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AN L-DOPA-LIKE DEPRESSOR ACTION OF L-*THREO*-DIHYDROXYPHENYL-SERINE IN THE RAT CAUDAL VENTROLATERAL MEDULLA

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Summary

We have proposed that L-3,4-dihydroxyphenylalanine (L-DOPA) is a neurotransmitter and/or neuromodulator in the central nervous system (1). In this study, we investigated whether or not L-*threo*-dihydroxyphenylserine (L-*threo*-DOPS), a synthetic amino acid structurally related to L-DOPA, microinjected into the caudal ventrolateral medulla (CVLM) and the rostral ventrolateral medulla (RVLM) shows cardiovascular actions similar to those of L-DOPA in anesthetized rats. When L-*threo*-DOPS was microinjected into CVLM, it produced dose-dependent (0.01-3 ng) depressor and bradycardic responses. D-*threo*-DOPS (3 ng) produced no effect. The responses to L-*threo*-DOPS (1 ng) were almost completely blocked by L-DOPA methyl ester (1 μg), a competitive antagonist for L-DOPA, supporting the existence of an L-*threo*-DOPS-sensitive recognition site for L-DOPA in CVLM. Microinjection of L-*threo*-DOPS into RVLM, however, showed no effect (0.001-100 ng), which contrasted with the cardiopressor action of L-DOPA applied in RVLM. In RVLM, there may exist an L-*threo*-DOPS-insensitive recognition site for L-DOPA.

Key Words: L-DOPA, L-*threo*-dihydroxyphenylserine, neurotransmitter, caudal ventrolateral medulla, rostral ventrolateral medulla, central cardiovascular control, L-DOPA methyl ester

L-3,4-Dihydroxyphenylalanine (L-DOPA) has been believed traditionally to be an endogenous inert amino acid that exerts actions and effectiveness in Parkinson's disease *via* its conversion to dopamine (DA) by aromatic L-amino acid decarboxylase (AADC). Contrary to this generally accepted idea, we have proposed that L-DOPA is a neurotransmitter and/or neuromodulator in the central nervous system (1, 2).

If there exists a specific recognition site for L-DOPA or "L-DOPAergic receptor", we can expect to find specific antagonists or agonists for L-DOPA. In an effort to find a useful ligand, we have tested

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several L-DOPA analogues using impulse-evoked release of noradrenaline from rat hypothalamic slices as a screening system (3). In the presence or absence of AADC inhibitors, nanomolar L-DOPA facilitates the release of noradrenaline. This effect is antagonized by propranolol, but in a non-competitive fashion. This suggests that the recognition site for L-DOPA differs from β-adrenoceptors (3). In fact, L-DOPA does not displace a β-adrenoceptor ligand, [^{3}H]-dihydroalprenolol binding in rat brain membrane preparations (unpublished observation). In contrast, we found that L-DOPA methyl ester (DOPA ester), a carboxylic acid ester of L-DOPA, competitively antagonizes L-DOPA-induced facilitation of evoked noradrenaline release. Our studies on structure-activity relationship suggest that the activity of L-DOPA is related to such structural features as its catechol moiety in addition to amino and carboxy groups. These are exemplified by L-*threo*-dihydroxyphenyl serine (L-*threo*-DOPS), a synthetic amino acid precursor of noradrenaline. L-*threo*-DOPS at picomolar concentrations mimics the L-DOPA action, and this facilitatory action of L-*threo*-DOPS is seen in the presence of AADC inhibitor. Likewise, the recognition site for L-*threo*-DOPS differs from β-adrenoceptors, because this facilitation is antagonized competitively by DOPA ester but is antagonized non-competitively by propranolol (4). L-*threo*-DOPS thus seems to be an agonist for the L-DOPA recognition site. This idea is further supported by findings of the postsynaptic actions of L-*threo*-DOPS microinjected into depressor site of the nucleus tractus solitarii (NTS) of anesthetized rats. The L-*threo*-DOPS-induced cardiodepressor responses are dose-dependent, stereoselective and DOPA ester-sensitive but AADC inhibitor-insensitive (5).

The depressor NTS, the depressor caudal ventrolateral medulla (CVLM) and the pressor rostral ventrolateral medulla (RVLM) are all areas that are relevant to baroreflex and central regulation of blood pressure, and are important target cardiovascular centers for L-DOPA in the lower brainstem (1, 6-9), since 1) immunocytochemically tyrosine hydroxylase-positive, AADC-negative, L-DOPA-positive and DA-negative neurons and nerve fibers are seen in NTS, 2) basal L-DOPA release is in part tetrodotoxin-sensitive, Ca^{2+}-dependent in dialysates during microdialysis in NTS, CVLM and RVLM, 3) high K^+ evokes L-DOPA in a transmitter-like manner in dialysates in these brain areas, 4) unilateral microinjection of L-DOPA into NTS and CVLM or RVLM produces cardiodepressor or pressor responses, all of which are antagonized by DOPA ester ipsilaterally microinjected, and 5) DOPA ester alone bilaterally microinjected into NTS, CVLM or RVLM, demonstrates opposite cardiovascular responses to L-DOPA, suggesting a tonic function of endogenously released L-DOPA in these areas. L-DOPA is probably a neurotransmitter of the primary baroreceptor afferents terminating in NTS (1, 9). There exists biochemical and functional evidence for a baroreceptor-aortic nerve-NTS-mediated depressor L-DOPAergic and monosynaptic relay terminating in CVLM, and a posterior hypothalamic nucleus-mediated cardiopressor L-DOPAergic and monosynaptic relay terminating in RVLM (1). For example, electrical lesion of the right NTS produces a selective decrease of 40% in the L-DOPA contents of the ipsilateral CVLM punched out without modifications of those of DA, noradrenaline and adrenaline (1).

In the present study, we attempted to clarify whether or not L-*threo*-DOPS, when microinjected into the depressor sites of CVLM or into the pressor sites of RVLM in anesthetized rats, produces depressor or pressor actions similar to those of L-DOPA, and characterized the pharmacological actions.

Methods

Materials

Male Wistar rats weighing 250-350 g were anesthetized i.p. with 1.2 g/kg urethane, paralyzed i.m. with 1 mg/kg D-tubocurarine and artificially ventilated at a rate of 70-90 beats/min and a volume of 2.5-3.5 ml with a respirator (Shinano, SN-480, Tokyo, Japan), keeping the rectal temperature at

36.5-37.5 °C with a temperature controller (BAS CMA/150). The femoral artery was cannulated for recording systolic/diastolic blood pressure (BP) and heart rate (HR). Rats were placed in a stereotaxic apparatus with the head fixed at 45°. The dorsal surface of the lower brainstem was exposed by a limited occipital craniotomy. Drugs used for microinjections were L-DOPA, L-glutamate monosodium, (Nacalai, Kyoto, Japan), D- and L-*threo*-DOPS (Sumitomo Pharmaceuticals Co., Osaka, Japan), and DOPA ester (Sigma, USA).

Microinjection experiments

A glass micropipette (outside diameter 40-50 μm) was inserted into CVLM (0-0.5 mm rostral and 1.8-2.2 mm lateral to the caudal tip of the area postrema and 2.5 mm beneath the dorsal surface of the medulla) and RVLM (1.8-2.0 mm rostral, 1.8-2.0 mm lateral and 2.5-3.0 mm beneath), respectively. Drugs dissolved in 10 mM phosphate-buffered saline (pH 7.4) were injected within two seconds. At the end of the experiments, the injection site was marked by injecting 50 μl Evans blue dye solution. The brain was removed, frozen sections were cut (50 μm) with a cryostat (Leica, Leitz 1720) and the injection site was identified.

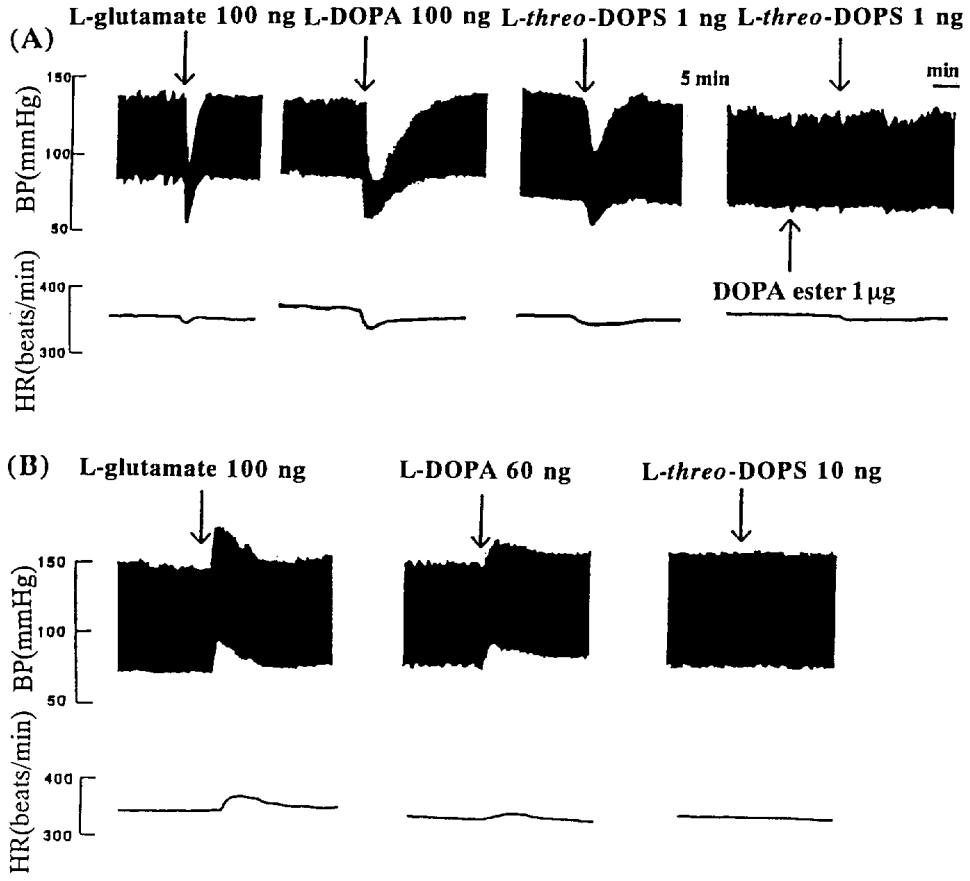

Fig. 1

Representative traces of blood pressure (BP) and heart rate (HR) in rats microinjected with L-glutamate, L-DOPA and L-*threo*-DOPS into CVLM (A) or RVLM (B) and antagonism by L-DOPA methyl ester (DOPA ester) against L-*threo*-DOPS in CVLM (A).

Statistical analysis

The data were expressed as means ± SEM. The statistical significance was calculated using Student's *t* -test.

Results

The resting mean BP and HR before microinjection experiments, was 88 ± 2 mmHg and 357 ± 6 beats/min (n = 33), respectively. To identify depressor site in CVLM or pressor site in RVLM, we confirmed cardiodepressor or cardiopressor action by microinjecting L-glutamate (100 ng) into these areas (Fig. 1A, B). In comparison, L-DOPA actions in CVLM and RVLM (7, 8) were also confirmed (Fig. 1A, B). When delivered at the depressor sites of CVLM, L-*threo*-DOPS (0.01-3 ng) elicited dose-dependent hypotension and bradycardia (Fig. 1A, Table I). The peak effect was seen at 3 ng for both parameters. The onset was within 1-2 seconds. D-*threo*-DOPS produced no effect (Table I). DOPA ester (1 μg), unilaterally microinjected into CVLM 1 min previously, blocked completely hypotensive and bradycardic responses to 1 ng L-*threo*-DOPS (Fig. 1A, Table I), while saline did not affect these responses (Table I).

TABLE I

Dose-dependent and Stereoselective Depressor Action of L-*threo*-DOPS Microinjected into Unilateral CVLM, and Antagonism by DOPA ester against L-*threo*-DOPS.

Drugs	Dose (n g)	n	Δ Mean BP	Δ Mean HR
L-*threo*-DOPS	0.01	5	- 8 ± 1	- 3 ± 1
	0.1	5	- 14 ± 1	- 10 ± 1
	1	5	- 18 ± 1	- 13 ± 1
	3	5	- 27 ± 2	- 16 ± 2
	10	5	- 13 ± 2	- 5 ± 1
D-*threo*-DOPS	3	3	- 1 ± 1 *	- 1 ± 1 *
Saline + L-*threo*-DOPS	1	4	- 20 ± 3	- 14 ± 2
DOPA ester 1 μg + L-*threo*-DOPS	1	4	- 3 ± 1 **	- 2 ± 1 **

Antagonism by DOPA ester (1 μg) against L-*threo*-DOPS-induced decreases in blood pressure (BP, mmHg) and heart rate (HR, beats/min) was determined 2 min after ipsilateral microinjection of saline or DOPA ester. Values are mean ± SEM from n experiments.

*P < 0.01, compared to L-*threo*-DOPS 3 ng alone (unpaired *t*-test).

**P < 0.01, compared to L-*threo*-DOPS 1 ng with saline (paired *t*-test).

When delivered at the pressor sites of RVLM, L-*threo*-DOPS (0.001-100 ng, n = 20) produced no effect, while both L-glutamate and L-DOPA produced hypertensive and tachycardiac responses (Fig. 1B). The negative result with L-*threo*-DOPS in RVLM was confirmed by three investigators in our group.

Discussion

We demonstrated that L-*threo*-DOPS microinjected into CVLM produced cardiodepressor action similar to that of L-DOPA. This effect is probably neither due to its conversion to noradrenaline nor due to activation of catecholaminergic receptors, because 1) the effect of L-*threo*-DOPS was blocked by DOPA ester, a competitive antagonist for L-DOPA (3) and was stereoselective in nature in common with many receptors, 2) the effective concentration range of 0.01 to 3 ng for L-*threo*-DOPS in CVLM (see Fig. 1A, Table I) is extremely lower than that for noradrenaline. Noradrenaline even at 100 ng microinjected into the same area decreases blood pressure levels only by 10 mmHg and the onset of depressor responses to noradrenaline was far slower compared with that to L-*threo*-DOPS (7). Further evidence is that L-*threo*-DOPS itself does not displace the specific binding of α_2-adrenoceptor and DA D_1, D_2 receptor ligands in the rat or guinea pig brain (10, 11) and DOPA ester also produces no or negligible effects on that of β-adrenoceptor and D_2 receptor ligands or of α_2-adrenoceptor ligand in the rat brain membrane preparations (3). It was confirmed that depressor responses to L-DOPA (100 ng) micronjected into NTS were not affected by premicroinjection with propranolol (100 ng) and sulpiride (100 ng) with negligible inhibition by yohimbine (100 ng) (9).

When microinjected into RVLM, however, L-*threo*-DOPS produced no effect. This provides marked contrast to our present and previous studies showing that L-*threo*-DOPS microinjected into NTS or CVLM mimics the cardiodepressor actions of L-DOPA (5) (Table II). This was an

TABLE II

Comparison between L-DOPA and L-*threo*-DOPS Microinjected into NTS, CVLM, and RVLM of Anesthetized Rats.

L-DOPA

	BP · HR	Dose (ng)	Antagonism by DOPA ester
NTS	↓	10 - 100	+
CVLM	↓	10 - 100	+
RVLM	↑	30 - 300	+

L-*threo*-DOPS

	BP · HR	Dose (ng)	Antagonism by DOPA ester
NTS	↓	0.01 - 3	+
CVLM	↓	0.01 - 3	+
RVLM	→	0.001 - 100	not tested

unexpected finding, because the pharmacological characteristics of the actions of L-*threo*-DOPS and L-DOPA by themselves are similar in several aspects. Both the *in vivo* cardiovascular actions of L-*threo*-DOPS and L-DOPA are antagonized by DOPA ester. Both the facilitatory actions on hypothalamic noradrenaline release are antagonized competitively by DOPA ester, and non-competitively by propranolol (4). Finally, the efficacy of L-*threo*-DOPS appears to be higher than that of L-DOPA. In the hypothalamic slices, for instance, picomolar concentrations of L-*threo*-DOPS mimic the action of nanomolar concentrations of L-DOPA (3, 4). In the NTS and CVLM, 3 ng L-*threo*-DOPS elicits hypotensive and bradycardic responses almost equal to those to 30 ng L-DOPA (6, 7) (Table II).

Based on our findings in CVLM, RVLM and NTS (5), the overall actions of L-*threo*-DOPS in the lower brainstem may be cardiodepressive. This idea is consistent with the finding that intracerebroventricular administration of L-*threo*-DOPS produces depressor effect in anesthetized rat (12). However, L-*threo*-DOPS has, in fact, been used for the treatment of disorders related to hypotension (13). The therapeutic effect of L-*threo*-DOPS is probably due to replenishment of noradrenaline in peripheral sympathetic neurons (12, 14).

In NTS, CVLM and RVLM, there exist tonically functioning L-DOPA systems to mediate cardiovascular control (1). In NTS, it is highly probable that L-DOPA is a neurotransmitter of the primary baroreceptor afferents terminating in NTS (1, 9). Our finding that L-*threo*-DOPS was inactive in RVLM might reflect some differences in constituents of neuronal circuits, neurotransmitters and/or receptors in this area. It is probable that there exists L-*threo*-DOPS-insensitive L-DOPA recognition site in RVLM. Alternatively, an endogenous L-*threo*-DOPS-like substance may exist in the central nervous system and act on a recognition site in NTS and CVLM different from that for L-DOPA.

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