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L-Dopa-like Regulatory Actions of L-*threo*-3,4-Dihydroxyphenylserine on the Release of Endogenous Noradrenaline Via Presynaptic Receptors in Rat Hypothalamic Slices

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Abstract—Effects of L-*threo*-3,4-dihydroxyphenylserine (L-*threo*-DOPS) on the spontaneous release and the stimulus(2 Hz)-evoked release of endogenous noradrenaline were studied in rat hypothalamic slices with functioning L-aromatic amino acid decarboxylase (AADC) and with AADC inhibition. In non-inhibited slices, spontaneous release was not modified by L-*threo*-DOPS at 1 pM–100 nM, tended to increase at 1–10 μM and increased at 100 μM. Noradrenaline tissue content slightly increased at 100 μM. Stimulated release was concentration-dependently facilitated at 1–1000 pM and tended to decrease gradually from a maximum at 10 nM–10 μM. Under AADC inhibition, spontaneous release concentration-dependently increased at 10–100 μM by 60% of the increase seen in slices without AADC inhibition. Increase in noradrenaline tissue content was abolished. L-*threo*-DOPS produced a triphasic pattern on stimulated release; concentration-dependent facilitation at 1–1000 pM similar to that seen in slices with functional AADC, no facilitation at 10–1000 nM, and a concentration-dependent increment at 10–100 μM. The facilitation at 1 nM was stereoselective and was antagonized by (−)-propranolol 10 nM, and no facilitation at 100 nM was restored to the maximum by yohimbine 10 nM, DG-5128 10 nM or *S*-sulpiride 1 nM. Furthermore, L-*threo*-DOPS (1–1000 pM)-induced facilitation was competitively antagonized by L-dopa methyl ester, a competitive antagonist for L-dopa, with a pA_2 value of 13·6, whereas it was noncompetitively antagonized by (−)-propranolol.

L-Dihydroxyphenylalanine (L-dopa) is believed to be a pharmacologically inert amino acid which exerts its actions via its conversion to dopamine by L-aromatic amino acid decarboxylase (AADC). Contrary to this generally accepted idea, depolarizing stimuli released endogenous L-dopa in a transmitter-like manner from rat striatal slices (Goshima et al 1988; Misu et al 1990). The transmitter-like basal release of endogenous L-dopa was seen under physiological conditions in striata of freely moving rats (Nakamura et al 1992). On the other hand, the nanomolar concentrations of exogenous L-dopa stereoselectively facilitate the impulse-evoked release of endogenous dopamine and noradrenaline via presynaptic β-adrenoceptors from rat brain slices even under essentially complete inhibition of AADC (Goshima et al 1986, 1990, 1991b; Misu et al 1986). Facilitation of the dopamine release was also seen in striatal slices from 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-treated C57 Black mouse model for parkinsonism (Arai et al 1990; Goshima et al 1991a). In rat hypothalamic slices, this facilitation of the noradrenaline release was competitively antagonized by L-dopa methyl ester, a prodrug for L-dopa (Cooper et al 1987), whereas it was noncompetitively antagonized by (−)-propranolol (Goshima et al 1991b). Furthermore, picomolar concentrations of L-dopa stereoselectively potentiated the isoprenaline-induced facilitation of the evoked noradrenaline release (Goshima et al 1991c). This potentiation was selectively antagonized by L-dopa methyl ester, whereas (−)-propranolol antagonized both the facilitation by isoprenaline alone and its potentiation by L-dopa. Thus, L-dopa may act on the recognition site for itself, leading to potentiation of the activities of presynaptic β-adrenoceptors. On the other hand, in rat brain slices with functional AADC, submicromolar to higher concentrations of L-dopa produced its conversion to dopamine (Goshima et al 1986, 1990; Misu et al 1986). However, in slices under AADC inhibition, moderate micromolar concentrations of L-dopa inhibited the evoked release of noradrenaline and dopamine via presynaptic D_2 receptors without increases in the spontaneous dopamine release. We have proposed that L-dopa is an endogenous neuroactive substance in rat CNS.

L-*threo*-3,4-Dihydroxyphenylserine (L-*threo*-DOPS) has characteristics similar to L-dopa and has a catechol moiety, and amino- and carboxy-groups essential to the agonist activity of L-dopa (Goshima et al 1991b). L-*threo*-DOPS is decarboxylated to yield noradrenaline by AADC and the actions are thought to be via its decarboxylation (Bartholini et al 1975; Inagaki & Tanaka 1978; Kato et al 1987). L-*threo*-DOPS is effective in treating akinesia and in arresting Parkinson's disease (Narabayashi et al 1981). Micromolar concentrations of L-*threo*-DOPS inhibited the evoked release of [^{3}H]noradrenaline and higher concentrations increased spontaneous [^{3}H]noradrenaline efflux in guinea-pig cortical and hypothalamic slices (Nishino et al 1987), whereas picomolar concentrations mimicked the action of L-dopa to facilitate the evoked release of endogenous noradrenaline in rat hypothalamic slices (Goshima et al 1991b).

Thus, we have attempted to clarify whether or not picomolar to submillimolar concentrations of L-*threo*-DOPS mimic the actions of L-dopa on the release of endogenous noradrenaline and to characterize these actions in rat hypothalamic slices with and without functioning AADC.

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Materials and Methods

Male Sprague-Dawley rats, 7–8 weeks old, were decapitated and their brains were placed on ice. The hypothalamus was dissected out and sliced sagitally 0·3 mm in thickness using a McIlwain tissue chopper. Four slices, approximately 30 mg, were transferred to a glass chamber (8 × 11·5 mm) and superfused with Krebs-Henseleit medium bubbled with 95% O_2–5% CO_2 in an overflow manner at the rate of 0·45 mL min^{-1} at 37°C, in the presence of cocaine 20 μM, an uptake blocker for valid measurement of the release of endogenous noradrenaline (Ueda et al 1983). The composition of the medium was as follows (mM): NaCl 113, $NaHCO_3$ 25, $CaCl_2$ 2·52, KCl 4·75, KH_2PO_4 1·18, $MgSO_4$ 1·19, glucose 11·2, disodium EDTA 0·029 and ascorbic acid 0·29. Supramaximal electrical field stimulation (2 Hz, 2 ms, 25 V, 3 min, alternative polarity) was performed 60 (S_1) and 90 (S_2) min after the start of superfusion through platinum spiral electrodes set up at the two ends of the chamber, using an electrical stimulator with an isolator (SEN-3201 and SS-201, Nihon Kohden, Tokyo, Japan). Superfusates were collected every 3 min. The stimulus-evoked release (S) of noradrenaline during S_1 and S_2 periods of stimulation was calculated as the total minus the basal release for 9 min. The basal release was estimated by interpolation between the amounts in a sample immediately before and in the 4th sample after stimulation. The amount of noradrenaline in the former sample was regarded as the spontaneous release (Sp). Cocaine 20 μM and 3-hydroxybenzylhydrazine (NSD-1015) 20 μM was applied 60 and 20 min before S_1 and continued throughout the experiments. L- or D-*threo*-DOPS was applied to the medium 15 min before S_2, and the effects were evaluated by the ratios of the noradrenaline release, S_2/S_1 and Sp_2/Sp_1, and the tissue contents of noradrenaline at the end of the superfusion experiments. Pretreatment with L-dopa methyl ester or (−)-propranolol was initiated 10 min before and that with yohimbine, 2-[2-(4,5-dihydro-1H-imidazol-2-yl)-1-phenyl ethyl] pyridine dihydrochloride sesquihydrate (DG-5128) or *S*-sulpiride was initiated 60 min before S_1 and continued throughout the experiments.

Measurement of noradrenaline was made as described previously (Ueda et al 1983). Superfusates were transferred to polypropylene test tubes containing activated alumina 10 mg, disodium EDTA 10 mg and dihydroxybenzylamine 2 pmol as an internal standard, 0·12 mL of 1·5 M Tris-HCl buffer (pH 8·6) was added to the tubes and the preparations were placed in a mixer for 10 min. The supernatant was discarded and the alumina was washed three times with water. Adsorbed noradrenaline was eluted from the alumina with 0·1 mL of 0·1 M HCl. Eluted noradrenaline was measured by HPLC with an electrochemical detector (ECD). Details of the chromatographic conditions are as follows: column, $5C_{18}$ (4·6 × 250 mm); mobile phase, 0·1 M phosphate potassium buffer (pH 6·0) containing 11% methanol, 0·9 mM (−)-heptane-sulphonate sodium and 1 mM disodium EDTA; applied potential, 500 mV vs Ag/AgCl; flow rate, 1·0 mL min^{-1}. After the superfusion experiments, the slices were homogenized in 0·5 mL of 0·1 M $HClO_4$, centrifuged (12000 rev min^{-1}) and 0·15 mL portions of the supernatant were used for the determination of the tissue content of noradrenaline.

Drugs used were NSD-1015 (Aldrich, Inc., Milwaukee, WI, USA), D- and L-*threo*-DOPS (Sumitomo Pharmaceuticals Co. Ltd, Osaka, Japan), L-dopa methyl ester (Research Biochemicals Inc., Natick, MA, USA), yohimbine (Nakalai Tesque Ltd, Kyoto, Japan), DG-5128 (Daiichi Pharmaceutical Co. Ltd, Tokyo, Japan), *S*-sulpiride (Mitsui Pharmaceuticals Inc., Tokyo, Japan), and (−)-propranolol hydrochloride (Imperial Chemical Industries, Wilmslow, Cheshire, UK). The chromatographic peaks of noradrenaline were not interfered with by these drugs at concentrations below 0·1 mM.

Data shown are mean ± s.e.m. Statistical significance was calculated using unpaired Student's *t*-test (two-tailed) and Dunnett's multiple comparison test.

Results

Spontaneous release, evoked release and tissue content of endogenous noradrenaline in rat hypothalamic slices

The release of endogenous noradrenaline in superfusates was consistently detectable with HPLC-ECD. The spontaneous release was stabilized 60 min after the start of superfusion. The spontaneous release and the evoked release at S_1 in the presence of 20 μM cocaine was 2·26 ± 0·12 and 6·06 ± 0·38 pmol (mg wet wt)$^{-1}$ min^{-1} (n = 34), respectively. The control ratios, Sp_2/Sp_1 and S_2/S_1 were 0·68 ± 0·02 and 0·83 ± 0·05 (n = 6), respectively. At the end of the superfusion experiments, the tissue content of noradrenaline was 6·27 ± 0·23 pmol (mg wet wt)$^{-1}$ (n = 6). Pretreatment with cocaine and 20 μM NSD-1015, an AADC inhibitor, produced no modifications of Sp_1 and S_1 (2·37 ± 0·11 and 5·77 ± 0·42 pmol (mg wet wt)$^{-1}$ min^{-1}, n = 40, respectively), Sp_2/Sp_1 and S_2/S_1 (0·68 ± 0·03 and 0·76 ± 0·03, n = 6), or the tissue content of noradrenaline (5·83 ± 0·24 pmol (mg wet wt)$^{-1}$, n = 5) at the end of superfusion experiments.

Effects of L-threo-*DOPS on the spontaneous release, evoked release and tissue content of endogenous noradrenaline in the absence and presence of NSD-1015 20 μM*

In control slices (Fig. 1A), L-*threo*-DOPS at 1 pM–1 nM facilitated the evoked release of noradrenaline in a concentration-dependent manner without modification of the spontaneous release. At 0·1–10 μM, however, the evoked release tended to decrease gradually from the maximal facilitation at 1 nM with a tendency of an increase in the spontaneous release. The highest concentration of L-*threo*-DOPS 100 μM again tended to increase the evoked release of noradrenaline with a marked increase in the spontaneous release. Only this concentration slightly increased the tissue content of noradrenaline (Table 1).

In the presence of 20 μM NSD-1015 (Fig. 1B), the concentration-effect curve for L-*threo*-DOPS on the evoked release of noradrenaline showed a triphasic pattern. The concentration-dependent facilitation at 1 pM–1 nM without modifications of the spontaneous release was similar to that in control slices. On the other hand, D-*threo*-DOPS 1 nM produced no modifications of the spontaneous and evoked release of noradrenaline. No facilitation was seen at 10–1000 nM without modification of the spontaneous release and the values at 10–1000 nM differed from the peak facilitation at 1 nM. The value at 100 nM was also different from that in control slices. There was again a concentration-dependent

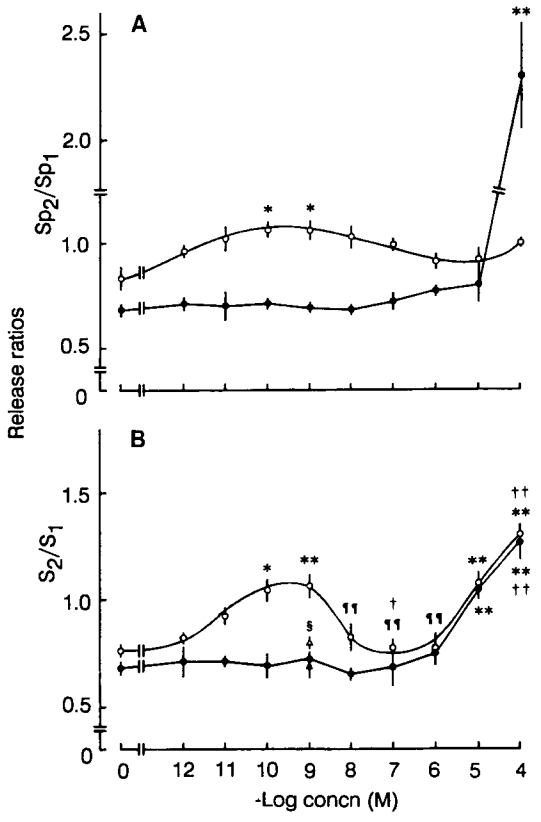

FIG. 1. Log concentration-effect curves for L-*threo*-DOPS on the spontaneous release (Sp, ●) and the evoked release (S, ○) of endogenous noradrenaline from rat hypothalamic slices in the absence (A) and presence (B) of NSD-1015 20 μM. The slices were superfused with Krebs-Henseleit medium containing 20 μM cocaine. Electrical field stimulation by biphasic pulses of 2 Hz, 2 ms, 25 V for 3 min was done twice, 60 (S_1) and 90 (S_2) min, after the start of superfusion. L- or D-*threo*-DOPS was applied 15 min before S_2 for 27 min and the effect was evaluated by calculating the ratio of Sp and S immediately before and during the S_2 and S_1 periods of stimulation, Sp_2/Sp_1 and S_2/S_1, respectively, as indicated in ordinates. In B, 20 μM NSD-1015 was applied 20 min before S_1 and was present throughout the experiments. The effect of D-*threo*-DOPS on Sp (▲) and S (△) is also shown. Each point represents the mean ±s.e.m. (n = 3–11). *$P < 0.05$, **$P < 0.01$, compared with the corresponding control at 0 M; ¶¶$P < 0.01$, compared with the value at 1 nM (Dunnett's multiple comparison test); §$P < 0.05$, compared with L-*threo*-DOPS; †$P < 0.05$, ††$P < 0.01$, compared with the corresponding value in A (Student's *t*-test).

increment of the evoked release of noradrenaline at 10–100 μM with a moderate and concentration-dependent increase in the spontaneous release. This increase in the spontaneous release at the highest concentration was approximately 60% of that in control slices. This highest concentration-induced increase in the tissue content of noradrenaline was abolished by pretreatment with NSD-1015 (Table 1).

Effects of (−)-propranolol, yohimbine, DG-5128 and S-sulpiride on the L-threo-*DOPS (1 nM)-induced facilitation of the evoked release of noradrenaline in the presence of NSD-1015 20 μM*

Pretreatment with (−)-propranolol 10 nM completely antagonized L-*threo*-DOPS (1 nM)-induced facilitation of the evoked release of noradrenaline (Fig. 2). On the other hand, yohimbine 10 nM, DG-5128 10 nM or *S*-sulpiride 1 nM

Table 1. Effect of L-*threo*-DOPS on the tissue noradrenaline after the end of superfusion experiments in the absence (control) and presence of NSD-1015 20 μM. Each value represents the mean ±s.e.m. (n = 3–11). Other details are as in Fig. 1.

	Tissue content of noradrenaline (pmol (mg wet wt)$^{-1}$)	
Pretreatment	Control	NSD-1015
L-*threo*-DOPS (nM)		
0	6·27 ± 0·23	5·83 ± 0·24
10^{-3}	6·83 ± 0·49	6·01 ± 0·70
10^{-2}	5·90 ± 0·59	5·68 ± 0·28
10^{-1}	6·08 ± 0·64	5·73 ± 0·22
1	6·25 ± 0·22	6·01 ± 0·24
10	6·55 ± 0·18	6·06 ± 0·21
10^2	6·06 ± 0·18	5·72 ± 0·13
10^3	6·12 ± 0·31	5·82 ± 0·08
10^4	6·30 ± 0·48	6·04 ± 0·58
10^5	7·41 ± 0·40*	5·87 ± 0·34†

* $P < 0.05$, compared with the value of 0 nM (Dunnett's multiple comparison test), † $P < 0.05$, compared with the corresponding control (Student's *t*-test).

produced no modification. These pretreatments produced no effect on Sp_1, S_1, Sp_2/Sp_1, S_2/S_1 and the tissue contents of noradrenaline.

Effects of yohimbine, DG-5128 and S-sulpiride on the L-threo-*DOPS (100 nM)-induced facilitation of the evoked release of noradrenaline in the presence of NSD-1015 20 μM*

The respective pretreatment with yohimbine 10 nM, DG-5128 10 nM and *S*-sulpiride 1 nM unmasked the facilitatory effect of L-*threo*-DOPS 100 nM on the evoked noradrenaline

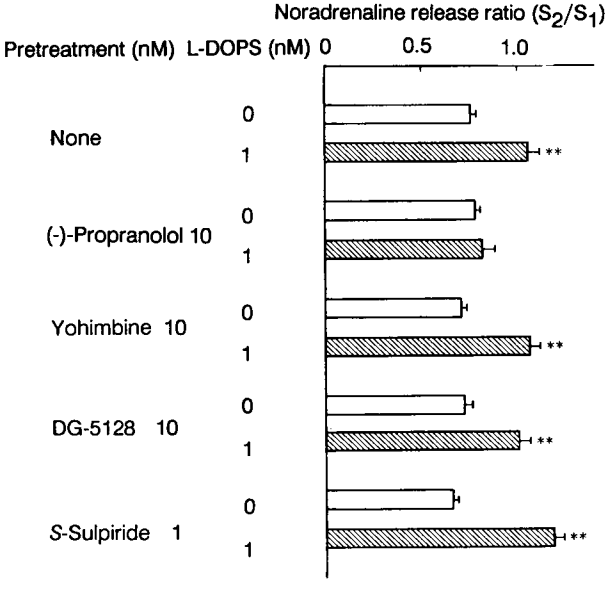

FIG. 2. Antagonizing effects of (−)-propranolol, yohimbine, DG-5128 and *S*-sulpiride against the L-*threo*-DOPS (L-DOPS, 1 nM)-induced facilitation of the evoked release of endogenous noradrenaline from rat hypothalamic slices in the presence of NSD-1015 20 μM. Pretreatment with 10 nM (−)-propranolol was initiated 10 min before and with 10 nM yohimbine, 10 nM DG-5128 or 1 nM *S*-sulpiride was initiated 60 min before S_1 and continued throughout the experiments. Data shown are mean ±s.e.m. (n = 4–11). **$P < 0.01$, compared with the corresponding 0 nM (Student's *t*-test). Other details are as in Fig. 1.

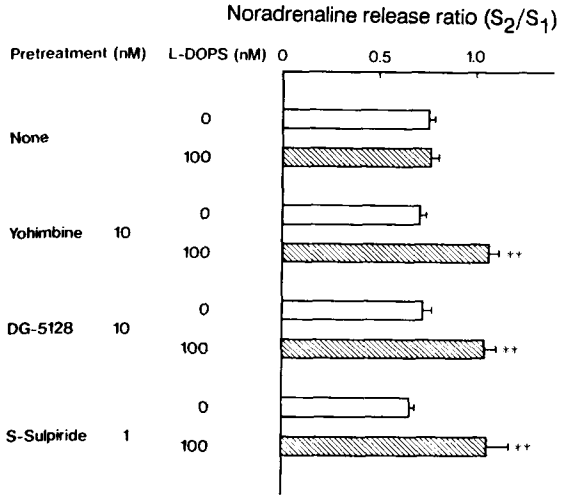

FIG. 3. Yohimbine-, DG-5128- and *S*-sulpiride-induced restoration of the facilitatory effect of 100 nM L-*threo*-DOPS on the evoked release of endogenous noradrenaline from rat hypothalamic slices in the presence of NSD-1015 20 μM. Data shown are mean ±s.e.m. (n=4–11). $^{**}P<0.01$, compared with the corresponding 0 nM (Student's *t*-test). Other details are as in Fig. 2.

release from a control level to the level of the maximal facilitation at 1 nM (Fig. 3).

Competitive antagonism by L-dopa methyl ester against L-threo-DOPS-induced facilitation of the evoked release of noradrenaline in the presence of NSD-1015 20 μM

The concentration-facilitation curve for L-*threo*-DOPS at 1–1000 pM was progressively shifted to the right in the presence of increasing concentrations of L-dopa methyl ester 0·03, 0·1, 0·3 and 1 pM (Fig. 4A). The Schild plots gave a straight line with a slope of 1·00. The pA_2 extrapolated from the Schild plots was 13·65 (Fig. 4B).

Noncompetitive antagonism by (−)-propranolol against L-threo-DOPS-induced facilitation of the evoked release of noradrenaline in the presence of NSD-1015 20 μM

(−)-Propranolol 0·1, 1 and 10 nM progressively reduced the L-*threo*-DOPS-induced maximum facilitation of the evoked release of the noradrenaline, without the rightward shift of the curve (Fig. 5).

Discussion

Picomolar concentrations of L-*threo*-DOPS mimicked the action of the nanomolar concentrations of L-dopa to facilitate the evoked release of noradrenaline and dopamine from rat brain slices (Goshima et al 1986, 1990, 1991b; Misu et al 1986) as follows.

L-*threo*-DOPS concentration-dependently facilitated the evoked release of endogenous noradrenaline in rat hypothalamic slices with functional AADC and even with AADC inhibition. The results with AADC inhibition are consistent with previous findings (Goshima et al 1991b). These results indicate that this facilitation is not via the conversion to noradrenaline by AADC (Bartholini et al 1975; Inagaki & Tanaka 1978; Kato et al 1987) but is due to L-*threo*-DOPS itself, since this facilitation was seen at concentrations lower than those needed to elicit increases in the spontaneous noradrenaline release as an indicator of its conversion, and NSD-1015 (20 μM) inhibits AADC noncompetitively by 99·6% in these slices (Goshima et al 1990). However, our findings are not consistent with those showing no effects of nanomolar concentration of L-*threo*-DOPS on the evoked release of ^3H from guinea-pig cortical or striatal slices

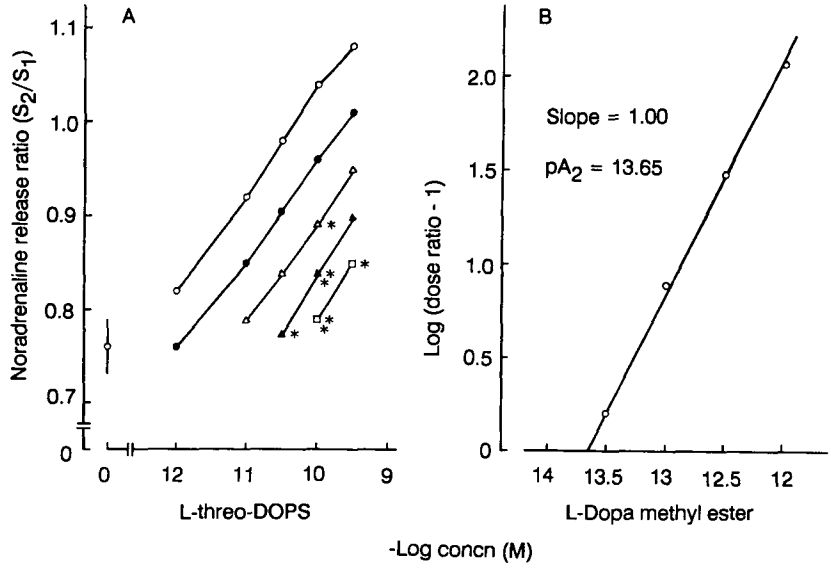

FIG. 4. Competitive antagonism by L-dopa methyl ester against L-*threo*-DOPS-induced facilitation of the evoked release of endogenous noradrenaline from rat hypothalamic slices in the presence of NSD-1015 20 μM. Pretreatment with L-dopa methyl ester was initiated 10 min before S_1. A. Concentration-release curves for L-*threo*-DOPS in the absence (○, control) and presence of L-dopa methyl ester at 0·03 (●), 0·1 (△), 0·3 (▲) and 1 pM (□). Each point is the mean (n = 3–11). The bars of s.e.m. are omitted for clarity. $^{*}P<0.05$, $^{**}P<0.01$, compared with the corresponding control (Dunnett's multiple comparison test). Other details are as in Fig. 1. B. Schild plots for the antagonism in A. The regression of log (dose ratio (r) − 1) on -log concn (L-dopa methyl ester) (M) yields a straight line with a slope = 1·00, and a pA_2 of 13·65.

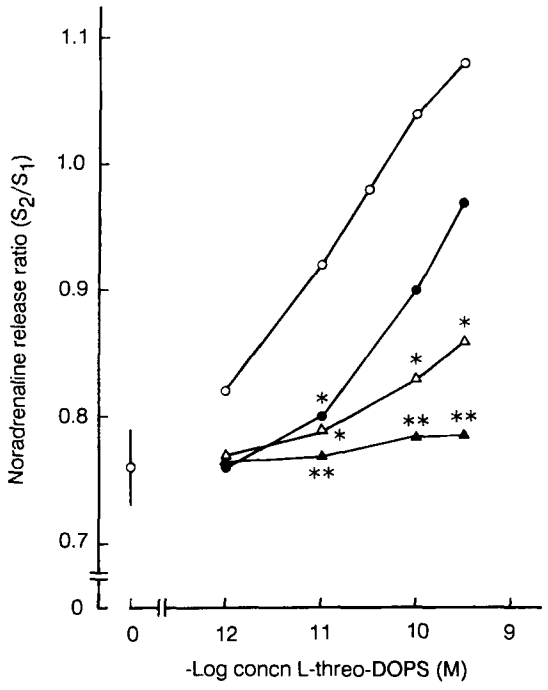

FIG. 5. Noncompetitive antagonism by (−)-propranolol against the L-*threo*-DOPS-induced facilitation of the evoked release of endogenous noradrenaline from rat hypothalamic slices in the presence of NSD-1015 20 μM. Concentration-release curves for L-*threo*-DOPS in the absence (○, control) and presence of (−)-propranolol at 0·1 nM (●), 1 nM (△) and 10 nM (▲). (−)-Propranolol was initiated 10 min before S_1. Each point is the mean ($n = 3$–11). $^*P < 0.05$, $^{**}P < 0.01$, compared with the corresponding control (Dunnett's multiple comparison test). Other details are as in Fig. 4.

preloaded with [^{3}H]noradrenaline or [^{3}H]dopamine (Nishino et al 1987). The reason for this discrepancy is not clear.

L-*threo*-DOPS produced this facilitation in a propranalol-sensitive manner. This amino acid facilitates the noradrenaline release via presynaptic β-adrenoceptors on the noradrenergic nerve terminals (Misu & Kubo 1986). These adrenoceptors tonically and stereoselectively function in rat hypothalamus (Ueda et al 1983, 1985).

L-Dopa methyl ester, a prodrug for L-dopa (Cooper et al 1987) and a potent competitive antagonist acting on the recognition site for L-dopa itself to facilitate the evoked noradrenaline release (Goshima et al 1991b), also antagonized competitively the L-*threo*-DOPS-induced facilitation. This interaction is due to the actions of two drugs themselves, since the experiments were done under almost complete AADC inhibition (Goshima et al 1990). Furthermore,(−)-propranolol antagonized noncompetitively the L-*threo*-DOPS-induced facilitation, as did this antagonist against L-dopa. There exists a recognition site for L-*threo*-DOPS, which differs from presynaptic β-adrenoceptors. All results indicate the similarity between L-*threo*-DOPS and L-dopa. However, Schild plots for the antagonism by L-dopa methyl ester gave a pA_2 of 13·6 against L-*threo*-DOPS and of 8·9 against L-dopa (Goshima et al 1991b), although previous experiments were done under different conditions. It is uncertain whether or not the recognition site for L-*threo*-DOPS differs from that for L-dopa. Importantly, this recognition site for L-*threo*-DOPS is stereoselective in nature, in common with that for L-dopa, and has many receptors, since D-*threo*-DOPS produced no facilitation.

At the higher concentrations, L-*threo*-DOPS produced a complex pattern. In slices with functional AADC, the spontaneous noradrenaline release tended to increase at micromolar concentrations and increased at the highest concentration (100 μM). Only this concentration slightly increased the tissue noradrenaline content. These increases are less marked, compared with L-dopa (Goshima et al 1986); the spontaneous dopamine release increases at concentrations of L-dopa higher than 0·1 μM and the tissue dopamine content markedly increases at 10 μM. L-*threo*-DOPS is a weaker substrate for AADC compared with L-dopa (Inagaki & Tanaka 1978, Kato et al 1987). L-*threo*-DOPS (100 μM)-induced increase in the spontaneous release of endogenous noradrenaline is consistent with the finding by Nishino et al (1987) that these high concentrations produced an increase in the spontaneous efflux of [^{3}H]noradrenaline in guinea-pig cortical and hypothalamic slices. They who suggested that this increase is mainly due to the conversion to noradrenaline and a displacement mechanism. This increase in endogenous noradrenaline, however, was suppressed by approximately 60% by NSD-1015. The minor 40% component reflects the conversion to noradrenaline by AADC (Bartholini et al 1975; Inagaki & Tanaka 1978; Kato et al 1987) and the displacement mechanism. The slight increase in the tissue noradrenaline content was also abolished by NSD-1015. The major 60% component of the increase in the spontaneous release remaining after AADC inhibition might be explained by actions other than the conversion. A probable factor seems to be a competitive inhibitory action of L-*threo*-DOPS itself on monoamine oxidase A (Naoi & Nagatsu 1986).

In slices with functional AADC, L-*threo*-DOPS (10 nM–100 μM) did not increase the evoked noradrenaline release but tended to decrease it from the peak facilitation (at 1 nM) at the moderate concentrations and again tended to increase at the highest concentration. This parameter may continue to increase via activation of presynaptic β-adrenoceptors or via its conversion to noradrenaline. Possible inhibitory mechanisms may reduce the evoked noradrenaline release. Indeed, under essentially complete AADC inhibition (Goshima et al 1990), the evoked noradrenaline release decreased from the peak to a control level at moderate concentrations (10–1000 nM). Furthermore, pretreatment with yohimbine or DG-5128, a selective α_2-adrenoceptor antagonist (Muramatsu et al 1983), or with *S*-sulpiride, a selective D_2-receptor antagonist (Kebabian & Calne 1979), restored the action of L-*threo*-DOPS (100 nM) from the control level to the peak. Presynaptic inhibitory α_2-adrenoceptors and D_2 receptors on the noradrenergic nerve terminals function tonically in rat hypothalamus (Ueda et al 1983; Misu et al 1985). L-*threo*-DOPS itself seems to reduce the evoked noradrenaline release via α_2-adrenoceptors or D_2 receptors. A level of the L-*threo*-DOPS-induced modification of the evoked noradrenaline release may be determined by balance between decrease via these inhibitory receptors and increase via facilitatory β-adrenoceptors and via the possible conversion to noradrenaline suggested by a tendency of increase in the spontaneous noradrenaline release. These apparent inhibitory actions of L-*threo*-DOPS show a similarity in some degree to those of L-dopa (Goshima et al 1986; Misu et al 1986). However,

moderate micromolar concentrations of L-dopa consistently inhibit the evoked release of noradrenaline and dopamine from rat brain slices via stereoselective presynaptic D_2 receptors under AADC inhibition. The present results are apparently consistent with the findings that L-*threo*-DOPS (5 μM) inhibited the evoked release of [^{3}H]noradrenaline, and the inhibition tended to be antagonized by yohimbine or *S*-sulpiride in guinea-pig cortical slices (Nishino et al 1987). Nishino et al (1987) suggested that as noradrenaline and dopamine are displaced by L-*threo*-DOPS, and then act on inhibitory α_2- and D_2 autoreceptors. Our interpretation is as follows. L-*threo*-DOPS itself reduces the evoked noradrenaline release via presynaptic inhibiting α_2-adenoceptors or D_2 receptors. At the higher concentrations (10–100 μM) of L-*threo*-DOPS, the evoked noradrenaline release increased under almost complete AADC inhibition (Goshima et al 1990). In general, however, the evoked release of a transmitter cannot be exactly estimated under the conditions accompanying modifications of the spontaneous release. This apparent increase may be mainly due to the inhibitory action of L-*threo*-DOPS itself on monoamine oxidase A (Naoi & Nagatsu 1986) but not due to the conversion to noradrenaline.

In conclusion, in rat hypothalamic slices, picomolar to-nanomolar concentrations of L-*threo*-DOPS itself, facilitate the evoked noradrenaline release via presynaptic β-adrenoceptors. The recognition site for L-*threo*-DOPS differs from these adrenoceptors. The nanomolar to micromolar concentrations inhibit the evoked noradrenaline release via presynaptic α_2- or D_2 receptors. These biphasic regulatory actions may occur without decarboxylation in a manner similar to L-dopa. The higher concentrations partially increase the spontaneous release and tissue content of noradrenaline via its conversion and major increases in the spontaneous and evoked noradrenaline release may be explained by other actions.

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