Involvement of Brain Dopamine in Prolactin Secretion Induced by a Synthetic Met⁵-enkephalin Analogue in Rats

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

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

Abstract

The effect of a potent opioid peptide FK 33-824, [D-Ala², MePhe⁴, 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 µg/100 g BW i.p.) caused a rapid and dose-related increase in plasma PRL in urethane-anesthetized male rats. Naloxone (125 µg/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.), α-methyl-p-tyrosine (30 mg/100 g BW i.p.) or pimozide (50 µg/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 µg/rat, i.c.v.) nor diphenhydramine (100 µg/100 g BW i.v.) treatment influenced the plasma PRL response to FK 33-824. FK 33-824 (10⁻⁸–5 × 10⁻⁵ M) did not stimulate PRL release from dispersed anterior pituitary cells *in vitro* nor attenuate the inhibitory effect of dopamine (5 × 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; Tashjian *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 Met⁵-enkephalin, [D-Ala², MePhe⁴, 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 ($23 \pm 1^\circ\text{C}$) 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 cm^2) at 37°C for 48 h under a water-saturated atmosphere of 5% CO_2 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 (5×10^6) 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% O_2 and 5% CO_2 . 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 (4×10^5) was added to each culture well (16 mm) in a Falcon multiwell culture plate, which was incubated at 37°C under 5% CO_2 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 (Fujisawa 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 $\mu\text{g}/\text{rat}$) was administered intraventricularly 1 week before the experiment as described by Spampinato *et al.* (1979). Pimozide (50 $\mu\text{g}/100$ g BW *i.v.*) was injected 30 min before, and naloxone (125 $\mu\text{g}/100$ g BW *i.v.*) and DPH (100 $\mu\text{g}/100$ 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 $\mu\text{g}/100$ g BW), a synthetic Met⁵-enkephalin analogue, caused a dose-dependent increase in plasma PRL in urethane-anesthetized rats (Fig. 1). The PRL response to FK 33-824 (10 $\mu\text{g}/100$ g BW *i.v.*) was blunt-

Fig. 1. Effects of FK-33-824 (1, 10 and 100 $\mu\text{g}/100\text{ g BW i.v.}$) on plasma PRL levels in urethane-anesthetized rats. Values represent the mean $\pm$ SE of 5-7 rats.

Fig. 2. Effects of naloxone (125 $\mu\text{g}/100\text{ g BW i.v.}$) on PRL release induced by FK 33-824 (10 $\mu\text{g}/100\text{ g BW i.v.}$) in urethane-anesthetized rats. Means $\pm$ SE are shown.

Fig. 3. Effects of FK 33-824 (10 $\mu\text{g}/100\text{ g BW i.v.}$) 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 $\pm$ SE are shown.

ed by naloxone (125 $\mu\text{g}/100\text{ g BW i.v.}$), 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 $\mu\text{g}/100\text{ g BW}$) 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 α -MT (30 mg/100 g BW i.p.) and DPH (100 μ g/100 g BW i.v.) on PRL release induced by FK 33-824 (10 μ g/100 g BW i.v.) α -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 α -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 μ g/100 g BW i.v.) on plasma PRL levels in rats pretreated with pimozone (50 μ g/100 g BW i.v.). Pimozone 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 pimozone. 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 pimozone are shown by a dotted line. Means $\pm$ SE are shown.

plasma PRL in these animals (Fig. 5).

Pretreatment with 5,6-DHT, a drug toxic to the serotonergic system, did not significantly change plasma PRL increase induced by FK 33-824 (Fig. 6). When DPH (100 μ g/100 g BW i.v.), a histamine antagonist, was injected 3 min before the injection of FK 33-824, plasma PRL responses to FK 33-824 were not affected (Fig. 4).

In vitro Studies

PRL release from monolayer-cultured pituitary cells was stimulated by TRH (5×10^{-7} M) and inhibited by dopamine (5×10^{-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

(5×10^{-7} – 5×10^{-5} M) failed to increase PRL release from dispersed pituitary cells in the perfused column, whereas TRH (5×10^{-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 μ g/rat i.c.v.) pretreatment on plasma PRL response to FK 33-824 (10 μ g/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 $\pm$ SE are shown.

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

	Dose (M)	Mean ($\pm$ SE) PRL concentration (ng/ml)
Experiment 1.		
Control		2109 $\pm$ 55
TRH	5×10^{-7}	2992 $\pm$ 84*
Dopamine	5×10^{-6}	363 $\pm$ 27**
FK 33-824	10^{-8}	2221 $\pm$ 26 ^a
FK 33-824	10^{-7}	2230 $\pm$ 64 ^a
FK 33-824	10^{-6}	2278 $\pm$ 51 ^a
Experiment 2.		
Control		2692 $\pm$ 122
TRH	5×10^{-7}	4393 $\pm$ 229*
Dopamine	5×10^{-7}	1572 $\pm$ 147*
FK 33-824	10^{-6}	1875 $\pm$ 145 ^b
+dopamine	5×10^{-7}	

* $p < 0.05$, 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 (5×10^{-7} and 10^{-6} M) and FK 33-824 (5×10^{-7} – 5×10^{-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 RPL 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 RPL 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 hypophyseal 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). We

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 α -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.*, 1977b), 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.

Acknowledgements

We are indebted to the NIAMDD and Endo Labs. for the gift of rat PRL radioimmunoassay kit and naloxone, respectively. This work was supported in part by grant from other Ministry of Education and the Ministry of Health and Welfare, Japan.

References

Algeri, A., A. Consolazione, G. Calderini, G. Achilli,

- E. Puche Canas and S. Garattini (1978). Effect of the administration of (D-Ala)²methionine-enkephalin on the serotonin metabolites in rat brain. *Experientia* **34**, 1488-1489.
- Björklund, A., H-G. Baumgarten and A. Nöbin (1974). Chemical lesioning of central monoamine axons by means of 5,6-dihydroxytryptamine and 5,7-dihydroxytryptamine. *Adv. Biochem. Psycho-Pharmacol.* **10**, 13-33.
- Bowers, C. Y., H. G. Friesen, P. Hwang, H. J. Guyda and K. Folkers (1971). Prolactin and thyrotropin release in man by synthetic pyroglutamyl-histidyl-prolinamide. *Biochem. Biophys. Res. Commun.* **45**, 1033-1041.
- Bruni, J. F., D. van Vugt, S. Marshall and J. Meites (1977). Effects of naloxone, morphine and methionine enkephalin on serum prolactin, luteinizing hormone, follicle stimulating hormone, thyroid stimulating hormone and growth hormone. *Life Sci.* **21**, 461-466.
- Cox, B. M., A. Goldstein and C. H. Li (1976). Opioid activity of a peptide, β -lipotropin-(16-91), derived from β -lipotropin. *Proc. Natl. Acad. Sci. U.S.A.* **73**, 1821-1823.
- Deyo, S. N., R. M. Swift and R. J. Miller (1979). Morphine and endorphins modulate dopamine turnover in rat median eminence. *Proc. Natl. Acad. Sci. U.S.A.* **76**, 3006-3009.
- Donoso, A. O., A. M. Banzan and M. I. Borzino (1976). Prolactin and luteinizing hormone release after intraventricular injection of histamine in rats. *J. Endocrinol.* **68**, 171-172.
- Dupont, A., L. Cusan, L. Ferland, A. Lemay and F. Labrie (1979). Evidence for a role of endorphins in the control of prolactin secretion. In: Central Nervous System Effects of Hypothalamic Hormone and Other Peptides (Collu, R., A. Barbeau, J. R. Ducharme and J. G. Rochefort eds.), Raven Press, New York. pp. 283-300.
- Dupont, A., L. Cusan, F. Labrie, D. H. Coy and C. H. Li (1977). Stimulation of prolactin release in the rat by intraventricular injection of β -endorphin and methionine-enkephalin. *Biochem. Biophys. Res. Commun.* **75**, 76-82.
- Edén, S., P. Bolle and K. Modigh (1979). Monoaminergic control of episodic growth hormone secretion in the rat: Effects of reserpine, α -methyl-tyrosine, p-chlorophenyl-alanine, and haloperidol. *Endocrinology* **105**, 523-529.
- Enjalbert, A., M. Ruberg, S. Arancibia, M. Priam and C. Kordon (1979). Endogenous opiates block dopamine inhibition of prolactin secretion *in vitro*. *Nature* **280**, 595-597.
- Ferland, L., K. Fuxe, P. Eneroth, J. A. Gustafsson and P. Skett (1977). Effects of methionine-enkephalin on prolactin release and catecholamine levels and turnover in the median eminence. *Eur. J. Pharmacol.* **43**, 89-90.
- Gudelsky, G. A. and J. C. Porter (1979). Morphine-

- and opioid peptide-induced inhibition of the release of dopamine from tuberoinfundibular neurons. *Life Sci.* **25**, 1697–1702.
- Hughes, J., T. W. Smith, H. W. Kosterlitz, L. A. Fothergill, B. A. Morgan and H. R. Moriss (1975). Identification of two related pentapeptides from the brain with potent opiate agonist activity. *Nature* **258**, 577–579.
- Iwasaki, Y., Y. Kato, S. Ohgo, H. Abe, H. Imura, F. Hirata, S. Senoh, T. Tokuyama and O. Hayashi (1978). Effects of indoleamines and their newly identified metabolites on prolactin release in rats. *Endocrinology* **103**, 254–258.
- Kamberi, I. A., R. S. Mical and J. C. Porter (1971). Effects of melatonin and serotonin on the release of FSH and prolactin. *Endocrinology* **88**, 1288–1293.
- Kato, Y., Y. Iwasaki, H. Abe, S. Ohgo and H. Imura (1978a). Effects of endorphins on prolactin and growth hormone secretion in rats. *Proc. Soc. Exp. Biol. Med.* **158**, 431–436.
- Kato, Y., Y. Iwasaki, J. Iwasaki, H. Abe, N. Yanaihara and H. Imura (1978b). Prolactin release by vasoactive intestinal polypeptide in rats. *Endocrinology* **103**, 554–558.
- Kato, Y., N. Matsushita, H. Katakami and H. Imura (1980). Effect of a synthetic enkephalin analogue on prolactin secretion in man and rats. In: *Advances in Prolactin* (L. Hermite, M. and J. L. Judd eds.) Karger, Basel. pp. 54–60.
- Koenig, J., M. A. Mayfield, R. J. Coppings, S. M. McCann and L. Krulich (1980). Role of central nervous system neurotransmitters in mediating the effects of morphine on growth hormone- and prolactin-secretion in the rat. *Brain Res.* **197**, 453–468.
- Kuhr, M. J., C. B. Pert and S. H. Snyder (1973). Regional distribution of opiate receptor binding in monkey and human brain. *Nature* **245**, 447–450.
- Libertun, C. and S. M. McCann (1976). The possible role of histamine in the control of prolactin and gonadotropin release. *Neuroendocrinology* **20**, 110–120.
- Lien, E. L., R. L. Fenichel, V. Garsky, D. Sarantakis and N. H. Grant (1976). Enkephalin-stimulated prolactin release. *Life Sci.* **19**, 837–840.
- Macleod, R. M. and J. E. Lehmeyer (1974). Studies on the mechanism of the dopamine-mediated inhibition of prolactin secretion. *Endocrinology* **94**, 1077–1085.
- Matsushita, N., Y. Kato, H. Katakami, A. Shimatsu, N. Yanaihara and H. Imura (1981a). Stimulation of growth hormone release by vasoactive intestinal polypeptide from human pituitary adenoma *in vitro*. *J. Clin. Endocrinol. Metab.* **53**, 1297–1301.
- Matsushita, N., Y. Kato, H. Katakami, A. Shimatsu and H. Imura (1981b). Inhibition by naloxone of prolactin release induced by L-5-hydroxytryptophan in rats. *Proc. Soc. Exp. Biol. Med.* **168**, 282–285.
- Meites, J., J. F. Bruni, D. A. van Vugt and A. F. Smith (1979). Relation of endogenous opioid peptides and morphine to neuroendocrine function. *Life Sci.* **24**, 1325–1336.
- Mulder, G. H. and P. G. Smelik (1977). A superfusion system technique for the study of the sites of action of glucocorticoids in the rat hypothalamus-pituitary-adrenal system *in vitro* I: Pituitary cell superfusion. *Endocrinology* **100**, 1143–1152.
- Rivier, C., W. Vale, N. Ling, M. Brown and R. Guillemin (1977a). Stimulation *in vivo* of the secretion of prolactin and growth hormone by β -endorphin. *Endocrinology* **100**, 238–241.
- River, C. M. Brown and W. Vale (1977a). Effect of neurotensin, substance P and morphine sulfate on the secretion of prolactin and growth hormone in the rat. *Endocrinology* **100**, 751–754.
- Roemer, D., H. H. Buescher, R. C. Hill, J. Pless, W. Bauer, F. Cardinaux, A. Closse, D. Hauser and R. Huguenin (1977). A Synthetic enkephalin analogue with prolonged parenteral and oral analgesic activity. *Nature* **268**, 547–549.
- Ruberg, M., W. H. Rotsztein, S. Arancibia, J. Besson and A. Enjalbert (1978). Stimulation of prolactin release by vasoactive intestinal polypeptide (VIP). *Eur. J. Pharmacol.* **51**, 319–320.
- Shaar, C. J., R. C. A. Fredrickson, N. B. Dininger and L. Jackson (1977). Enkephalin analogues and naloxone modulate the release of growth hormone and prolactin—Evidence for regulation by an endogenous opioid peptide in brain. *Life Sci.* **21**, 853–860.
- Simantov, R. and S. H. Snyder (1977). Opiate receptor binding in the pituitary gland. *Brain Res.* **124**, 178–184.
- Spampinato, S., V. Locatelli, D. Cocchi, L. Vincentini, S. Bajustz, S. Ferri and E. E. Müller (1979). Involvement of brain serotonin in the prolactin-releasing effect of opioid peptides. *Endocrinology* **105**, 163–170.
- Taché, Y., G. Charpnet, M. Chrétien and R. Collu (1979). Role of serotonergic pathways in hormone changes induced by opioid peptides. In: *Central Nervous System Effects of Hypothalamic Hormones and Other Peptides* (Collu, R., A. Barbeau, J. R. Ducharme and J. G. Rochefort eds.) Raven Press, New York. pp. 301–303.
- Takahara, J., A. Arimura and A. V. Schally (1974). Suppression of prolactin release by a purified porcine PIF preparation and catecholamines infused into a rat hypophyseal portal vessel. *Endocrinology* **95**, 462–465.
- Tashjian, A. H. Jr. N. J. Barowsky and D. K. Jensen (1971). Thyrotropin releasing hormone: Direct evidence for stimulation of prolactin production by pituitary cells in culture. *Biochem. Biophys. Res. Commun.* **43**, 516–523.
- van Loon, G. R. and E. B. de Souza (1978). Effects of β -endorphin on brain serotonin metabolism. *Life Sci.* **23**, 971–978.
- van Loon, G. R., D. Ho and C. Kim (1980). β -endo-

- rphine-induced decrease in hypothalamic dopamine turnover. *Endocrinology* **106**, 76-80.
- van Vugt, D. A., J. F. Bruni, P. W. Sylvester, H. T. Chen, T. Ieiri and J. Meites (1979). Interaction between opiates and hypothalamic dopamine on prolactin release. *Life. Sci.* **24**, 2361-2368.
- von Graffenried, B., E. del Pozo, J. Roubicek, E. Krebs, W. Pöldinger, P. Brumeister and L. Kerp (1978). Effects of the synthetic enkephalin analogue FK 33-824 in man. *Nature* **272**, 729-730.