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# Central Inhibitory Action of TRH on Prolactin Secretion in the Rat[1] (42056)

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*Abstract.* Intravenous (iv) injection of FK33-824 ([D-Ala$^2$, MePhe$^4$, Met-(*O*)$^5$-ol]-enkephalin, 8 and 16 nmole/100 g body wt), a potent Met$^5$-enkephalin analog, and domperidone (1.2, 2.4, and 24 nmole/100 g body wt), a dopamine antagonist, resulted in a dose-related increase in plasma prolactin (PRL) levels in urethane-anesthetized male rats. PRL release induced by FK33-824 (16 nmole/100 g body wt, iv) was inhibited by intraventricular (icv) injection of TRH (0.6 nmole/rat). DN-1417 (γ-butyrolactone-γ-carbonyl-histidyl-prolinamide citrate, 0.6 nmole/rat, icv), a TRH analog, also blunted PRL release induced by FK33-824. PRL release induced by a smaller dose of domperidone (1.2 nmole/100 g body wt, iv) was blunted by TRH and DN-1417, whereas both peptides failed to suppress elevated PRL levels induced by larger doses of domperidone. These results suggest that TRH not only stimulates PRL secretion by acting directly at the pituitary, but has an inhibitory action on PRL release through activation of the central dopaminergic mechanism. 

Accumulating evidence suggests that hypothalamic hormones are widely distributed in the central nervous system (CNS) and exert different effects from their direct action on the pituitary (1). We have previously reported that growth hormone (GH) secretion was stimulated in the rat by central administration of somatostatin (SRIF) which is a hypothalamic inhibitor of GH release from the pituitary (2).

Thyrotropin-releasing hormone (TRH) stimulates the release of prolactin (PRL) as well as thyroid-stimulating hormone (TSH) from rat pituitary cells (3). TRH is also capable of antagonizing the behavioral effect of CNS acting drugs (4, 5) and of suppressing the morphine-induced release of PRL (6). Since the locomotor stimulant action of TRH in rats has been reported to be due to dopamine release in the mesolimbic brain (7), TRH may act in the CNS as a modulator of dopamine neurotransmission.

Dopamine is currently viewed as a physiological PRL-inhibiting factor (PIF) which is released from hypothalamic neurons into hypophyseal portal blood (8). Opioid peptides and dopamine antagonists stimulate PRL release by possibly inhibiting hypothalamic dopamine release (9–14) and by blocking the dopamine action at the pituitary (15, 16), respectively.

In the present study, we attempted to study the involvement of dopamine in the central action of TRH on PRL secretion in the rat.

**Materials and Methods.** *Animals.* Wistar strain male rats weighing 220–240 g (Japan Animal Co., Osaka) were maintained in a temperature controlled room (23 ± 1°C) on a 12 hr dark:12 hr 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 into a jugular vein in a volume of 100 μl/100 g body wt as previously described (17). Saline solution was injected in control animals.

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.

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*PRL radioimmunoassay.* PRL concentrations in plasma were measured by specific radioimmunoassay (18) using a kit supplied by National Hormone and Pituitary Program, Baltimore, Maryland. NIAMDD rat prolactin RP-1 was used as the standard.

*Drugs.* Synthetic TRH and a TRH analog, DN-1417 (γ-butyrolactone-γ-carbonyl-histidyl-prolinamide citrate) were supplied from Tanabe Seiyaku Company, Osaka and Takeda Chemical Industry Company, Osaka, respectively. A Met$^5$-enkephalin analog, FK33-824 ([D-Ala$^2$, MePhe$^4$, Met(*O*)$^5$-ol]-enkephalin) and domperidone were obtained from Sandoz, Basel, and Kyowa Hakko Kogyo Company, Tokyo, respectively.

*Statistical analysis.* Statistical differences were evaluated by one-way analysis of variance in combination with Duncan's new multiple range test. A $P$ value less than 0.05 was considered significant.

**Results.** As shown in Fig. 1, intravenous injection of FK33-824 (8 and 16 nmole/100 g body wt), a potent Met$^5$-enkephalin analog, resulted in a significant and dose-related increase of plasma PRL levels in the rat. When TRH (0.6 nmole/rat) or DN-1417 (0.6 nmole/rat) was injected intraventricularly immediately before the injection of FK33-824 (16 nmole/100 g body wt, iv), the PRL response to FK33-824 was significantly inhibited (Fig. 2). Basal plasma PRL levels were not influenced by a single intraventricular injection of TRH, DN-1417 or saline in these animals.

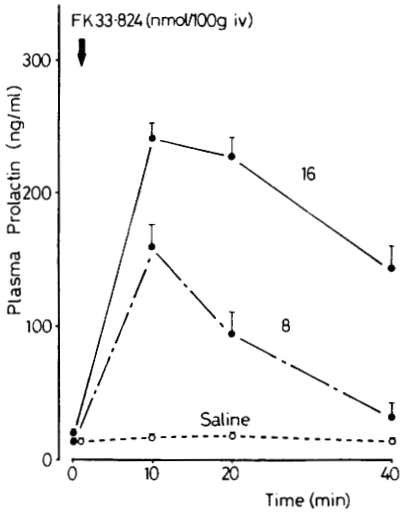


FIG. 1. Effect of intravenous injection of FK33-824 (8 and 16 nmole/100 g body wt) on plasma prolactin levels in urethane-anesthetized rats. Mean (±SE) values of eight to nine rats are shown. Saline (100 μl/100 g body wt, iv) was injected in control animals.

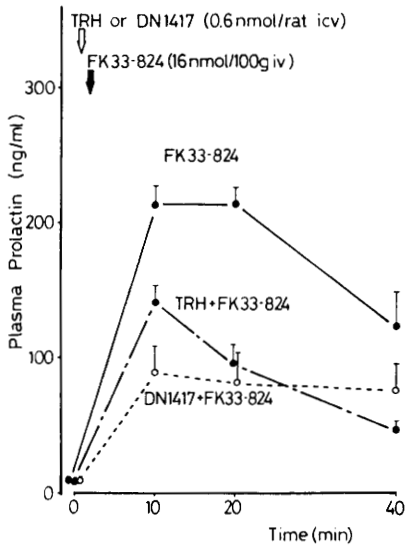


FIG. 2. Effects of intraventricular injection of TRH (0.6 nmole/rat) and DN-1417 (0.6 nmole/rat) on prolactin release induced by FK33-824 (16 nmole/100 g body wt, iv) in urethane-anesthetized rats. Mean (±SE) values of five to seven rats are shown. Saline (10 μl/rat, icv) was injected in control rats.

As shown in Fig. 3, plasma PRL levels were increased in a dose-related manner by intravenous injection of domperidone (1.2, 2.4, and 24 nmole/100 g body wt), a dopamine antagonist. When TRH (0.6 nmole/rat) or DN-1417 (0.6 nmole/rat) was injected intraventricularly immediately before the injection of a small dose of domperidone (1.2 nmole/100 g body wt, iv), plasma PRL response was significantly suppressed (Fig. 4). In contrast, the elevated plasma PRL levels induced by larger doses of domperidone were not suppressed by TRH or DN-1417 (data not shown).

**Discussion.** TRH is widely distributed in the brain (19). TRH stimulates the secretion of TSH and PRL from the pituitary (3). Extrapituitary actions of central TRH have been reported (4–6, 20). In the present study, we demonstrated that intraventricular administration of synthetic TRH inhibited PRL release induced by FK33-824, a Met$^5$-enke-

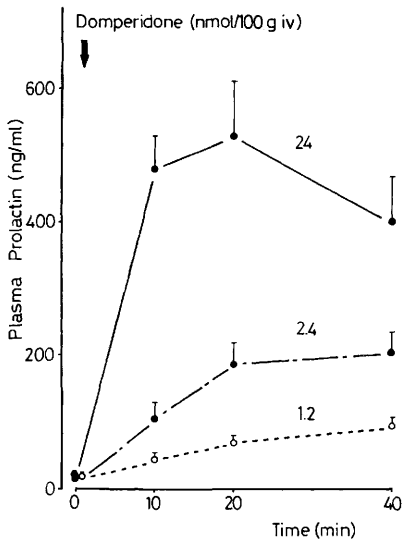


FIG. 3. Effect of intravenous injection of domperidone (1.2, 2.4, and 24 nmole/100 g body wt) on plasma prolactin levels in urethane-anesthetized rats. Mean (±SE) values of six rats are shown.

phalin analog, in the rat. These findings are in line with the previous report of Collu *et al.* (6) that morphine-induced PRL release is antagonized by intraperitoneal injection of TRH (10 mg/kg body wt) in the rat.

We further demonstrated that intraventricular injection of DN-1417, a TRH analog, blunted PRL release induced by FK33-824. Since DN-1417 has a potent central action but less direct stimulating effects on pituitary TSH and PRL release than TRH (20), these findings suggest a potential inhibitory role of central TRH in regulating PRL secretion in the rat.

Intraventricular administration of TRH and DN-1417 also suppressed PRL release induced by a small dose of domperidone. However, higher doses of domperidone abolished the inhibitory action of TRH and DN-1417 on PRL secretion in the rat. Domperidone is a peripheral dopamine antagonist which does not cross the blood–brain barrier but blocks dopamine receptors at the pituitary (15, 21). It is plausible, therefore, that a partial blockade of dopamine could be antagonized by the action of TRH and DN-1417, possibly by increasing the release of endogenous dopamine from the hypothalamus. When dopamine receptors are blocked by larger doses of domperidone, TRH and DN-1417 do not inhibit PRL secretion. We also found that DN-1417 ($10^{-7}$ *M*) stimulated dopamine release from perifused rat hypothalamic fragments *in vitro* (Y. Kabayama, and Y. Kato, unpublished observation). These findings suggest that central TRH has an inhibitory action on PRL secretion possibly by stimulating the release of hypothalamic dopamine into hypophyseal portal blood, indicating a potential physiological role of central TRH in ultrashort feedback control of the anterior pituitary gland, although TRH stimulates PRL secretion by acting at the pituitary.

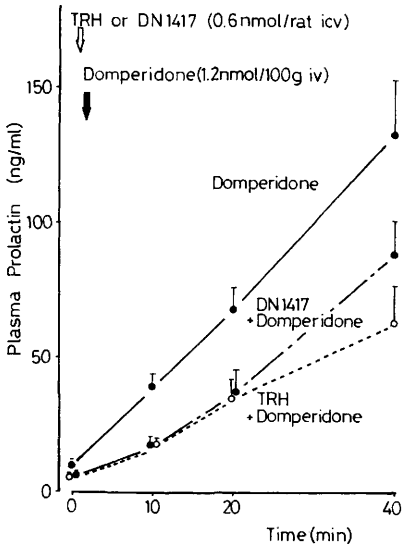


FIG. 4. Effects of intraventricular injection of TRH (0.6 nmole/rat) and DN-1417 (0.6 nmole/rat) on prolactin release induced by domperidone (1.2 nmole/100 g body wt, iv) in rats. Mean (±SE) values of 12–13 rats are shown. Saline (10 μl/rat, icv) was injected in a control group.

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