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Short communication

Depressor action of L-*threo*-dihydroxyphenylserine in the rat nucleus tractus solitarii

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Abstract

Microinjections of L-*threo*-dihydroxyphenylserine (L-*threo*-DOPS, 0.1–3 ng), a synthetic precursor amino acid of noradrenaline, into the medial area of the nucleus tractus solitarii produced dose-dependent depressor and bradycardic responses in anesthetized rats treated with or without i.p. 3-hydroxybenzylhydrazine, a central inhibitor of L-aromatic amino acid decarboxylase. D-*threo*-DOPS (3 ng) produced no effect. L-Dihydroxyphenylalanine (L-DOPA) methyl ester (1 μg), a competitive antagonist of L-DOPA, microinjected into the nucleus tractus solitarii, blocked the depressor and bradycardic responses to L-*threo*-DOPS. L-*threo*-DOPS itself produces vasodepressor actions without its conversion to noradrenaline, probably via a recognition site for L-DOPA in the rat nucleus tractus solitarii.

Keywords: L-*threo*-DOPS (L-*threo*-dihydroxyphenylserine); L-DOPA (L-dihydroxyphenylalanine); Nucleus tractus solitarii; L-DOPA methyl ester; Neurotransmitter; (Rat)

1. Introduction

We have proposed that L-3,4-dihydroxyphenylalanine (L-DOPA) is an endogenous neurotransmitter or neuromodulator in the central nervous system (Misu et al., 1995). Endogenous L-DOPA is released in vitro and in vivo in a tetrodotoxin-sensitive and Ca^{2+}-dependent manner from striata (Nakamura et al., 1992), caudal and rostral ventrolateral medulla (Yue et al., 1993; Misu et al., 1995), and the nucleus tractus solitarii of rats (Yue et al., 1994). In addition, exogenously applied L-DOPA itself produces various types of pre- and postsynaptic responses (Misu et al., 1995).

As for presynaptic actions, nanomolar L-DOPA stereoselectively facilitated the impulse-evoked release of endogenous noradrenaline from superfused rat hypothalamic slices via presynaptic β-adrenoceptors and picomolar concentrations potentiated the activities of these adrenoceptors (Misu et al., 1995). These effects were also observed under essentially complete inhibition of L-aromatic amino acid decarboxylase, the enzyme catalyzing the conversion of L-DOPA to dopamine (Goshima et al., 1991). The action of L-DOPA was antagonized non-competitively by propranolol, a β-adrenoceptor antagonist, whereas the action was antagonized competitively by L-DOPA methyl ester (Goshima et al., 1991). L-DOPA and L-DOPA methyl ester did not displace specific binding of a β-adrenoceptor ligand in a rat membrane preparation. These findings suggest that there exists a recognition site for L-DOPA itself, which differs from that of β-adrenoceptors (Goshima et al., 1991). However, little is known about the characteristics of the recognition site because there are few agonists or antagonists available.

In an effort to find a useful ligand, we have tested several L-DOPA analogues using noradrenaline release from rat hypothalamic slices as a screening system (Goshima et al., 1991). L-*threo*-Dihydroxyphenylserine (L-*threo*-DOPS), a synthetic precursor of noradrenaline, has structural features similar to those of L-DOPA, in that both possess a catechol moiety, and amino and carboxy groups (Bartholini et al., 1975). We found that picomolar L-*threo*-DOPS mimicked the action of L-DOPA to facilitate the release of noradrenaline (Goshima et al., 1991). This L-*threo*-DOPS-induced facilitation was also seen when its conversion to noradrenaline was prevented by the inhibition of L-aromatic amino acid decarboxylase; again, this effect was antagonized competitively by L-DOPA methyl ester and was antagonized non-competitively by propra-

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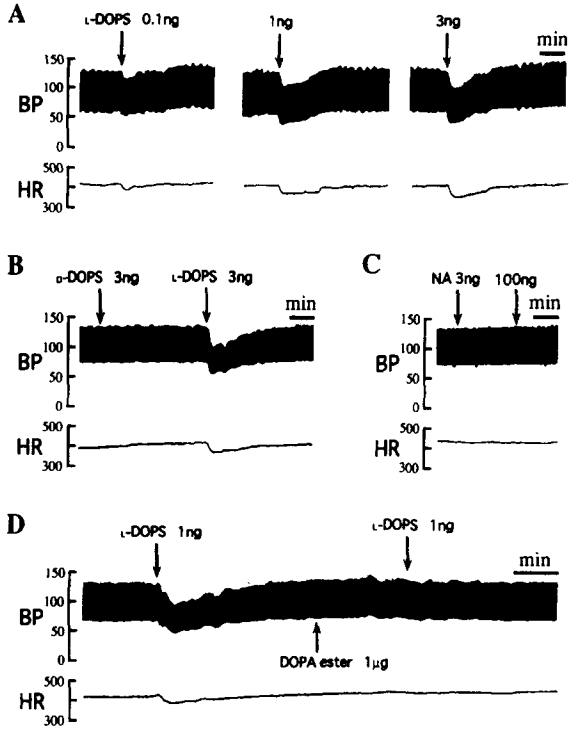

Fig. 1. Representative traces of blood pressure (BP, mm Hg) and heart rate (HR, beats/min) in four individual rats (A–D) microinjected with L-*threo*-DOPS (L-DOPS) (A), D-*threo*-DOPS (D-DOPS) (B) and noradrenaline (NA) (C) into the unilateral nucleus tractus solitarii, and antagonism by ipsilateral injection of L-DOPA methyl ester (DOPA ester) against L-DOPS (D).

nolol (Yue et al., 1992). These findings indicated that L-*threo*-DOPS had pharmacological properties similar to those of L-DOPA on the evoked release of endogenous noradrenaline from hypothalamic slices.

In addition, a postsynaptic depressor response was elicited under inhibition of L-aromatic amino acid decarboxylase by microinjection of L-DOPA into the nucleus tractus solitarii in anesthetized rats, since the response was also seen after destruction of noradrenergic fibers with i.c.v. 6-hydroxydopamine (Kubo et al., 1992; Yue et al., 1994). This effect was not mimicked by dopamine and was blocked by L-DOPA methyl ester microinjected into the area. This gives us evidence for the presence of a postsynaptic recognition site for L-DOPA responsible for cardiovascular actions in the nucleus tractus solitarii. The site was stereoselective in nature in common with many receptors, since D-DOPA produced no effect (Kubo et al., 1992). We further extended our studies to demonstrate that endogenously released L-DOPA itself tonically functions to activate depressor neurons for regulation of blood pressure in the nucleus tractus solitarii (Yue et al., 1994). In the present study, we investigated whether or not L-*threo*-DOPS itself, when microinjected into the depressor sites of the nucleus tractus solitarii of anesthetized rats, produces a depressor action similar to that of L-DOPA, and characterized the pharmacological actions.

2. Materials and methods

Male Wistar rats weighing 250–350 g were anesthetized with urethane (1.2 g/kg, i.p.), paralyzed with D-tubocurarine (1 mg/kg, i.m.) and artificially ventilated at a rate of 80–90 breaths/min and a volume of 2.5–3.5 ml with a respirator (Shinano, SN-480, Tokyo, Japan). The femoral artery was cannulated for recording systolic/diastolic blood pressure and heart rate. In some rats, 3-hy-

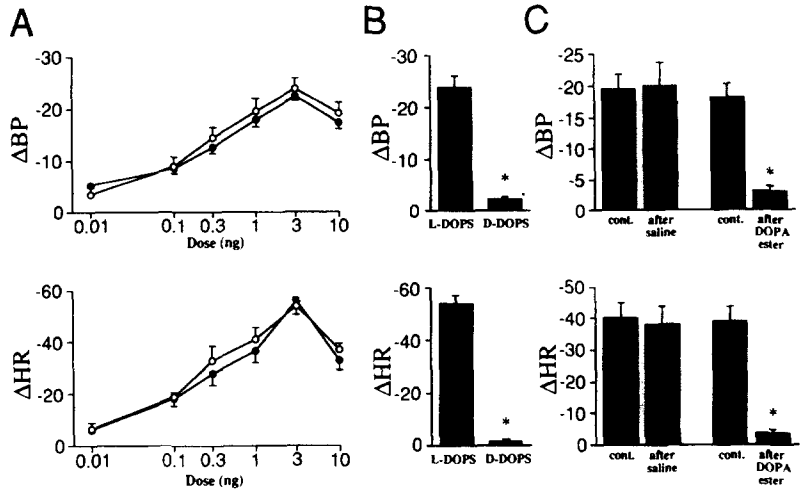

Fig. 2. A: Dose-effect curves for depressor and bradycardic responses to microinjections of L-*threo*-DOPS into the unilateral nucleus tractus solitarii in rats treated with (●) or without (○) NSD-1015 (100 mg/kg, i.p.). B: Stereoselective effect of *threo*-DOPS (L- or D-DOPS, 3 ng). * $P < 0.01$ (unpaired *t*-test), compared to L-DOPS. C: Effects of saline vehicle and DOPA ester (1 μg) on depressor and bradycardic responses to microinjections of L-*threo*-DOPS (1 ng). The effects were determined 2 min after ipsilateral microinjection of saline or DOPA ester. * $P < 0.01$ (paired *t*-test), compared to the corresponding control (cont.). Upper and lower vertical axes show Δmean blood pressure (ΔBP, mm Hg) and Δheart rate (ΔHR, beats/min), respectively. Values are means ± S.E.M. from 5 separate experiments.

droxybenzylhydrazine (NSD-1015, 100 mg/kg, Aldrich, WI, USA) was injected i.p. 30 min before microinjections to inhibit brain L-aromatic amino acid decarboxylase (Kubo et al., 1992). Rats were placed in a stereotaxic apparatus (Narishige, Tokyo, Japan) with the head fixed at 45°. The dorsal surface of the lower brainstem was exposed by a limited occipital craniotomy. A glass micropipette pulled to an outside diameter of 40–50 μm was introduced into the unilateral nucleus tractus solitarii. The coordinate for the depressor site of the nucleus tractus solitarii was 0.6 mm rostral and 0.6 mm lateral to the caudal tip of the area postrema and 0.6 mm beneath the dorsal surface of the brainstem (Yue et al., 1994). Drugs dissolved in 10 mM phosphate-buffered saline (pH 7.4) were given in a volume of 50 nl in 2 s. At the end of experiments, the injection site was marked by injecting 50 nl of Evans blue solution. The brain was removed, frozen sections (50 μm) were cut and the injection site was identified.

Drugs used for microinjections were L-glutamate (monosodium salt, Nacalai Tesque, Kyoto, Japan), D- and L-*threo*-DOPS (Sumitomo Pharmaceuticals Co., Osaka, Japan), L-DOPA methyl ester (Sigma, USA) and L-noradrenaline (Sigma, USA).

The data are given as means ± S.E.M. and the statistical significance of difference was calculated using Student's *t*-test.

3. Results

In normal rats with intact brain L-aromatic amino acid decarboxylase, the mean arterial pressure was 86 ± 3 mm Hg and heart rate was 400 ± 12 beats/min ($n = 10$). To identify depressor sites in the nucleus tractus solitarii, we first microinjected the excitatory amino acid glutamate (30 ng) into the nucleus tractus solitarii (Kubo et al., 1992). When delivered at the depressor sites of the unilateral nucleus tractus solitarii, L-*threo*-DOPS (0.1–3 ng) elicited dose-dependent decreases in blood pressure and heart rate (Fig. 1A and Fig. 2A). The peak effect was seen at 3 ng for both parameters (Fig. 2A). Saline ($n = 16$) or D-*threo*-DOPS (Fig. 1B and Fig. 2B) produced no effect. Noradrenaline (3 and 100 ng, $n = 5$) produced no effect (Fig. 1C). L-DOPA methyl ester (1 μg), microinjected 2 min previously, markedly inhibited depressor and bradycardic responses to 1 ng L-*threo*-DOPS, while saline vehicle did not affect the responses (Fig. 1D and Fig. 2C).

In rats with inhibition of L-aromatic amino acid decarboxylase produced by i.p. NSD-1015, the mean arterial pressure was 87 ± 6 mm Hg and heart rate was 406 ± 13 beats/min ($n = 5$). The inhibition of L-aromatic amino acid decarboxylase did not modify the dose-effect curves for L-*threo*-DOPS obtained with intact L-aromatic amino acid decarboxylase (Fig. 2A).

4. Discussion

It is highly probable that L-DOPA is a neurotransmitter of the primary baroreceptor afferents terminating in the nucleus tractus solitarii (Yue et al., 1994). The present experiments provide the first evidence that L-*threo*-DOPS, when microinjected into the medial area of the nucleus tractus solitarii of anesthetized rats, produced a L-DOPA-like action, namely dose-dependent decreases in blood pressure and heart rate. L-*threo*-DOPS is believed to exert its actions via its conversion to noradrenaline in peripheral and/or central noradrenergic neurons (Bartholini et al., 1975; Birkmayer et al., 1983). The effect of L-*threo*-DOPS in this study, however, is probably not due to its conversion to noradrenaline, because (1) this effect was not modified by almost complete L-aromatic amino acid decarboxylase inhibition with NSD-1015, a potent central inhibitor of L-aromatic amino acid decarboxylase in vitro (Goshima et al., 1990) and in vivo (Carlsson, 1964); (2) the effective doses of L-*threo*-DOPS were 0.1 to 3 ng, while the minimum dose of noradrenaline microinjected into nucleus tractus solitarii needed to elicit depressor responses is 1 μg (Kubo and Misu, 1981); (3) noradrenaline at 3 and 100 ng produced no effect. Further, L-*threo*-DOPS itself does not displace the specific binding of α_2-adrenoceptor and dopamine D_1, D_2 receptor ligands in the rat or guinea pig brain (Cha et al., 1991; Nishino et al., 1987). It is thus unlikely that cardiodepressor responses elicited by L-*threo*-DOPS might be due to its action on α_2-adrenoceptors or dopamine D_1, D_2 receptors within the depressor site of the nucleus tractus solitarii. More importantly, the effect of L-*threo*-DOPS was stereoselective in nature, and was blocked by L-DOPA methyl ester, a competitive antagonist for L-DOPA. In our previous experiments using superfused rat hypothalamic slices, L-DOPA methyl ester antagonized in a competitive fashion the action of L-DOPA and L-*threo*-DOPS on the evoked release of noradrenaline (Goshima et al., 1991; Yue et al., 1992). When microinjected into the nucleus tractus solitarii, L-DOPA methyl ester antagonized the depressor responses induced by L-DOPA but not by glutamate (Kubo et al., 1992), another candidate for a neurotransmitter of the primary baroreceptor afferents (Misu et al., 1995). Thus, our present findings provide evidence that L-*threo*-DOPS is an agonist for a L-DOPA recognition site in the nucleus tractus solitarii. Alternatively, an endogenous L-*threo*-DOPS-like substance may exist in the central nervous system.

Some of the aspects of a L-DOPA recognition site in the nucleus tractus solitarii appear to be common to those of a L-DOPA recognition site in rat hypothalamic slices (Goshima et al., 1991; Misu et al., 1995). In both preparations, L-*threo*-DOPS mimicked the action of L-DOPA. Both the actions of L-DOPA and L-*threo*-DOPS were antagonized by L-DOPA methyl ester in a competitive fashion. Finally, the potencies of L-*threo*-DOPS appeared

to be higher than those of L-DOPA. For example, in rat hypothalamic slices, picomolar concentrations of L-*threo*-DOPS mimicked the action of nanomolar concentrations of L-DOPA to facilitate the evoked release of noradrenaline (Goshima et al., 1991; Yue et al., 1992). In the nucleus tractus solitarii, 3 ng L-*threo*-DOPS elicited hypotensive and bradycardic responses almost equal to those elicited by 30 ng L-DOPA (Kubo et al., 1992).

L-*threo*-DOPS is effective in treating akinesia and in arresting Parkinson's disease (Narabayashi et al., 1981) and has been used for orthostatic hypotension which is sometimes encountered with Parkinson's disease (Birkmayer et al., 1983). From this point of view, it is important to know whether or not L-*threo*-DOPS microinjected into the rostral ventrolateral medulla of rat induces cardiopressor responses. This issue arises from our previous finding that L-DOPA microinjected into the same area induced hypertensive and tachycardiac responses in anesthetized rats. These responses to L-DOPA were antagonized by L-DOPA methyl ester (Yue et al., 1993).

In conclusion, L-*threo*-DOPS microinjected into the rat nucleus tractus solitarii stereoselectively produces hypotensive and bradycardic responses. This action is not due to its conversion to noradrenaline but due to its agonistic property on the L-DOPA recognition site. This experimental system may provide a useful screening method for L-DOPA ligands.

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