Pharmacological Study of TA-0910, a New Thyrotropin-Releasing Hormone (TRH) Analog (II): Involvement of the DA System in the Locomotor Stimulating Action of TA-0910

Michio Yamamura, Kiyoshi Kinoshita, Hiroyuki Nakagawa and Ryuichi Ishida

Department of Pharmacology, Safety Research Laboratory, Tanabe Seiyaku Co., Ltd., 18-89, 3-chome, Kashima, Yodogawa-ku, Osaka 532, Japan

Received June 12, 1990 Accepted October 11, 1990

ABSTRACT—The mechanism of the locomotor stimulating action of a new thyrotropin-releasing hormone (TRH) analog, TA-0910, was studied in rats. The locomotor stimulating action of TA-0910 (3 mg/kg) was inhibited by haloperidol or a-methyl-p-tyrosine (α-MT); slightly inhibited by phenoxybenzamine, prazosin, clonidine, or naloxone; not affected by propranolol, metergoline, or a low dose of scopolamine; and was enhanced by a high dose of scopolamine. The locomotor activity was increased by TA-0910 (0.3 mg/kg) in combination with methamphetamine, apomorphine, or L-DOPA under pretreatment with pargyline. A low dose of apomorphine inhibited the increase in locomotor activity induced by TA-0910 (3 mg/kg). The increase in locomotion was most notable and dose-dependent with the injection of 20 ng or more in the nucleus accumbens. The intravenous administration of TA-0910 produced dose-dependent and significant hyperlocomotion at 1 mg/kg or more. In the rats lesioned unilaterally in the nigrostriatal dopamine (DA) pathway by 6-hydroxydopamine, TA-0910 induced ipsilateral circling behavior at 3 mg/kg or more. This circling behavior was inhibited by haloperidol or α-MT. These results suggest that the locomotor stimulating action of TA-0910 is mediated primarily via the dopaminergic neuron, especially the nucleus accumbens of the mesolimbic DA system. Other possible mechanisms are also discussed.

Thyrotropin-releasing hormone (TRH) not only has hormonal actions in the hypothalamus-pituitary-thyroid axis but also has a variety of CNS actions independent of its hormonal action. This tripeptide has been shown to potentiate an excitatory action of L-3,4-dihydroxyphenylalanine (L-DOPA) under pretreatment with pargyline, a monoamineoxidase inhibitor, in both normal and hypophysectomized rats (1, 2). The hyperlocomotion and stereotyped behavior induced by dopamine (DA) agonists such as methamphetamine and apomorphine are considered to be derived from balanced stimulation of the nigrostriatal and centrolimbic dopaminergic systems (3, 4), and the circling behavior induced by these DA agonists in animals with unilateral nigrostriatal lesion is suggested to be derived from predominant stimulation of one of the two dopaminergic systems (5, 6).

Behavioral pharmacological studies have shown that TRH increases the locomotor activity of mice and rats (7-9) and induces ipsilateral circling behavior in rats with uni-
lateral nigrostriatal lesion (10, 11). Biochemical studies have demonstrated that TRH accelerates DA turnover and promotes DA release in rats (12–16).

A new TRH analog TA-0910 [1-methyl-(S)-4,5-dihydroorotyl-L-histidyl-L-prolineamide] is known to have about 30 and 100 times more potent CNS actions in rodents than TRH when administered parenterally (17) and orally (18), respectively, and about 50 times less potent TSH releasing activity in rats than TRH (17). In this study, to clarify the mechanism of the locomotor stimulating action of TA-0910, the effects of various drugs on TA-0910-induced hyperlocomotion and the effects of intracerebral injection of TA-0910 on locomotor activity were examined in rats. Furthermore, to elucidate the mode of action of TA-0910 on dopaminergic systems, we also studied the effect of TA-0910 on the circling behavior in rats with unilateral lesion of the nigrostriatal dopaminergic pathway by 6-hydroxydopamine (6-OHDA).

MATERIALS AND METHODS

Animals

Male Slc:Wistar rats (Japan SLC, Inc.) weighing 270–330 g were used for the locomotion study; and male Jcl-SD rats (Clea Japan, Inc.) weighing 155–190 g were used for the circling behavior study, because in this strain of rats with unilateral irreversible lesion of the nigrostriatal dopaminergic pathway by 6-OHDA, circling behaviors were known to be provoked at any time (semipermanently) after administration of DA agonists (10). Each rat was housed in one compartment (15 × 25 × 14 cm) of a stainless steel 5-compartment wire-mesh cage (mesh: 6 mm, 75 × 25 × 14 cm). The animals were kept in an animal room maintained at 23 ± 1°C with 55 ± 5% humidity and illuminated for 12 hr (6:30–18:30). The rats were allowed free access to pellet diets (CRF-1, Oriental Yeast, Co., Ltd.) and tap water.

Drugs and their preparation

TA-0910 [1-methyl-(S)-4,5-dihydroorotyl-L-histidyl-L-prolineamide tetrahydrate: Lot No. 503090] and TRH (L-pyroglutamyl-L-histidyl-L-prolineamide-L-tartrate monohydrate: Lot No. 111419A) were synthesized in the Research Laboratory of Applied Biochemistry, Tanabe Seiyaku Co., Ltd. Other drugs used for the study were haloperidol (Dainippon), DL-α-methyl-p-tyrosine methylster hydrochloride (α-MT, Sigma), phenoxybenzamine hydrochloride (Tokyo Kasei), prazosin (privately synthesized), clonidine hydrochloride (Boehringer Ingelheim), DL-propranolol hydrochloride (Nacalai Tesque), metergoline (Farmitalia Carlo Erba), naloxone hydrochloride (Sigma), scopolamine hydrobromide (Sigma), methamphetamine hydrochloride (Dainippon), apomorphine hydrochloride (Messrs. Meferlane Amith), 6-hydroxydopamine hydrobromide (6-OHDA, Sigma), and pentobarbital sodium (Nacalai Tesque). All were dissolved in physiological saline (Otsuka) except for α-MT and metergoline, which were suspended in 0.5% carboxymethyl cellulose (CMC), and haloperidol, which was dissolved in distilled water. TA-0910 and TRH were administered intravenously (i.v.) or intraperitoneally (i.p.) at 2 ml/kg and intracerebrally (i.c.) at the volume of 0.5 μl on each side. Control rats were treated with an equal volume of physiological saline, i.e., i.v., or i.e., and pentobarbital sodium (Nacalai Tesque) was dissolved in ice-cold physiological saline containing 0.2% ascorbic acid (Wako Pure Chemicals) before use.

Drug administration

The D2 dopamine receptor antagonist haloperidol (0.1 mg/kg), α-adrenaline receptor antagonist phenoxybenzamine (5 mg/kg), α1-adrenaline receptor antagonist prazosin (0.3 mg/kg), α2-adrenaline receptor agonist clonidine (0.1 mg/kg), β-adrenaline receptor antagonist propranolol (10 mg/kg), serotonin (5-HT) receptor antagonist metergoline (0.5 mg/kg), opioid receptor antagonist naloxone (2 mg/kg), and muscarinic acetylcholine (ACh) receptor antagonist scopolamine (0.1 and 1
mg/kg) were administered i.p. 0.5, 4, 1, 0.25, 0.5, 0.5, 0.25, and 0.5 hr, respectively, before administration of TA-0910 (3 mg/kg, i.p.). Methamphetamine (0.2 mg/kg), apomorphine (1 mg/kg), and L-DOPA (20 mg/kg) were administered i.p. immediately before administration of a low dose of TA-0910 (0.3 mg/kg, i.p.). L-DOPA was administered 3 hr after administration of pargyline (50 mg/kg, i.p.). A low dose of apomorphine (0.2 mg/kg) was administered i.p. immediately before administration of a high dose of TA-0910 (3 mg/kg, i.p.).

Measurement of locomotor activity

A rat was placed in a transparent acrylic regin cage (24 × 37 × 29 cm) mounted on the top of an ANIMEX (Muromachi Kikai, DSE), with a sensitivity of 20 μA to mainly count large horizontal or vertical movements, consisting of ambulation and rearing. After acclimation for 60 min, the drug (TA-0910 or TRH) was injected i.v., i.p., or i.c. The locomotor activity was measured for 60 min starting immediately after the drug administration. All experiments were carried out between 9:00 - 18:00 in a sound-proof and uniformly illuminated laboratory room maintained at 23 ± 1 °C with 55 ± 5% humidity.

Implantation of a guide cannula for microinjection of drugs into the brain

The rats were fixed on a stereotaxic instrument (Narishige, ST-7) under pentobarbital-Na anesthesia (50 mg/kg, i.p.). A stainless steel guide cannula (0.5-mm outer and 0.25-mm inner diameters) was implanted 1 mm above the target injection site according to the brain atlas of Pellegrino and Cushman (19) and fixed onto the skull with screws and dental cement. Figure 1 shows the stereotaxic coordinates of the injection sites. The guide cannula for the raphe nuclei was implanted on the midline and those for other sites, bilaterally. A stainless steel injection cannula (0.2-mm outer and 0.08-mm inner diameters) was inserted into the guide cannula, and the drug solution was injected at a volume of 1 μl in the raphe nuclei and 0.5 μl on each side of the other sites, and at a rate of 0.5 μl/30 sec using a microsyringe (TERMO®). After injection, a stainless steel stylet (0.2 mm outer diameter) was inserted 0.5 mm deeper than the tip of the guide cannula to prevent its occlusion by infiltration of blood or tissue fluid. The rats were used repeatedly at 1-week intervals from 7 days after cannula implantation.

Lesions of the unilateral nigrostriatal dopaminergic pathway by 6-OHDA and measurement of circling behavior

The rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and fixed on a stereotaxic instrument (Narishige, ST-7), and the injection needle (0.4 mm outer and 0.2 mm inner diameters) was inserted into the dopaminergic pathway (A:4.6, L:1.8, H:-2.2) in the unilateral nigrostriatal system according to the brain atlas of König and Klippel (20). 6-OHDA (8 μg/4 μl) was injected at 1 μl/min using a microsyringe (TERMO®). The animals that showed apparent contralateral circling behavior following i.p. injection of 0.3 mg/kg of apomorphine 1 week after 6-OHDA treatment were selected and used repeatedly at intervals of 1 week or more. Each rat was placed in a hard polyethylene pail (40 cm diameter, 50 cm height) with its floor covered with wood shavings, and the drugs were administered after 10-min acclimation. The number of circling behaviors was counted for every 5 min at 5-min intervals, a total of 30 min, until 60 min after administration. Circling toward the lesioned side was regarded as ipsilateral, and that to the other side regarded as contralateral.

Verification of the site of cannula implantation

At the end of all experiments, the rats with the implanted guide cannula and those injected with 6-OHDA were perfused with 10% formalin solution through the left cardiac ventricle under sodium pentobarbital (40 mg/kg, i.p.) anesthesia. The brains were removed and kept in 10% formalin for at least 7 days and then sectioned to verify the side under binocu-
lar. The tips of all guide cannula for intracerebral injection were confirmed to have been in proper sites of the brain (Fig. 1).

Statistical analyses

The values obtained were expressed in the tables and figures as the mean ± standard error. The differences between the groups were examined first by the Kruskal-Wallis test and then by Dunnett type multiple comparison concerning the locomotive activity and by Scheffé type multiple comparison or Mann-Whitney U-test concerning the circling behavior of rats with nigrostriatal lesion.

RESULTS

Effects of various drugs on TA-0910-induced increases in locomotor activity

The increase in the locomotor activity induced by TA-0910 (3 mg/kg, i.p.) was significantly suppressed by haloperidol (0.1 mg/kg) or α-MT (30 mg/kg) at doses at which neither alone significantly affected the locomotor activity. Prazosin (3 mg/kg), phenoxybenzamine (5 mg/kg), clonidine (0.1 mg/kg), and naloxone (2 mg/kg) showed a tendency to suppress the TA-0910-induced increase in locomotor activity. Propranolol (10 mg/kg) and metergoline (0.5 mg/kg) had no effect on the locomotor stimulating action of TA-0910. Scopolamine (0.1 mg/kg) at a dose which had no effect on activity per se showed no significant effect on the locomotor stimulating action of TA-0910, but enhanced the action at a dose which increases the locomotor activity (Fig. 2).

The locomotor activity was remarkably in-

Fig. 1. Photomicrograph of locations of the cannula tips for intracerebral injection, according to the atlas of Pellegrino and Cushman (19). Abbreviations: PLHA: posterior lateral hypothalamic area (A, +4.6; L, +1.8; H, -3.2); CPU: corpus striatum (A, +7.8; L, +3.0; H, +1.0); ACB: nucleus accumbens (A, +9.2; L, +1.5; H, 0.0); RA: raphe nuclei (A, -2.2; L, 0.0; H, -6.3); LC: locus ceruleus (A, -1.5; L, +1.3; H, -3.3); HPC: hippocampus (A, +3.2; L, +2.5; H, +2.0).
creased by administration of low dose TA-0910 (0.3 mg/kg, i.p.) in combination with methamphetamine (0.2 mg/kg), apomorphine (1 mg/kg), or L-DOPA (20 mg/kg) after pretreatment with pargyline (50 mg/kg). A low dose of apomorphine (0.2 mg/kg) significantly suppressed the increase in locomotor activity induced by TA-0910 (3 mg/kg, i.p.) (Table 1).

**Effect on locomotor activity after intracerebral injection**

The effects of intracerebral injection of TA-

---

![Graph](image)

**Fig. 2.** Effect of pretreatment with various drugs on increased locomotor activity induced by TA-0910 in rats. Each value represents the mean ± S.E. from 10 rats. Recording of locomotor activity was started immediately after the TA-0910 (3 mg/kg) administration. Abbreviations: HPD: haloperidol, α-MT: α-methyl-p-tyrosine, PBZ: phenoxybenzamine, PZS: prazosin, CLN: clonidine, PPL: propranolol, MTG: metergoline, NLX: naloxone, SCP: scopolamine. ++P < 0.01, compared with saline alone. *P < 0.05, **P < 0.01, compared with saline with TA-0910 (3 mg/kg) (Dunnett type multiple comparison test).

**Table 1.** Effects of TA-0910 combined with methamphetamine, apomorphine and L-DOPA on locomotor activity in rats

| First injection | Dose mg/kg, i.p. | Saline | Methamphetamine | Apomorphine | L-DOPA* |
|-----------------|-----------------|--------|-----------------|-------------|---------|
| Second injection | Control | — | 263 ± 20 | 367 ± 34 | 107 ± 10 | 779 ± 100 | 216 ± 42 |
| | TA-0910 | 0.3 | 436 ± 64 | 737 ± 81** | NT | 1521 ± 122** | 707 ± 156* |
| | TA-0910 | 3 | 910 ± 98 ++ | NT | 366 ± 68* | NT | NT |

Groups of 8 rats were used. NT: not tested. *The rat was pretreated with pargyline (50 mg/kg, i.p.). Results are presented as the total mean counts for 60 min ± S.E. ++P < 0.01: compared with saline/control values; *P < 0.05, **P < 0.01: compared with saline/TA-0910 values (Dunnett type multiple comparison test).
0910 and TRH on locomotor activity are shown in Fig. 3. The locomotor activity was increased significantly by injection of 20 ng or more of TA-0910 into the nucleus accumbens and the posterior lateral hypothalamic area. When injected into the nucleus accumbens, TA-0910 (6–200 ng) exerted the most potent and dose-dependent stimulating action on the locomotor activity. However, no dose-dependence was observed in the locomotor stimulating action by injection into the posterior lateral hypothalamic area. The locomotor stimulating action was observed after injection of TA-0910 into the locus ceruleus and raphe nuclei at 60 ng or more or the hippocampus at 200 ng, but these increases were not dose-dependent and were less potent than those induced by the intranucleus accumbens or -post-

![Fig. 3. Effect of intracerebral and intravenous injection of TA-0910 and TRH on locomotor activity in rats. The number of rats is shown in parentheses. Each value is the percentage of basal locomotor activity obtained 7 days before the 1st drug-injection. Locomotor activity (counts/60 min) of control: TA-0910: CPU = 247 ± 50, ACB = 237 ± 38, PLHA = 260 ± 55, HPC = 280 ± 45, RA = 329 ± 55, LC = 238 ± 40, i.v. = 209 ± 34, TRH: CPU = 172 ± 42, ACB = 227 ± 38, PLHA = 238 ± 45, HPC = 217 ± 33, RA = 226 ± 29, LC = 227 ± 26, i.v. = 212 ± 23. *P < 0.05, **P < 0.01, compared with the respective basal locomotor activity (Dunnett type multiple comparison test).]
terior lateral hypothalamic area injection. The intra-corpus striatum injection had no obvious effect on the locomotor activity.

TRH (0.6–20 μg), like TA-0910, increased the locomotor activity most markedly when injected into the nucleus accumbens. The stimulating action was dose-dependent and significant at 6 μg or more. The locomotor stimulating action of TRH after intracerebral injection was in the following order of effectiveness: Nucleus accumbens > posterior lateral hypothalamic area > hippocampus > raphe nuclei ≅ locus ceruleus. As, in the case of TA-0910, TRH also had no significant effect on the locomotor activity when injected into the corpus striatum.

The intravenous administration of TA-0910 and TRH caused a dose-dependent and significant increase in locomotor activity at 1 mg/kg or more and 30 mg/kg or more, respectively (Fig. 3).

Effect of TA-0910 and TRH on circling behavior in rats with 6-OHDA-induced unilateral nigrostriatal lesion

In rats with unilateral lesion of the nigrostriatal dopaminergic pathway by injection of 6-OHDA, administration of apomorphine (0.1–1 mg/kg) induced dose-dependent contra-

lateral circling behavior toward the intact side (Fig. 4). The contralateral circling behavior induced by apomorphine began at 3–5 min, reached a peak at 10–20 min, and disappeared at 30–60 min after administration. On the other hand, methamphetamine (0.1–1 mg/kg) induced dose-dependent ipsilateral circling behavior toward the lesioned side (Fig. 4). The circling behavior caused by methamphetamine began about 5 min after administration and lasted for over 60 min with a peak about 30 min after administration.

Ipsilateral circling behavior similar to that induced by methamphetamine was observed after administration of TA-0910 or TRH. The frequency of circling behavior of 30 min at 5 min intervals until 60 min after administration of 1, 3, 10, and 30 mg/kg of TA-0910 was 6.7 ± 1.8, 24.7 ± 8.1, 31.7 ± 9.5, and 44.2 ± 8.8, respectively, and that after administration of 30, 100, and 300 mg/kg of TRH was 1.5 ± 0.3, 14.8 ± 3.9, and 18.3 ± 5.2, respectively (Fig. 5). The ipsilateral circling behavior induced by 3–30 mg/kg of TA-0910 began 5 min after administration and lasted for over 60 min. The maximum effect was observed between 30 and 40 min after administration. The ipsilateral circling behavior induced by TRH (100–300 mg/kg) began immediately after

![Graph](image_url)

**Fig. 4.** Circling behavior induced by methamphetamine and apomorphine in rats with unilateral 6-OHDA lesions. The number of rats is shown in parentheses. **P < 0.01, compared with the saline control (Scheffé type multiple comparison test).
administration, reached a peak after 5–15 min, and disappeared after 35–40 min.

Ipsilateral circling behavior induced by TA-0910 (10 mg/kg), TRH (100 mg/kg), and methamphetamine (1 mg/kg) were almost entirely prevented by pretreatment with haloperidol (1 mg/kg) or α-MT (250 mg/kg) (Figs. 6 and 7). On the other hand, the contralateral circling behavior induced by apomorphine (0.3 mg/kg) was markedly inhibited by pretreatment with haloperidol but not by pretreatment with α-MT (Fig. 7).

DISCUSSION

The locomotor stimulating action induced by a high dose (3 mg/kg, i.p.) of TA-0910 was...
specifically inhibited by pretreatment with haloperidol, a-MT or a low dose of apomorphine (0.2 mg/kg), whereas that action induced by a low dose (0.3 mg/kg, i.p.) of TA-0910 was significantly enhanced by methamphetamine (0.2 mg/kg), apomorphine (1 mg/kg), or L-DOPA (20 mg/kg) after pretreatment with pargyline. The mechanism of its locomotor stimulating action, therefore, is considered to be mainly due to activation of the central dopaminergic system.

In the present study, the locomotor stimulating action of TA-0910 was slightly suppressed by pretreatment with phenoxybenzamine, prazosin, and naloxone. Accordingly, the results do not rule out the possibility that TA-0910 may be acting via the noradrenaline and opioid mechanisms. This is supported by the reports that clonidine-induced inhibition of locomotor activity is antagonized by TRH and its analog CG-3509, CG-3703, and RX77368; that the locomotor stimulating action of TRH, CG-3509 and CG-3703 is inhibited by pretreatment with prazosin (21); and that the action of TRH is inhibited by naloxone (22). On the other hand, the locomotor stimulating action of TA-0910 was not affected by a low dose (0.1 mg/kg) but was enhanced by a high dose (1 mg/kg) of scopolamine. Since the dopaminergic and cholinergic systems are known to function reciprocally in the nucleus accumbens and corpus striatum (23-25), the dopaminergic system is considered to be activated as a result of inhibition of the muscarinic cholinergic system by a high dose of scopolamine. The stimulating action of TA-0910 as well as TRH was most pronounced when the drugs were injected into the nucleus accumbens. This strongly suggests that the mesolimbic DA system, especially the nucleus accumbens, is important in the locomotor stimulating action of TA-0910. This speculation is supported by the fact that injection of DA into the nucleus accumbens markedly stimulated locomotor activity and that this stimulating effect was counteracted by intraperitoneal or intra-nucleus accumbens injection of haloperidol and pimozide (26).

The locomotor activity slightly increased when TA-0910 or TRH was injected in the corpus striatum, which is another dopaminergic nerve terminal (nigro-striatal dopaminergic system). Since the doses of TA-0910 and TRH required for increasing the locomotor activity were about 1/10 of those required for induction of ipsilateral circling behavior in rats (17), TA-0910 and TRH are considered to preferentially act on the nucleus accumbens rather than on the corpus striatum. These results are consistent with the earlier reports that the corpus striatum is related to the development of circling behaviors or stereotyped behavior (27, 28) and that the nucleus accumbens is related to increased locomotor activity in rodents (15, 21, 29, 30).

The nucleus accumbens, which is one of the terminals of the mesolimbic system, is considered to be the main action site of neuroleptics. Since the nucleus accumbens appears to be closely associated with emotional and mental function in humans (31-33), TA-0910 may enhance the psychomotor responses (motivation, volition, interest, expression of emotions, and contactness). Messerano and King (34), on the other hand, reported that the locomotor activity was not increased by injection of TRH into the caudate nucleus or septal area, but it was clearly increased by injection into the hypothalamus in rats. In our present study, TA-0910 injected into the posterior lateral hypothalamic area produced hyperactivity from a low dose of 20 ng, although no clear dose-dependence was observed. Since the hypothalamus posterior is involved in the hypothalamic activating system suggested by Murphy and Gellhorn (35), the increased locomotor activity after injection into the posterior lateral hypothalamic area may be a phenomenon secondary to the increase in the level of consciousness. The locomotor activity was also increased by injection into the locus ceruleus, which is rich in noradrenergic neurons, but this effect was smaller than that caused by injection in the nucleus accumbens and lacked dose-dependence. However, since the hyperactivity induced by intraperitoneal
administration of TA-0910 was partly inhibited by phenoxybenzamine, prazosin, or clonidine, the locus ceruleus may be partially involved in this hyperactivity. The locomotor activity was increased by injection into the raphe nuclei, which are rich in 5-HT-mediated neurons, or the hippocampus, which is the nerve terminals of the ACh system, but this increase was slightly dose-dependent, and was not inhibited by i.p. administration of metergoline or scopolamine. Therefore, these two sites are not considered to have major roles in TA-0910-induced hyperactivity. At 1 mg/kg or more of intravenous administration or 20 ng or more of the intra-nucleus accumbens injection, TA-0910 significantly and dose-dependently enhanced locomotor activity in rats. Similar effects were observed after intravenous administration of 30 mg/kg or the intra-nucleus accumbens injection of 6μg TRH. These results indicate that the locomotor stimulating action of TA-0910 is similar in quality but different in quantity as compared to that of TRH. Furuuchi et al. showed that, after intravenous administration of 1 mg/kg [14C]-TA-0910 to rats weighing about 200 g (brain weight about 1.39 g), the brain contained about 44 ng (about 0.02% of the amount administered) of the agent (S. Furuuchi et al., personal communication). Therefore, considering the doses of the above intravenous and intracerebral administration, the locomotor stimulating action of TA-0910 is thought to be mediated via the central nervous system.

In the rats with 6-OHDA induced lesion of the unilateral DA pathway, TA-0910, TRH and methamphetamine produced ipsilateral circling behavior, whereas apomorphine produced contralateral circling behavior. The ipsilateral circling behavior was inhibited by pretreatment with haloperidol or α-MT. On the other hand, the contralateral circling behavior was inhibited by pretreatment with haloperidol, but not with α-MT. This indicates that both TA-0910 and TRH, like methamphetamine, probably produce ipsilateral circling behavior by inhibiting DA uptake and/or enhancing DA release in the dopaminergic nerve terminals of the corpus striatum, not by direct action on DA receptors, like apomorphine. However, the fact that TRH does not inhibit the uptake of [14C]-DA in the rat corpus striatum slices (14, 36) suggests promotion of DA release from pre-synaptic sites. In this connection, TRH at high concentrations (10\(^{-5}\) to 10\(^{-3}\) M) is reported to promote the release of [14C]-DA from slices of the rat nucleus accumbens and corpus striatum by the superfusion (13, 15, 26), but there is also a report that TRH did not promote the release of [3H]-DA from slices of the rat corpus striatum by the same method (37). The results are not consistent. Kawashima et al. showed that TA-0910 and TRH increase the 3-methoxytyramine contents in the corpus striatum and nucleus accumbens of rats pretreated with pargyline, although they do not promote the release of [3H]-DA from the superfused slices of rat nucleus accumbens or corpus striatum, suggesting that TA-0910 and TRH promote DA release in the same way (K. Kawashima et al., personal communication). These observations suggest that TA-0910 and TRH promote the release of DA from pre-synaptic sites, but their promotive activity is considered to be far weaker than that of methamphetamine, because TA-0910 and TRH induced ipsilateral circling behavior at about 30 and 1,000 times higher doses than methamphetamine, respectively. On the other hand, TRH is reported to inhibit DA autoreceptors and, thus, promote the release of DA and increase locomotion (38). In our study, the locomotor stimulating action of TA-0910 was markedly inhibited by low dose (0.2 mg/kg) of pargyline, at which apomorphine stimulates DA autoreceptors, and by α-MT, which reduces endogenous dopamine. Therefore, TA-0910 may have an inhibitory effect on DA autoreceptors and a promoting effect on DA synthesis. TRH is also suggested to enhance the sensitivity of DA receptors at post-synaptic sites (10, 39). Since the locomotor activity was markedly increased by the administration of a low dose (0.3 mg/kg) of TA-0910 in combination with L-DOPA after pretreatment with pargyline, TA-0910 may also
enhance the sensitivity of post-synaptic DA receptors.

In conclusion, the locomotor stimulating action of TA-0910 is probably mediated primarily via activation of the mesolimbic DA system including the nucleus accumbens.

REFERENCES

1 Plotnikoff, N.P., Prange, A.J., Jr., Breese, G.R., Anderson, M.S. and Wilson, I.C.: Thyrotropin-releasing hormone: Enhancement of DOPA activity by a hypothalamic hormone. Science 178, 417–418 (1972)

2 Plotnikoff, N.P., Prange, A.J., Jr., Breese, G.R. and Wilson, I.C.: Thyrotropin releasing hormone: Enhancement of DOPA activity in thyroidectomized rats. Life Sci. 14, 1271–1278 (1979)

3 Costall, B., Marsden, C.D., Naylor, R.J. and Pycock, C.J.: Stereotyped behavior patterns and hyperactivity induced by amphetamine and apomorphine after 6-hydroxydopamine lesions of extrapyramidal and mesolimbic nuclei. Brain Res. 123, 89–111 (1979)

4 Ernst, A.M. and Smelik, P.G.: Site of action of dopamine and apomorphine on compulsive gnawing behaviour in rats. Experientia 22, 837–838 (1966)

5 Ungerstedt, U.: Stereotaxic mapping of the monoamine pathways in the rat brain. Acta Physiol. Scand. Suppl. 367, 1–48 (1971)

6 Kelly, P.H., Seviour, P.W. and Iversen, S.D.: Amphetamine and apomorphine response in the rat following 6-OHDA lesions of the nucleus accumbens septi and corpus striatum. Brain Res. 94, 507–522 (1975)

7 Ushijima, I., Yamada, K., Furukawa, T., Noda, Y., Todoroki, M. and Inanaga, K.: Biphasic effect of thyrotropin-releasing hormone on exploratory behavior in mice. Arch. Int. Pharmacodyn. Ther. 247, 257–263 (1980)

8 Andrews, J.S. and Sahgal, A.: The effects of thyrotropin-releasing hormone, metabolites and analogues on locomotor activity in rats. Regul. Pept. 7, 97–109 (1983)

9 Ervin, G.N., Schmitz, S.A., Nemeroff, C.B. and Prange, A.J., Jr.: Thyrotropin-releasing hormone and amphetamine produce different patterns of behavioral excitation in rats. Eur. J. Pharmacol. 72, 35–43 (1981)

10 Fukuda, N., Miyamoto, M., Narumi, S., Nagai, Y., Shima, T. and Nagawa, Y.: Thyrotropin-releasing hormone (TRH): Enhancement of dopamine dependent circling behavior and its own circling-inducing effect in unilateral striatal lesioned animals. Folia Pharmacol. Japon. 75, 251–270 (1979) (Abs. in English)

11 Jerussi, T.P. and Glick, S.D.: Drug-induced rotation in rats without lesions: Behavioral and neurochemical indices of a normal asymmetry in nigro-striatal function. Psychopharmacology (Berlin) 47, 249–260 (1976)

12 Marel, K. and Haubrich, D.R.: Thyrotropin-releasing hormone-increased catecholamines in brains of thyroidectomized rats. Biochem. Pharmacol. 26, 1817–1818 (1977)

13 Sharp, T., Bennett, G.W. and Marsden, C.A.: Thyrotropin-releasing hormone analogues increase dopamine release from slices of rat brain. J. Neurochem. 39, 1763–1766 (1982)

14 Narumi, S., Nagai, Y. and Nagawa, Y.: Thyrotropin-releasing hormone (TRH): Action mechanism of an enhanced dopamine release from rat striatal slices. Folia Pharmacol. Japon. 75, 239–250 (1979) (Abs. in English)

15 Heal, D.J. and Green, A.R.: Administration of thyrotropin releasing hormone (TRH) to rats releases dopamine in the n. accumbens but not n. caudatus. Neuropharmacology 18, 23–31 (1979)

16 Griffiths, E.C., Mc Dermott, J.R. and Smith, A.I.: Mechanism of brain inactivation of centrally-acting thyrotropin-releasing hormone (TRH) analogues: a high performance liquid chromatography study. Regul. Pept. 5, 1–11 (1982)

17 Suzuki, M., Sugano, H., Matsumoto, K., Yamamura, M. and Ishida, R.: Synthesis and central nervous system actions of thyrotropin-releasing hormone analogues containing a dihydroorotic acid moiety. J. Med. Chem. 33, 2130–2137 (1990)

18 Yamamura, M., Kinoshita, K., Nakagawa, H., Tanaka, Y., Maeda, K. and Ishida, R.: Pharmacological study of TA-0910, a new thyrotropin-releasing hormone (TRH) analog, (I): Effects on the central nervous system by administration. Japan. J. Pharmacol. 53, 451–461 (1990)

19 Pellegrino, L.J. and Cushman, A.J.: Stereotaxic Atlas of the Rat Brain. Appleton-Century-Crofts, New York (1967)

20 König, J.F.R. and Klippel, R.A.: The Rat Brain. A Stereotaxic Atlas of the Forebrain and Lower Parts of the Brain Stem. Williams and Wilkins, Baltimore (1963)

21 Miyamoto, M., Narumi, S., Nagai, Y., Saji, Y. and Nagawa, Y.: A TRH analog (DN-1447): Motor stimulation with rearing related to catecholaminergic mechanism in rats. Neuropharmacology 23, 61–72 (1984)
22 Lin, M.T., Chan, H.K., Chen, C.F. and Teh, G.W.: Involvement of both opiate and catecholaminergic receptors in the behavioral excitation provoked by thyrotropin-releasing hormone: Comparisons with amphetamine. Neuropharmacology 22, 463–469 (1983)

23 Costall, B., Naylor, R.J. and Olley, J.E.: Catalepsy and circling behaviour after intracerebral injections of neuroleptic, cholinergic and anti-cholinergic agents into the caudate-putamen, globus pallidus and substantia nigra of rat brain. Neuropharmacology 11, 645–663 (1972)

24 Keller, H.H., Bartholini, G. and Pletscher, A.: Drug-induced changes of striatal cholinergic and dopaminergic functions in rats with spreading depression. J. Pharm. Pharmacol. 25, 433–436 (1973)

25 Stoof, J.C., Russhen, F.T., Verheijden, P.F.H.M. and Hoogland, P.V.J.M.: A comparative study of the dopamine- acetylcholine interaction in telencephalic structures of the rat and of a reptile, the lizard Gekko gekko. Brain Res. 404, 273–281 (1987)

26 Miyamoto, M., Narumi, S., Nagai, Y., Shima, T. and Nagawa, Y.: Thyrotropin-releasing hormone: Hyperactivity and mesolimbic dopamine system in rats. J. Pharmacol. 29, 335–347 (1979)

27 Asher, I.M. and Aghajanian, G.K.: 6-Hydroxydopamine lesions of olfactory tubercles and caudate nuclei. Effect on amphetamine-induced stereotyped behavior in rats. Brain Res. 82, 1–12 (1974)

28 Costall, B. and Naylor, R.J.: The role of telencephalic dopaminergic systems in the mediation of apomorphine-stereotyped behaviour. Eur. J. Pharmacol. 24, 8–24 (1973)

29 Miyamoto, M. and Nagawa, Y.: Mesolimbic involvement in the locomotor stimulant action of thyrotropin-releasing hormone (TRH) in rats. Eur. J. Pharmacol. 44, 143–152 (1977)

30 Kalivas, P.W., Stanley, D. and Prange, A.J., Jr.: Interaction between thyrotropin-releasing hormone and the mesolimbic dopamine system. Neuropharmacology 26, 33–38 (1987)

31 Powell, E.W. and Leman, R.B.: Connections of the nucleus accumbens. Brain Res. 105, 389–403 (1976)

32 Stevens, J.: An anatomy of schizophrenia? Arch. Gen. Psychiatry 27, 177–189 (1973)

33 Iversen, L.L.: Dopamine receptors in the brain: A dopamine-sensitive adenylate cyclase models synaptic receptors, illuminating antipsychotic drug action. Science 188, 1084–1089 (1975)

34 Masserano, J.M. and King, C.: TRH increase locomotor activity in rats after injection into the hypothalamus. Eur. J. Pharmacol. 69, 217–219 (1981)

35 Murphy, J.P. and Gellhorn, E.: The influence of hypothalamic stimulation on chronically induced movements and action potentials of the cortex. J. Neurophysiol. 8, 341–364 (1945)

36 Horst, W.D. and Spirt, N.: A possible mechanism for the anti-depressant activity of thyrotropin releasing hormone. Life Sci. 15, 1073–1082 (1974)

37 Kerwin, R.W. and Pycock, C.J.: Thyrotropin-releasing hormone stimulates release of $[^3]$H] dopamine from slices of rat nucleus accumbens in vitro. Br. J. Pharmacol. 67, 323–325 (1979)

38 Funatsu, K., Tejima, S. and Inanaga, K.: Interaction between TRH receptor and dopamine auto-receptor in the rat limbic forebrain and striatum. Bull. Japan. Neurochem. Soc. 24, 412–414 (1985) (in Japanese)

39 Narumi, S., Nagai, Y., Saji, Y. and Nagawa, Y.: A possible mechanism of action of thyrotropin-releasing hormone (TRH) and its analog DN-1417 on the release of dopamine from the nucleus accumbens and striatum in rats. Japan. J. Pharmacol. 39, 425–435 (1985)