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Biphasic actions of L-DOPA on the release of endogenous noradrenaline and dopamine from rat hypothalamic slices

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1 Effects of L-DOPA on the release of endogenous noradrenaline and dopamine from rat hypothalamic slices evoked by electrical field stimulation at 5 Hz were investigated in the absence and presence of *p*-bromobenzyloxyamine (NSD-1055), a DOPA-decarboxylase inhibitor.

2 In the absence of NSD-1055, L-DOPA produced a facilitation of impulse-evoked release of noradrenaline at 0.1 μM but not at 1 and 10 μM, and had no effect on the spontaneous release. On the other hand, L-DOPA 0.1 to 10 μM dose-dependently increased the spontaneous release of dopamine and the highest concentration only increased the evoked release and tissue content of dopamine.

3 In the presence of NSD-1055 10 μM, the increase in the spontaneous release of dopamine was prevented and L-DOPA produced biphasic regulatory effects on the evoked release of noradrenaline and dopamine, a facilitation at 0.1 μM and an inhibition at 1 μM. The facilitation was antagonized by (−)-propranolol 0.1 μM, but not by the (+)-isomer, whereas the inhibition was antagonized by S-sulpiride 1 nM, but not by the **R**-isomer.

4 In conclusion, L-DOPA appears to produce biphasic actions on the release of endogenous noradrenaline and dopamine from rat hypothalamic slices, not through its conversion to dopamine but through presynaptic regulatory mechanisms, an inhibition via dopamine receptors at a micromolar concentration and a facilitation via β-adrenoceptors at the lower concentration.

Introduction

The release of endogenous catecholamines from rat hypothalamic slices is regulated by presynaptic catecholamine receptors (Ueda *et al.*, 1983a,b; 1984; 1985; Goshima *et al.*, 1985a; Misu *et al.*, 1985). Stereoselective and tonically functioning presynaptic facilitatory β-adrenoceptors and inhibitory dopamine receptors on the noradrenergic neurone terminals (Ueda *et al.*, 1983a; 1985; Misu *et al.*, 1985) and on the dopaminergic neurone terminals (Ueda *et al.*, 1983a,b; 1984) have been shown to exist in the rat hypothalamus. The present work is concerned with the actions of exogenously applied L-DOPA on these receptors. It is generally accepted that the effects of L-DOPA are mediated through its conversion to dopamine by an aromatic amino acid decarboxylase (DOPA-decarboxylase) (Hefti & Melamed, 1980), and that this conversion initiates the resultant phenomena such as accumulation of striatal dopamine in rats (Ponzio *et al.*, 1983) and humans (Lloyd *et al.*, 1975), displacement of [^{3}H]-dopamine (Ng *et al.*, 1970), impulse-evoked release of radioactive dopamine (Ng *et al.*, 1971) from rat brain slices and increases in the content of the dopamine metabolite, 3-methoxytyramine, formed in the synaptic cleft, in the rat striatum (Ponzio *et al.*, 1983). Through this conversion, L-DOPA was thought to induce via presynaptic dopamine receptors, an inhibition of cardiac acceleration elicited by electrical stimulation of right postganglionic cardiac sympathetic nerves in dogs (Lokhandwala & Buckley, 1978) and also a decreased firing rate of cells in the zona compacta and ventral tegmental areas of rats (Bunney *et al.*, 1973), since these actions were similar to those of dopamine (Lokhandwala & Buckley, 1978) and apomorphine (Bunney *et al.*, 1973) and disappeared in the presence of DOPA-decarboxylase inhibitors.

On the other hand, little is known about the effects of L-DOPA which differ from those of dopamine (Sharma & Fahn, 1979). We demonstrated that dopamine produced biphasic presynaptic actions on the release of noradrenaline from rat hypothalamic slices, a facilitation via β-adrenoceptors at micromolar concentrations and an inhibition via dopamine receptors at the lower concentrations (Misu *et al.*, 1985).

Table 1 Effects of L-DOPA on the release of endogenous noradrenaline and dopamine from rat hypothalamic slices

Experimental group	μM	n	Release: Noradrenaline S_2/S_1	Noradrenaline Sp_2/Sp_1	Dopamine S_2/S_1	Dopamine Sp_2/Sp_1
a Control		14	0.86 ± 0.05	0.80 ± 0.04	0.91 ± 0.07	0.71 ± 0.04
L-DOPA	0.01	6	1.00 ± 0.09	0.80 ± 0.04	0.93 ± 0.06	0.67 ± 0.10
	0.1	5	1.21 ± 0.14*	0.74 ± 0.03	1.15 ± 0.10	1.40 ± 0.15**
	1	5	0.90 ± 0.10	0.87 ± 0.08	1.03 ± 0.15	3.91 ± 1.22**
	10	5	0.94 ± 0.14	0.71 ± 0.10	5.34 ± 0.80**	21.51 ± 3.91**
b Control		9	0.80 ± 0.07	0.85 ± 0.03	0.78 ± 0.06	0.83 ± 0.08
L-DOPA	0.01	6	0.92 ± 0.06	0.81 ± 0.04	0.72 ± 0.09	0.79 ± 0.14
	0.1	7	1.10 ± 0.10*	0.76 ± 0.05	1.05 ± 0.08*	0.66 ± 0.05
	1	8	0.55 ± 0.07*	0.88 ± 0.06	0.54 ± 0.05*	0.76 ± 0.03

(a) Slices were superfused with Krebs medium containing cocaine 20 μM. Electrical field stimulations (5 Hz, 2 ms, 30 mA, 3 min) were performed at 60 (S_1) and 90 (S_2) min after the start of superfusion. L-DOPA was applied to the medium 15 min before S_2 for 27 min and the effect was evaluated by calculating the ratio of the evoked release (S) and the spontaneous release (Sp) during and immediately before the S_2 and S_1 periods of stimulation, S_2/S_1 and Sp_2/Sp_1, respectively. (b) The experiments were identical to (a) except that NSD-1055 10 μM was applied to medium at the start of superfusion and was present throughout the experiments. Values are mean ± s.e. from *n* estimations. Statistical significance: $*P<0.05$ and $**P<0.01$, compared with control.

However, in the present experiments we found that the same lower concentration of L-DOPA as that of dopamine inversely facilitated the release of noradrenaline. Hence, we further investigated whether or not L-DOPA produces biphasic actions via presynaptic regulatory mechanisms on the release of noradrenaline and dopamine in the absence and presence of *p*-bromobenzyloxyamine (NSD-1055), a DOPA-decarboxylase inhibitor (Hirata *et al.*, 1983). Part of this work was presented at the Ninth Annual Meeting of Japan Neuroscience Society (Goshima *et al.*, 1985b).

Methods

Male Sprague-Dawley (8 weeks old) rats were decapitated and their brains placed on ice. The hypothalamus was dissected out using the method of Glowinski & Iversen (1966) and cut sagittally into six pieces. These slices were transferred to a glass chamber (10 mm × 5 mm) and superfused in an overflow manner at a rate of 0.45 ml min^{-1} at 37°C with Krebs-Henseleit medium, bubbled with 95% O_2 and 5% CO_2, in the presence of cocaine 20 μM as described previously (Ueda *et al.*, 1983a,b). The Krebs-Henseleit solution had the following composition (mM): NaCl 113, $NaHCO_3$ 25, KCl 4.75, KH_2PO_4 1.18, $CaCl_2$ 2.52, $MgSO_4$ 1.19, glucose 11.1, disodium EDTA 0.029 and ascorbic acid 0.29.

Electrical field stimulation was by impulses of 5 Hz, 2 ms duration and 30 mA for 3 min through platinum spiral electrodes set up at the 2 ends of the chamber, using an electrical stimulator with an isolator (SEN-3201 and SS-120J, Nihon Kohden), at 30 (S_0 as a test stimulation), 60 (S_1) and 90 (S_2) min after the start of superfusion and 3 min samples of the superfusates were successively collected throughout the experiments. The impulse-evoked release (S) of noradrenaline and dopamine during the S_1 and S_2 periods of stimulation was estimated by calculating the total amount released minus the basal release for 9 min. The spontaneous release (Sp) during S_1 and S_2 was estimated as the amount released for 3 min immediately before stimulation. L-DOPA was added to the medium 15 min before S_2, and the effects were evaluated by means of the ratios S_2/S_1 and Sp_2/Sp_1. Pretreatment with NSD-1055 or the antagonists used was initiated at the start of superfusion and was continued throughout the experiments.

Measurement of noradrenaline and dopamine was made as described previously (Ueda *et al.*, 1983a,b). Samples of superfusates were transferred to polypropylene test tubes containing activated alumina 10 mg, disodium EDTA 10 mg and dihydroxybenzylamine 10 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 placed in a mixer for 15 min. The supernatant was discarded and the alumina washed three times with water. Adsorbed catecholamines were eluted from the alumina with 100 μl of 0.1 N HCl. Eluted catecholamines were measured by high-performance liquid chromatography with an electro-

chemical detector (Yanako). Details of the chromatographic data are as follows: column, $_5C_{18}$ (4.6 mm × 150 mm); mobile phase, 0.1 M phosphate potassium buffer (pH 5.8) containing 7% of methanol, 0.02% of *l*-heptane-sulphonate sodium and 1 mM disodium EDTA; applied potential, 700 mV vs Ag/AgCl; and flow rate, 1.5 ml min^{-1}. The lower limit of sensitivity of catecholamines was 0.1 pmol. After the superfusion experiments, the slices were homogenized in 1 ml of 0.1 N $HClO_4$, centrifuged and aliquots of 100 μl of supernatant used for the determination of the tissue content of catecholamines.

Drugs used were L-DOPA and NSD-1055 (Nakarai), (−)- and (+)-propranolol hydrochloride (ICI) and S- and R-sulpiride (Mitsui). The peaks of noradrenaline and dopamine were not interfered with by application of these drugs.

Student's *t* test was used to evaluate the data.

Results

Spontaneous and evoked release and tissue content of endogenous noradrenaline and dopamine in rat hypothalamic slices in the absence and presence of NSD-1055

Endogenous noradrenaline and dopamine released in the samples of superfusates were consistently detectable with high-performance liquid chromatography with an electrochemical detector and stabilized 60 min after the start of superfusion. In control preparations, the spontaneous release (Sp_1) of noradrenaline and dopamine for 3 min immediately before the S_1 period of electrical field stimulation in the presence of cocaine 20 μM was 0.022 ± 0.002 and 0.013 ± 0.001 pmol mg^{-1} wet wt. ($n = 14$), respectively. The evoked release (S_1) of noradrenaline and dopamine during the S_1 stimulation period at 5 Hz was 0.038 ± 0.006 and 0.035 ± 0.004 pmol mg^{-1} wet wt. ($n = 14$), respectively. The ratio Sp_2/Sp_1 and S_2/S_1 of noradrenaline and dopamine was slightly attenuated (Table 1a). The tissue content of noradrenaline and dopamine in slices after the superfusion experiments was 4.27 ± 0.34 and 3.52 ± 0.18 pmol mg^{-1} wet wt. ($n = 14$), respectively. Pretreatment with NSD-1055 10 μM ($n = 9$) produced no effects on Sp_1 (0.025 ± 0.002 and 0.012 ± 0.002 pmol mg^{-1} wet wt.), S_1 (0.046 ± 0.005 and 0.027 ± 0.003 pmol mg^{-1} wet wt.) or on ratios Sp_2/Sp_1 and S_2/S_1 (Table 1b) of noradrenaline and dopamine, respectively, or on the tissue content of noradrenaline (3.61 ± 0.2 pmol mg^{-1} wet wt.). However, pretreatment with NSD-1055 decreased the tissue content of dopamine (2.38 ± 0.27 pmol mg^{-1} wet wt., $P < 0.01$, compared to control).

Electrically-evoked release of endogenous noradrenaline and dopamine was Ca^{2+}-dependent and tetrodotoxin-sensitive (Ueda *et al.*, 1983a,b).

Effects of L-DOPA on the release of noradrenaline and dopamine in the absence and presence of NSD-1055

In the absence of NSD-1055 (Table 1a), the dose-response relationship for the effect of L-DOPA on the stimulation-evoked release of noradrenaline showed a biphasic pattern, a facilitation at 0.1 μM but no effect at 1 and 10 μM; no effects on the spontaneous release were observed. On the other hand, L-DOPA produced dose-dependent increases in the spontaneous release of dopamine (6.95 ± 0.52 vs control 3.52 ± 0.18 pmol mg^{-1} wet wt., $P < 0.01$), but it produced no effect on at 10 μM this increase was significant. Only the highest concentration of L-DOPA increased the tissue content of dopamine (6.95 ± 0.52 vs control 3.52 ± 0.18pmol-mg^{-1} wet wt., $P < 0.01$), but it produced no effect on the tissue content of noradrenaline (3.55 ± 0.34 vs 4.27 ± 0.34 pmol mg^{-1} wet wt.).

In the presence of NSD-1055 10 μM (Table 1b), L-DOPA-induced increases in the spontaneous release of dopamine were prevented and L-DOPA produced biphasic regulatory effects on the evoked release of both noradrenaline and dopamine, a facilitation at 0.1 μM and an inhibition at 1 μM, respectively, without affecting the spontaneous release.

Stereoselective antagonism by propranolol of L-DOPA-induced facilitation of the evoked release of noradrenaline and dopamine in the presence of NSD-1055

L-DOPA (0.1 μM)-induced facilitation of the evoked release of noradrenaline and dopamine was antagonized by pretreatment with (−)-propranolol 0.1 μM, respectively, but no effects on the spontaneous release were observed. A significant dissociation was seen between the actions of the (−)- and (+)-isomers of propranolol (Figure 1). This pretreatment produced no effect on Sp_1, S_1 or the ratio Sp_2/Sp_1 of both catecholamines.

Stereoselective antagonism by sulpiride of L-DOPA-induced inhibition of the evoked release of noradrenaline and dopamine in the presence of NSD-1055

L-DOPA (1 μM)-induced inhibition of the evoked release of noradrenaline and dopamine was antagonized by pretreatment with S-sulpiride 1 nM, without an effect on the spontaneous release. Again, a significant dissociation was seen between the actions of the S- and R-isomers of sulpiride (Figure 2). This pretreatment produced no effect on Sp_1, S_1 or the ratio Sp_2/Sp_1 of both catecholamines.

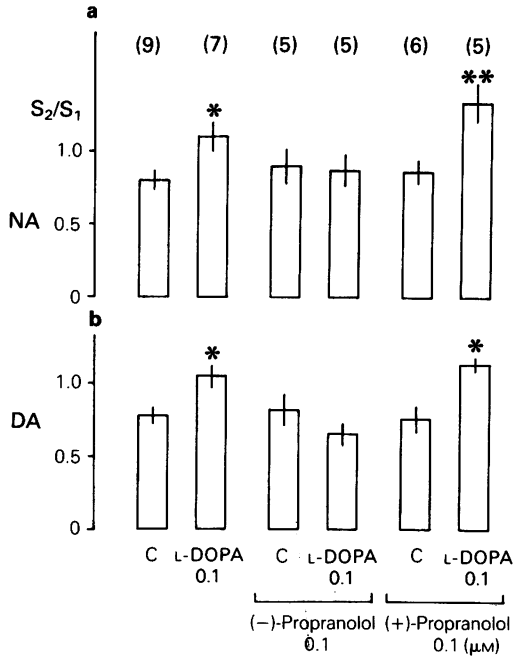

Figure 1 Stereoselective antagonism by propranolol of L-DOPA (0.1 μM)-induced facilitation of the evoked release of endogenous noradrenaline (NA) and dopamine (DA) from rat hypothalamic slices in the presence of NSD-1055. Electrical field stimulation was by impulses of 5 Hz, 2 ms, 30 mA for 3 min in the presence of cocaine 20 μM at 60 (S_1) and 90 (S_2) min after the start of superfusion. Ordinates show ratios of amounts of NA (a) and dopamine (b) released during the S_2 and S_1 periods of stimulation. L-DOPA 0.1 μM was added to the medium 15 min before S_2. NSD-1055 10 μM and (−)- or (+)-propranolol 0.1 μM was added at the start of superfusion and was present throughout the experiments. Each column represents the mean, and vertical lines show s.e., of number of estimations shown in parentheses. Statistical significance: $*P<0.05$, $**P<0.01$, compared to corresponding control (C).

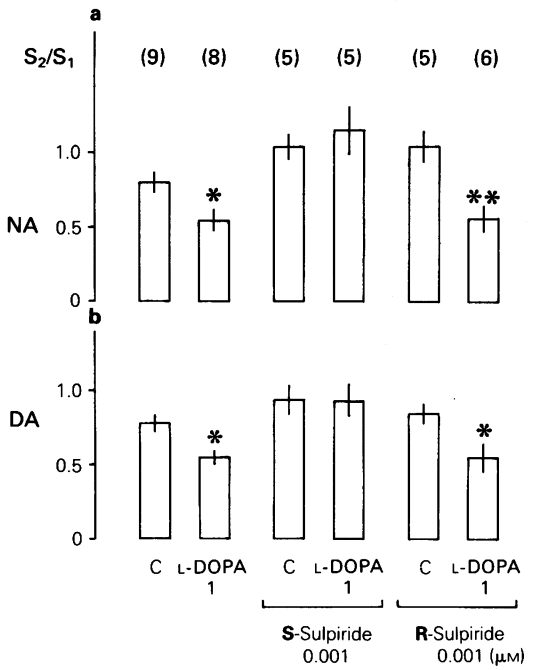

Figure 2 Stereoselective antagonism by sulpiride of L-DOPA (1 μM)-induced inhibition of the evoked release of endogenous (a) noradrenaline (NA) and (b) dopamine (DA) from rat hypothalamic slices in the presence of NSD-1055. **S-** or **R-Sulpiride** was added at the start of superfusion and was present throughout the experiments. Other details are similar to those in Figure 1.

Discussion

In rat hypothalamic slices, L-DOPA produced biphasic regulatory effects on the impulse-evoked release of endogenous noradrenaline and dopamine, an inhibition at micromolar concentrations and a facilitation at the lower concentration (0.1 μM), but no increase in the spontaneous release of either catecholamine was observed in the presence of NSD-1055, a DOPA-decarboxylase inhibitor. The facilitatory actions of L-DOPA were antagonized by (−)-propranolol, a non-selective β-adrenoceptor antagonist, but not by the (+)-isomer and the inhibitory actions were antagonized by S-sulpiride, a relatively selective D_2-dopamine receptor antagonist (Kebabian & Calne, 1979), but not by the **R**-isomer. The lower concentration of L-DOPA also similarly facilitated the impulse-evoked release of noradrenaline without increasing the spontaneous release in the absence of NSD-1055. Our findings clearly demonstrate that these actions of L-DOPA are mediated via the activation of stereoselective presynatic facilitatory β-adrenoceptors and inhibitory dopamine receptors. The presynaptic nature of the effects of L-DOPA is further supported by the findings that the impulse-evoked release of noradrenaline and dopamine is Ca^{2+}-dependent and tetrodotoxin-sensitive (Ueda *et al.*, 1983a,b) and that both types of presynaptic receptors exist mainly on the noradrenergic neurone terminals (Ueda *et al.*, 1983a; 1985; Misu *et al.*, 1985) and on the dopaminergic neurone terminals (Ueda *et al.*, 1983a,b; 1984) in the rat hypothalamus, respectively.

These actions of L-DOPA obtained in the present experiments do not appear to be as a result of its conversion to dopamine, but are mainly due to L-DOPA, since in the presence of NSD-1055, all of these actions were still observed whereas increases in the spontaneous release of dopamine were completely prevented. Furthermore, facilitation of the impulse-evoked release of noradrenaline was not affected by NSD-1055. This idea is further supported by the finding that the dose-response relationship, and the pharmacological characterization, of the biphasic regulatory effects of L-DOPA on the release of noradrenaline were completely inverse compared to those of exogenously applied dopamine on the same parameter (Misu *et al.*, 1985). Our findings are the first to provide evidence that some of the effects of L-DOPA are via presynaptic facilitatory and inhibitory receptors, probably different from those of converted dopamine such as the action on peripheral presynaptic dopamine receptors (Lokhandwala & Buckley, 1978), and are not consistent with the generally accepted idea that the actions of L-DOPA are manifested through its conversion to dopamine (Ng *et al.*, 1970; 1971; Bunney *et al.*, 1973; Lloyd *et al.*, 1975; Hefti & Melamed, 1980; Ponzio *et al.*, 1983), except for the dose-dependent increases in the spontaneous release of dopamine and increases in the evoked release and tissue content of dopamine induced by the highest concentration of L-DOPA (10 μM) in the absence of NSD-1055. The increases in the spontaneous release of dopamine are probably due to the conversion of L-DOPA to dopamine and reflect intact DOPA-decarboxylase activity in our slices and may, in part, explain the findings that high concentrations of L-DOPA induce displacement of [^{3}H]-dopamine in rat brain slices (Ng *et al.*, 1970). The prevention of the conversion of L-DOPA to dopamine by NSD-1055 and NSD-1055-induced decrease in the tissue content of dopamine, as a result of decrease in newly synthesized dopamine, show that adequate concentrations of the drug were used. (Hirata *et al.*, 1983).

The conversion of L-DOPA to dopamine and the probable resultant increase in the amounts of dopamine and noradrenaline available for impulse-evoked release may interfere with the manifestation of the inhibitory actions of L-DOPA, at a micromolar concentration, on the release of both catecholamines in the absence of NSD-1055. In general, the impulse-evoked release of a transmitter cannot be exactly estimated under the conditions accompanying modifications of the spontaneous release of the transmitter. This idea may also reflect the negative finding concerning the facilitatory effect of L-DOPA (0.1 μM) on the impulse-evoked release of dopamine in the absence of NSD-1055. One of the objectives of using NSD-1055 was to determine, by preventing increases in the spontaneous release of dopamine, whether or not L-DOPA can produce biphasic regulatory effects especially on the impulse-evoked release of dopamine; this was found to occur in the presence of NSD-1055.

Even when the spontaneous release of dopamine was increased in the absence of NSD-1055, the highest concentration of L-DOPA (10 μM) increased the impulse-evoked release of dopamine and this finding may, in part, be consistent with that of the impulse-evoked release of radioactive dopamine from rat brain slices (Ng *et al.*, 1971) and of the increase in the content of 3-methoxytyramine in the rat striatum (Ponzio *et al.*, 1983). Only the same high concentration of L-DOPA increased the tissue content of dopamine which partly explains the accumulation of striatal dopamine observed in rats (Ponzio *et al.*, 1983) and humans (Lloyd *et al.*, 1975).

Biphasic presynaptic actions of L-DOPA on the release of noradrenaline and dopamine, an inhibition via dopamine receptors at a micromolar concentration and a facilitation via β-adrenoceptors at the lower concentration in rat hypothalamic tissues with low DOPA-decarboxylase activity, and the facilitation of the release of noradrenaline in tissues with intact DOPA-decarboxylase activity may be relevant to pharmacological, therapeutic and/or untoward actions of L-DOPA.

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