Vol. 107, No. 2, 1982 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 30, 1982 Pages 735-741

STIMULATION OF PROLACTIN SECRETION IN THE RAT BY α-NEO-ENDORPHIN, β-NEO-ENDORPHIN AND DYNORPHIN

Norio Matsushita, Yuzuru Kato, Akira Shimatsu, Hideki Katakami
Masahiko Fujino*, Hisayuki Matsuo** and Hiroo Imura

Second Medical Clinic, Department of Medicine, Kyoto University Faculty of Medicine, Sakyo-ku, Kyoto 606; *Chemical Research Division, Takeda Chemical Industries, Ltd., Yodogawa-ku. Osaka 532; and **Department of Biochemistry Miyazaki Medical College, Kiyotake, Miyazaki 889-16, Japan

Received June 18, 1982

SUMMARY: Intraventricular injections of α-neo-endorphin, β-neo-endorphin and dynorphins (dynorphin[1-13], dynorphin[1-17], dynorphin[1-8]) resulted in an increase in plasma prolactin levels in urethane-anesthetized rats. Dynorphin [1-13] was the most potent to stimulate prolactin release among these opioid peptides. Plasma prolactin responses to these stimuli were blunted by naloxone an opiate antagonist. In in vitro studies, prolactin release from perfused pituitary cells was stimulated by α-neo-endorphin, and the effect was blunted by naloxone, whereas neither β-neo-endorphin nor dynorphin[1-13] affected prolactin release. These results suggest that newly identified "big" Leu-enkephalins in the brain stimulate prolactin secretion in the rat and that α-neo-endorphin has a possible direct action on the pituitary.

Since the isolation of two morphin-like pentapeptides, Leu-enkephalin and Met-enkephalin, a variety of opioid peptides have been demonstrated in the hypothalamus and the pituitary, suggesting their potential roles in regulating the secretion of pituitary hormones. Recent studies have shown that Leu-enkephalin, Met-enkephalin and related peptides such as β-endorphin and α-endorphin stimulate prolactin (PRL) and growth hormone (GH) secretion in the rat (1,2,3,4).

However, the action of newly identified "big" Leu-enkephalin such as α-neo-endorphin (5), β-neo-endorphin (6) and dynorphin (7) on the hypothalamo-pituitary function remains to be fully elucidated. α-Neo-endorphin and β-neo-endorphin were discovered in the porcine hypothalamus, and dynorphin was first

0006-291X/82/140735-07$01.00/0

isolated from the porcine pituitary. They are also highly concentrated in the rat hypothalamus as well as in the pituitary. The N-terminal tridecapeptide of pituitary dynorphin (dynorphin[1-13]) was found to be as potent in bioassays as naturally occuring dynorphin (dynorphin[1-17]) (7). Dynorphin-related peptide, PH-8P (dynorphin[1-8]), was also isolated from the hypothalamus (8). The complete structure[1] of these opioid peptides have been reported (6,9,10).

The stimulation of PRL secretion by dynorphin[1-13] was previously reported in a preliminary form (11). In this paper we report the effects of α-neo-endorphin, β-neo-endorphin and dynorphins (dynorphin[1-13], dynorphin-[1-17], dynorphin[1-8]) on PRL secretion from the rat anterior pituitary *in vivo* and *in vitro*.

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 (lights on 0600 - 1800). Laboratory chow (Oriental Yeast Co., Tokyo) and tap water were given *ad libitum*.

In vivo experiments: After overnight fasting, animals were anesthetized with urethane (150 mg/100 g body wt., ip). Test substances were injected into the lateral ventricle in a volume of 10 µl/rat or into the exposed jugular vein in a volume of 0.1 ml/100 g body wt., and 0.6 ml blood samples were withdrawn from the jugular vein immediately before and 10, 20 and 40 min after the injection, as described previously (12). The plasma was promptly separated and stored at -20°C until assayed.

In vitro experiments: The animals were decapitated and the anterior pituitary glands were removed promptly. They were minced 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). The cells were suspended in Ham's F10 medium containing 15% horse serum and 2.5% FCS. 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. Then the cells were transferred to the superfusion chamber.

The superfusion system employed in the present study was described previously (13). In brief, dispersed pituitary cells ($5x10^6$) were placed on a Sephadex G-25 column packed on a 2.5 ml disposable syringe (Terumo), which was perfused with Krebs-Ringer bicarbonate buffer containing 10 mM glucose and 0.1% bovine serum albumin (Sigma) (pH 7.4), and a constant flow rate of 330 µl/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

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[1] α-neo-endorphin Tyr-Gly-Gly-Phe-Leu-Arg-Lys-Tyr-Pro-Lys
β-neo-endorphin Tyr-Gly-Gly-Phe-Leu-Arg-Lys-Tyr-Pro
dynorphin[1-17] Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-Tyr-Asp-Asn-Gln
dynorphin[1-13] Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys
dynorphin[1-8] Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile
Leu-enkephalin Tyr-Gly-Gly-Phe-Leu

immersed in a water bath at 37°C. After a preperfusion period of 60 min, the effluent was collected as 5 min fractions and stored at -20°C until assayed. Test substances were dissolved in the fresh gassed medium and infused at an interval of time into the chamber without changing the flow pressure.

PRL radioimmunoassay: PRL concentrations in plasma and the effluent from the cell column were measured by specific radioimmunoassay (12) using the kit supplied by the National Institute of Arthritis, Metabolism and Digestive Diseases. NIAMDD rat RP-1 was used as the standard preparation

Drugs: Synthetic α-neo-endorphin, β-neo-endorphin, dynorphin[1-13] and Leu-enkephalin were purchased from Protein Research Foundation, Osaka. Dynorphin-[1-17] and dynorphin[1-8] were synthesized by one of us (M.F.) and the homogeneity of the synthetic products was confirmed. Synthetic TRH and LHRH were supplied by Tanabe Pharmaceutical Co., Osaka. Naloxone hydrochloride was obtained from Endo Labs., New York. The drugs were dissolved in physiological saline for *in vivo* experiments, and in Krebs-Ringer bicarbonate buffer for *in vitro* experiments.

Statistical analysis: Significance of changes in hormone concentrations were evaluated by one way analysis of variance in combination with Duncan's new multiple range test.

RESULTS

In vivo studies: Intraventricular injection of α-neo-endorphin and β-neo-endorphin, in doses of 0.6 and 6 nmol per rat, caused a dose-related increase in plasma PRL levels in the rat (Fig. 1). Plasma PRL levels were also raised by the intraventricular administration of dynorphin[1-13], dynorphin[1-8], dynorphin[1-17] and Leu-enkephalin (Figs. 2 and 3). Physiological saline injection did not change plasma PRL levels in control animals. Plasma PRL

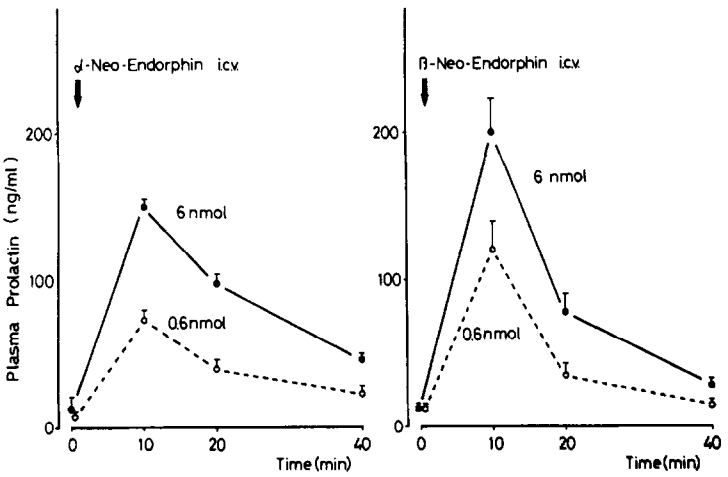

Fig. 1. Effects of α-neo-endorphin and β-neo-endorphin on plasma prolactin levels in the rat. α-neo-endorphin and β-neo-endorphin were injected intraventricularly in a dose of 0.6 and 6 nmol per rat. All values are the mean ± SE of five to seven animals.

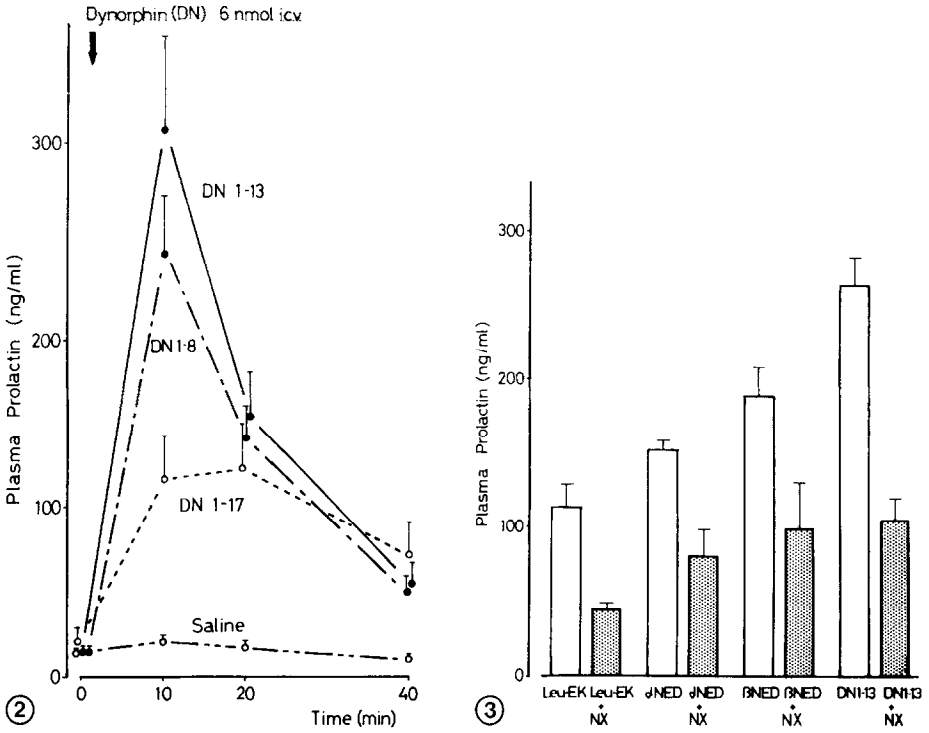

Fig. 2. Effects of dynorphins (DN) on plasma prolactin levels in the rat. Dynorphin 1-13 (DN1-13), dynorphin 1-8 (DN1-8) and dynorphin 1-17 (DN1-17) were injected intraventricularly in a dose of 6 nmol per rat. Saline solution (10 µl/rat) was injected intraventricularly in a control group. All values are the mean ± SE of six to seven animals.

Fig. 3. Effects of naloxone (NX) on plasma prolactin increase induced by Leu-enkephalin (Leu-EK), α-neo-endorphin (αNED), β-neo-endorphin (βNED) and dynorphin 1-13 (DN1-13) in the rat. Naloxone was injected intravenously in a dose of 125 µg/100 g body wt. 3 min before the injection of the peptides, which was administered intraventricularly in a dose of 6 nmol per rat. All values are the mean ± SE of peak values obtained in six to seven animals.

responses to these stimuli were significantly suppressed by naloxone, an opiate antagonist, which was injected 3 min before the administration of these peptides (Fig. 3). Dynorphin[1-13] was the most potent in stimulating PRL release among these opioid peptides when compared on a mola basis.

In vitro studies: The effects of the peptides on rat PRL release from dispersed pituitary cells were also studied in vitro with a superfusion method. After the 60 min perfusion period, the secretion of PRL from the pituitary cell column was stable during the 6 h perfusion period. The infusion of TRH (10^{-11}-10^{-7}M) as 6 min pulses caused a rapid increase in PRL levels in the effluent, and the response was quite reproducible (Fig. 4). LHRH (10^{-7}-10^{-5}M) had no effect on PRL release, suggesting the specificity of the PRL responses in

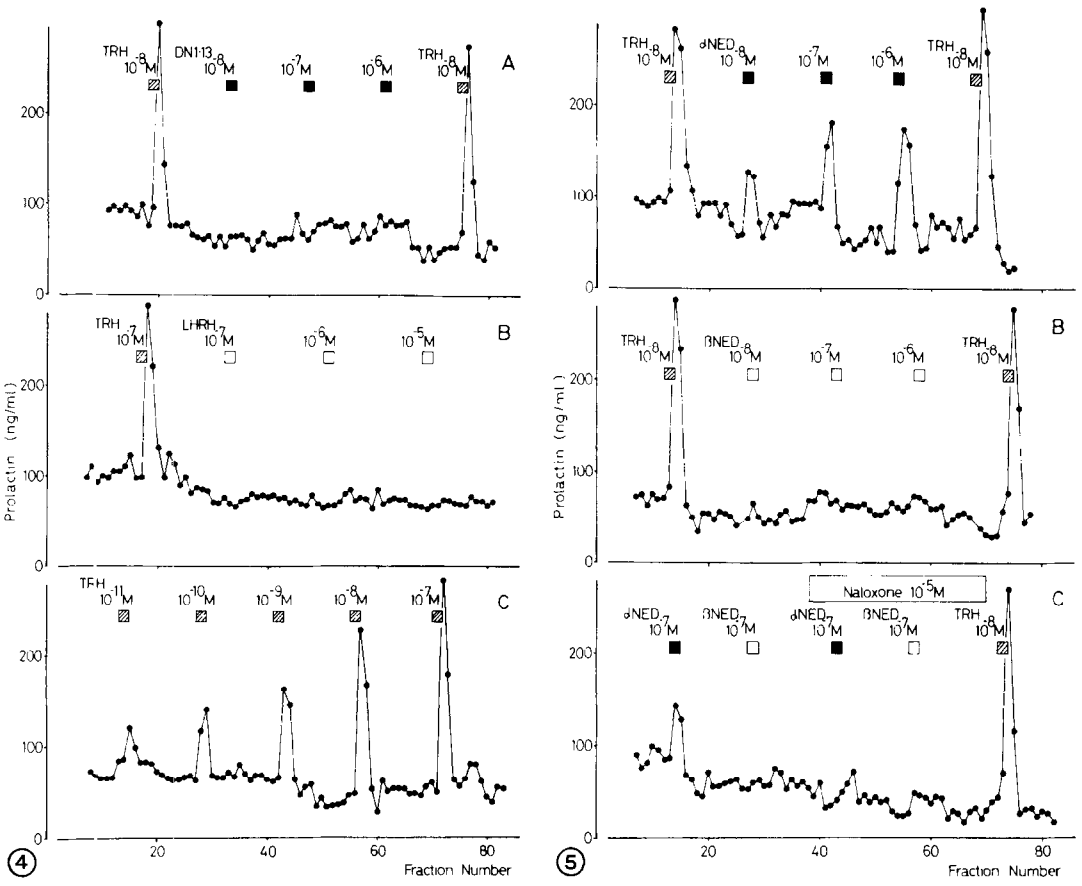

Fig. 4. Effects of dynorphin[1-13] (DN1-13) (10^{-8}-10^{-6}M), LHRH (10^{-7}-10^{-5}M) and TRH (10^{-11}-10^{-7}M) on prolactin release from the perfused rat anterior pituitary cells. All drugs were infused for 6 min at the concentrations indicated.

Fig. 5. Effects of α-neo-endorphin (αNED) (10^{-8}-10^{-6}M), β-neo-endorphin (βNED) (10^{-8}-10^{-6}M) and TRH (10^{-8}M) on prolactin release from the perfused rat anterior pituitary cells. All drugs were infused for 6 min at the concentrations indicated. In the bottom panel, αNED (10^{-7}M) and βNED (10^{-7}M) were added before and during the infusion of naloxone (10^{-5}M) for 30 min.

this system. The addition of α-neo-endorphin (10^{-8}-10^{-6}M) to the perfusion medium stimulated PRL secretion in a dose-related manner, although the potency of α-neo-endorphin in stimulating PRL release was much smaller than that of TRH on a molar basis (Fig. 5). PRL release induced by α-neo-endorphin was inhibited by naloxone (10^{-5}M), which was concomitantly perfused for 30 min. PRL release from the pituitary cells was not influenced by β-neo-endorphin or dynorphin[1-13] (Figs. 4 and 5). Leu-enkephalin had no effect on PRL release from the pituitary cell column (data not shown).

DISCUSSION

We first demonstrated that α-neo-endorphin and β-neo-endorphin stimulated PRL secretion in the rat *in vivo*. α-Neo-endorphin, but not β-neo-endorphin, unexpectedly stimulated PRL release from the perfused anterior pituitary cells. This finding is not explained by an artificial response, since the response was dose-related and reproducible, and other peptides than TRH did not affect PRL release in our system *in vitro*.

It is generally accepted that opioid peptides such as enkephalins and β-endorphin have no direct effect on the pituitary but act via the hypothalamus by increasing serotonin and decreasing dopamine metabolism, which could stimulate PRL release from the pituitary (4). However, opioid receptors are known to exist not only in the hypothalamus but also in the pituitary (14). In our experiments, the direct action of α-neo-endorphin was considerably suppressed by naloxone, an opiate receptor antagonist. It is possible, therefore, that the direct action of α-neo-endorphin may involve the specific opiate receptor-mediated process in the pituitary.

We have confirmed the stimulating effect of dynorphin[1-13] on rat PRL secretion *in vivo* (11,15). We further demonstrated that dynorphin[1-17] is less potent than dynorphin[1-8] and dynorphin[1-13] in raising plasma PRL levels in the rat at the dose examined, whereas dynorphin[1-17] was found to be as potent as dynorphin[1-13] in bioassays using the guinea pig ileum preparation and mouse vas deferens (7).

A particular physiological role for each of these "big" enkephalins might be speculated by the receptor selectivity. Wüster *et al* (16) revealed that dynorphin[1-13] and α-neo-endorphin[1-8] may preferentially interact with κ-type opiate receptors in the brain, and that the C-terminal sequence of opioid peptides is a critical determinant of receptor preference. Although the receptor preference of α-neo-endorphin has not been elucidated, the C-terminal lysine might have an important role for the action of α-neo-endorphin.

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