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Effect of L-Threo-3,4-dihydroxyphenylserine (L-DOPS) on Catecholamine Levels in Plasma and Cerebrospinal Fluid (CSF) in Anesthetized Rats (42467)

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Abstract. The effect of L-threo-3,4-dihydroxyphenylserine (L-DOPS) on norepinephrine (NE) levels in plasma and CSF was examined in urethane-anesthetized rats. Intravenous injection of L-DOPS (0.5, 1, and 10 mg/100 g body wt) raised plasma NE levels in a dose-related manner whereas CSF NE levels were significantly increased only by the largest dose of L-DOPS. Intracerebroventricular injection of L-DOPS (50 and 250 μg/rat) dose-relatedly raised CSF NE levels whereas plasma NE levels were slightly increased by a larger dose of L-DOPS. These findings may indicate that L-DOPS stimulates central noradrenergic mechanisms in the rat although a large dose of L-DOPS is required for peripheral administration.

L-Threo-3,4-dihydroxyphenylserine (L-DOPS) is an unphysiological amino acid which is converted to natural (−)-norepinephrine (NE) *in vivo* by aromatic L-amino acid decarboxylase (1, 2). L-DOPS is now available as a therapeutic reagent for orthostatic hypotension (3, 4) and akinesia of Parkinsonism (5). However, the effect of L-DOPS on NE levels in the central nervous system (CNS) remains to be fully elucidated. Bartholini *et al.* (2) reported that intracerebroventricular (icv) injection of L-DOPS resulted in an increase in NE concentrations in the rat cerebrum whereas intraperitoneal (ip) injection of L-DOPS failed to change cerebral NE. (+)-L-Erythro-DOPS, a stereoisomer of L-DOPS, raised (+)-NE levels in the brain, especially the caudate nucleus and the hypothalamus, when it was injected ip in the rat (2). In contrast, Cuello *et al.* (6) found that ip injection of L-DOPS decreased rather than increased NE concentrations in the rat hypothalamus. In the present study, we examined the effect of L-DOPS injected icv or intravenously (iv) on NE levels in the plasma and cerebrospinal fluid (CSF) of rats.

Materials and Methods. *Animals.* Wistar strain male rats (Japan Animal Co., Osaka) weighing 250–300 g were used throughout the experiments. They were maintained in a temperature (23 ± 1°C)- and humidity (50–60%)-controlled room on a 12-hr dark and 12-hr light schedule (lights on 0600–1800 hr). Laboratory chow (Oriental Yeast Co., Tokyo) and tap water were given *ad libitum.*

After overnight fasting, the animals were anesthetized with urethane (150 mg/100 g body wt, ip). The test substance or acidified saline solution as a control was injected into the lateral ventricle in a volume of 10 μl/rat or into the exposed jugular vein in a volume of 100 μl/100 g body wt as previously described (7).

Blood samples (0.6 ml) were withdrawn from the jugular vein immediately before and 10, 20, and 40 min after injection. The plasma was promptly separated. CSF samples (100 μl) were obtained from the rat by suboccipital puncture with a microsyringe (Ito Co., Tokyo).

Catecholamine assay. Catecholamines in the plasma and CSF were extracted with alumina, and NE and epinephrine (E) concentrations were measured by high-performance liquid chromatography with electrochemical detection (HPLC-ECD) using a modification of the method described previously (8). In brief, 30 mg alumina, 3 ml of 0.1 *M* Tris–HCl buffer (pH 8.6), 1 mg 3,4-dihydroxybenzilamine (DHBA) as an internal standard, 100 μl 5% EDTA-Na-$Na_2S_2O_5$, and the sample plasma (400 μl) or CSF (100 μl) were mixed in a siliconized glass tube. The tube was vigorously shaken for 20 min and the alumina was washed three times with distilled water. Subsequently, the alumina was applied with 3 ml distilled water to a 0.2-μm pore-sized mi-

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crofilter MF-1 (BAS) and centrifuged at 3000 rpm for 5 min for dehydration. Catecholamines were eluted from the alumina by 150 μl HCl. Each eluate (100 μl) was applied to a stainless steel HPLC system (L-4000S, Yanagimoto Co., Kyoto) consisting of a single-piston pump with pulse damper, loop injection valve, and Yanapak ODS-T reverse-phase column connected to a thin-layer voltammetric detector (VMD 501, Yanagimoto Co.).

Analysis of plasma catecholamines was performed with 0.1 *M* citrate buffer (pH 4.5) containing 0.15% octane sulfonic acid sodium salt and 5% methanol at a flow rate of 0.8 ml/min. Citrate buffer (0.1 *M*) containing 0.07% octane sulfonic acid sodium salt was used for analysis of CSF catecholamines. NE and E concentrations in the samples were determined by calculating the ratio of each peak height to DHBA in the chromatogram. Catecholamine peak was identified by the retention time of the standard catecholamine. Catecholamine peak was confirmed by the sample and standard catecholamines having the same relative current ratio when the applied potential of the working electrode was changed as reported by Warnhoff (9). The minimum detectable quantity of NE and E was 70 pg/ml and the mean recovery was 93%.

Drugs. L-DOPS was obtained from Sumitomo Pharmaceuticals Co. (Osaka, Japan) and dissolved in physiological saline acidified with 0.1 *N* HCl.

Statistical analysis. Statistical differences were evaluated by one-way analysis of variance in combination with Duncan's new multiple range test. Student's *t* test also was used when applicable. A *P* value less than 0.05 was considered significant.

Results. Intravenous injection of L-DOPS (0.5, 1, and 10 mg/100 g body wt) resulted in a dose-related increase in plasma NE with a peak value at 10–20 min after the injection (Fig. 1). The mean ± SE peak plasma NE levels after L-DOPS injection were 3.6 ± 0.1, 5.4 ± 1.0, and 74.7 ± 20.7 ng/ml, respectively. Acid saline injection, the control substance, did not change plasma NE levels, with mean peak values of 0.6 ± 0.1 ng/ml. Plasma E levels were not significantly changed by iv injection of L-DOPS.

Intracerebroventricular injection of L-DOPS (250 μg/rat) caused a slight but significant increase in plasma NE levels, with a peak value at 10 min after the injection compared with acidified saline (mean ± SE: 1.7 ± 0.2 ng/ml vs 0.7 ± 0.1 ng/ml, $P < 0.05$) (Fig. 2). A lower dose of L-DOPS (50 μg/rat) injected icv did not change plasma NE levels. Plasma E levels were not affected by icv injection of L-DOPS.

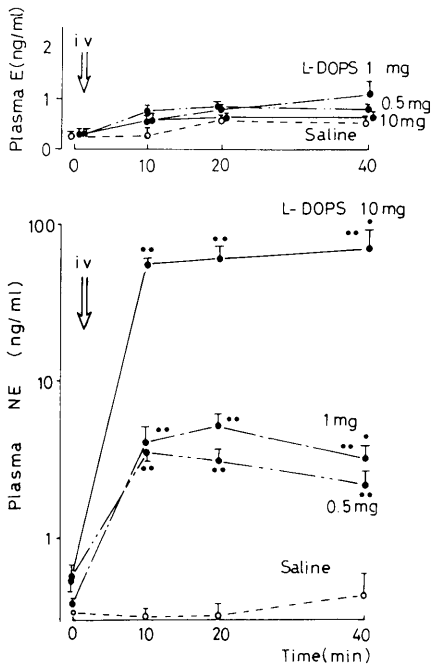

FIG. 1. Effect of iv injection of L-DOPS on plasma NE and E levels in urethane-anesthetized rats. L-DOPS was injected into the external jugular vein in doses of 0.5, 1, and 10 mg/100 g body wt. Acidified saline solution (100 μl/100 g body wt) was injected iv in a control group. Mean (±SE) values of five to seven rats are shown. $^{**}P < 0.01$ vs saline control; $^{*}P < 0.05$ vs lower doses.

Intraventricular injection of L-DOPS (50 and 250 μg/rat) caused a dose-related rise in NE levels in the CSF obtained 40 min after the injection and the mean ± SE values were 3.3 ± 0.5 and 11.6 ± 2.9 ng/ml, respectively ($P < 0.01$ vs saline control 1.3 ± 0.2 ng/ml) (Fig. 3). Intravenous injection of L-DOPS (10 mg/100 g body wt) also caused a slight but significant increase in CSF NE levels (2.2 ± 0.2 ng/ml vs saline control 1.5 ± 0.2 ng/ml, $P < 0.05$). CSF NE levels were not significantly changed by iv injection of lower doses of L-DOPS (0.5 and 1 mg/100 g body wt). CSF E levels were not detectable in these animals.

Discussion. In the present study, we first demonstrated that iv injection of L-DOPS rapidly raised plasma NE levels in anesthetized rats. These findings are in the line with previous reports which indicated that L-DOPS is converted to natural NE by aromatic L-amino acid decarboxylase (1, 2). We further demonstrated that CSF NE levels were significantly raised by icv injection of a much smaller dose of L-DOPS (50 μg/rat). Although NE levels in the brain tissue were not measured in the present study, an increase in the CSF levels of NE might lead to an increase in the uptake of NE into nerve terminals, resulting in an increase in the concentration of NE in nerve terminals. It is possible, therefore, that L-DOPS is converted to NE and stimulates noradrenergic mechanisms not only in the periphery but also in the brain of the rat.

We showed that iv injection of the largest dose of L-DOPS examined (10 mg/100 g body wt) yielded a slight but significant increase in CSF NE levels. The relative low levels of NE in the CSF, following iv injection of L-DOPS, could be explained by (i) poor penetration of L-DOPS across the blood–brain barrier, (ii) rapid conversion of L-DOPS to NE in the periphery and poor penetration of NE across the blood–brain barrier, (iii) conversion of L-DOPS to NE followed by rapid catabolism of NE in the brain, or (iv) conversion of L-DOPS to NE in the brain followed by a rapid uptake

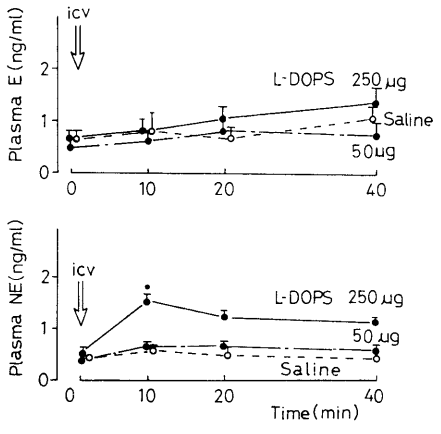

FIG. 2. Effect of icv injection of L-DOPS on plasma NE and E levels in urethane-anesthetized rats. L-DOPS was injected into the lateral ventricle in a dose of 50 and 250 μg/rat. Acidified saline solution (10 μl/rat) was injected in a control group. $^*P < 0.05$ vs saline control.

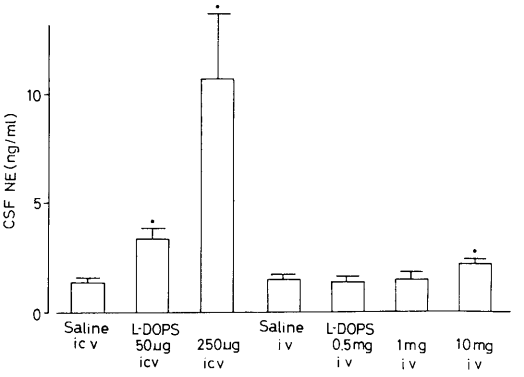

FIG. 3. Effect of L-DOPS injected iv or icv on CSF NE levels in urethane-anesthetized rats. L-DOPS was injected iv in doses of 0.5, 1, and 10 mg/100 g body wt or icv in doses of 50 and 250 μg/rat. CSF samples were obtained 40 min after the injection. $^*P < 0.05$ vs saline control and lower doses.

of NE into nerve terminals. Creveling *et al.* (10) reported that approximately 0.3% of the radioactivity of DL-threo-erythro-DOPS-^{14}C, a radiolabeled analog of L-DOPS, was taken rapidly in the mouse brain to form NE-^{14}C after iv injection and that the level of labeled NE decreased slowly with detectable amounts after 3 hr. However, further studies are required to conclude that L-DOPS crosses the blood–brain barrier poorly.

L-DOPS is now used therapeutically (3–5). We demonstrated that icv injection of L-DOPS raises the level of NE in the CSF without altering the plasma levels of NE. These findings may indicate that central administration of L-DOPS could be considered for the purpose of increasing NE activity in the central nervous system.

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1. Blasnko H, Burn JH, Lanemann H. The formation of noradrenaline from dihydroxyphenylserine. Brit J Pharmacol **5**:431–437, 1950.
2. Bartholini G, Constantinidis J, Puig M, Tissot R, Pletscher A. The steroisomers of 3,4-dihydroxyphenylserine as precursors of norepinephrine. J Pharmacol Exp Ther **193**:523–532, 1975.
3. Suzuki T, Higa S, Sakada S, Hayashi A, Yamamura Y, Takaba Y, Nakajima A. Orthostatic hypotension in familial amyloid polyneuropathy: DL-Threo-3,4-hydroxyphenylserine. Neurology **31**:1323–1326, 1981.

4. Suzuki T, Higa S, Tsuge I, Sakoda S, Hayashi A, Yamamura Y, Takaba T, Nakajima A. Effect of infused L-threo-3,4-dihydroxy-phenylserine on adrenergic activity in patients with familial amyloid polyneuropathy. Eur J Clin Pharmacol **17**:429–435, 1980.
5. Narabayashi H, Kondo T, Hayashi A, Suzuki T, Nagatsu T. L-Threo-3,4-dihydroxyphenylserine treatment for akinesia and freezing of Parkinsonism. Proc Japan Acad **57**:523–532, 1981.
6. Cuello AC, Scapagnini U, Licko U, Preziosi P, Ganong WF. Effect of dihydroxyphenylserine on the increase in plasma corticosterone in rats treated by α-methyl-*p*-tyrosine. Neuroendocrinology **13**:115–120, 1973/1974.
7. Kato T, Iwasaki Y, Abe H, Ohgo S, Imura H. Effects of endorphins on prolactin and growth hormone secretion in rats. Proc Soc Exp Biol Med **158**:431–436, 1978.
8. Kabayama Y, Kato Y, Tojo K, Shimatsu A, Ohta H, Imura H. Central effects of DN1417, a novel TRH analogue, on plasma glucose and catecholamines in conscious rats. Life Sci **36**:1287–1294, 1985.
9. Warnhoff M. Simultaneous determination of norepinephrine, dopamine, 5-hydroxytryptamine and their main metabolites in rat brain using high-performance liquid chromatography with electrochemical detection. J Chromatogr **307**:271–281, 1984.
10. Creveling CR, Daly J, Tokuyama T, Witkop B. The combined use of α-methyl-tyrosine and threo-dihydroxyphenylserine—Selective reduction of dopamine levels in the central nervous system. Biochem Pharmacol **17**:65–70, 1968.

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