STIMULATION OF PROLACTIN RELEASE IN THE RAT BY INTRAVENTRICULAR INJECTION OF β-ENDORPHIN AND METHIONINE-ENKEPHALIN

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SUMMARY: A single dose of 0.5 μg β-endorphin injected intraventricularly in unanesthetized male rats bearing chronic intraventricular and intrajugular cannulae led to a 7-fold stimulation of plasma prolactin (PRL) levels 10 to 20 min after injection of the peptide, whereas a dose of 500 μg methionine-enkephalin (met-enkephalin) led to only a 4-fold stimulation of plasma PRL levels. Combined with our previous data, the present findings show that β-endorphin and met-enkephalin have a more potent and more rapid stimulatory effect on PRL than GH release after intracerebral injection. These data suggest a possible role of endogenous opiate-like peptides in the control of both PRL and GH secretion.

Following reports of the presence of endogenous opiate activity in brain (1-3), the pentapeptide Tyr-Gly-Gly-Phe-Met (met-enkephalin) has been isolated from porcine (4) and calf (5) brain. The sequence of this peptide is the same as the N-terminal of the COOH-fragment (β-LPH_{61-91}, also called β-endorphin) of β-lipotropin first isolated from sheep pituitaries (6). Both met-enkephalin and β-endorphin have potent morphine-like effects (4, 7, 8) and bind to the opiate receptor (9-11).

We have recently found that β-endorphin and met-enkephalin injected into lateral cerebral ventricles stimulate growth hormone secretion in the rat (12). Since morphine can stimulate the release of both GH (13, 14) and prolactin (15, 16; Cusan and Labrie, unpublished observations), we studied the effect of β-endorphin and met-enkephalin on PRL release. The present

Abbreviations: PRL, prolactin; GH, growth hormone; met-enkephalin, methionine-enkephalin.

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data show for the first time that the two endogenous opioid peptides are more potent stimuli of PRL than GH release.

Materials and Methods

Adult male Sprague-Dawley rats (obtained from Canadian Breeding Farms, St. Constant, Quebec) weighing 225-275 g upon arrival were kept in a sound-attenuated and temperature-controlled room (24 ± 2°C) and exposed to 14 hr light/10 hr dark cycles (lights on at 5:00 and off at 19:00 hr). Purina rat chow and water were available ad libitum.

In order to minimize stress-induced inhibition of GH release, animals were handled twice a day for 7 to 10 days before insertion of a catheter (Venocath No. 18 Abbott) into the right superior vena cava under Surital (50 mg/kg, ip) anesthesia. The animals were then implanted stereotaxically with a metallic cannula (guage 25) in the left lateral ventricle according to the coordinates (A=5.4, D=2, D=3) described by De Groot (17). The cannula was fixed to the skull with a polymerizing acrylate (Yates Flash Acrylic). This chronic technique permits a minimum of manipulations of the animal at the time of the experiment performed 2 days after implantation of the cannulae.

Animals were then injected intravenously through the intrajugular catheter with 0.2 ml of sheep somatostatin antiserum, kindly supplied by Dr. A. Arimura, 5 min before injection of the indicated doses of β-endorphin or met-enkephalin or the vehicle alone (40 µl of 0.9% NaCl) over a 3-min period (18). β-Endorphin and met-enkephalin were synthesized by the solid-phase method as described (10, 19). Samples of 0.7 ml blood were then withdrawn into heparinized syringes at the indicated time intervals. A volume of 0.7 ml of saline containing heparin was injected after each blood sampling to minimize extracellular volume changes. Plasma was separated by centrifugation at 1200 x g for 40 min at 2-4°C and kept at 20°C until assayed.

Plasma GH and PRL were measured in duplicate by double-antibody radioimmunoassay (20, 21) using rat hormones (GH-1-2, PRL-I-1, GH-RP-1 and PRL-RP-1) and rabbit antisera (anti-GH-S-2 and anti-PRL-S-2) kindly provided by Dr. A. F. Parlow for the National Institute of Arthritis and Metabolic Diseases, Rat Pituitary Hormone Program. Purified goat anti-rabbit γ-globulins were a product of Endocrinolab Ltd., Quebec.

Radioimmunoassay data were analysed with a Hewlett-Packard desk-top calculator using a program written in this laboratory and based on model II of Rodbard and Lewald (22). Data are expressed as mean ± S.E.M. Statistical significance was evaluated according to the multiple-range test of Duncan-Kramer (23).

Results

As illustrated in Fig. 1, the intraventricular injection of 0.5 to 25 µg of β-endorphin (β-LPH_{61-91}) led to a rapid and significant stimulation of PRL

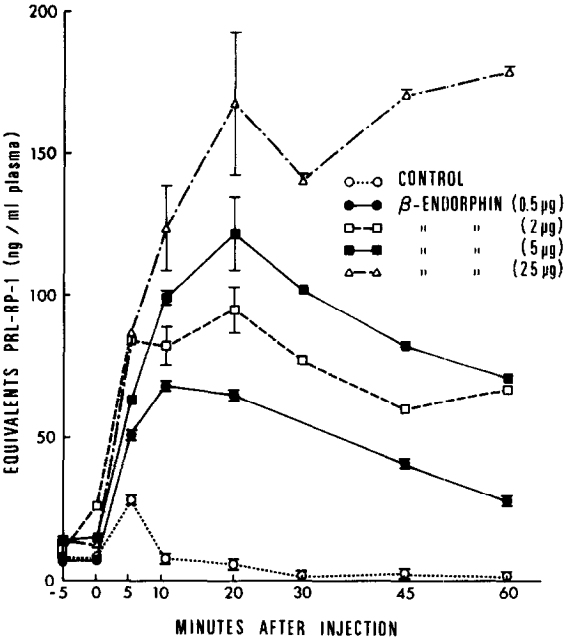

Fig. 1 Effect of increasing doses of β-endorphin on plasma prolactin (PRL) levels in the rat. Adult male rats bearing intraventricular and intrajugular cannulae were injected intravenously with 0.2 ml of sheep somatostatin antiserum 5 min before the intraventricular injection of the indicated amounts of synthetic β-endorphin. PRL measurements were performed at the indicated times intervals after administration of β-endorphin. Data are presented as mean ± S.E.M. of triplicate measurements of pooled plasma samples from 8 to 9 animals per group.

release in unanesthetized freely-moving rats. With the 0.5 μg dose, a marked rise was already evident 5 min after injection of the peptide and a maximal stimulation (approximately 7-fold) was measured after 10 min with a slow decrease of plasma hormone levels at later time intervals. The higher doses of β-endorphin (2.5 and 25 μg) led to a progressive increase of PRL release, a 30- to 60-fold increase being measured between 20 and 60 min after injection of 25 μg of the peptide.

Fig. 2A shows that methionine-enkephalin, the NH_2-terminal penta-peptide of β-endorphin, was much less potent than β-endorphin to stimulate

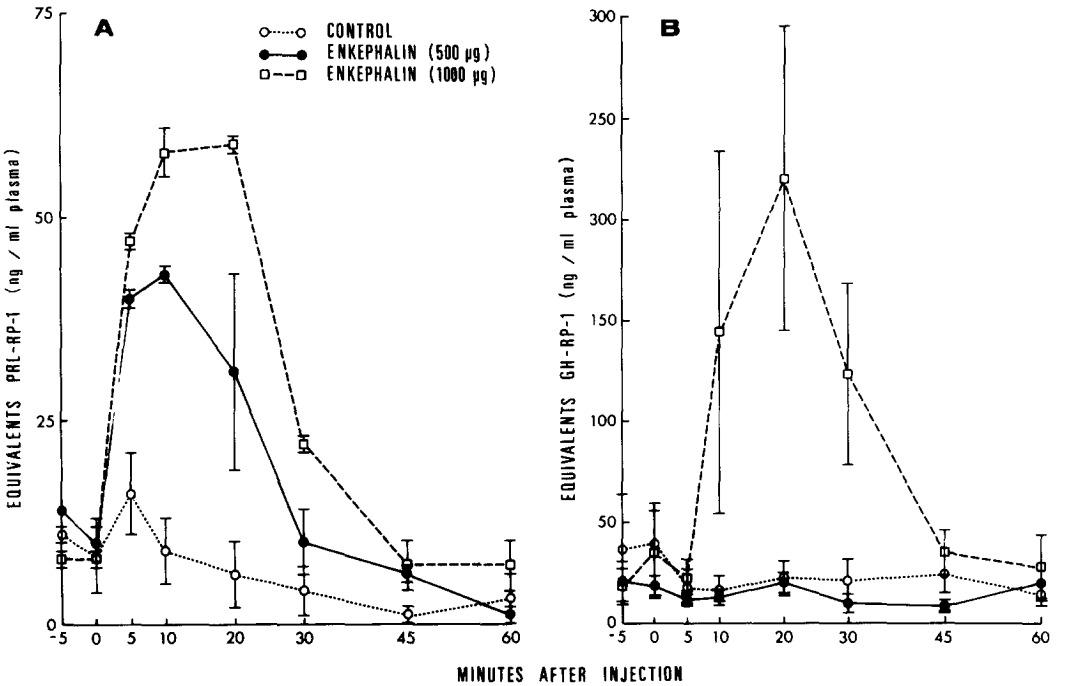

Fig. 2 Effect of 0.5 or 1.0 mg of methionine-enkephalin on plasma PRL (A) and GH (B) levels in the rat. The experiment was performed as described in Fig. 1. Data are expressed as mean ± S.E.M. of triplicate measurements of individual plasma samples obtained.

PRL release. In fact, at the 500 to 1000 µg doses, methionine-enkephalin led to approximately 4- to 6-fold increases of plasma PRL levels, respectively. Maximal stimulation was measured 10 to 20 min after injection of the pentapeptide with a rapid return to basal levels between 30 and 45 min. It can be seen in Fig. 2B that stimulation of GH release was observed only at the 1000 µg dose, thus indicating a greater sensitivity of the PRL than GH responses to the opioid peptide.

Discussion

Using the sensitive in vivo model which minimizes the inhibitory effect of endogenous somatostatin on GH secretion (18), we have recently

found that β-endorphin and met-enkephalin injected intraventricularly can be potent stimuli of GH release in the rat (12). The present data demonstrate that β-endorphin and met-enkephalin injected intraventricularly can be more potent stimuli for PRL release than GH release.

The stimulatory effect of both β-endorphin and met-enkephalin[1] was first detected on PRL release both as a function of time after injections with various doses of the peptides. While the effect of both peptides on PRL release was found 5 min after injection, the effect on plasma GH levels could be measured only at 10 min. Moreover, the effect of β-endorphin appeared somewhat more long-lasting on PRL than GH release: while plasma GH levels decreased by about 80% between 30 and 60 min after injection of 25 μg of β-endorphin (12), no significant decrease of plasma PRL levels was measured up to 60 min (Fig. 1).

β-Endorphin and met-enkephalin do however display markedly different activities in the various bioassays used. In the guinea pig ileum assay, β-endorphin has been found to be equipotent (24) or 4 to 5 times more potent (25) than met-enkephalin. The in vivo analgesic activity of met-enkephalin injected centrally appears to be of short duration (8, 26) while that of β-endorphin is long-lasting (27, 28). Our previous findings (12) and the present data indicate that β-endorphin is 500 to 2000 times more potent than met-enkephalin to induce GH and PRL release.

Since the stimulatory effect of morphine (13, 14), β-endorphin (12) and met-enkephalin (12, Fig. 2) is observed in animals where circulating somatostatin should be neutralized by excess somatostatin antiserum (18, 29), it is likely that observed increased GH release is due to stimulated release of GH-releasing activity (GH-RH) from the hypothalamus (12).

1 After completion of the manuscript, two reports (34, 35) which described the effect of met-enkephalin on prolactin release in rats, came to our attention.

Increasing evidence suggests a physiological role of dopamine as inhibitor of PRL secretion (30, 31). It is thus possible that opiates and opioid peptides exert at least part of their marked stimulatory effect on PRL release through inhibition of dopamine release from nerve endings in the external layer of the median eminence. Indeed, it was found recently that β-endorphin inhibits striatal dopamine release in vitro (32) . Such a mechanism is further supported by the observation that stimulation of dopamine receptors by apomorphine or L-Dopa led to inhibition of morphine analgesia in mice while the dopamine antagonists haloperidol and pimozide had the opposite effect (33).

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