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INHIBITION OF PROLACTIN SECRETION BY GASTRIN RELEASING PEPTIDE (GRP) IN THE RAT[1]

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Abstract. Synthetic gastrin releasing peptide (GRP) injected intraventricularly (1 μg/rat), but not intravenously, suppressed rat prolactin (PRL) release induced by a Met-enkephalin analog, FK33-824 (10 μg/100 g body wt., iv). GRP also blunted PRL release induced by a dopamine antagonist, domperidone (1 μg/100 g body wt., iv). In contrast, GRP did not suppress elevated plasma PRL levels sustained by a large dose of domperidone (10 μg/100 g body wt., iv). GRP (10^{-5} M) had no effect on PRL release from superfused pituitary cells *in vitro*. These results suggest that GRP inhibits PRL secretion in the rat by acting through the brain to stimulate the dopaminergic mechanism.

Gastrin-releasing peptide (GRP) is a 27-amino acid peptide recently isolated from porcine gut (1). GRP has a C-terminal decapeptide fragment almost identical to amphibian bombesin, and bombesin-like peptide and receptors are highly concentrated in rat hypothalamus (2,3). Yanaihara *et al.* (4) showed characteristic distribution of immunoreactive GRP in mammalian brain and intestinal tissues, suggesting that GRP is one of the brain-gut peptides. A variety of neuropeptides such as substance P, neurotensin, VIP and opioids stimulate pituitary prolactin (PRL) secretion in the rat. However, the effect of GRP on hypothalamo-pituitary function remains to be elucidated.

In the present study, we report the inhibitory action of GRP on PRL secretion in the rat.

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

After overnight fasting, the animals were anesthetized with urethane (150 mg/100 g body wt., ip). Test substances were injected into a lateral ventricle in a volume of 10 μl/rat or a jugular vein in a volume of 0.1 ml/100 g body wt. as described previously (5). In control animals, saline solution was injected intraventricularly or intravenously. Blood samples of 0.6 ml were withdrawn from the jugular vein immediately before and 10, 20 and 40 min after the injection. Plasma samples were promptly separated and stored at -20°C until assayed.

In the *in vitro* experiments, PRL release from dispersed pituitary cells were studied by the superfusion method described previously (6). In brief, dispersed anterior pituitary cells (5×10^{-6}) were placed on a Sepadex G-25 column and superfused with Krebs-Ringer bicarbonate buffer containing 10 mM glucose

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(PH 7.4) at a constant flow rate of 0.3 ml/min using a peristaltic pump. Throughout the experiments the superfusion medium was gassed with 95% O_2 and 5% CO_2, and immersed in a water bath at 37°C. The effluent was collected in 5 min fractions and stored at -20°C until assayed.

GRP was synthesized by one of us (N.Y.) and the homogeneity of the synthetic products was confirmed by HPLC. FK33-824 and domperidone were obtained from Endo Labs., New York, and Kyowa Hakko Kogyo Co., Tokyo, respectively. Synthetic TRH was supplied by Tanabe Seiyaku Co., Osaka. The drug was dissolved in physiological saline for *in vivo* experiments and in freshly gassed Krebs-Ringer bicarbonate buffer for *in vitro* experiments.

PRL concentrations in plasma and in the effluent were measured by specific radioimmunoassay (5,6) using a kit supplied by the National Institute of Arthritis, Metabolism and Digestive Diseases. NIAMDD-rat prolactin RP-1 was used as the standard. Analysis of variance in combination with Duncan's new multiple range test was used for the statistical evaluation.

Results. As shown in Fig. 1, intravenous injection of FK33-824 (10 µg/100 g body wt.), a potent Met-enkephalin analog, resulted in a significant increase of plasma PRL levels in the rat ($p<0.01$ *vs* basal levels and saline control). When GRP (1 µg/rat) was injected intraventricularly immediately before the injection of FK33-824, the PRL response to FK33-824 was suppressed ($p<0.01$). Plasma PRL levels were not influenced by the intraventricular injection of GRP or saline alone. Intravenous injection of GRP did not affect the plasma PRL response to FK33-824.

As shown in Fig. 2, plasma PRL levels were raised by intravenous injection of domperidone (1 µg/100 g body wt.), a peripheral dopamine antagonist ($p<0.01$ *vs* basal levels and saline control). When GRP (1 µg/rat) was injected intraventricularly immediately before the injection of antagonist, plasma PRL response was significantly suppressed ($p<0.01$ *vs* control). In contrast, the elevated plasma PRL levels induced by a large dose of domperidone (10 µg/100 g body wt., iv) were not suppressed by GRP (1 µg/rat, icv), which was injected intraventricularly 15 min after the injection of of domperidone (Fig. 3).

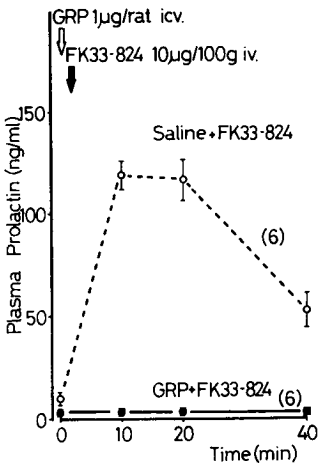

Fig. 1. Effect of GRP on prolactin release induced by FK33-824 in the rat. GRP was injected intraventricularly in a dose of 1 µg per rat. FK33-824 (10 µg/100 g body wt.) was injected into the jugular vein immediately after the injection of GRP or saline solution (10 µl/rat icv). All values are the mean±SE of six rats as indicated in parenthesis.

In the *in vitro* studies, after a preperfusion period of 120 min, the basal

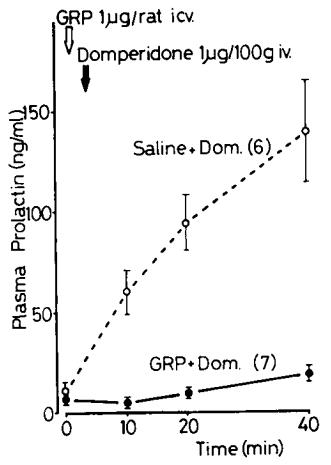

Fig. 2. Effect of GRP on prolactin release induced by domperidone in the rat. GRP (1 µg/rat) or saline (10 µl/rat) was injected i.c.v. immediately before the i.v. injection of domperidone (1 µg/100 g body wt.). Mean (±SE) values of six rats are shown.

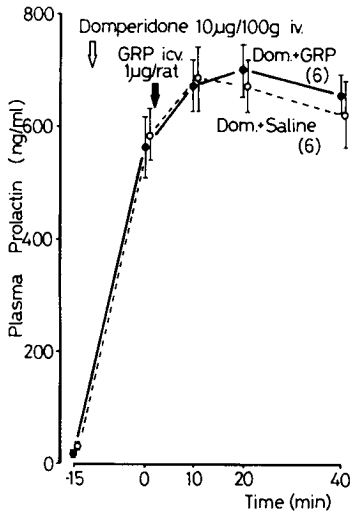

Fig. 3. Effect of GRP on prolactin release induced by a large dose of domperidone in the rat. GRP (1 µg/rat) or saline solution (10 µl/rat) was injected i.c.v. 15 min after the i.v. injection of domperidone (10 µg/100 g body wt.). All values are the mean±SE of six rats.

secretion of PRL from the dispersed pituitary cells was 82±7 ng/ml (mean±SE) and it rose rapidly to 310±12 ng/ml when TRH (10^{-8}M) was infused in 6 min pulses. GRP infusion (10^{-5}M) did not affect the basal PRL secretion or the TRH-induced PRL release in vitro, whereas dopamine infusion (10^{-7}M) markedly suppressed both the basal secretion and the TRH-induced release of PRL in this system.

Discussion. This study is the first to demonstrate that intraventricular administration of GRP inhibited PRL release induced by FK33-824 in the rat. FK33-824 stimulates PRL secretion in both anesthetized and conscious rats by acting through opiate receptors in the central nervous system; brain amines, at least dopamine, mediate the action of the opioid peptide on PRL release (7). The evidence presented suggest that GRP blocks the action of FK33-824 by acting on the opiate receptor. Another possibility is that, like bombesin, which has been shown not to act at the level of the opiate receptor in the brain (3), GRP may also be acting at a specific GRP receptor on the dopamine neuron to stimulate dopamine release.

The evidence, however, does not favor the former suggestion since GRP also suppressed PRL release induced by a small dose of domperidone. In addition, pretreatment with the high dose of domperidone, which caused the maximum increase in plasma PRL levels, completely blocked the inhibitory action of GRP on PRL secretion in the rat. Domperidone is a dopamine receptor blocking agent which does not cross the blood brain barrier (8). It is possible, therefore, that a partial blockade of dopamine receptors by a small dose of domperidone could be antagonized by the action of GRP and when dopamine receptors are blocked by a large dose of domperidone, GRP has no inhibitory action on PRL secretion. This suggests that GRP inhibits PRL secretion by possibly acting in the central nervous system to stimulate the release of dopamine. The fact that GRP does not alter basal PRL levels does not conflict with the latter suggestion, since dopamine concentrations in hypophysial portal blood are high enough to inhibit basal PRL secretion and exogenous dopamine does not further decrease plasma PRL levels in urethane-anesthetized rats (9).

GRP shares a common C-terminal decapeptide with bombesin. It is known that synthetic GRP, like bombesin, induces hypothermia, hyperglycemia and adrenal epinephrine secretion in the rat (10). Along with the same line with our findings, Tache et al. (11) demonstrate that the central administration of bombesin has a potent inhibitory action on PRL release induced by acute stress in the conscious rat. Güller et al. very recently reported that synthetic GRP stimulated LH and suppressed TSH secretion in the rat. However, physiological significance of GRP in the hypothalamo-pituitary axis remains to be further investigated.

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