Involvement of Brain Dopamine in Prolactin Secretion
Induced by a Synthetic Met\(^{5}\)-enkephalin Analogue in Rats

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Abstract

The effect of a potent opioid peptide FK 33-824, \([D-Ala^2, MePhe^4, Met(O)^5-ol]\) enkephalin, on prolactin (PRL) release from rat anterior pituitary was investigated in vivo and in vitro. Systemic administration of FK 33-824 (1, 10 and 100 \(\mu\)g/100 g BW i.p.) caused a rapid and dose-related increase in plasma PRL in urethane-anesthetized male rats. Naloxone (125 \(\mu\)g/100 g BW i.v.) abolished PRL responses to FK 33-824. In the rat pretreated with either reserpine (2 mg/100 g BW i.p.), \(\alpha\)-methyl-p-tyrosine (30 mg/100 g BW i.p.) or pimozide (50 \(\mu\)g/100 g BW i.v.), basal plasma PRL levels were elevated and FK 33-824 injection did not further increase plasma PRL. In contrast, neither 5,6-dihydroxy-tryptamine (50 \(\mu\)g/rat, i.c. v.) nor diphenhydramine (100 \(\mu\)g/100 g BW i.v.) treatment influenced the plasma PRL response to FK 33-824. FK 33-824 (10\(^{-8}\)-5 \(\times\) 10\(^{-5}\) M) did not stimulate PRL release from dispersed anterior pituitary cells in vitro nor attenuate the inhibitory effect of dopamine (5 \(\times\) 10\(^{-7}\) M). These results suggest that central dopaminergic mechanisms are involved in PRL release induced by the opioid peptide.

It has been widely accepted that the secretion of prolactin (PRL) is controlled mainly by the tonic inhibition of the hypothalamus via PRL release inhibiting factor (PIF), and dopamine seems to be the most important PIF (Macleod and Lehmeyer, 1974; Takahara et al.; 1974). On the other hand, there are such substances in the brain as serotonin (Iwasaki et al., 1978; Kamberi et al., 1971), histamine (Donoso et al., 1976; Libertun et al., 1976), TRH (Bower et al., 1971; Tashijian et al., 1971) and VIP (Kato et al., 1978b; Ruberg et al., 1978) that seems to play a stimulatory role in PRL release. Recently, morphinomimetic peptides have been isolated from the mammalian central nervous system (Cox et al., 1976; Hughes et al., 1975) and subsequently they have been proved to stimulate PRL secretion (Bruni et al., 1977; Dupont et al., 1977; Ferland et al., 1977; Kato et al., 1978a; Rivier et al., 1977a). However, the interaction of these opioid peptides with brain amines and other neuropeptides has not been fully elucidated. In the present experiments, we studied the relationship between brain amines and opioid peptides in regulating PRL secretion using a synthetic analogue of Met\(^{5}\)-enkephalin, \([D-Ala^2, MePhe^4, Met(o)^5-ol]\) enkephalin, which has a long-lasting analgesic action and a potent stimulating effect on PRL secretion (Kato et al., 1980; Roemer et al., 1977; von Graffenried et al., 1978).

Materials and Methods

Animals

Wistar strain male rats weighing 200–220 g (Japan Animal Co., Osaka) were maintained in a temperature...
controlled room (23 ± 1°C) on a 12 h dark : 12 h light schedule (light on 0600-1800). Laboratory chow (Oriental Yeast Co., Tokyo) and tap water were given ad libitum.

In vivo Experiments
After overnight fasting, they were anesthetized with urethane (150 mg/100 g BW i.p.). Test substances were injected intravenously and blood samples of 0.6 ml were withdrawn from the jugular vein immediately before and 10, 20 and 40 min after the injection, as described previously (Kato et al., 1978b). Plasma samples were promptly separated and kept at −20°C until assayed.

Cell suspensions
The animals were decapitated, and the anterior pituitary glands were promptly removed. They were chopped with a razor blade into small pieces, and rinsed several times with phosphate-buffered saline (PBS). They were incubated with PBS containing 0.25% trypsin (Difco) at 37°C for 20 min. The dispersed pituitary cells were washed with Hank’s solution containing 10% fetal calf serum (FCS). Then the cells were suspended in Ham’s F 10 medium containing 15% horse serum and 2.5% FCS.

Superfusion System
The cells were then incubated in Falcon plastic flasks (75 cm²) at 37°C for 48 h under a water-saturated atmosphere of 5% CO₂ and 95% air. The cells were resuspended in Ham’s F 10 medium and transferred to the superfusion chamber. A sample was used for vital staining and counting. Cell viability proved to be more than 90%. The superfusion system employed in this study was a modification (Matsushita et al., 1981a) of the method described by Mulder and Smelik (1977). Dispersed cells (5 × 10⁶) were placed on a Sephadex G-25 column packed in a 2.5 ml disposable syringe (Terumo) and perfused with Krebs Ringer Bicarbonate buffer containing 10 mM glucose (pH 7.4) at a constant flow rate of 0.33 ml/min using a peristaltic pump. Throughout the experiments, the perfusion medium was gassed with 95% O₂ and 5% CO₂. The cell column and the perfusion medium were immersed in a water bath at 37°C. After a preperfusion period of 60 min, the effluent was collected as 2 min fractions and stored at −20°C until assayed.

Monolayer Culture Methods
A 1 ml suspension of dispersed cells (4 × 10⁶) was added to each culture well (16 mm) in a Falcon multi-well culture plate, which was incubated at 37°C under 5% CO₂ and 95% air for 96 h. The cells which were attached to the wells were then washed twice with 1 ml fresh Ham’s F 10 medium without serum. Then the medium was replaced by 1 ml of Ham’s F 10 medium to which test substances or vehicle (control) had been added. After further incubation at 37°C for 4 h, the medium was removed, diluted with 2% bovine serum albumin (BSA) in PBS, and kept at −20°C until assayed.

PRL Radioimmunoassay
PRL concentration in plasma and in the incubation medium were measured by specific radioimmunoassay (Matsushita et al., 1981b) using the kit supplied by the NIAMDD. NIAMDD rat PRL RP-1 was used as the standard preparation.

Drugs
Reserpine (Nakarai Chemicals Co., Kyoto) was first dissolved in a few drops of chloroform and then diluted in physiological saline. Alpha-methyl-p-tyrosine (α-MT, Nakarai Chemicals) was dissolved in 0.5 N NaOH to which 0.5 N HCl was added to bring the pH to 9.0. Pimozide (Fujiisawa Pharmaceutical Co., Osaka) was dissolved in a small volume of 0.1 m tartaric acid and then diluted in physiological saline. 5,6-dihydroxytryptamine (5,6-DHT, Sigma, St. Louis) was dissolved in physiological saline containing ascorbic acid (1 mg/ml). Naloxone (Endo Labs., New York), diphenhydramine (DPH, Kanto Chemicals Co., Tokyo) and FK 33-824 (Sandoz, Basel) were dissolved in physiological saline. Reserpine (2 mg/100 g BW i.p.) was given 14 h before the experiment. α-MT (30 mg/100 g BW i.p.) was administered in three divided doses 14, 9 and 5 h before experiments. 5,6-DHT (50 μg/rat) was administered intraventricularly 1 week before the experiment as described by Spampinato et al. (1979). Pimozide (50 μg/100 g BW i.v.) was injected 30 min before, and naloxone (125 μg/100 g BW i.v.) and DPH (100 μg/100 g BW i.v.) were injected 3 min before the injection of FK 33-824. For the in vitro experiments, synthetic TRH (Tanabe Pharmaceutical Co., Osaka), dopamine hydrochloride (Inovan®, Kyowahakko Co., Tokyo), and FK 33-824 were used. These drugs were dissolved in the appropriate medium in the concentrations indicated in the text.

Statistical Analysis
Statistical evaluation was performed with Duncan’s new multiple range test, Student’s t test and paired t test as appropriate.

Results
In vivo Studies
Intravenous injection of FK 33-824 (1, 10 and 100 μg/100 g BW), a synthetic Met- enkephalin analogue, caused a dose-dependent increase in plasma PRL in urethane-anesthetized rats (Fig. 1). The PRL response to FK 33-824 (10 μg/100 g BW i.v.) was blun-
Fig. 1. Effects of FK-33-824 (1, 10 and 100 µg/100 g BW i.v.) on plasma PRL levels in urethane-anesthetized rats. Values represent the mean±SE of 5–7 rats.

Fig. 2. Effects of naloxone (125 µg/100 g BW i.v.) on PRL release induced by FK 33-824 (10 µg/100 g BW i.v.) in urethane-anesthetized rats. Means±SE are shown.

Fig. 3. Effects of FK 33-824 (10 µg/100 g BW i.v.) on plasma PRL levels in rats pretreated with reserpine (2 mg/100 g i.p.). Reserpine was injected 14 h before the experiments. PRL responses to FK 33-824 in reserpine-treated rats are shown by a solid line and those in control rats treated by vehicle solutions are shown by a dotted line. Means±SE are shown.

ed by naloxone (125 µg/100 g BW i.v.), a specific opiate antagonist, which was injected 3 min before the injection of FK 33-824 (Fig. 2).

Pretreatment with reserpine, a depletor of brain catecholamines and serotonin, resulted in a rise in basal plasma PRL levels, and FK 33-824 did not further increase plasma PRL (Fig. 3). A significant increase in plasma PRL induced by FK 33-824 was not obtained in rats treated with α-MT, an inhibitor of catecholamine biosynthesis, in which basal plasma PRL levels were elevated (Fig. 4).

Intravenous injection of pimozide (50 µg/100 g BW) raised plasma PRL levels within 30 min, and the high plasma PRL concentration were maintained throughout the experiment. FK 33-824 injection failed to increase
Fig. 4. Effects of \( \alpha \)-MT (30 mg/100 g BW i.p.) and DPH (100 \( \mu \)g/100 g BW i.v.) on PRL release induced by FK 33-824 (10 \( \mu \)g/100 BW i.v.) \( \alpha \)-MT was given in three divided doses 14, 9 and 5 h before the experiment. DPH was administered 3 min before FK 33-824 injection. PRL responses to FK 33-824 in rats pretreated with \( \alpha \)-MT are shown by a broken line. Solid line shows plasma PRL levels in the rats after the administration of FK 33-824 and DPH. Control rats are shown by a dotted line. Means \( \pm \) SE are shown.

Fig. 5. Effects of FK 33-824 (10 \( \mu \)g/100 g BW i.v.) on plasma PRL levels in rats pretreated with pimozide (50 \( \mu \)g/100 g BW i.v.). Pimozide or vehicle was injected 30 min before FK 33-824 or saline injection. Solid line shows plasma PRL levels after FK 33-824 injection in rats pretreated with pimozide. Broken line shows PRL responses to FK 33-824 in rats pretreated with vehicle solution. Plasma PRL levels after saline injection in rats pretreated with pimozide are shown by a dotted line. Means \( \pm \) SE are shown.

In vitro Studies

PRL release from monolayer-cultured pituitary cells was stimulated by TRH (5 \( \times \) 10\(^{-7}\) M) and inhibited by dopamine (5 \( \times \) 10\(^{-7}\) M). FK 33-824 had no significant effect on basal PRL release from primary monolayer-cultured pituitary cells, and it also failed to attenuate the suppressive effect of dopamine on PRL release at the dose used (Table 1). FK 33-824 (5 \( \times \) 10\(^{-7}\)–5 \( \times \) 10\(^{-5}\) M) failed to increase PRL release from dispersed pituitary cells in the perfused column, whereas TRH (5 \( \times \) 10\(^{-7}\)–10\(^{-6}\) M) caused a rapid and remarkable increase in PRL in the effluent. (Fig. 7).

Discussion

The present study clearly demonstrated that intravenous injection of FK 33-824 causes a dose-related rise in plasma PRL levels, and the response is blocked by naloxone, a specific opiate antagonist. These findings support...
Fig. 6. Effect of 5,6-DHT (50 µg/rat i.c.v.) pretreatment on plasma PRL response to FK 33-824 (10 µg/100 g BW i.v.) in the rat. 5,6-DHT was administered 1 week before experiment. PRL response to FK 33-824 in control rats are shown by a dotted line and those in rats pretreated with 5,6-DHT are shown by a solid line. Means ± SE are shown.

Table 1. Effects of FK 33-824, TRH and dopamine on PRL release from cultured rat pituitary cells

<table>
<thead>
<tr>
<th>Dose (M)</th>
<th>Mean (±SE) PRL concentration (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2109±55</td>
</tr>
<tr>
<td>TRH 5x10^-7</td>
<td>2992±84*</td>
</tr>
<tr>
<td>Dopamine 5x10^-6</td>
<td>363±27**</td>
</tr>
<tr>
<td>FK 33-824 10^-8</td>
<td>2221±26*</td>
</tr>
<tr>
<td>FK 33-824 10^-7</td>
<td>2230±64*</td>
</tr>
<tr>
<td>FK 33-824 10^-6</td>
<td>2278±51*</td>
</tr>
<tr>
<td>Control</td>
<td>2692±122</td>
</tr>
<tr>
<td>TRH 5x10^-7</td>
<td>4393±229*</td>
</tr>
<tr>
<td>Dopamine 5x10^-7</td>
<td>1572±147*</td>
</tr>
<tr>
<td>FK 33-824 10^-6</td>
<td>1875±145</td>
</tr>
</tbody>
</table>

*p < 0.05, Compared to each control
**p < 0.01, Compared to each control

a Not significant, compared to control
b Not significant, compared to dopamine

Fig. 7. Effects of TRH (5 x 10^-7 and 10^-6 M) and FK 33-824 (5 x 10^-7 - 5 x 10^-5 M) on PRL secretion from perfused pituitary cells. All drugs were infused for 4 min at the concentrations indicated.
the earlier hypothesis obtained with endorphins such as β-endorphine, α-endorphin, Met5-enkephalin and Leu5-enkephalin (Bruni et al., 1977; Dupont et al., 1977; Ferland et al., 1977; Kato et al., 1978a; Rivier et al., 1977a) that PRL secretion is stimulated by opioid peptides via opiate receptors in the rat.

Opiate receptors are known to exist in the hypothalamus and in the pituitary (Kuhar et al., 1973; Simantov and Snyder, 1977). Lien et al. (1976) have reported that Leu5-enkephalin increased PRL release from rat pituitary cell cultures, suggesting a direct action of the opioid peptide on the pituitary. Enjalbert et al. (1979) noted that endorphins blocked dopamine inhibition of PRL secretion in vitro. In contrast, subsequent studies have shown that endorphins do not stimulate PRL secretion from the pituitary in vitro (Meites et al., 1979; Rivier et al., 1977a; Shaar et al., 1978). In our study, FK 33-824 had no stimulatory effect on PRL secretion by rat anterior pituitary cells in vitro, and it did not attenuate the suppressive effect of dopamine. Our results suggest the involvement of the central nervous system in the action of FK 33-824. However, the possible direct action of other endogenous opioid peptides cannot be completely ruled out when the results of the present study are considered.

We found that pretreatment with reserpine (a depletor of catecholamine and serotonin), α-MT (an inhibitor of catecholamine biosynthesis), and pimozide (a dopamine receptor blocker) inhibit the PRL response to FK 33-824 in the rat. Impaired PRL response to FK 33-824 is not simply explained by raised basal plasma PRL levels in these animals after pretreatment, since PRL release is further stimulated by other stimuli such as 5-HTP in rats pretreated with reserpine or α-MT (Matsushita et al., 1981b). These results seem to indicate that the central dopaminergic system essential for PRL secretion induced by FK 33-824 and that FK 33-824 might stimulate PRL secretion by reducing the central dopaminergic tone which tonically inhibits the PRL release from the anterior pituitary gland. Dopaminergic mediation of the endorphin-induced PRL release has been also suggested by several investigators using the dopamine receptor blocker (Dupont et al., 1979; van Vugt et al., 1979). A decrease in the turnover of dopamine in the hypothalamus (Deyo et al., 1979; Ferland et al., 1977; van Loon et al., 1980) and a reduction in the dopamine concentration in the hypophysial portal blood induced by opioid peptides (Gudelsky et al., 1979) also suggest the involvement of dopamine in the PRL response to opioid peptides.

In contrast to these data suggesting a central dopaminergic involvement in PRL release induced by opioid peptides, Spampinato et al. (1979) failed to demonstrate a reduction in the PRL response to the opioid peptide following α-MT treatment. Similar results were reported by Koenig et al. (1980) in morphine-induced PRL secretion in the rat. However, the regimens of α-MT administration used by them differed from ours. The dose and the time schedule they used may not be sufficient to reduce the central dopaminergic activity (Edén et al., 1979).

It has been reported that opioid peptides increase brain serotonin turnover (Algeri et al., 1978; van Loon et al., 1978). Spampinato et al. (1979) have recently demonstrated that treatment with 5,6-DHT, a drug neurotoxic to serotonergic nerve, almost completely abolished the rise in plasma PRL induced by the opioid peptide. Koenig et al. (1980) have reported that morphine-induced PRL release is inhibited by 5,7-DHT treatment. These results suggest serotonergic involvement in the PRL release induced by the opioid peptide. However, Spampinato et al. (1979) and Taché et al. (1979) failed to demonstrate the reduction in PRL response to morphine and opioid peptides following treatment with PCPA, an inhibitor of serotonin biosynthesis. In the present study, the PRL response to FK 33-824 was not impaired by 5,6-DHT treatment, which is known to degenerate the central serotonergic system (Björklund et al., 1974).
also found that specific destruction of the serotonergic neural system by 5,6-DHT in combination with PCPA also did not affect the PRL response to FK 33-824 (data not shown). Furthermore, we have recently reported that L-5HTP-induced PRL release was obtained in rats pretreated with either reserpine or \( \alpha \)-MT (Matsushita et al., 1981b), whereas under these conditions FK 33-824-induced PRL release was blunted. These findings rather indicate that the serotonergic mechanisms are not involved in PRL release induced by the opioid peptide. Further studies are needed to elucidate the interaction between serotonin and opioid peptides in stimulating PRL secretion.

Intraventricular injection of histamine causes a dose-dependent rise in PRL secretion and the response is blocked by diphenhydramine, a histamine H\( _{1} \)-receptor blocker, in the rat (Donoso et al., 1979; Libertun et al., 1976). Since both diphenhydramine and naloxone suppress PRL release induced by morphine (Rivier et al., 1977), we investigated the potential role of histamine in FK 33-824-induced PRL release in the rat. However, the lack of any modification by DPH in the PRL response to FK 33-824 suggests that the effect of FK 33-824 on PRL secretion is not mediated through histaminergic pathways. These results seem to indicate that the morphine and FK 33-824 action modes are different. Further studies are required to elucidate the role of histamine in PRL secretion induced by the endogenous opioid peptide.

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