different manner in the rat.

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