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

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Abstract—Slices of the nucleus accumbens, the terminus of the mesolimbic dopaminergic system and the striatum, the terminus of the nigrostriatal dopaminergic system obtained from rats, were used for analyzing the dopamine releasing effects of TRH and its analog DN-1417 (*γ*-butyrolactone-*γ*-carbonyl-L-histidyl-L-prolinamide citrate). (1) Dopamine release: The addition of DN-1417 (5×10^{-5} M) or TRH (5×10^{-4} M) stimulated the release of prelabelled [^3H]-dopamine (DA) from the superfused nucleus accumbens slices, and 100 or 20 times higher concentrations of each compound stimulated DA release from the striatal slices. The omission of Ca^{2+} from the superfusion medium or the addition of ouabain (5×10^{-3} M), a ($\text{Na}^+ + \text{K}^+$) stimulated adenosine triphosphate (ATP) phosphohydrolase [$(\text{Na}^+ + \text{K}^+)\text{-ATPase}$] inhibitor, almost completely abolished the DN-1417- or TRH-induced DA releasing effect. (2) $^{45}\text{Ca}^{2+}$ uptake: The addition of DN-1417 (10^{-4} M) or TRH (10^{-4} M) stimulated $^{45}\text{Ca}^{2+}$ uptake into the nucleus accumbens slices in a time-dependent manner from 1 to 5 min at 30°C . On the other hand, both drugs (10^{-4} M) had no effect on $^{45}\text{Ca}^{2+}$ uptake into the striatal slices. A Ca^{2+} ionophore, A-23187 (10^{-6} M), stimulated $^{45}\text{Ca}^{2+}$ uptake into slices of the nucleus accumbens and striatum. Ouabain abolished the DN-1417-, TRH- and A-23187-induced effects. (3) Cyclic AMP formation: The addition of DN-1417 (10^{-4} M) or TRH (10^{-4} and 10^{-3} M) accelerated cyclic AMP formation in the nucleus accumbens, but not in the striatal slices. A Ca^{2+} antagonist, methoxyverapamil (D-600, 10^{-6} M) almost completely abolished the DN-1417- and TRH-effects. (4) ($\text{Na}^+ + \text{K}^+$)-ATPase activity: The addition of DN-1417 (10^{-5} and 10^{-4} M) or TRH (10^{-4} M) activated ($\text{Na}^+ + \text{K}^+$)-ATPase in the P_1 fraction (cell debris, nuclei) of the nucleus accumbens, but had little effect on the same activity in the striatum. (5) [^3H]-TRH binding: The addition of 10^{-6} to 10^{-4} M of DN-1417 and TRH inhibited concentration-dependently [^3H]-TRH binding in the nucleus accumbens and striatal slices. Ouabain (5×10^{-3} M) almost completely abolished [^3H]-TRH binding in both types of slices. These results suggest that DN-1417 and TRH activate ($\text{Na}^+ + \text{K}^+$)-ATPase and stimulate Ca^{2+} uptake in the presynaptic sites and release DA which stimulates cyclic AMP formation in the postsynaptic sites of the mesolimbic dopaminergic system mainly and causes several central nervous actions.

There are nigrostriatal and mesolimbic pathways in the central nervous dopaminergic system (1, 2). The former, which originates from the cell bodies of A 8 and A 9 (substantia nigra) and terminates in the striatum, is thought to be related to stereotypy

and circling behavior. The latter, which originates from the A 10 cell body and terminates in the nucleus accumbens and olfactory tubercle, is thought to be related to locomotor activity (3–5).

In a behavioral study in rats, the locomotor

stimulatory action of a TRH analog, DN-1417 (γ -butyrolactone- γ -carbonyl-L-histidyl-L-prolinamide citrate), was about ten times more potent than that of TRH. This action seemed to be mediated through a dopaminergic mechanism that enhanced the release of dopamine (DA) from the nucleus accumbens, the terminus of nerves in the mesolimbic DA system (6, 7). In rats with a unilateral lesion in the nigrostriatal DA pathway produced by 6-hydroxydopamine (6-OHDA), 10 times higher doses of DN-1417 and TRH than the doses used in the locomotor experiments, produced circling toward the side of the lesion. This action appeared to be mediated through enhancement of DA release from the striatum, the terminus of nerves in the nigrostriatal DA system.

In the present study, to analyze further the behavioral study described above, the following neurochemical experiments were done using slices of the nucleus accumbens and striatum: [^3H]-DA release, $^{45}\text{Ca}^{2+}$ uptake, cyclic AMP formation, $(\text{Na}^+ + \text{K}^+)$ -stimulated adenosine triphosphate (ATP) phosphohydrolase [$(\text{Na}^+ + \text{K}^+)$ -ATPase] and [^3H]-TRH binding. The proposed mechanisms of action of TRH and its analog DN-1417 on the release of DA from the nucleus accumbens are suggested in this report.

Materials and Methods

Animals: Male Sprague-Dawley (SD) rats (8–10 weeks old) were purchased from Japan Clea Laboratories. They were decapitated, and the nucleus accumbens and striatum were quickly removed and sliced to a thickness of 0.5 mm with a McIlwain tissue chopper.

Release of [^3H]-DA from slices: [^3H]-DA release from brain slices was measured using the method reported previously with a minor modification (7). Slices of the nucleus accumbens and striatum were suspended in 95% O_2 –5% CO_2 saturated Krebs-Ringer phosphate buffer (KRPB) containing 0.1 mM pargyline, 1 mM ascorbic acid and 10 mM glucose, and centrifuged at 2,000 g for 5 min at 4°C. Slices of 40 mg wet weight were put into a test tube containing 2 ml of KRPB, incubated with 1 $\mu\text{Ci}/3.7 \times 10^{-8}$ mol of

[^3H]-DA for 60 min at 37°C, repeatedly rinsed with fresh KRPB, and centrifuged three times. Slices, thus preloaded with [^3H]-DA, were superfused with KRPB at a constant rate of 0.2 ml/min in a superfusion apparatus. The radioactivities of [^3H] in the superfusate effluents collected every 10 min for 30 min and every 5 min from 30 to 100 min and those in the supernatant (3,000 g for 10 min at 4°C) of 5% trichloroacetic acid (TCA) homogenized slices were counted using a liquid scintillation spectrometer with ACS® II scintillation.

$^{45}\text{Ca}^{2+}$ uptake into slices: Slices (0.5 mm thick, 30–40 mg wet weight) of the nucleus accumbens and striatum were preincubated for 15 min at 30°C in 95% O_2 –5% CO_2 saturated KRPB without Ca^{2+} and then further incubated from 1 to 5 min at 30°C with $^{45}\text{CaCl}_2$ (1 $\mu\text{Ci}/344$ nmol) and [^3H]-mannitol (1 $\mu\text{Ci}/58.8$ nmol) according to the method of Blaustein and Ector (8). After the incubation was stopped by adding 30 mM EGTA, the slices were centrifuged at 2,000 g for 5 min at 4°C and then rinsed with fresh KRPB twice. The radioactivities of [^3H] and [^{45}Ca] in slices dissolved in 0.2 N NaOH were counted by liquid scintillation. A 10 μl aliquot of the same solution was assayed for protein using the Folin reagent (9). To exclude $^{45}\text{Ca}^{2+}$ uptake into the extracellular space, the uptake of [^3H]-mannitol was subtracted from the total $^{45}\text{Ca}^{2+}$ uptake.

Cyclic AMP formation: Cyclic AMP formation in the slices was determined by the method of Krueger et al. (10). The slices, 2.5 mm thick, were chopped twice using a McIlwain tissue chopper, suspended in KRPB and centrifuged at 2,000 g for 5 min. Wet slices were weighed and resuspended in fresh KRPB to a final volume of 30–40 mg/ml. A 0.5 ml aliquot of these suspensions was added to tubes, each containing 0.1 ml of KRPB with one of the drugs, and the preparations were incubated for 15 min at 37°C. After the reaction was terminated by placing the tube in boiling water for 3 min, the supernatant obtained by centrifugation (3,000 g for 5 min) was assayed for cyclic AMP by a radioimmunoassay method using a Yamasa assay kit.

ATPase assay: Enzyme suspensions com-

prised of 100 μ g protein of P₁ (cell debris, nuclei, 800 g for 10 min), P₂ (crude mitochondria, 12,000 g for 30 min) or P₃ (crude microsome, 100,000 g for 60 min) fractions of the nucleus accumbens and the striatum were used. ATPase activities were measured in a reaction medium consisting of the enzyme suspensions, 5 mM disodium ATP, 5 mM MgCl₂ and 100 mM Tris-acetate buffer (pH 7.5) with or without 100 mM NaCl and 20 mM KCl in a final volume of 1.0 ml. The mixtures were incubated at 37°C for 20 min, and the enzymatic reactions were terminated by adding 0.5 ml of an ice-cold TCA solution. The inorganic phosphate liberated during the incubation was estimated according to the method of Takahashi (11).

[³H]-TRH binding: Slices (0.5 mm thick, wet weight 40 mg) and [³H]-TRH (0.5 μ Ci/20 nmol) in KRBP containing 50 μ g bacitracin were incubated for from 15 to 60 min at 37°C. The nonspecific binding was assayed in the presence of 10⁻³ M TRH. Specific binding was the total binding minus the nonspecific binding. After the incubation was stopped by adding ice-cold KRBP, the slices were centrifuged at 2,000 g for 5 min at 4°C and then rinsed with fresh KRBP twice. The radioactivities of [³H] in the slices dissolved in 0.2 N NaOH were counted by liquid scintillation. A 10 μ l aliquot of the same solution was assayed for protein using the Folin reagent (9).

Agents used: γ -Butyrolactone- γ -carbonyl-L-histidyl-L-prolinamide citrate (DN-1417, Lot No. I 615-02), thyrotropin-releasing hormone tartrate (TRH, Lot No. T-8-2), and methoxyverapamil (D-600, Synthesized at Takeda, Chemical Lab.); A-23187 (Calbiochem); ouabain octahydrate, bacitracin (Sigma); adenosine 5' triphosphate (ATP) 2 Na (Böhringer Mannheim); MgCl₂, NaCl, KCl, Tris, trichloroacetic acid (TCA), sodium molybdate, isobutyl alcohol and ascorbic acid (Wako); glycoetherdiamine-N,N,N',N'-tetraacetic acid (EGTA, Dojinyakugaku); ACS® II (Amersham); cyclic AMP assay kit (Yamasa Shoyu); and [³H]-dopamine (26.8 Ci/mmol), ⁴⁵CaCl₂ (2.9 Ci/mmol), [³H]-mannitol (17 Ci/mmol) and [³H]-TRH (100 Ci/mmol) (New England Nuclear).

Statistics: Statistical comparisons between

different treatments were made using Student's *t*-test (two-tailed).

Results

Effects on the release of [³H]-dopamine (DA): The effects of DN-1417, TRH and KCl on the release of [³H]-DA from slices of the nucleus accumbens and striatum were investigated by means of the superfusion method. As shown in Fig. 1a, DN-1417 (10⁻⁵, 5 $\times$ 10⁻⁵ and 10⁻⁴ M) and TRH (10⁻⁴, 5 $\times$ 10⁻⁴ and 10⁻³ M) concentration-dependently enhanced the release of [³H]-DA from the superfused nucleus accumbens slices after a stable spontaneous DA release was established. One hundred and 20 times higher concentrations of DN-1417 (10⁻³ and 5 $\times$ 10⁻³ M) and TRH (10⁻³ and 10⁻² M) enhanced the release of preloaded [³H]-DA from the striatal slices (Fig. 1b). The addition of KCl (5 $\times$ 10⁻² M) stimulated the DA release from slices of the nucleus accumbens and striatum to the same extent.

As shown in Fig. 2, the omission of Ca²⁺ from the superfusion medium almost completely abolished the DN-1417 (5 $\times$ 10⁻⁵ M)- and TRH (5 $\times$ 10⁻⁴ M)-induced DA releasing effects from the nucleus accumbens slices. A Ca²⁺ ionophore, A-23187 (10⁻⁶ M), stimulated the DA release from the nucleus accumbens slices (Fig. 3a). The addition of ouabain (5 $\times$ 10⁻³ M), a (Na⁺+K⁺) stimulated adenosine triphosphate (ATP) phosphohydrolase [(Na⁺+K⁺)-ATPase] inhibitor, almost completely abolished the DN-1417 (10⁻⁴ M)-, TRH (10⁻³ M)- and A-23187 (10⁻⁶ M)-induced DA releasing effects from the nucleus accumbens slices (Fig. 3b).

Effects on the uptake of Ca²⁺: The addition of DN-1417 (10⁻⁴ M) or TRH (10⁻⁴ M) stimulated ⁴⁵Ca²⁺ uptake into the nucleus accumbens slices time-dependently from 1 to 5 min at 30°C incubation (Fig. 4a). On the other hand, neither of the drugs (10⁻⁴ M) had an effect on ⁴⁵Ca²⁺ uptake into the striatal slices (Fig. 4b). A-23187 (10⁻⁶ M) stimulated ⁴⁵Ca²⁺ uptake into slices of the nucleus accumbens and striatum. DN-1417 (10⁻⁶–10⁻⁴ M) and TRH (10⁻⁶–10⁻⁴ M) stimulated ⁴⁵Ca²⁺ uptake into the nucleus accumbens slices concentration-dependently at 30°C for 5-min incubation (Table 1).

Fig. 1. Effects of TRH and DN-1417 on the release of [3 H]-dopamine (DA) from a) the nucleus accumbens and b) striatal slices in rats by superfusion. The drug was added from 75 to 90 min after the start of the superfusion. All values are the mean $\pm$ S.E.M. of 6 determinations. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. each control. a, b) $\bullet$: Control, $\blacksquare$: 5×10^{-2} M KCl, $\triangle$: 10^{-5} M DN-1417, ∇ : 5×10^{-5} M DN-1417, $\blacktriangle$: 10^{-4} M DN-1417, $\blacktriangledown$: 10^{-4} M TRH, $\diamond$: 5×10^{-4} M TRH, $\blacklozenge$: 10^{-3} M TRH, b) ∇ : 10^{-3} M DN-1417, $\blacktriangle$: 5×10^{-3} M DN-1417, $\diamond$: 10^{-3} M TRH, $\blacklozenge$: 10^{-2} M TRH.

$$\% \text{ release of } [^3\text{H}]\text{-DA} = \frac{[^3\text{H}]\text{-DA in 5-min incubation medium}}{\text{Total } [^3\text{H}]\text{-DA (slices+all incubation media)}} \times 100$$

Ouabain (5×10^{-3} M) abolished the DN-1417 (10^{-4} M)-, TRH (10^{-4} M)- and A-23187 (10^{-6} M)-induced effects.

Effects on cyclic AMP formation: As shown in Table 2, the addition of DN-1417 (10^{-6} – 10^{-4} M) or TRH (10^{-5} – 10^{-3} M) accelerated cyclic AMP formation in the nucleus accumbens, but not in the striatal slices. A-23187 (10^{-6} M) accelerated cyclic AMP formation in the nucleus accumbens slices (Table 3). A Ca^{2+} antagonist, D-600 (10^{-6} M), almost completely blocked the DN-1417 (10^{-4} M)-, TRH (10^{-3} M)- and A-23187 (10^{-6} M)-induced effects (Table 3).

Effects on ATPase activities: As shown in Table 4, the subcellular distribution of ($\text{Na}^{+} +$

K^{+})-ATPase activities in the nucleus accumbens was as follows: crude mitochondria (P_2) > crude microsome (P_3) > cell debris (P_1) > supernatant (S). The addition of DN-1417 (10^{-4} M) activated ($\text{Na}^{+} + \text{K}^{+}$)-ATPase in the P_1 fraction, but not in the P_2 , P_3 and S fractions. DN-1417 (10^{-4} M) also had no effect on Mg^{2+} -dependent ATPase (Mg^{2+} -ATPase) activities in all fractions. Ouabain (5×10^{-3} M) inhibited ($\text{Na}^{+} + \text{K}^{+}$)-ATPase activities in the P_1 , P_2 and P_3 fractions to about 60 to 80% of the control. This result suggests that the ($\text{Na}^{+} + \text{K}^{+}$)-ATPase in these fractions is mostly ouabain sensitive. The addition of DN-1417 (10^{-6} – 10^{-4} M) or TRH (10^{-6} – 10^{-4} M) concentration-

dependently activated ($\text{Na}^+ + \text{K}^+$)-ATPase in the P_1 fraction of the nucleus accumbens, but had little effect on the activity in the P_1

fraction of the striatum (Table 5).

Effects on [^3H]-TRH binding: The total and non-specific binding (in the presence of TRH 10^{-3} M) of [^3H]-TRH in the nucleus accumbens (Fig. 5a) and striatal slices (Fig. 5b) increased time-dependently from 15 to 60 min at 37°C , while the specific binding of [^3H]-TRH (total binding minus nonspecific binding) plateaued for 30 min at 37°C . Ouabain (5×10^{-3} M) markedly blocked the specific binding of [^3H]-TRH during the incubation time in slices of the nucleus accumbens and striatum. The addition of 10^{-6} to 10^{-4} M of DN-1417 or TRH concentration-dependently inhibited the specific binding of [^3H]-TRH in the nucleus accumbens and striatal slices incubated at 37°C for 30 min. The potency of DN-1417 was about twice that of TRH (Fig. 6).

These results suggest that DN-1417 and TRH activate ($\text{Na}^+ + \text{K}^+$)-ATPase and stimulate Ca^{2+} uptake in the presynaptic sites and release DA which stimulates cyclic AMP formation in the postsynaptic sites of the mesolimbic dopaminergic system mainly and causes several central nervous actions (Fig. 7).

Discussion

In an earlier behavioral study in rats, DN-

Fig. 2. Effect of Ca^{2+} omission from the medium on TRH- and DN-1417-induced [^3H]-dopamine release from the nucleus accumbens slices in rats. All values are the mean $\pm$ S.E.M. of 6 determinations. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. control. $\bullet$: Control.

Fig. 3. Effect of ouabain on DN-1417-, TRH- and A-23187-induced release of [^3H]-dopamine from rat nucleus accumbens slices by superfusion. All values are the mean $\pm$ S.E.M. of 8 determinations. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. control. $\bullet$: Control.

1417 and TRH stimulated locomotor actions mediated via enhancing the release of DA from the nucleus accumbens. In rats with a unilateral lesion in the nigrostriatal DA pathway produced by 6-OHDA, 10 times

higher doses of DN-1417 and TRH than those used in the locomotor experiments produced a circling toward the side with lesion by enhancing the release of DA from the striatum (6). In addition, the bilateral microinjection into the nucleus accumbens of a small dose of DN-1417 (0.5 μ g) or TRH (10 and 20 μ g) markedly enhanced locomotor activity in rats, whereas the unilateral intra-striatal injection of DN-1417 (50 μ g) or TRH (50 μ g) did not induce any circling behavior in intact rats (7). These observations suggest that DN-1417 and TRH have a different affinity for the nucleus accumbens and striatum.

In the present investigation, an *in vitro* study demonstrated that DN-1417 (5×10^{-5} M) or TRH (5×10^{-4} M) enhanced the release of preloaded [3 H]-DA from the nucleus accumbens slices; 100 or 20 times higher concentrations of each compound, respectively, were needed to release DA from the striatal slices. This biochemical evidence supports the results of the behavioral study mentioned above (6, 7). The omission of Ca^{2+} from the superfusion medium or the addition of ouabain, a ($\text{Na}^+ + \text{K}^+$)-ATPase inhibitor, almost completely abolished the DN-1417- and TRH-induced DA releasing effects in the nucleus accumbens slices. The effects of DN-1417 and TRH on $^{45}\text{Ca}^{2+}$ uptake and ($\text{Na}^+ + \text{K}^+$)-ATPase in slices of the nucleus accumbens and striatum were investigated. The addition of DN-1417 or TRH stimulated $^{45}\text{Ca}^{2+}$ uptake into the nucleus accumbens,

Fig. 4. Effects of DN-1417, TRH and A-23187 on $^{45}\text{Ca}^{2+}$ uptake into the nucleus accumbens and striatal slices of rat brain. All values are the mean of 6 determinations. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. each control. ●: Control, ▲: 10^{-4} M DN-1417, ◆: 10^{-4} M TRH, ■: 10^{-6} M A-23187.

Table 1. Effect of ouabain on DN-1417-, TRH- and A-23187-induced $^{45}\text{Ca}^{2+}$ uptake into rat nucleus accumbens slices

Treatments (M)	$^{45}\text{Ca}^{2+}$ uptake (pmol/mg protein/5 min)			
	Normal medium		Ouabain 5×10^{-3} M	
		%		%
Control	596 $\pm$ 41	(100)	602 $\pm$ 59	(100)
DN-1417	10^{-6}	608 $\pm$ 56 (102)	N.D.	
	10^{-5}	707 $\pm$ 117 (119)	N.D.	
	10^{-4}	856 $\pm$ 37*** (143)	746 $\pm$ 82 (124)	
TRH	10^{-6}	676 $\pm$ 67 (113)	N.D.	
	10^{-5}	672 $\pm$ 71 (113)	N.D.	
	10^{-4}	867 $\pm$ 45*** (145)	668 $\pm$ 67 (111)	
A-23187	10^{-6}	869 $\pm$ 25*** (146)	754 $\pm$ 76 (125)	

*** $P < 0.001$ vs. control group. All values are the mean $\pm$ S.E. of 8 experiments.

Table 2. Effects of DN-1417 and TRH on cyclic AMP formation in slices of nucleus accumbens and striatum of rat brain

Treatments	Concentration (M)	Cyclic AMP formation (pmol/mg protein/15 min)			
		Nucleus accumbens		Striatum	
			%		%
Control		21.3±1.9	(100)	17.9±2.3	(100)
DN-1417	10 ⁻⁶	25.8±1.7	(121)	20.2±2.6	(113)
	10 ⁻⁵	22.9±2.3	(108)	17.2±2.0	(96)
	10 ⁻⁴	30.7±2.3**	(144)	19.6±1.9	(109)
TRH	10 ⁻⁵	31.0±5.3	(145)	16.4±1.5	(92)
	10 ⁻⁴	30.3±3.3*	(142)	19.2±2.6	(107)
	10 ⁻³	28.9±2.2*	(136)	20.9±2.4	(117)

*P<0.05, **P<0.01, when compared to the control group. Each value is the mean±S.E. of 8 determinations.

Table 3. Effect of D-600 on DN-1417-, TRH- and A-23187-induced cyclic AMP formation in rat nucleus accumbens slices

Treatments	Concentration (M)	Cyclic AMP formation (pmol/mg protein/15 min)			
		Normal medium		D-600 (10 ⁻⁶ M)	
			%		%
Control		20.8±2.2	(100)	27.4±2.2	(100)
DN-1417	10 ⁻⁶	22.4±2.2	(108)	N.D.	
	10 ⁻⁵	21.6±1.2	(104)	N.D.	
	10 ⁻⁴	29.8±1.2**	(143)	23.6±0.6	(86)
TRH	10 ⁻⁵	19.2±1.4	(92)	N.D.	
	10 ⁻⁴	21.4±1.2	(103)	N.D.	
	10 ⁻³	26.8±0.8*	(129)	22.8±1.2	(83)
A-23187	10 ⁻⁶	28.2±1.2*	(136)	25.0±1.0	(91)

*P<0.05, **P<0.01 vs. control group. All values are the mean±S.E. of 8 experiments. N.D.: Not determined.

Table 4. Effect of DN-1417 on the activity of ATPase in subcellular fractions from rat nucleus accumbens

Fractions	ATPase activity (μmoles/mg protein/hr)			
	Protein levels (%)	Mg ²⁺	Na ⁺ +K ⁺	Ouabain (5 mM)+Na ⁺ +K ⁺
Control				(% inhibition)
Cell debris	14	10.6±2.3	8.7±1.7	3.5±0.6 (60)
Mitochondria	41	12.4±1.8	14.6±3.0	3.6±1.2 (75)
Microsome	24	11.2±2.0	10.8±4.1	3.4±1.0 (68)
Supernatant	21	2.0±0.8	8.6±1.7	4.7±0.6 (45)
DN-1417 (10 ⁻⁴ M)			(% of control)	
Cell debris	14	10.5±2.5	17.2±3.2* (198)	3.2±1.5 (81)
Mitochondria	41	10.0±1.3	19.4±4.0 (133)	3.6±1.6 (81)
Microsome	24	11.4±2.2	10.8±3.3 (100)	4.4±0.8 (60)
Supernatant	21	2.7±1.6	8.6±2.2 (100)	4.8±0.9 (44)

All values are the mean±S.E. of 5 duplicate experiments. *P<0.05 vs. control.

Table 5. Effects of DN-1417 and TRH on the activity of ATPase in cell debris fractions of nucleus accumbens and striatum of rat brain

Treatments	Concentration (M)	ATPase activity (μ moles/mg protein/hr)			
		Nucleus accumbens		Striatum	
		Mg ²⁺	Na ⁺ +K ⁺	Mg ²⁺	Na ⁺ +K ⁺
				%	%
Control		8.2 $\pm$ 1.0	3.3 $\pm$ 0.3 (100)	9.5 $\pm$ 1.6	4.8 $\pm$ 1.0 (100)
DN-1417	10 ⁻⁶	8.2 $\pm$ 0.9	3.9 $\pm$ 0.9 (118)	9.9 $\pm$ 1.5	4.4 $\pm$ 0.5 (92)
	10 ⁻⁵	8.2 $\pm$ 0.6	5.8 $\pm$ 0.9* (176)	10.2 $\pm$ 1.6	4.7 $\pm$ 1.3 (98)
	10 ⁻⁴	8.9 $\pm$ 1.0	7.7 $\pm$ 0.9** (233)	11.1 $\pm$ 2.5	6.6 $\pm$ 1.6 (138)
TRH	10 ⁻⁶	8.6 $\pm$ 0.9	3.1 $\pm$ 0.2 (94)	8.9 $\pm$ 1.2	4.6 $\pm$ 1.2 (96)
	10 ⁻⁵	8.2 $\pm$ 0.8	4.5 $\pm$ 1.2 (136)	10.6 $\pm$ 1.9	6.7 $\pm$ 1.7 (140)
	10 ⁻⁴	8.2 $\pm$ 0.5	6.7 $\pm$ 1.5* (203)	9.7 $\pm$ 1.2	7.8 $\pm$ 2.0 (172)

All values are the mean $\pm$ S.E. of 4 duplicate experiments. *P<0.05, **P<0.01 vs. each control group.

Fig. 5. Effect of ouabain on [³H]-TRH binding in a) the nucleus accumbens and b) striatal slices in rats. All values are the mean $\pm$ S.E.M. of 6 determinations. *P<0.05, **P<0.01, ***P<0.001 vs. specific binding. ○: Total binding, ●: Non-specific binding, ▽: Specific binding, ▲: Specific binding in the presence of ouabain (5 $\times$ 10⁻³ M).

Fig. 6. Effects of DN-1417 and TRH on [³H]-TRH specific binding in a) the nucleus accumbens and b) striatal slices in rats. All values are the mean $\pm$ S.E.M. of 6 determinations. Incubation condition: 37°C for 30 min. △▲: TRH, ○●: DN-1417.

Fig. 7. Proposed mechanism of action of DN-1417 and TRH at presynaptic and postsynaptic sites in the nucleus accumbens and striatum in rats.

but not the striatal slices. Ouabain abolished the DN-1417- and TRH-induced effects. The addition of DN-1417 or TRH activated (Na⁺+K⁺)-ATPase in the P₁ fraction of the nucleus accumbens, but had little effect on the same activity in the P₁ fraction of the striatum. Ouabain also inhibited [³H]-TRH binding in both the nucleus accumbens and striatal slices, but DN-1417 or TRH did not inhibit [³H]-ouabain binding (S. Narumi et al., unpublished observation). From these results, it is speculated that TRH and ouabain do not bind at the same site, and the ouabain blocked some process of [³H]-TRH binding.

Kerwin and Pycock reported that TRH stimulates the release of prelabelled DA from superfused nucleus accumbens, but not superfused striatal slices (12). Horst and Spirt reported that TRH enhances DA release from the synaptosomal fraction of the rat striatum (13). Tashjian et al. demonstrated that TRH stimulates prolactin release from cultured pituitary cells and a Ca²⁺ antagonist, CoCl₂, or a Ca²⁺ chelator, EGTA, blocks TRH-induced prolactin release (14). Gourdji demonstrated that ouabain inhibits TRH binding and blocks the release of prolactin from the same pituitary cells (15). A similar finding regarding the TRH effect on the DA release from the nucleus accumbens slices in rats was obtained in the present study. Burt and Taylor reported that the potency of TRH

binding in the nucleus accumbens is similar to that in the pituitary in sheep (16).

Agarwal et al. reported that intraperitoneal (i.p.) treatment with TRH (2–8 mg/kg) twice a day for 5–15 days increases the tyrosine hydroxylase activity of the brain stem and 5-hydroxytryptamine (5-HT) and DA levels of the cerebral cortex dose- and time-dependently (17). From the fact that TRH, unlike a tricyclic antidepressant, has no effect on [³H]-NE uptake, Reigle et al. stated that the antidepressant effect of TRH is mostly due to an increased synthesis and release of monoamines (18). Green et al. reported that TRH attenuates α -methyl-P-tyrosine induced DA decline, suggesting a decreased DA turnover probably due to an increased DA release of rat brain (19). We reported that DN-1417 (5–20 mg/kg, i.p.) produces marked decreases in the levels of DA, norpinephrine (NE) and 5-HT, especially DA and 5-HT, in various regions of rat brain, and in high dose (20 mg/kg), it increases DA metabolites, especially 3-methyltyramine (3-MT), in the nucleus accumbens and striatum in correspondence with the decreases in DA; the increases in 3-MT in the nucleus accumbens are more marked than that in the striatum (20). 3-MT is the first metabolite of DA after it is released into the synaptic cleft; this reaction is catalyzed by catechol-O-methyltransferase (COMT), which is apparently located at the postsynaptic membranes; 3-MT is then metabolized to homovanillic acid (HVA) by monoamine oxidase (MAO). The increases in 3-MT reflect DA release from the nerve terminal in the nucleus accumbens and striatum. These *in vivo* results are quite consistent with the present findings; the stimulatory effect of DN-1417 on DA release is more sensitive in the nucleus accumbens than in the striatum. We reported that *in vivo* or *in vitro* treatment with DN-1417 increases cyclic AMP levels in various brain regions. Pretreatment with pimozide, a DA receptor blocker, almost completely abolished the DN-1417-induced effect (21). These changes do not appear to be related to activation of adenylate cyclase by DN-1417. DN-1417 seems to exert some central nervous system action by increasing the levels of cyclic AMP through endogenous

dopaminergic activation. In the present report, the effects of DN-1417 and TRH on the different sensitivities of DA release between the nucleus accumbens and the striatum are reflected in the cyclic AMP formation.

The addition of 10^{-6} to 10^{-4} M of DN-1417 or TRH concentration-dependently inhibited the [^3H]-TRH binding in the nucleus accumbens and striatal slices to almost the same extent. This result suggests that DN-1417 and TRH have almost the same affinity for the nucleus accumbens and striatum, but both drugs have a different sensitivity in the process after the TRH binding [($\text{Na}^+ + \text{K}^+$)-ATPase and Ca^{2+} uptake] in both types of slices. With respect to the brain distribution of immunoreactive DN-1417 after intravenous administration of 2.5 mg/kg, the nucleus accumbens, hypothalamus and hippocampus showed a higher concentration, whereas the striatum showed rather lower levels than other brain regions (22).

In conclusion, DN-1417 and TRH activate ($\text{Na}^+ + \text{K}^+$)-ATPase and stimulate Ca^{2+} uptake in mesolimbic dopaminergic sites and release DA which stimulates cyclic AMP formation in the post synaptic sites and caused several central nervous system actions.

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