Possible Involvement of Endogenous Opioid Peptides in Prolactin Secretion Induced by α_2-Adrenergic Stimulation in Rats (42962)

HIROYUKI KOSHIYAMA, YUZURU KATO,* AKIRA SHIMATSU, YOSHIO MURAKAMI, NAOKI HATTORI, YASUHIRO ISHIKAWA, AND HIROO IMURA

Second Division, Department of Internal Medicine, Kyoto University Faculty of Medicine, Kyoto 606, Japan and First Division, Department of Medicine, Shimane Medical University, Izumo 693, Japan*

Abstract. **Noradrenergic mechanisms have a stimulatory role in regulating prolactin (PRL) secretion in the rat. We investigated the mechanism by which the α_2-adrenergic system stimulates PRL release in urethane-anesthetized male rats. Intracerebroventricular injection of norepinephrine (2 μg/rat) or epinephrine (100 ng and 1 μg/rat) caused an increase in plasma PRL levels. The PRL increase induced by epinephrine was much greater than that by norepinephrine. Intracerebroventricular injection of phentolamine (1 μg/rat), an α-antagonist, blunted the plasma PRL increase induced by epinephrine (100 ng intracerebroventricularly). Plasma PRL levels were increased by intravenous injection of α_2-agonists, clonidine (15 μg/100 g of body wt), and xylazine (200 μg/100 g of body wt). Plasma PRL increase induced by clonidine or xylazine was suppressed by intravenous injection of naloxone (125 μg/100 g of body wt), an opiate antagonist. These findings suggest that α_2-adrenergic mechanisms stimulate pituitary PRL secretion, at least partly, by activating endogenous opioid peptides in the rat.**

[P.S.E.B.M. 1989, Vol 192]

Several lines of evidence suggest that brain noradrenergic systems play a stimulatory role in regulating prolactin (PRL) secretion. Intraventricular injection of norepinephrine (NE) (1) or microinjection of NE and epinephrine into the mediobasal hypothalamus (2) stimulates PRL release in conscious male rat. L-*threo*-3,4-dihydroxyphenylserine, a synthetic compound which is directly converted to NE, stimulates PRL release in conscious and urethane-anesthetized male rats (3). Clonidine, an α_2-agonist, also stimulates PRL secretion in conscious ovariectomized rats (4–6) or conscious and urethane-anesthetized male rats (7–9). On the other hand, phenoxybenzamine and phentolamine, α_2-antagonists, suppress the proestrous PRL surge in ovariectomized, estrogen-treated rats (10). Blockade of NE systhesis by diethyldithiocarbamate also prevents estrogen-induced PRL release in ovariectomized rats (11). Furthermore, selective destruction of ascending noradrenergic pathways by 6-hydroxydopamine blunts both stress-induced PRL release in male rats (12) and proestrous PRL surges in female rats (13). However the mechanisms by which noradrenergic systems stimulate PRL secretion remain to be elucidated. In this study, we report that endogenous opioid peptides may be involved in PRL release induced by α_2-adrenergic stimulation.

Received December 5, 1988. [P.S.E.B.M. 1989, Vol 192]
Accepted May 15, 1989.

0037-9727/89/1922-0105$2.00/0

Materials and Methods

Wistar strain male rats weighing 250–300 g (Japan Animal Co., Osaka, Japan) were used throughout the experiments. They were maintained in a temperature-controlled room (23 ± 1°C) on a 12-hr dark and 12-hr light schedule (lights on 0600–1800). Laboratory chow (Oriental Yeast Co., Tokyo, Japan) and tap water were given *ad libitum*. After overnight fasting, animals were anesthetized with urethane (150 mg/100 g of body wt ip). Fifty minutes after anesthesia, test substances were injected into the lateral ventricle in a volume of 10 μl/rat or into an exposed external jugular vein in a volume of 100 μl/100 g of body wt. Blood samples of 0.6 ml were withdrawn from the other jugular vein 20 min before, immediately before, and 10, 20, and 40 min after the injection of test substances as previously described (14). Plasma was promptly separated and kept at −20°C until assayed. Plasma PRL levels were measured by specific radioimmunoassay using a kit supplied by the NIDDK as previously described (14). NIDDK-

rat PRL RP-3 was used as the standard. The intraassay and interassay coefficients of variations were 6.8 and 8.7%, respectively.

Clonidine HCl and naloxone HCl were kindly supplied from C. H. Boeringer Japan Co. (Osaka, Japan) and Endo Laboratories (New York, NY), respectively. Norepinephrine bitartrate, epinephrine bitartrate, phentolamine mesylate (REGITINE), and xylazine HCl were purchased from Wako Chemical Co. (Osaka, Japan), Tokyo Kasei Co. (Tokyo, Japan), Ciba-Geigy Japan Co. (Tokyo, Japan), and Bayer Japan Co. (Tokyo, Japan), respectively. Drugs were dissolved in physiologic saline immediately before use.

Statistical differences were evaluated by one-way analysis of variance with Duncan's new multiple range test and Student's *t* test as appropriate.

Results

As shown in Figure 1, intracerebroventricular (icv) injection of epinephrine (100 ng and 1 μg/rat) caused a dose-related increase in plasma PRL levels. Intracerebroventricular injection of NE (1 μg/rat) also caused an increase of plasma PRL levels. However, the plasma PRL increase induced by epinephrine was much greater than that by NE. Simultaneous icv injection of phentolamine (1 μg/rat) blunted the plasma PRL increase induced by epinephrine (100 ng/rat icv; mean ± SE peak PRL: phentolamine + epinephrine 4.5 ± 0.7 ng/ml vs vehicle + epinephrine 14.2 ± 4.3 ng/ml, $P < 0.05$).

Intravenous (iv) injection of clonidine (15 μg/100 g of body wt) and xylazine (200 μg/100 g of body wt) resulted in a rise in plasma PRL levels as shown in Figures 2 and 3. Intravenous injection of naloxone (125 μg/100 g of body wt) did not cause any change in plasma PRL levels. Pretreatment with naloxone suppressed the plasma PRL increase induced by clonidine as shown in Figure 2. Naloxone also partially blocked the PRL increase induced by xylazine (Fig. 3).

Discussion

We showed that icv injection of epinephrine and NE stimulated PRL secretion. We used the urethane-anesthetized male rats as an experimental model, since the basal PRL levels are stable under anesthesia and stimulatory effects of various substances such as L-*threo*-3,4-dihydroxyphenylserine, leumorphin, and galanin (3, 14, 15) in PRL secretion were observed in both

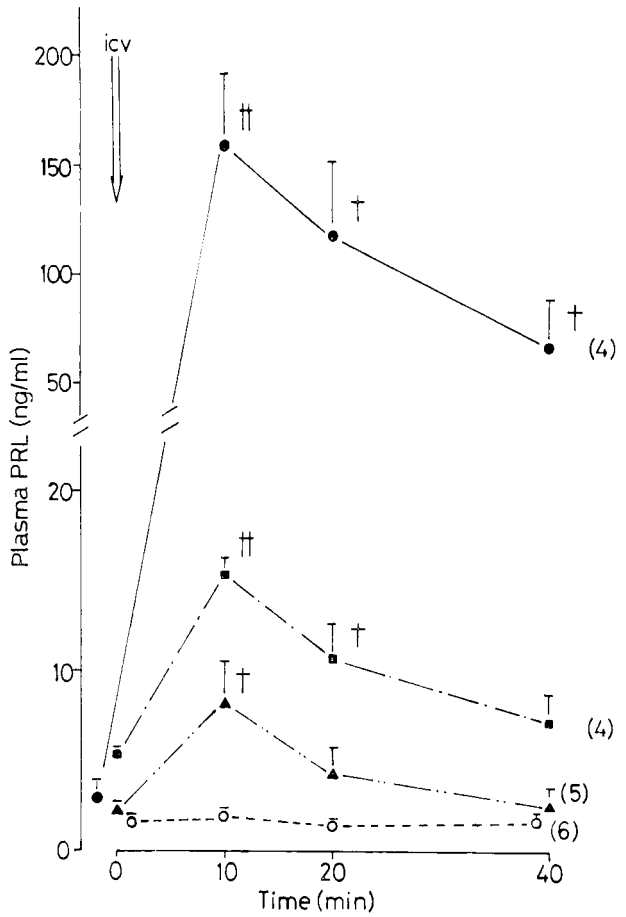

Figure 1. Effect of epinephrine (100 ng and 1 μg/rat icv) and norepinephrine (1 μg/rat icv) on plasma PRL levels in urethane-anesthetized male rats. Saline was injected icv in a control group. Mean ± SE values are shown. Numbers of animals are shown in parentheses. ●:epinephrine, 1 μg; ■:epinephrine 100 ng; ▲:NE, 1 μg; and ○:saline. †$P < 0.05$ and††$P < 0.01$ vs basal levels.

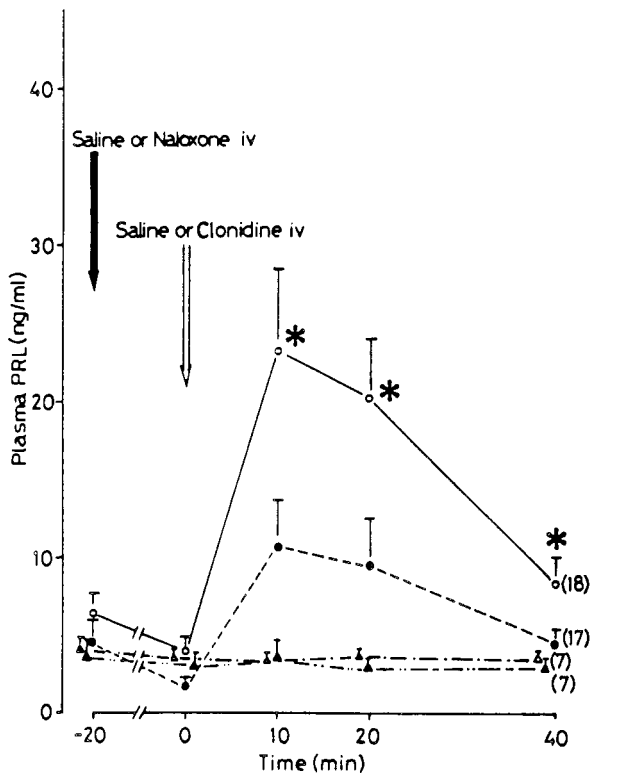

Figure 2. Effect of naloxone (125 μg/100 g of body wt iv 20 min before) and clonidine (15 μg/100 g of body wt iv) on plasma PRL levels in urethane-anesthetized rats. Mean ± SE values are shown. Numbers of animals are shown in parentheses. ○:saline + clonidine, ●:naloxone + clonidine, ▲:naloxone + saline, and △:saline + saline. *$P < 0.05$ vs naloxone + clonidine group and naloxone + saline group.

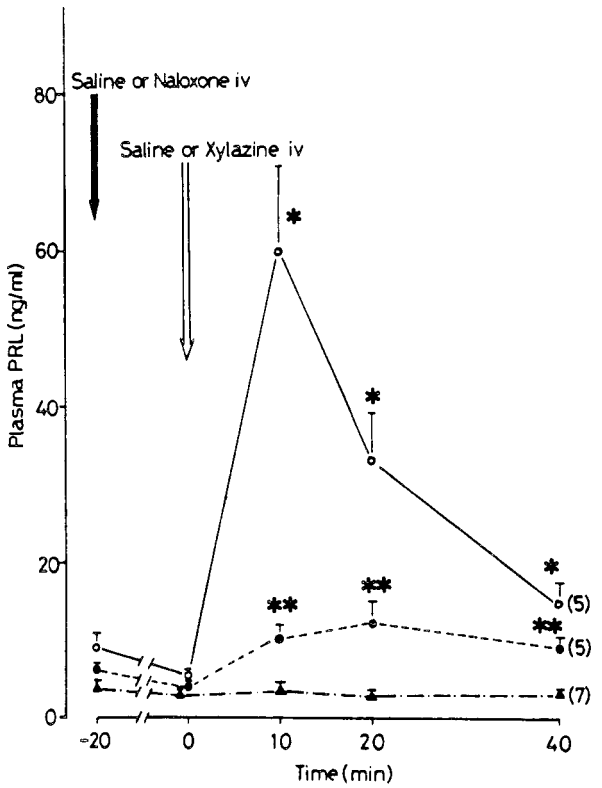

Figure 3. Effect of naloxone (125 μg/100 g of body wt iv 20 min before) and xylazine (200 μg/100 g of body wt iv) on plasma PRL levels in urethane-anesthetized rats. Mean ± SE values are shown. Numbers of animals are shown in parentheses. ○:saline + xylazine, ●:naloxone + xylazine, and ▲:naloxone + saline. *$P < 0.01$ vs naloxone + xylazine group and naloxone + saline group. **$P < 0.05$ vs naloxone + saline group.

anesthetized and conscious rats. NE and clonidine stimulate PRL release in both urethane-anesthetized and conscious rats (1, 2, 4–9). Therefore, urethane anesthesia itself may not alter PRL response to α-adrenergic stimulants. The peak plasma PRL after the injection of epinephrine was much greater than that of NE, and phentolamine blunted the plasma PRL increase induced by epinephrine. Epinephrine is known to be more potent as α_2-agonist than NE (16). These findings suggest that α-adrenergic mechanisms are involved in epinephrine- and NE-induced PRL release, although we cannot exclude the influences of local vasoconstriction and cardiovascular changes induced by icv injection of epinephrine and NE. It is less likely that the systemic effect of α_2-agonists may alter PRL secretion, since clonidine and xylazine stimulated PRL release in a dose-related manner (7 and our unpublished data). Lien *et al.* (17) observed the inhibition of PRL secretion at a small dose of clonidine (5 μg/100 g of body wt). The discrepancy may be explained by the dose of clonidine used. Clonidine may mainly activate postsynaptic α_2-receptors at the dose used in this study (15 μg/100 g of body wt), since it can stimulate PRL release in epinephrine- and NE-depleted rats treated with dopamine-β-hydroxylase inhibitors (6, 9).

We demonstrated that PRL increase induced by clonidine and xylazine was blunted by iv injection of naloxone, an opiate antagonist. The α_2-adrenergic system is known to interact with and to stimulate the endogenous opioid system (18–21). Recently, we have reported that clonidine decreased the dopamine levels in the mediobasal hypothalamus and that naloxone blocked mediobasal hypothalamus dopamine decrease induced by clonidine (22). Endogenous opioid peptides are known to inhibit the activity of tuberoinfundibular dopamine, which is a major PRL-inhibiting factor (23, 24). It was also reported that both an α-antagonist (10) and naloxone (25) blocked the proestrous PRL surge. Taken together, the present study indicates that α_2-adrenergic mechanisms may stimulate PRL secretion, at least partly, through the activation of endogenous opioid peptides. Further studies are required to elucidate the physiologic role of α_2-adrenergic receptors in regulating PRL secretion.

This work was supported in part by grants from the Ministry of Education, Science and Culture and the Ministry of Health and Welfare, Japan.

1. Vijayan E, McCann SM. Re-evaluation of the role of catecholamines in control of gonadotropin and prolactin release. Neuroendocrinology **25**:150–165, 1978.
2. Day TA, Jervois PM, Menadue MF, Willoughby JO. Catecholamine mechanisms in medio-basal hypothalamus influence prolactin but not growth hromone secretion. Brain Res **253**:213–219, 1982.
3. Koshiyama H, Kato Y, Ishikawa Y, Murakami Y, Inoue T, Imura H. Stimulation of prolactin secretion by L-3,4-dihydroxyphenylserine (L-DOPS) via central norepinephrine in the rat. Life Sci **41**:983–988, 1987.
4. Lawson DM, Gala RR. The influence of adrenergic, dopaminergic, cholinergic and serotonigergic drugs on plasma prolactin levels in ovariectomized, estrogen-treated rats. Endocrinology **96**:313–318, 1975.
5. Stevens RW, Lawson DM. The influence of estrogen on plasma prolactin levels induced by thyrotrophin releasing hormone (TRH), clonidine and serotonin in ovariectomized rats. Life Sci **20**:261–266, 1977.
6. Negro-Vilar A, Ojeda SR, Advis JP, McCann SM. Evidence for noradrenergic involvement in episodic prolactin and growth hormone release in ovariectomized rats. Endocrinology **105**:86–91, 1979.
7. Ruch W, Marbach P, Jaton AL, Bucher B, Doepfner W. Adrenergic and dopaminergic control of growth hormone secretion in urethane anesthetized adult male rats. Horm Res **9**:208–224, 1978.
8. Durand D, Martin JB, Brazeau P. Evidence for a role of α-adrenergic mechanisms in regulation of episodic growth hormone secretion in the rat. Endocrinology **100**:722–728, 1977.
9. Terry JC, Martin JB. Evidence for α-adrenergic regulation of episodic growth hormone and prolactin secretion in the undisturbed male rat. Endocrinology **108**:1869–1873, 1981.
10. Subramanian MG, Gala RR. The influence of cholinergic, adrenergic and serotonergic drugs on the afternoon surge of plasma prolactin in ovariectomized, estrogen-treated rats. Endocrinology **98**:842–848, 1976.
11. Carr LA, Conway PM, Voogt JL. Role of norepinephrine in the

release of prolactin induced by suckling and estrogen. Brain Res **133**:305–314, 1977.
12. Fenske M, Wuttke W. Effects of intraventricular 6-hydroxydopamine injection on serum prolactin and LH levels: Absence of stress-induced pituitary release. Brain Res **104**:63–70, 1976.
13. Langelier P, McCann SM. The effects of interruption of the ventral noradrenergic pathway on the proestrous discharge of prolactin in the rat. Proc Soc Exp Biol Med **154**:553–557, 1977.
14. Koshiyama H, Kato Y, Inoue T, Murakami Y, Ishikawa Y, Yanaihara N, Imura H. Central galanin stimulates pituitary prolactin secretion in rats: possible involvement of hypothalamic vasoactive intestinal polypeptide. Neurosci Lett **75**:49–54, 1987.
15. Tojo K, Kato Y, Ohta H, Matsushita N, Shimatsu A, Kabayama Y, Inoue T, Yanaihara N, Imura H. Stimulation by leumorphin of prolactin secretion from the pituitary in rats. Endocrinology **117**:1169–1174, 1985.
16. Berthelesen S, Pettinger WA. A functional basis for classification of α-adrenergic receptors. Life Sci **21**:595–606, 1977.
17. Lien EL, Morrison A, Kassarich J, Sullivan D. Alpha-2-adrenergic control of prolactin release. Neuroendocrinology **44**:184–189, 1986.
18. Agajanian GK. Tolerance of locus coeruleus neurones to morphine and suppression of withdrawal response by clonidine. Nature **276**:186–188, 1978.
19. Farsang C, Kunos G. Naloxone reverses the antihypertensive effect of clonidine. Br J Pharmacol **67**:161–164, 1979.
20. Kunos G, Farsang C. β-Endorphin: Possible involvement in the antihypertensive effect of central α-receptor activation. Science **211**:82–84, 1981.
21. Pettibone J, Mueller G. Clonidine releases immunoreactive β-endorphin from rat pars distalis. Brain Res **221**:409–414, 1981.
22. Koshiyama H, Kato Y, Shimatsu A, Imura H. Effects of clonidine and naloxone on the dopamine levels in the rat mediobasal hypothalamus measured by in vivo voltammetry. Neurosci Lett **99**:328–332, 1989.
23. Gudelsky GA, Porter JC. Morphine- and opioid peptide-induced inhibition of the release of dopamine from tuberoinfundibular neurons. Life Sci **25**:1697–1702, 1979.
24. Van Loon GR, Ho D, Kim C. β-Endorphin-induced decrease in hypothalamic dopamine turnover. Endocrinology **106**:76–80, 1980.
25. Ieiri T, Chen HT, Campbell GA, Meites J. Effects of naloxone and morphine on the proestrous surge of prolactin and gonadotropins in the rat. Endocrinology **106**:1568–1570, 1980.