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Further Evidence that Central Neurotensin Inhibits Pituitary Prolactin Secretion by Stimulating Dopamine Release from the Hypothalamus[1] (42286)

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Abstract. Intracerebroventricular (icv) injection of neurotensin (NT) (2 μg/rat) suppressed prolactin (PRL) release induced by L-5-hydroxytryptophan (1 mg/100 g body wt, iv), prostaglandin E_2 (1 μg/rat, icv), and FK33-824 (10 μg/100 g body wt, iv), a Met^5-enkephalin analog, in urethane-anesthetized or conscious rats. In contrast, NT did not suppress elevated plasma PRL levels sustained by a large dose of domperidone (10 μg/100 g body wt, iv), a peripheral dopamine antagonist. In *in vitro* experiments, NT (10^{-5} *M*) stimulated dopamine release from perifused rat hypothalamic fragments. These results suggest that central NT inhibits PRL secretion by stimulating dopamine release from the hypothalamus into hypophysical portal blood in the rat.

Neurotensin (NT), a tridecapeptide originally isolated from bovine hypothalamus (1, 2), is widely distributed throughout the central nervous system (CNS), especially in the median eminence and the preoptic area in the rat (3–6). It has been reported that NT exhibits different effects on pituitary prolactin (PRL) secretion depending on the method of administration. Intravenous (iv) injection of NT raised plasma PRL levels in rats (7–10). In contrast, intracerebroventricular (icv) injection of NT suppressed basal plasma PRL levels and PRL release induced by stress and serotoninergic agents in the rat (8–10).

Koenig *et al.* (10) found that interruption of dopamine transmission by either α-methyl-*p*-tyrosine (αMT) or spiperidol led to a blockage of the inhibitory effect of NT on PRL secretion and suggested that the central dopaminergic system mediates the PRL-inhibiting effect of NT. In the present study, we first studied the central effect of NT on rat PRL secretion induced by such stimuli as L-5-hydroxytryptophan (L-5-HTP) (12–14), prostaglandin E (15, 16), FK33-824 [D-Ala^2, $MePhe^4$, $Met(O)^5$-ol] (17, 18) and domperidone (19). We further investigated the effect of NT on hypothalamic dopamine release in rats *in vitro*.

Materials and Methods. *Animals.* Wistar strain male rats (Japan Animal Co., Osaka, Japan) weighing 250–300 g were maintained in a temperature- (23 ± 1°C) and humidity- (50–60%) controlled room on a 12-h dark: 12-h light schedule (lights on 0600–1800 h). Laboratory chow (Oriental Yeast Co., Tokyo, Japan) and tap water were given *ad libitum*.

In vivo experiments: Anesthetized rats. After an overnight fast, the animals were anesthetized with urethane (150 mg/100 g body wt, ip). Test substances or physiological saline solution as a control were injected into the lateral ventricle in a volume of 10 μl/rat or into the exposed jugular vein in a volume of 100 μl/100 g body wt as previously described (20, 21). Blood samples (0.6 ml) were withdrawn from the exposed jugular vein 15 min before, immediately before, and 10, 20, and 40 min after the injection as previously described (21). The plasma was promptly separated and stored at −20°C until assayed.

Conscious rats. In each animal, a chronic indwelling catheter (Silastic) was inserted into the right atrium via the external jugular vein and a polyethylene catheter (PE-10) was stereotaxically implanted into the right lateral ventricle as previously described (21). Test substances were injected icv or iv through the indwelling catheters. Blood samples of 0.4 ml

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were withdrawn from the intravenous catheters 20 min before, immediately before, and 10, 20, and 40 min after the injection of test substances. The plasma was promptly separated and stored at −20°C until assayed.

In vitro experiments. Animals were sacrificed by decapitation and hypothalamic fragments, defined by the anterior margin of the optic chiasm, the anterior margin of the mammillary bodies, the lateral hypothalamic sulci, and a depth of 1.5 mm from the attachment of the stalk, were dissected. The fragments were bisected midsagitally and 20 hypothalamic halves (10 hypothalami equivalent) were placed on a small Sephadex G-25 column, which was perifused at a flow rate of 330 μl/min by the method described previously (22). The perifusion medium consisted of Krebs–Ringer bicarbonate buffer containing 10 m*M* glucose, 0.1% bovine serum albumin (BSA), and 100 μ*M* bacitracin (Sigma, St. Louis, Mo.), saturated with a 95% O_2–5% CO_2 mixture. Fractions eluting from the perifusion column were collected every 5 min. The hypothalamic fragments were stimulated with 10 min pulses of drugs and KRBG as a control without any change of flow pressure. High potassium (K^+, 20 m*M*) was also added as 5 min pulses at the end of the experiment to examine the viability of the tissue. For measurement of catecholamines, 400 μl of each fraction was promptly mixed in ice-cold tubes containing 100 μl of a solution of 5% EDTA-Na_2 and $Na_2S_2O_5$.

Drugs. NT and L-5-HTP were purchased from Protein Research Foundation (Mino, Japan) and Nakarai Chemical Company (Kyoto, Japan), respectively. Prostaglandin E_2 (PGE_2) and FK33-824 were obtained from Ono Pharmaceutical Company (Osaka, Japan) and Sandoz (Basel), respectively. Domperidone was supplied by Kyowa Hakko Company (Tokyo, Japan). PGE_2 was first dissolved in 95% ethanol and diluted with physiological saline. Other drugs were dissolved in physiological saline for *in vivo* experiments and in the perifusion medium freshly gassed with 95% O_2–5% CO_2 for *in vitro* studies.

Radioimmunoassay of PRL. Plasma PRL concentrations were measured by specific radioimmunoassay (21) using the kit supplied by the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases (NIADDK) (Bethesda, Md.). NIADDK rat prolactin RP-1 was used as the standard. The minimum detectable quantity of plasma rat PRL was 1.0 ng/ml, and intraassay and interassay variations were 5 and 7.5%, respectively.

Catecholamine assay. Catecholamines of each fraction were extracted with alumina (23) and measured by high-performance liquid chromatography with electrochemical detection (HPLC-ECD) as described previously (24). In brief, 30 mg alumina, 4 ml of 0.1 *M* Tris–HCl buffer (pH 8.6), 2 ng 3,4-dihydroxybenzilamine (DHBA) as an internal standard, and a mixture of 400 μl sample and 100 μl 5% EDTA-Na_2-$Na_2S_2O_5$ were mixed in a siliconized glass tube. The tube was vigorously shaken for 20 min and the alumina was washed three times with distilled water. Subsequently, the alumina was applied with 3 ml distilled water on 0.2 μm pore sized microfilter MF-1 (BAS, West Lafayette, Ind.) and centrifuged at 3000 rpm for 5 min for dehydration. Catecholamines were eluted from the alumina by 200 μl 0.2 *N* HCl.

Of each eluate 100 μl was evaluated using the stainless-steel HPLC system (L-4000S, Yanagimoto Co., Kyoto) consisting of a single piston pump with pulse damper, loop injection valve and a C_{18}-reversed-phase column (Yanapak ODS-T) which was connected to a thin-layer voltammetric detector (Yanako VMD 501, Yanagimoto Co., Kyoto) with two glassy carbon working electrodes operated at 0.525 and 0.700 V vs an Ag/AgCl reference electrode. Analysis was performed with 0.1 *M* citrate buffer (pH 4.5) containing 0.08% 1-citrate sulfonic acid (Sigma) and 10% methanol at a flow rate of 1.0 ml/min at a column temperature of 25–28°C. External standards were run at regular intervals to check the sensitivity of the detector. The dopamine content (DA) of each sample was determined by calculating each peak height and relative ratio of DHBA in the chromatogram. By this method, the mean ± SE recoveries for DA and DHBA were 83.4 ± 1.0 and 95.2 ± 3.7%, respectively. The minimum detectable quantity of DA in a sample was 87 pg/ml. The interassay coefficient of variation of DA was 3.3%.

Statistical analysis. Statistical differences were evaluated by one-way analysis of variance and Student's *t* test. The Wilcoxon test was also used to evaluate the maximum DA release

in vitro expressed as a percentage of the mean of five basal values just before adding the drug.

Results. As shown in Fig. 1 (left panel), iv injection of L-5-HTP (1 mg/100 g body wt), a precursor of serotonin, resulted in an increase of plasma PRL levels in urethane-anesthetized rats. When NT (2 μg/rat) was injected icv 2 min before the injection of L-5-HTP, the plasma PRL response to L-5-HTP was blunted (means ± SE peak values: NT + L-5-HTP 42.0 ± 3.2 ng/ml vs saline + L-5-HTP 384.8 ± 91.5 ng/ml, $P < 0.05$). Intracerebroventricular injection of NT (2 μg/rat) eliminated plasma PRL increase induced by icv injection of PGE_2 (1 μg/rat) as shown in Fig. 1 (right panel) (NT + PGE_2 28.8 ± 5.7 ng/ml vs 119.5 ± 29.3 ng/ml, $P < 0.05$).

Iv injection of FK33-824 (10 μg/100 g body wt), a potent Met^5-enkephalin analog, caused an increase of plasma PRL levels in both urethane-anesthetized and conscious rats as shown in Fig. 2. The PRL response to FK33-824 was suppressed by NT (2 μg/rat, icv) which was injected 2 min before the injection of FK33-824 both in urethane-anesthetized rats (NT + FK33-824 105.2 ± 25.6 ng/ml vs saline + FK33-824 608.4 ± 77.1 ng/ml, $P < 0.01$) and in conscious rats (NT + FK33-824 9.6 ± 2.0 ng/ml vs saline + FK33-824 47.7 ± 3.9 ng/ml, $P < 0.01$).

Plasma PRL levels were raised by iv injection of a large dose of domperidone (10 μg/100 g body wt), a peripheral DA antagonist, and the elevated plasma PRL levels induced by domperidone were not suppressed by NT (2 μg/rat) which was injected icv 15 min after the injection of domperidone (Fig. 3) (domperidone + NT 357.0 ± 19.9 ng/ml vs domperidone + saline 359.6 ± 24.7 ng/ml, $P < 0.05$).

In *in vitro* studies, spontaneous release of DA was rather stable after a preperifusion period of 60 min with means ± SE basal values of 27.9 ± 3.1 pg/hypothalamus/5 min. The infusion of NT (10^{-5} *M*) for 10 min resulted in an increase in DA release from perifused rat hypothalamic fragments, whereas KRBG as a control did not affect DA release, as shown in Fig. 4 (left panel). When the maximum DA release was expressed as a percentage of each mean basal value, DA release after NT infusion was significantly greater than that of KRBG (Fig. 4, right panel) (% DA release: NT 165.6 ± 18.5% vs KRBG 76.7 ± 3.5%, P

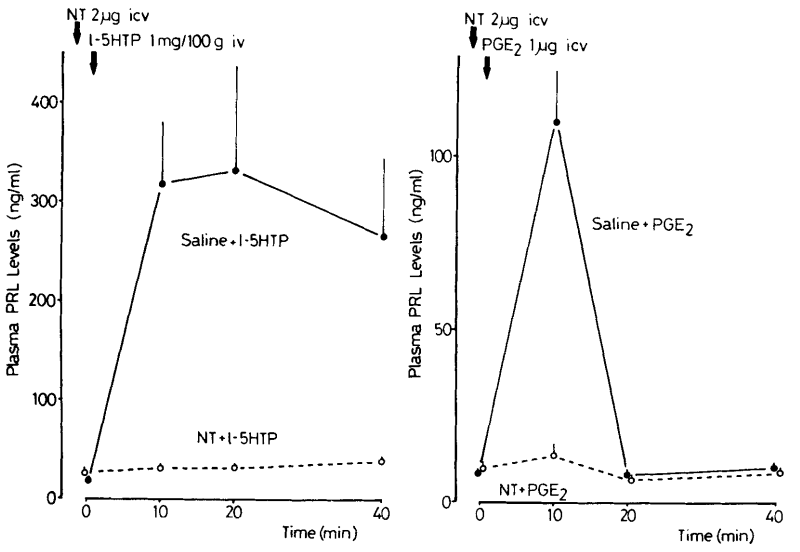

FIG. 1. Effect of neurotensin (NT) on prolactin (PRL) release induced by L-5-HTP (left panel) and PGE_2 (right panel) in urethane-anesthetized rats. NT (2 μg/rat, icv) or saline solution (10 μl/rat, icv) as a control was injected 2 min before the injection of L-5-HTP (1 mg/100 g body wt, iv) or PGE_2 (1 μg/rat, icv). All values are the means ± SE of four to six rats in each group. Statistical significance is shown by asterisks (* $P < 0.01$ and ** $P < 0.05$ vs saline control).

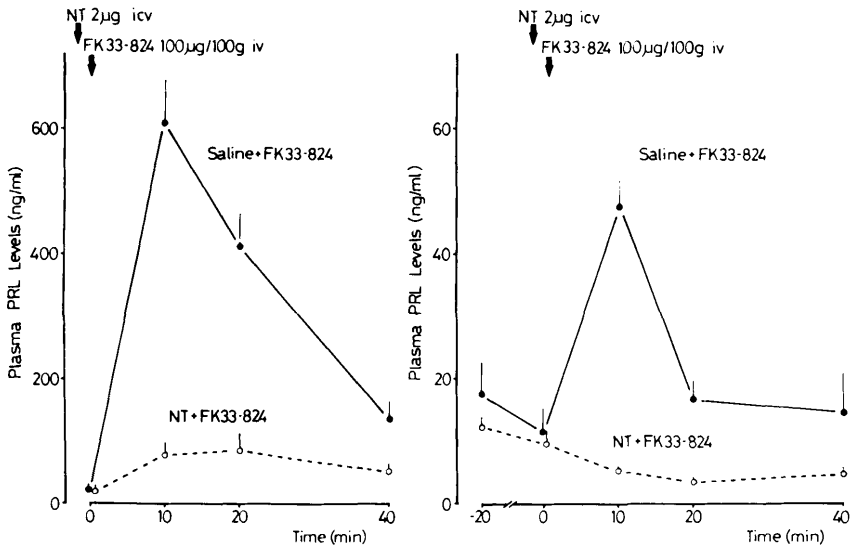

FIG. 2. Effect of NT on PRL release induced by FK33-824, a Met^5-enkephalin analog, in urethane-anesthetized rats (left panel) and in conscious rats (right panel). NT (2 μg/rat, icv) or saline was injected 2 min before the injection of FK33-824 (10 μg/100 g body wt, iv).

< 0.01). High potassium (20 mM) raised DA release to the same extent after the stimulation of the hypothalamic fragments by NT or KRBG.

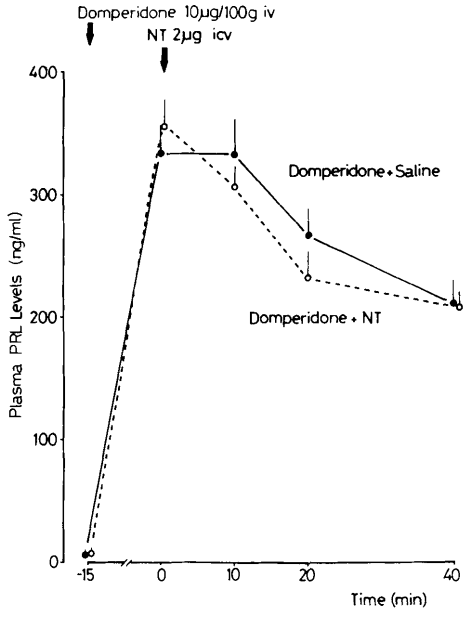

FIG. 3. Effect of NT on PRL release induced by domperidone in urethane-anesthetized rats. NT (2 μg/100 g body wt, icv) or saline was injected 15 min after the injection of domperidone (10 μg/100 g body wt, iv).

Discussion. We found in the present study that icv injection of NT suppressed PRL secretion induced by L-5-HTP, PGE_2, and FK33-824 in the rat. The results are in the same line with previous reports that icv injection of NT inhibited spontaneous PRL secretion (8–10) and PRL release induced by stress (11) and 5-HTP with fluoxetine (10) in the rat. The mechanisms by which these agents stimulate PRL secretion are different, however. Stimulation of hypothalamic vasoactive intestinal polypeptide (VIP) and/or peptide histidine isoleucine (PHI), putative PRL releasing factors, are involved in PRL secretion induced by 5-HTP (25–28), PGE (16), and stress (29), whereas inhibition of hypothalamic DA mechanisms is closely related to the stimulating action of FK33-824 on PRL secretion (18). Therefore, a final common pathway may be involved in the inhibitory effect of centrally administered NT on PRL release.

We also found that pretreatment with a large dose of domperidone, which caused the maximum increase in plasma PRL levels (19), blocked the inhibitory effect of NT on rat PRL secretion. Since domperidone is a peripheral DA receptor blocking agent which does not cross the blood–brain barrier (30), it is suggested that NT has no inhibitory effect on PRL secretion when DA receptors of pituitary lactotrophs are blocked by domperidone. The re-

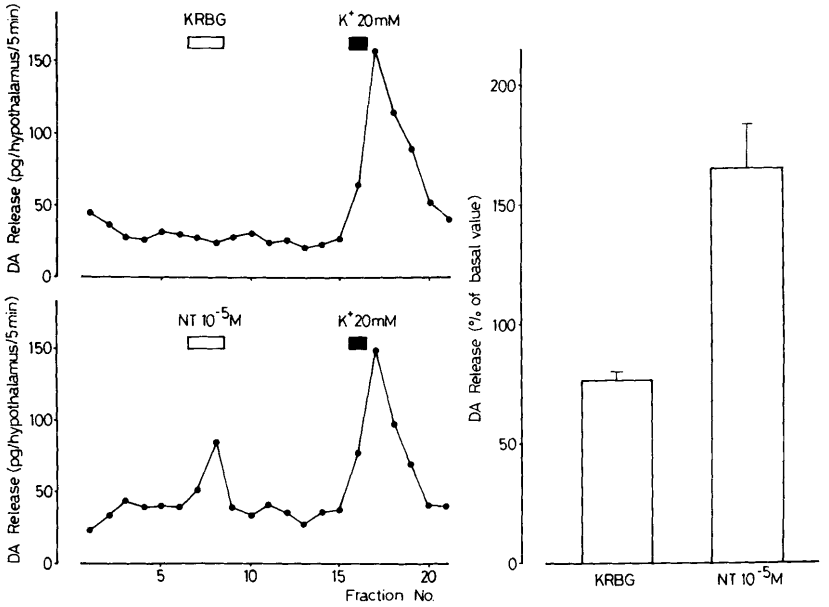

FIG. 4. Effect of NT on dopamine (DA) release from perifused rat hypothalamic fragments. NT (10^{-5} *M*) or KRBG as a control was infused for 10 min and K^+ (20 m*M*) was given as 5 min pulses. Left panel shows a representative experiment. The right panel shows the means ± SE of DA release which are expressed as percentage changes from each mean value of five fractions just before adding the stimulus.

sults are in good agreement with the findings of Koenig *et al.* (10). They reported that icv injection of NT decreased the resting PRL levels and PRL release induced by 5-HTP in combination with fluoxetine in intact rats but not in animals pretreated with αMT, an inhibitor of catecholamine biosynthesis, and spiperidol, an potent DA receptor blocker.

To investigate further the relationship between NT and central DA, we studied the effect of NT on DA release from the rat hypothalamus *in vitro*. Using the perifusion system, we first demonstrated that NT stimulated DA release from rat hypothalamic fragments *in vitro*. These findings strongly suggest that NT inhibits PRL secretion by acting through the CNS to stimulate the release of DA, one of physiological PRL inhibiting factors (31), although the role of a newly identified PRL inhibiting peptide (32) cannot be ruled out.

We are indebted to the NIADDK for supplying materials for rat PRL radioimmunoassay. We would like to thank Sandoz Ltd. (Basel, Switzerland), Ono Pharmaceutical Company (Osaka, Japan) and Kyowa Hakko Company (Tokyo, Japan) for their donations of FK33-824, PGE_2, and domperidone, respectively.

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