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# Effect of Infused L-Threo-3,4-dihydroxyphenylserine on Adrenergic Activity in Patients with Familial Amyloid Polyneuropathy

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**Summary.** L-threo-3,4-dihydroxyphenylserine (DOPS), an immediate precursor amino acid of (-)-norepinephrine, was used as a pharmacological tool to investigate the pathophysiology of the peripheral sympathetic nervous system in Type 1 familial amyloid polyneuropathy. Patients with the well-established disorder showed an enhanced pressor response to L-threo-DOPS under conditions that produced no change in normal subjects. While octopamine induced a brisk pressor response, L-threo-DOPS produced a slow and prolonged change in blood pressure, with a marked concomitant increase in urinary excretion of norepinephrine. A slight increase in urinary excretion of total metanephrine was observed in both groups, but there was no significant increase in serum dopamine-$\beta$-hydroxylase activity. Since infusion of dilute norepinephrine into patients also produced a markedly hypersensitive response, the characteristic pressor response to L-threo-DOPS was indicative of denervation supersensitivity of adrenergic receptors to norepinephrine formed enzymatically from L-threo-DOPS.

**Key words:** L-threo-3,4-dihydroxyphenylserine, norepinephrine, octopamine, dopamine-$\beta$-hydroxylase, familial amyloid polyneuropathy denervation supersensitivity, autonomic dysfunction

Type 1 familial amyloid polyneuropathy is a dominantly inherited form of hereditary amyloidosis, characterized by polyneuropathy and severe autonomic dysfunction [1]. The lesions of the autonomic nervous system produce constipation, diarrhea, sphincter impairment, impotence in the male, postural hypotension, disordered cardiac conduction, hypohidrosis, trophic skin ulcers etc. It is often responsible for the death of patients. However, the pathophysiology of the disorder of the peripheral autonomic nervous system is mostly unknown.

In a previous paper [2], we demonstrated that the urinary excretion rate of catecholamines and serum dopamine-$\beta$-hydroxylase (DBH) activity were significantly reduced in patients with advanced disease, suggesting a deficiency of neurotransmitters in peripheral sympathetic nerves.

On the basis of this finding, L-threo-3,4-dihydroxyphenylserine (DOPS) and, for comparison, DL-threo-DOPS and octopamine were selected as pharmacologic tools to examine the pathophysiology of the peripheral sympathetic nervous system in the disease. As shown in Figure 1, L-threo-DOPS is an unphysiological precursor amino acid of natural (-)-norepinephrine [3]. It is converted to (-)-norepinephrine directly by aromatic L-amino acid decarboxylase, which is distributed in various tissues including peripheral sympathetic nerves. Octopamine, an endogenous precursor of norepinephrine, has a weak pressor activity [4].

This paper describes characteristic effects of infused L-threo-DOPS on blood pressure, serum DBH activity and urinary output of norepinephrine, which imply denervation supersensitivity of adrenergic receptors to norepinephrine in familial amyloid polyneuropathy.

## Materials and Methods

*Reagents.* L-threo-DOPS was synthesized in the laboratories of the Sumitomo Chemical Co. Ltd., Osaka, Japan. It had a negative rotation of $[\alpha]_D^{20} = -39.0$ (C=1.01, 1 N HCl). L-threo-DOPS 100 mg was packed into each ampule, which was flushed with nitrogen and stored in the dark at 4 °C until used.

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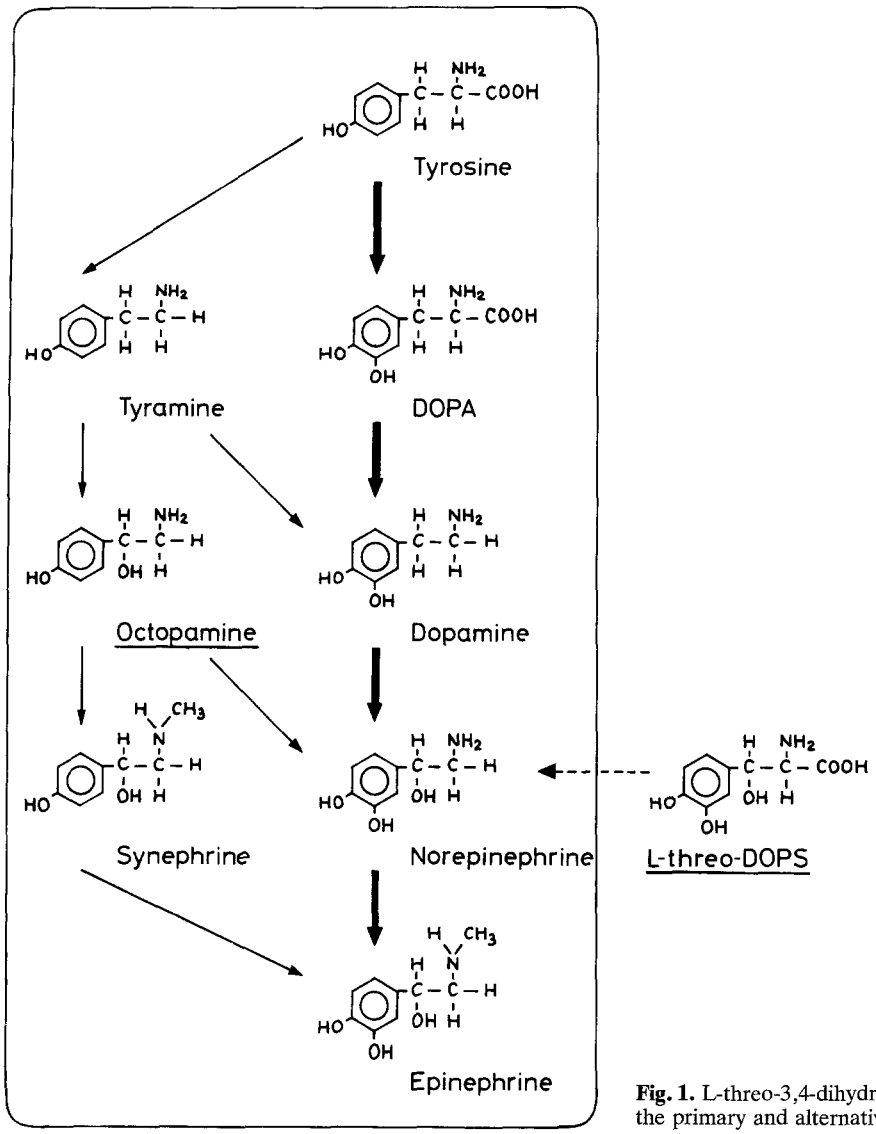


**Fig. 1.** L-threo-3,4-dihydroxyphenylserine (DOPS) and octopamine in the primary and alternative biosynthetic pathways of catecholamines

DL-threo-DOPS was purchased from Aldrich Chemical Co. Inc., Milwaukee, USA. A solution of DL-threo-DOPS 200 mg/40 ml 0.01 N HCl was sterilized by filtration through a Millipore filter. It was placed in ampules and stored in the dark at 4 °C until used. As described below, just prior to use it was diluted in sterile physiological saline. Contamination by norepinephrine assayed in our laboratory was found to be 0.0009% in L-threo-DOPS and 0.0268% in DL-threo-DOPS.

( ± )-p-octopamine HCl was kindly supplied as Norphen® ampules by Byk Gulden Lomberg GmbH, Konstanz, Federal Republic of Germany.

*Subjects.* Eight patients (six men and two women), from four affected families, with well-established Type 1 familial amyloid polyneuropathy were examined. They lived in Arao City, Kumamoto Prefecture [5]. The mean age at the time of examination was 40 y (range 24 to 50). The mean duration of illness was 6 years (range 4 to 10). The diagnosis of the disorder was established histopathologically in 6 cases by light-microscopy of biopsies. The other 2 cases were not confirmed histopathologically, but they were siblings of a patient who had died of the disorder, and they had similar neurological features. The normal group consisted of nine healthy subjects (seven men and two women; mean age 31 years, range 21 to 38); none of them had a history of neurological disorder, nor had they received drug treatment.

*Infusion Procedures.* The purpose of the infusion tests and their investigational nature were fully explained to the subjects.

The intravenous loading tests were performed with the subject supine in a darkened room. During the control period, each subject was infused with physiological saline. When his pulse rate and blood pressure had become stable, the infusate was changed to a solution of either of the norepinephrine precursors cited above.

L-threo-DOPS 100 mg dissolved in physiological saline 400 ml was infused into five patients and four normal subjects via the antecubital vein, at a constant rate (833 μg in 3.3 ml saline/min) over a period of 120 min.

Two patients, who were siblings, and two normal subjects were given DL-threo-DOPS 200 mg in 400 ml physiological saline, by intravenous infusion at a constant rate (1667 μg in 3.3 ml saline/min) over 120 min.

Five patients and four normal subjects were given (±)-p-octopamine HCl 50 mg in 400 ml physiological saline, by intravenous infusion at a constant rate (417 μg in 3.3 ml saline/min) over 120 min. Each infusion was carried out on a different day.

During the procedures and for 1 h afterwards, pulse rate and blood pressure were repeatedly measured. Blood was collected at 0, 30, 60, 120 and 180 min for determination of serum DBH activity. Urinary excretion of norepinephrine after intravenous infusion of L-threo-DOPS was followed over 24 h. Urine was collected in the presence of sodium metabisulfite at 3-h intervals for the first 9 h, and at the end of 24 h. The urine samples were immediately frozen and stored at −20 °C until analysed for catecholamines and their metabolites. According to Alexander et al. [6], a saline infusion of 15 ml/min (approximately 5 times as much as the infusion rate employed here) did not cause any significant change from normal in urinary norepinephrine + epinephrine, whereas plasma DBH activity fell significantly.

Intravenous infusion of dilute (±)-norepinephrine 0.2 to 3.0 μg/min [7] were performed in four patients and four normal subjects. Blood pressure and pulse were monitored during each infusion.

*Assay Procedures.* Serum DBH activity was determined by the spectrophotometric method [8]. The infusion of L-threo-DOPS necessitated use of three purification procedures in the determination of urinary norepinephrine; columns of borate gel were eluted with 0.05 N perchloric acid [9], and the eluate was passed through a column of Dowex 50 W, which was eluted with 1 N HCl 10 ml [10]. It was then diluted with water to 25 ml and was extracted with aluminum oxide according to Anton and Sayre [11]. Total metanephrine was assayed by the method of Pisano [12].

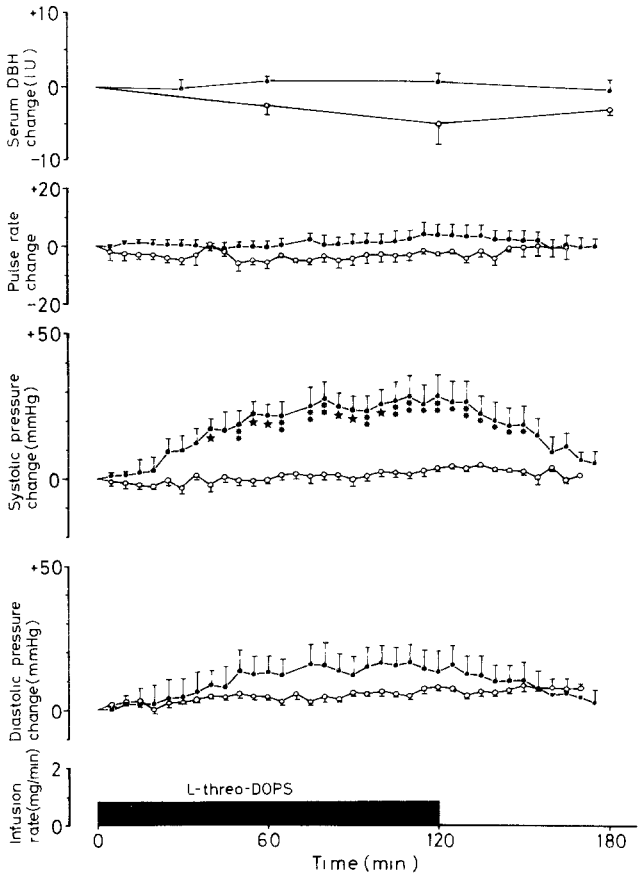


**Fig. 2.** Effect of infused L-threo-DOPS (833 μg/min) on blood pressure, pulse rate and serum DBH in 5 patients with familial amyloid polyneuropathy and 4 normal subjects; I: patients (mean + SEM); I: normal subjects (mean − SEM); * Significant difference ($p < 0.05$) from basal value
** $P < 0.02$ and ★ $P < 0.01$

*Statistical Calculations.* Data were analyzed by standard parametric techniques, such as Student's t test and paired t-tests; the level of significance was set at $p < 0.05$. Results are given as mean ± SEM.

## Results

### *Effects of L-threo- and DL-threo-DOPS Infusion on Pulse Rate, Blood Pressure and Serum DBH Activity*

The experimental design and a summary of the result of L-threo-DOPS infusion are shown in Figure 2. In normal subjects L-threo-DOPS had no effect on blood pressure, but in the patients (mean ± SEM mmHg in control period, systolic 105 ± 6; diastolic 74 ± 7) it gradually rose to a maximum increase of systolic 28 ± 6, diastolic 15 ± 8 mmHg, which was sustained throughout the infusion. The maximum

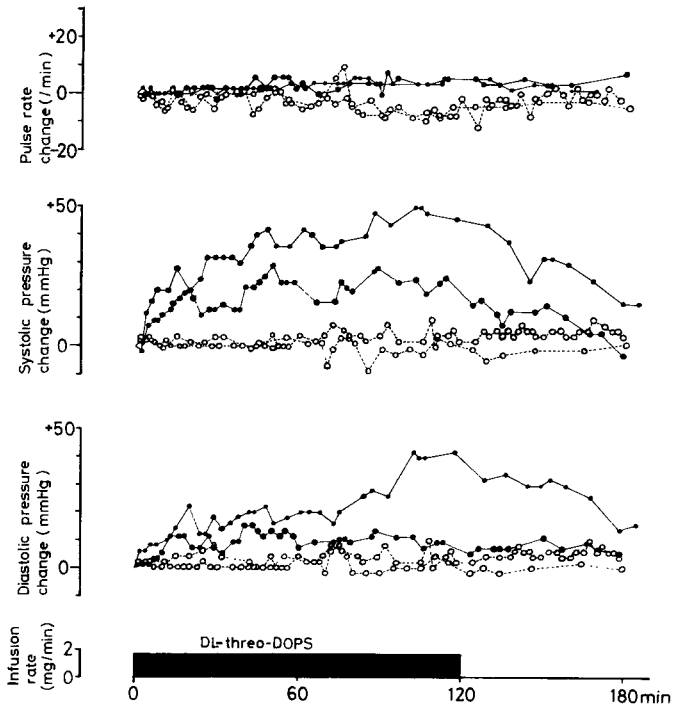


**Fig. 3.** Effect of infused DL-threo-DOPS (1667 μg/min) on blood pressure and pulse rate in 2 patients with familial amyloid polyneuropathy and 2 normal subjects; ●: patients, ○: normal subjects

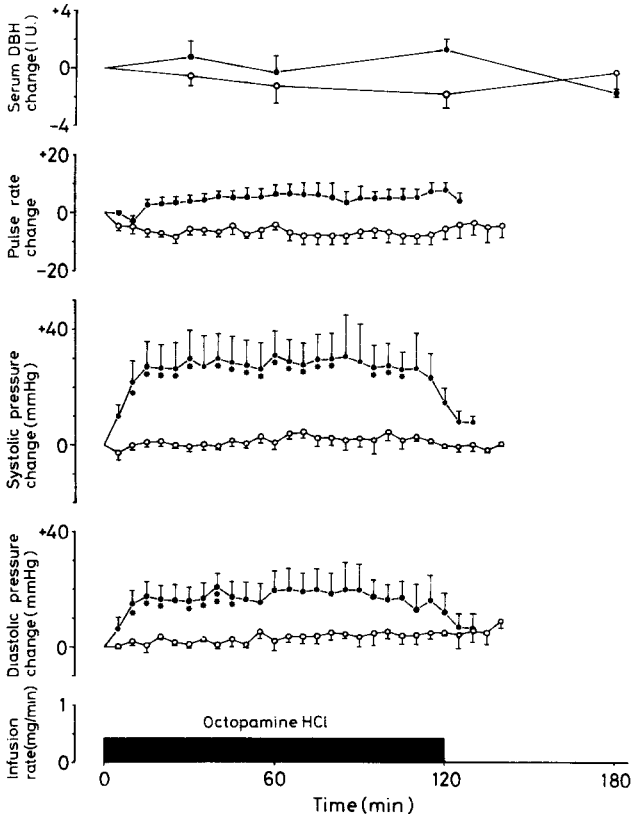


**Fig. 4.** Response of blood pressure, pulse rate and serum DBH to intravenous octopamine HCl (417 μg/min) in 5 patients with familial amyloid polyneuropathy and 4 normal subjects; ⟟ : patients (mean + SEM), ⟟: normal subjects (mean − SEM). * $P < 0.05$ ** $P < 0.02$

was reached after 80 min and the blood pressure returned to the initial value 60 min after cessation of the infusion. No change in pulse rate or serum DBH (mean ± SEM I. U. in control period, 35.0 ± 6.0 for normal subjects; 20.2 ± 8.1 for patients) was observed in either group. No subjective changes were reported during the test. Postural hypotension in the patients was not blocked, even during L-threo-DOPS infusion.

The experimental design and a summary of the results of DL-threo-DOPS infusion are summarised in Figure 3. It appears that twice as much of the DL-threo-DOPS gave essentially the same effects as those observed after the L-threo-DOPS infusion.

### *Effect of Octopamine Infusion on Pulse Rate, Blood Pressure and Serum DBH Activity*

The experimental design and results of the octopamine infusion are shown in Figure 4. In contrast to the normal subjects, who showed no response to octopamine, blood pressure in the patients (control period systolic 98 ± 6; diastolic 64 ± 6 mmHg) started to rise immediately, reached a plateau (pressor response, systolic 27 ± 9; diastolic 17 ± 5 mmHg) in 15 min and then returned toward the initial values immediately ater termination of the infusion. No change in pulse rate or serum DBH was found in either group.

### *Effect of L-threo-DOPS Infusion on Urinary Excretion of Norepinephrine and its Metabolites*

The urinary excretion rates of norepinephine and total metanephrine after intravenous infusion of L-threo-DOPS 100 mg are given in Table 1. As already described [2], the basal excretion rate of norepinephrine by the patients tended to be lower than by normal subjects. Following administration of L-threo-DOPS, an enormous increase in the urinary

excretion rate of norepinephrine was observed; to a maximal 157-fold increase in the first fraction (0–3 h) from normal subjects, and a 169-fold increase in the 2nd fraction (3–6 h) from the patients. Although excretion of norepinephrine by the patients was delayed during the first 6 h, there was no significant difference between the total amount of norepinephrine excreted during 24 h by normal subjects (1279 ± 459 μg) and by the patients (1138 ± 210 μg). This means that approximately 1.5% of the administered L-threo-DOPS was recovered as (-)-norepinephrine in urine during 24 h. Formation of (-)-norepinephrine during urine collection was negligible, although L-threo-DOPS could undergo non-enzymatic decarboxylation to (-)-norepinephrine.

L-threo-DOPS caused a relatively mild increase in the urinary excretion rate of total metanephrine: a maximal 2.5-fold increase in the first fraction from normal subjects, and a 1.9-fold increase in the second fraction (3–6 h) from the patients. There was no significant difference between the amount of total metanephrine excreted in the 24 h after administration of L-threo-DOPS by normal subjects (726 ± 43 μg) and by the patients (1047 ± 312 μg).

### *Effect of Dilute Norepinephrine Infusion on Blood Pressure*

The consequences of dilute norepinephrine infusion are illustrated in Figure 5. Patients showed a markedly hypersensitive response to norepinephrine within a range below the minimum infusion rate required to raise blood pressure in normal subjects.

## Discussion

3,4-Dihydroxyphenylserine has been a valuable pharmacological tool for introduction of an immediate precursor of norepinephrine into the central nervous system [13]. Until recently, however, experiments have been carried out with racemates, i. e. DL-erythro- or DL-threo-DOPS, of which only the L-threo isomer is decarboxylated to form (-)-norepinephrine [3]. There have been several reports of the action of each of the four stereoisomers on rat brain and peripheral organs [14–17], but there have been few accounts of the pharmacology of DOPS in humans [18]. DL-threo-DOPS has no known toxicological effect [19]. The present study is the first to examine the effect of L-threo-DOPS on the human peripheral sympathetic nervous system.

Patients with familial amyloid polyneuropathy showed an enhanced pressor response to infused L-threo-DOPS under conditions that produced no change in normal subjects, whereas, assuming that the increments in urinary norepinephrine (Table 1) reflected its circulating levels, the increase in plasma norephinephrine during the first 3 h might have been less in patients than in normal subjects. The characteristic time course of pressor responses to L-threo-DOPS (Fig. 2), which is quite different from that of octopamine (Fig. 4), and the marked increase in uri-

**Table 1.** Urinary Excretion Rates (mean ± SEM) of Norepinephrine and Total Metanephrine after Infusion of L-THREO-DOPS 100 mg over 2 h in 5 cases of Familial Amyloid Polyneuropathy and 4 normal subjects

| Time (h) | Norepinephrine (ng/min) | | Total Metanephrine (ng/min) | |
|---|---|---|---|---|
| | Controls | Patients | Controls | Patients |
| Before Loading | 32± 11 | 17± 5 | 465 ±145 | 592±209 |
| 0– 3 | 5010±1731 | 2708±898 | 1182*±138 | 798±284 |
| 3– 6 | 1533± 550 | 2868±805 | 663 ± 61 | 1133±327 |
| 6– 9 | 330± 126 | 1104±345 | 557 ±101 | 1508±579 |
| 9–24 | 42± 15 | 58± 17 | 432 ±121 | 490±212 |

* Significant change ($p < 0.02$) from basal value.

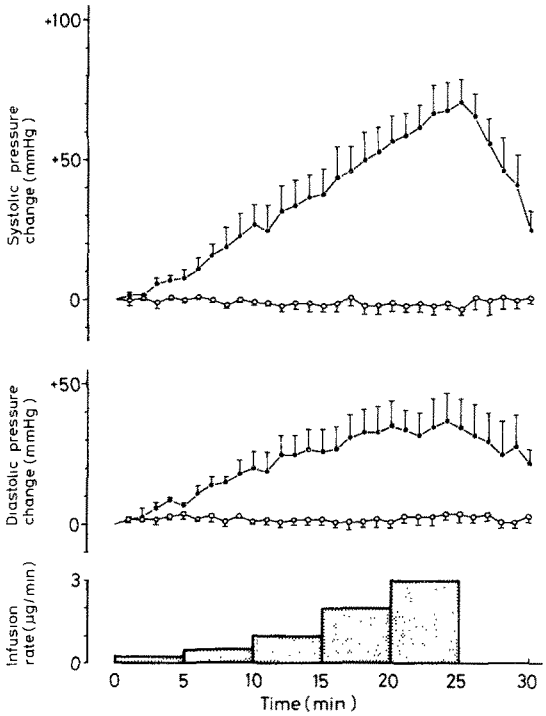


**Fig. 5.** Effect of infused dilute norepinephrine on blood pressure in 4 patients with familial amyloid polyneuropathy and 4 normal subjects; I: patients (mean + SEM), Î: normal subjects (mean − SEM)

nary excretion of norepinephrine indicate that conversion of L-threo-DOPS to (-)-norepinephrine was the primary metabolic event. In view of the histopathological appearances, which indicate that familial amyloid polyneuropathy is essentially a slow progressive degeneration of nerve fibers [20], and that the sympathetic disturbance is mainly postganglionic [21], the increased pressor response to L-threo-DOPS is suggestive of denervation supersensitivity of adrenergic receptors to norepinephrine [22]. The exaggerated pressor response in patients to infusion of the direct-acting agonist, norepinephrine, supports this interpretation. Exaggerated pressor and vasoconstrictor responses to infused norepinephrine were ascribed to denervation supersensitivity in idiopathic orthostatic hypotension [23–28], Shy-Drager syndrome [29], familial dysautonomia [30], pure pan-dysautonomia [31] and a case of non-hereditary primary amyloidosis [7].

It remains to be determined whether L-threo-DOPS is converted to (-)-norepinephrine in neuronal tissues or extraneuronally. At least, intravenous infusion of L-threo-DOPS induced no significant increase in serum DBH, which is released from sympathetic nerve terminals together with norepinephrine [32].

It has recently been reported that in vitro decarboxylation of L-threo-DOPS in the rat kidney was inhibited by the addition of D-threo-DOPS [17]. This property of aromatic L-amino acid decarboxylase has been confirmed in human kidney (unpublished observation). However, in the infusion tests reported above, the effect of L-threo- and DL-threo-DOPS on blood pressure was essentially the same. The explanation of this discrepancy remains unsolved, although part of pressor response to DL-threo-DOPS might have resulted from contamination of the sample by norepinephrine.

The metabolic fate of administered L-threo-DOPS is being investigated further, including measurement of the plasma levels of norepinephrine.

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## References

1. Glenner GG, Ignaczak TF, Page DL (1978) The inherited systemic amyloidosis and localized amyloid deposits. In: Stanbury JB, Wyngaarden JB, Fredrickson DS, (eds) The metabolic basis of inherited disease. 4th ed. McGraw-Hill, New York, pp 1308–1339
2. Suzuki T, Tsuge I, Higa S, Hayashi A, Yamamura Y, Takaba Y, Nakajima A (1979) Catecholamine metabolism in familial amyloid polyneuropathy. Clin. Genet. 16: 117–124
3. Patil PN, Miller DD, Trendelenburg U (1975) Molecular geometry and adrenergic drug activity. Pharmacol Rev 26: 323–392
4. Axelrod J, Saavedra JM (1977) Octopamine. Nature 265: 501–504
5. Araki S, Mawatari S, Ohta M, Nakajima A, Kuroiwa Y (1968) Polyneuritic amyloidosis in a Japanese family. Arch Neurol 18: 593–602
6. Alexander RW, Gill JR, Yamabe H, Lovenberg W, Keiser H (1974) Effects of dietary sodium and of acute saline infusion on the interrelationship between dopamine excretion and adrenergic activity in man. J Clin Invest 54: 194–200
7. Rubenstein AE, Yahr MD, Mytilineou C, Bajaj K (1978) Peripheral catecholamine depletion in amyloid autonomic neuropathy. Mt Sinai J Med 45: 782–789
8. Nagatsu T, Udenfriend S (1972) Photometric assay of dopamine-$\beta$-hydroxylase activity in human blood. Clin Chem 18: 980–983
9. Higa S, Suzuki T, Hayashi A, Tsuge I, Yamamura Y (1977) Isolation of catecholamines in biological fluids by boric acid gel. Anal Biochem 77: 18–24
10. Atack CV (1973) The determination of dopamine by modification of the dihydroxyindole fluorimetric assay. Br J. Pharmacol 48: 699–714
11. Anton AH, Sayre DF (1962) A study of the factors affecting the aluminum oxide-trihydroxyindole procedure for the analysis of catecholamines. J Pharmacol Exp Ther 138: 360–375
12. Pisano JJ (1960) A simple analysis for normetanephrine and metanephrine in urine. Clin Chim Acta 5: 406–414
13. Creveling GR, Daly J, Tokuyama T, Witkop B (1968) The combined use of $\alpha$-methyltyrosine and threo-dihydroxyphenylserine-selective reduction of dopamine levels in the central nervous system. Biochem Pharmacol 17: 65–70
14. Bartholini G, Constantinidis J, Puig M, Tissot R, Pletcher A (1975) The stereoisomers of 3,4-dihydroxyphenylserine as precursors of norepinephrine. J Pharmacol Exp Ther 193: 523–532
15. Hirai M, Matsuoka Y, Nakajima T, Sano I (1975) Effects of 3,4-dihydroxyphenylserine on the concentration of brain noradrenaline and the level of plasma growth hormon of rats. Med J Osaka Univ 26: 51–59
16. Fujiwara H, Inagaki C, Ikeda Y, Tanaka C (1976) Decarboxylation of stereoisomers of 3,4-dihydroxyphenylserine (DOPS) in vitro. Nippon Yakurigaku Zasshi 72: 891–898
17. Inagaki C, Fujiwara H, Tanaka C (1976) Inhibitory effect of (+)threo-3,4-dihydroxyphenylserine (DOPS) on decarboxylation of (−)threo-DOPS. Jpn J Pharmacol 26: 380–382
18. Gunne LM, Lidvall HF (1966) The urinary output of catecholamines in narcolepsy under resting conditions and following administration of dopamine, dopa and dops. Scand J Clin Lab Invest 18: 425–430

19. Gatlin LA, Gromes GJ, Hiranaka PK, Gallelli JF (1975) Investigational drug information. DL-threo-dihydroxyphenylserine. Drug Intell Clin Pharm 9: 655
20. Andrade C (1975) Hereditary amyloid neuropathy. In: Vinken PJ, Bruyn GW (eds) Handbook of clinical neurology. Vol 21. North-Holland Amsterdam, pp 119–143
21. Tsukagoshi H, Oguchi K, Shoji S, Yamasaki T, Nakamura H, Beppu H: Autonomic disturbance in familial amyloid polyneuropathy. Autonom Nerv Syst (Jpn) 12: 7–12
22. Burnstock G, Costa M (1975) Adrenergic neurons. Their organization, function and development in the peripheral nervous system. Chapman and Hall, London, pp 125–126
23. Barnett AJ, Wagner GR (1958) Severe orthostatic hypotension: Case report and description of response to sympathomimetic drugs. Am Heart J 56: 412–424
24. Hickler RB, Thomson GR, Fox LM, Hamlin JT (1959) Successful treatment of orthostatic hypotension with 9-alpha-fluorohydrocortisone. N Engl J Med 261: 788–791
25. Engelman K, Mueller PS, Horwitz D, Sjoerdsma A (1964) Denervation hypersensitivity of adipose tissue in idiopathic orthostatic hypotension. Lancet 2: 927–929
26. Chokroverty S, Barron KD, Katz FH, Del Greco F, Sharp JT (1969) The syndrome of primary orthostatic hypotension. Brain 92: 743–768
27. Burns RJ, Downey JA, Frewin DB, Whelan RF (1971) Autonomic dysfunction with orthostatic hypotension. Aust NZ J Med 1: 15–21
28. Kontos HA, Richardson DW, Norvell JE (1975) Norepinephrine depletion in idiopathic orthostatic hypotension. Ann Int Med 82: 336–341
29. Hohl RD, Frame B, Schatz IJ (1965) The Shy-Drager variant of idiopathic orthostatic hypotension. Am J Med 39: 134–141
30. Smith AA, Dancis J (1964) Exaggerated response to infused norepinephrine in familial dysautonomia. N Engl J Med 270: 704–707
31. Young RR, Asbury AK, Corbett JL, Adams RD (1975) Pure pan-dysautonomia with recovery. Brain 98: 613–636
32. Weinshilboum RM, Thoa NB, Johnson DG, Kopin IJ (1971) Proportional release of norepinephrine and dopamine-$\beta$-hydroxylase from sympathetic nerves. Science 174: 1349–1351

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