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Effects of a Synthetic Met[5]-enkephalin Analog on Plasma Luteinizing Hormone and Prolactin Levels in Conscious Orchiectomized Rats[1] (41314)

YUZURU KATO, SEIJI HIROTO, HIDEKI KATAKAMI, NORIO MATSUSHITA, AKIRA SHIMATSU, AND HIROO IMURA

Second Medical Clinic, Department of Medicine, Kyoto University, Faculty of Medicine, Kyoto 606, Japan

Abstract. The effects of a potent opioid peptide FK33-824, [D-Ala[2], MePhe[4], Met(O)[5]-ol]enkephalin, on luteinizing hormone (LH) and prolactin (PRL) secretion were investigated in conscious castrated male rats with chronically implanted indwelling cannulae. Intraventricular injection of FK33-824 (1, 10, and 100 ng/rat) resulted in a rapid and dose-related decrease in plasma LH whereas it increased plasma PRL levels in the rat. Similar results were obtained with β-endorphin (1 μg/rat). Intravenous injection of naloxone, an opiate antagonist, blunted the LH and PRL responses to FK33-824 and β-endorphin. Pretreatment with pimozide, a dopamine antagonist, blunted the PRL but not LH responses to FK33-824. Specific destruction of the serotoninergic neural system by 5,6-DHT in combination with PCPA did not affect the LH and PRL responses to FK33-824. These results suggest that the opioid peptide inhibits LH secretion and stimulates PRL release via opiate receptors in the central nervous system in conscious orchiectomized rats and that central dopaminergic mechanisms are involved in PRL release induced by the opioid peptide.

The recent identification of endogenous opioid peptides, which are concentrated in the hypothalamus and the pituitary gland, have suggested that opioid peptides play a role in regulating the secretion of pituitary hormones (1). Exogenous opioid peptides such as β-endorphin and Met[5]-enkephalin stimulate PRL release (2–6) and inhibit LH secretion (4, 7) in the rat. Opioid peptides also change the turnover and the release of brain dopamine (5, 8, 9) and serotonin (10, 11), which are known to influence the secretion of PRL and LH in opposite directions (12–16).

In the present study, we examined the effects of a potent Met[5]-enkephalin analog FK33-824 (17), Tyr–D-Ala–Gly-MePhe-Met(O)-ol, on plasma LH and PRL levels in conscious castrated male rats with chronically implanted indwelling cannulae. We also studied the interaction of the opioid peptide with brain dopamine and serotonin in the regulation of LH and PRL secretion in these animals.

Materials and Methods. Wistar strain male rats (Japan Animal Co., Osaka) weighing 200–250 g were maintained on Oriental laboratory chow (Oriental Yeast Co., Tokyo) and water *ad libitum* in an air-conditioned room under artificial lighting (light on 0600–1800 hr).

The animals were orchiectomized under chloral hydrate anesthesia (35 mg/100 g body wt, ip). A Silastic catheter was then implanted into the right atrium according to a modification of the technique of Szabo and Frohman (18). A PE-10 cannula was also implanted into the right lateral ventricle, using a modification of the method described by Altaffer *et al.* (19). Every 2–3 days, an extension tube (PE-50) was connected to the intraatrial catheter exteriorized over the back of the neck and rinsed with the heparinized saline (100 IU/ml), to maintain patency of the cannula and to adapt the animals to the blood sampling.

The experiments were begun 7 to 10 days after the surgery. When the same group of rats was used, each experiment was performed at an interval of 1 week. On the day of the experiment, 0.4-ml blood samples were withdrawn from the indwelling right atrial catheter into the heparinized tubercu-

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lin syringes every 10 min for 3 hr starting at 1100 hr. Test substances were given intraventricularly through the indwelling cerebroventricular cannula or intravenously through the right atrial catheter.

Plasma was promptly separated and stored at −20° until assayed. Blood cells were resuspended in saline and returned to the rats following the next blood sampling.

Plasma LH and PRL concentrations were measured by double antibody radioimmunoassay using the kits provided by NIAMDD, the rat pituitary hormone distribution program. NIAMDD-rat LH-RP-1 and rat PRL-RP-1 were used as the standard preparations. The minimum detectable quantities of LH and PRL were 5 and 1 ng/ml, respectively, and the coefficient of variation between assays averaged 12%. Analysis of variance in combination with Duncan's new multiple range test was used for statistical evaluation.

FK33-824 ([D-Ala^2, MePhe^4, Met(O)^5-ol]enkephalin, Sandoz, Basel) and human β-endorphin (Daiichi Pharmaceutical Co., Tokyo) were dissolved in physiological saline and injected intraventricularly in a volume of 10 μl per rat 1 hr after starting the experiment. Naloxone hydrochloride (Endo Labs., New York) dissolved in saline was injected intravenously 3 min before the injection of FK33-824 or β-endorphin. Pimozide (Fujisawa Pharmaceutical Co., Tokyo) was dissolved in 0.1 *M* tartaric acid, diluted with saline, and injected 20 min before the injection of FK33-824. 5,6-Dihydroxytryptamine (5,6-DHT, Sigma, St. Louis, Mo.) was dissolved in physiological saline containing 0.1% ascorbic acid. *para*-Chlorophenylalanine (PCPA, Nakarai Chemical Co., Kyoto) was dissolved in 0.5 *N* NaOH, which was adjusted to pH 9.0 by 0.5 *N* HCl. 5,6-DHT was injected intraventricularly 7 days before and PCPA was

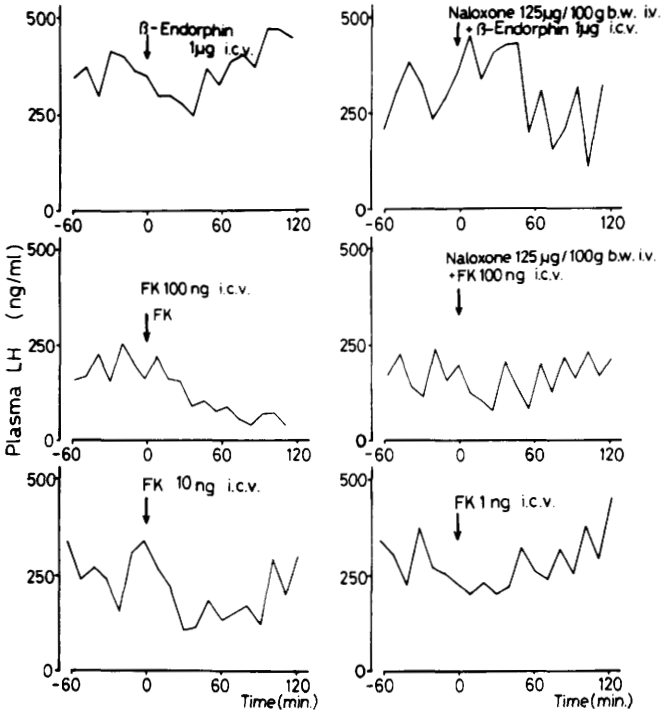

FIG. 1. Effects of the intraventricular injection of either β-endorphin (1 μg/rat) or FK33-824 (FK, 1, 10, and 100 ng/rat) on plasma LH levels in a representative conscious castrated male rat. Right upper and middle panels show the effect on plasma LH of naloxone (125 μg/100 g body wt) injected intravenously 3 min before β-endorphin (1 μg/rat, icv) or FK33-824 (100 ng/rat, icv) administration.

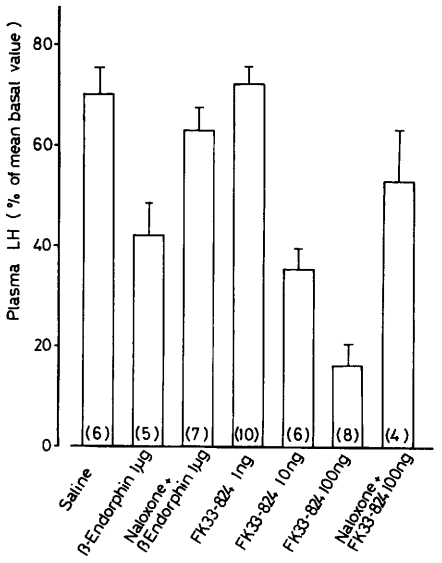

FIG. 2. Effects of the intraventricular injection of β-endorphin (1 μg/rat) and FK33-824 (1, 10, and 100 ng/rat) with or without intravenous injection of naloxone (125 μg/100 g body wt) on plasma LH levels in conscious castrated male rats. Plasma LH values are expressed as the maximum percentage change (basal %) for 60 min after the injection from each mean basal value of plasma LH before the injection. The numbers in parentheses refer to the number of animals in each test group. Statistical differences: saline vs β-endorphin, $P < 0.01$; β-endorphin vs naloxone + β-endorphin, $P < 0.05$; FK33-824 1 ng vs 10 ng, $P < 0.01$; FK33-824 10 ng vs 100 ng, $P < 0.05$; FK33-824 100 ng vs naloxone + FK33-824 100 ng, $P < 0.01$; β-endorphin vs FK33-824 100 ng, $P < 0.01$; saline vs FK33-824 1 ng, $P > 0.05$.

given ip in two divided doses at 48 and 24 hr before the experiment in the same rats as described by Collu *et al.* (20).

Results. When blood samples were collected every 10 min for 3 hr in conscious castrated male rats, plasma LH levels were elevated and pulsatile in nature whereas plasma PRL levels were rather stable. Intraventricular administration of saline (10 μl/rat) did not affect the pulsatile LH release nor plasma PRL levels.

As shown in Figs. 1 and 2, plasma LH levels were rapidly suppressed by β-endorphin (1 μg/rat) which was injected intraventricularly 1 hr after the start of the experiment. The intravenous injection of naloxone (125 μg/100 g body wt), a specific opiate antagonist, blunted the inhibitory effect of β-endorphin on LH release.

As shown in Figs. 1 and 2, LH release was inhibited by intraventricular injection of FK33-824 (10 and 100 ng/rat), a synthetic Met^5-enkephalin analog, whereas a smaller dose of FK33-824 (1 ng/rat) did not affect the spontaneous LH release. The intravenous injection of naloxone (125 μg/100 g body wt) blunted the LH response to FK33-824 (100 ng/rat).

When the maximum responses of plasma LH were compared within 60 min after drug injection, the inhibitory effect of FK33-824 on LH release was dose related and more potent than that of β-endorphin on a molar basis (Fig. 2).

On the other hand, plasma PRL levels were increased by β-endorphin (1 μg/rat) and FK33-824 (1, 10, and 100 ng/rat) in a dose-dependent manner in these animals (Fig. 3). The peak values of plasma PRL were obtained 10 min after the injection of the opioid peptides.

As shown in Fig. 4, the intravenous injection of pimozide (50 μg/100 g body wt), a specific dopamine antagonist, raised plasma PRL levels and FK33-824 (10 ng/rat, icv) did not further increase plasma PRL levels in these animals. Plasma LH levels and the LH response to FK33-824 were not influenced by pimozide administration.

Pretreatment with 5,6-DHT (50 μg/rat, icv), a drug toxic to the serotoninergic system, in combination with PCPA (60 mg/100 g body wt, ip, in two divided doses), an inhibitor of serotonin biosynthesis, did not affect either the LH or PRL responses to FK33-824 (10 ng/rat, icv) in conscious orchiectomized rats (Fig. 4).

Discussion. The present study demonstrates that intraventricular injections of FK33-824, a synthetic Met^5-enkephalin analog, as well as β-endorphin suppress plasma LH levels and stimulate PRL release in conscious, freely moving castrated male rats. The action of FK33-824 is dose related and is blocked by a specific opiate antagonist, naloxone, suggesting that the opioid peptide inhibits LH release and stimulates PRL secretion via central opiate receptors in the castrated rat. These results support earlier findings that endorphins

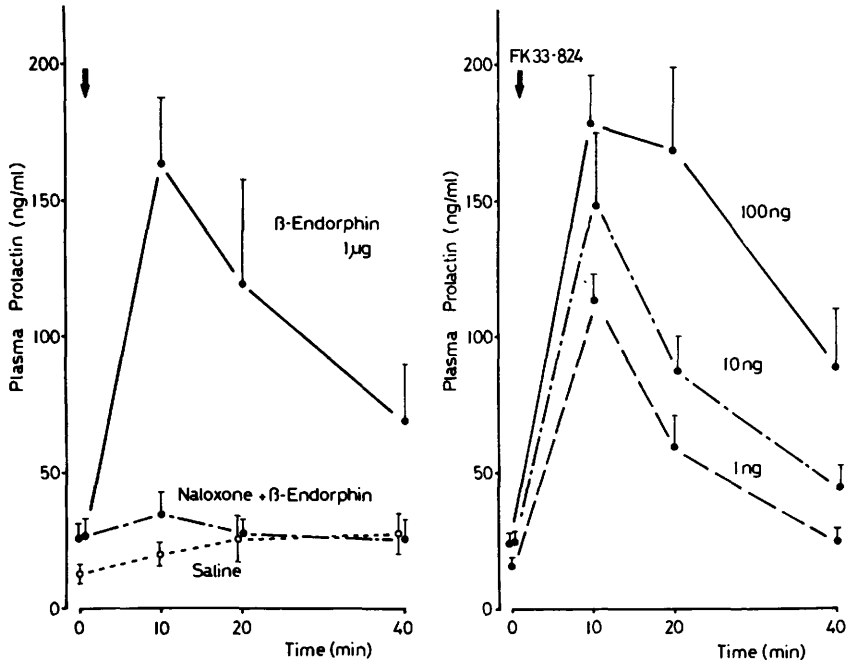

FIG. 3. Effects of the intraventricular injection of β-endorphin (1 μg/rat), FK33-824 (1, 10, and 100 ng/rat), and saline (10 μl/rat) on plasma PRL levels in conscious castrated male rats. Naloxone (125 μg/100 g body wt) was injected intravenously 3 min before β-endorphin (1 μg/rat) administration. All values are the mean ± SE of 5–10 animals in each group. The arrow indicates the time of injection of the opioid peptides.

such as Met^5-enkephalin and β-endorphin inhibit LH secretion in castrated rats (4, 7) and stimulate rat PRL release (2–6). The potent stimulating effect of FK33-824 on PRL secretion (21–23) and inhibitory action on plasma LH (22) also have been reported in human beings.

Opioid peptides stimulate the turnover of dopamine in the hypothalamus (5, 8) and the release of dopamine into the hypophysial portal vessels (9). Apomorphine, a dopamine receptor simulator, inhibits episodic LH release in ovariectomized rats (12). PRL secretion is known to be inhibited by brain dopamine (14). We then studied the possible involvement of dopamine on changes in plasma LH and PRL induced by FK33-824 in castrated rats. The present study shows that PRL release induced by FK33-824 is inhibited by pimozide, a dopamine antagonist, whereas the inhibition of LH release induced by the opioid peptide is not affected. These results suggest that central dopaminergic systems play a role in PRL secretion induced by the opioid peptide, but not in the LH response to FK33-824. The involvement of dopaminergic mechanisms in PRL secretion induced by opioid peptides has been suggested by previous reports (23–24). In contrast to these results, Spampinato *et al.* (26) failed to demonstrate a reduction of PRL responses to the opioid peptide after α-methyl-*p*-tyrosine treatment.

It has been reported that β-endorphin and [D-Ala]Met^5-enkephalin increase serotonin turnover in rat brain (10, 11). The intraventricular injection of serotonin inhibits LH secretion (13) and stimulates PRL release (15, 16) in the rat. These reports suggest a possible involvement of serotoninergic mechanisms in plasma LH and PRL changes induced by the opioid peptide. In the present study, however, the LH and PRL responses to FK33-824 were not influenced after specific destruction of serotoninergic neural systems by 5,6-DHT and PCPA in castrated rats. Spampinato *et al.* (26) and Tachè *et al.* (27) also failed to demonstrate the reduction of the PRL re-

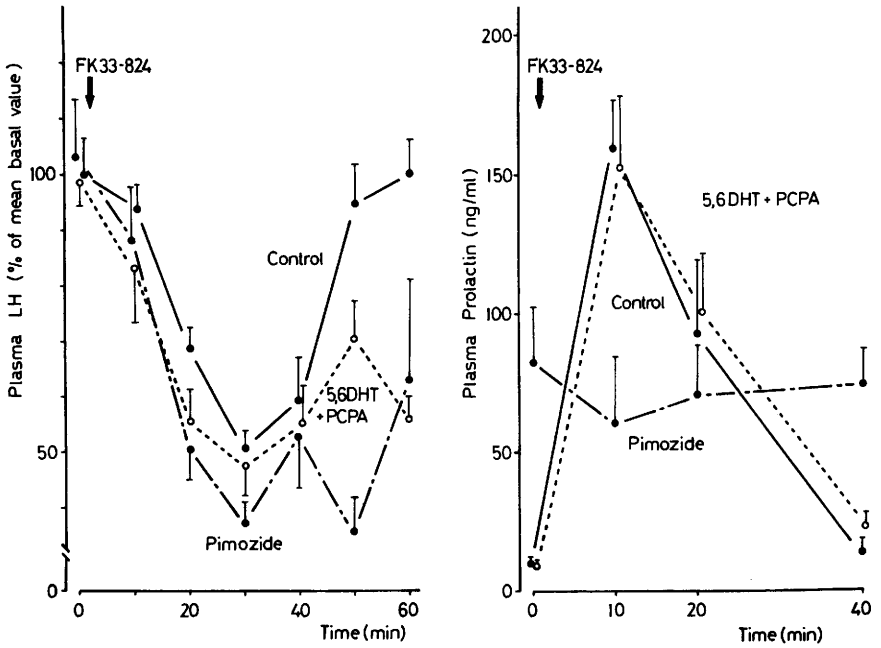

FIG. 4. Effects of the intraventricular injection of FK33-824 (10 ng/rat) on plasma LH (left panel) and PRL (right panel) levels in conscious castrated male rats pretreated with either pimozide or 5,6-DHT in combination with PCPA. Plasma LH values are expressed as percentage change (basal %) from each mean basal value of plasma LH obtained before the injection. All values are the mean ± SE of 5–9 animals in each group.

sponse to the opioid peptides by treatment with PCPA. In contrast, Spampinato *et al.* (26) have reported that 5,6-DHT treatment abolished the release of PRL induced by the opioid peptide in the rat.

Further studies are required to elucidate the role of serotonin in PRL secretion induced by the opioid peptide and the possible involvement of other neurotransmitters in the LH response to the opioid peptide in castrated rats.

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1. Meites J, Bruni JF, Van Vugt DA, Smith AF. Life Sci **24**:1325, 1979.
2. Dupont A, Cusan L, Labrie F, Coy DH, Li CH. Biochem Biophys Res Commun **75**:76, 1977.
3. Rivier C, Vale W, Ling N, Brown M, Guillemin R. Endocrinology **100**:238, 1977.
4. Bruni JF, Van Vugt DA, Marshall S, Meites J. Life Sci **21**:461, 1977.
5. Ferland L, Fuxe K, Eneroth P, Gustaffson JA, Skett P. Eur J Pharmacol **43**:89, 1977.
6. Kato Y, Iwasaki Y, Abe H, Ohgo S, Imura H. Proc Soc Exp Biol Med **158**:431, 1978.
7. Kinoshita F, Nakai Y, Katakami H, Kato Y, Yajima H, Imura H. Life Sci **27**:843, 1980.
8. Deyo SN, Swift RM, Miller RJ. Proc Nat Acad Sci USA **76**:3006, 1979.
9. Gudelsky GA, Porter JC. Life Sci **25**:1697, 1979.
10. Van Loon GR, De Sonza EB. Life Sci **23**:971, 1978.
11. Aligeri A, Consolazione A, Calderini G, Achill G, Puche Canas E, Garrattini S. Experientia **34**:1488, 1978.
12. Drouva SV, Gallo RV. Endocrinology **99**:651, 1976.
13. Schneider H, McCann SM. Endocrinology **86**:1122, 1970.
14. Takahara J, Arimura A, Schally AV. Endocrinology **95**:462, 1974.
15. Lu KH, Meites J. Endocrinology **93**:152, 1973.
16. Iwasaki Y, Kato Y, Ohgo S, Abe H, Hirata F, Senoh S, Tokuyama T, Hayaishi O. Endocrinology **103**:254, 1978.
17. Roemer D, Buescher HH, Hill RC, Press J, Bauer W, Cardinaux F, Closse A, Hauser D, Huguenin R. Nature (London) **268**:547, 1977.

18. Szabo M, Frohman LA. Endocrinology **101**:1023, 1977.
19. Altaffer FB, Verster FD, Hall S, Long CJ, D'Eucarnacao P. Physiol Behav **5**:119, 1970.
20. Collu R, Du Ruisseau P, Tache Y. Neuroendocrinology **28**:178, 1979.
21. Graffenried B von, del Pozo E, Roubicek J, Krebs E, Pöldinger W, Burmeister P, Kerp L. Nature (London) **272**:729, 1978.
22. Stubbs WA, Delitala G, Jones A, Jeffcoate WJ, Edwards CRW, Ratter SJ, Besser GM. Lancet **2**:1225, 1978.
23. Kato Y, Matsushita N, Katakami H, Imura H. In: Hubinont DO, ed. Progress in Reproductive Biology. Basel, Karger, Vol. 6:p54, 1980.
24. Dupont A, Cusan L, Ferland L, Lemay A, Labrie F. In: Collu R, Barbeau A, Ducharme JR, Rochefort JG, eds. Central Nervous System Effects of Hypothalamic Hormones and Other Peptides. New York, Raven Press, p283, 1979.
25. Van Vugt DA, Bruni JF, Sylvester PV, Chen HT, Ieiri T, Meites J. Life Sci **24**:2361, 1979.
26. Spampinato S, Locentelli V, Cocchi D, Vicentini L, Bajusz S, Ferri S, Müller EE. Endocrinology **105**:163, 1979.
27. Tachè Y, Chapenet G, Chretien M, Collu R. In: Collu R, Barbeau A, Ducharme JR, Rochefort JG, eds. Central Nervous System Effects of Hypothalamic Hormones and Other Peptides. New York, Raven Press, p301, 1979.

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