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RESEARCH ARTICLE

Substantial renal conversion of L-threo-3,4-dihydroxyphenylserine (droxidopa) to norepinephrine in patients with neurogenic orthostatic hypotension

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Abstract

Background The pressor effect of L-threo-3,4-dihydroxyphenylserine (L-DOPS, droxidopa, Northera™) results from conversion of L-DOPS to norepinephrine (NE) in cells expressing L-aromatic-amino-acid decarboxylase (LAAAD). After L-DOPS administration the increase in systemic plasma NE is too small to explain the increase in blood pressure. Renal proximal tubular cells abundantly express LAAAD. Since NE generated locally in the kidneys could contribute to the pressor effect of L-DOPS, in this study we assessed renal conversion of L-DOPS to NE.

Methods Ten patients who were taking L-DOPS for symptomatic orthostatic hypotension had blood and urine sampled about 2 h after the last L-DOPS dose. L-DOPS and NE were assayed by alumina extraction followed by liquid chromatography with electrochemical detection. Data were compared in patients off vs. on levodopa/carbidopa.

Results In patients off levodopa/carbidopa the ratio of NE/L-DOPS in urine averaged 63 times that in plasma ($p = 0.0009$ by t test applied to log-transformed data). In marked contrast, in the three patients on levodopa/carbidopa the ratio of NE/L-DOPS in urine did not differ from that in plasma.

Conclusion There is extensive renal production of NE from L-DOPS. Carbidopa seems to attenuate the conversion of L-DOPS to NE in the kidneys. Further research is needed to assess whether the proposed paracrine effect of L-DOPS in the kidneys contributes to the systemic pressor response.

Keywords Dihydroxyphenylserine · L-DOPS · Droxidopa · Norepinephrine · Orthostatic hypotension · Parkinson disease · Autonomic failure · Parkinsonism · Renal

Introduction

L-Threo-3,4-dihydroxyphenylserine (L-DOPS, droxidopa, Northera™) is a synthetic amino acid that is converted to norepinephrine (NE) in cells expressing the enzyme L-aromatic-amino-acid decarboxylase (LAAAD). L-DOPS is approved in the US to treat symptomatic orthostatic hypotension in conditions such as in Parkinson disease (PD), multiple system atrophy (MSA), pure autonomic failure (PAF), dopamine beta-hydroxylase deficiency, and non-diabetic autonomic neuropathy [15].

The mechanism of pressor action of L-DOPS is via its conversion to NE [15]; however, organ sites of the conversion are poorly understood. Plasma NE increases after administration of L-DOPS [15], but the increase is too small to explain the increase in blood pressure [8].

LAAAD is expressed abundantly in parenchymal cells of the liver, kidneys, and gut. We hypothesized that because of local LAAAD activity there is substantial renal conversion of L-DOPS to NE. We assessed this possibility from comparing the ratio of NE/L-DOPS in the urine to that in the plasma.

Patients with parkinsonian syndromes are often treated with the combination of levodopa/carbidopa, which remains the gold standard for managing symptomatic PD. Carbidopa augments the therapeutic effect of levodopa by inhibiting LAAAD outside the brain [18]. In so doing carbidopa might

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blunt renal NE production from L-DOPS. We, therefore, hypothesized that patients on both L-DOPS and levodopa/carbidopa have less renal conversion of L-DOPS to NE than do patients on L-DOPS alone.

We also compared the observed vs. expected urinary excretion rates of other catechols [13] after L-DOPS treatment.

Methods

Subjects

We studied ten patients (mean age 64.8 years, range 28–73 years, five men) at the NIH Clinical Center after they had given written informed consent to a research protocol approved by the NINDS Institutional Review Board.

Of the 10 patients, two carried a diagnosis of PAF, three PD, four MSA, and one autoimmunity-associated autonomic failure with sympathetic denervation (Table 1). Two patients with MSA and one with PD were on levodopa/carbidopa at the time of testing. For the other seven patients, levodopa had either never been prescribed or had been discontinued at least 3 days before the study, with baseline plasma dihydroxyphenylalanine (DOPA) concentrations within normal limits confirming discontinuation of levodopa/carbidopa.

Study design

Patients were tested on their usual dose of L-DOPS and other treatments. Blood and urine were collected 2–3 h after the last dose of L-DOPS. In order to calculate urinary excretion rates, the time since the previous urination was recorded. When this information was not available, we used the urinary excretion rates of catechols reported in the literature in patients off or on levodopa/carbidopa to estimate the time since last urination [6, 13, 19]. The time since last urination was obtained by rearrangement of the formula for urinary clearance of a circulating compound:

Table 1 Patient characteristics

Mean (SD) age, years	64.8 (5.8)
Female, *n* (%)	5 (50%)
White, *n* (%)	9 (90%)
Primary clinical diagnosis	
Pure autonomic failure	2
Parkinson disease with OH	3
Multiple system atrophy	4
Autoimmunity-associated autonomic failure with sympathetic denervation	1
Mean (SD) duration of illness, years	6.5 (1.6)
Mean (SD) daily dose of Droxidopa, mg	790 (427.6)

$$C = [U] \cdot \mathrm{VU}/[P],$$

where C = plasma clearance (L/min), $[U]$ = urine concentration (nmol/L), VU = urine volume (L), and $[P]$ = plasma concentration (nmol/L). C can be expressed in terms of the volume of plasma emptied per unit time, or VP/T. Since

$$C = \mathrm{VP}/T = [U] \cdot \mathrm{VU}/[P].$$

Rearrangement yields

$$T = \mathrm{VP}/C = (\mathrm{VP} \cdot [P])/(\mathrm{VU} \cdot [U]).$$

Expected urinary excretion rates of different catecholamines were found in the literature for L-DOPS, NE, dihydroxyphenylglycol (DHPG), dihydroxyphenylalanine (DOPA), dihydroxyphenylacetic acid (DOPAC), and dopamine (DA) [13]. Expected urinary excretion rates of DOPA and DA in patients taking levodopa were described previously [19].

Neurochemical assays

L-DOPS and NE in plasma and urine were assayed in our laboratory by alumina extraction followed by liquid chromatography with electrochemical detection, as described previously [10, 11].

Data analysis and statistics

Differences in neurochemical data between plasma and urine samples were compared by t tests for dependent means. Mean values were expressed ± 1 standard error of the mean (SEM). Although it was expected that L-DOPS would increase plasma NE, two-tailed tests were used. A p value less than 0.05 defined statistical significance. To account for inhomogeneity in the variance of the data, statistical tests were done on log-transformed data.

Results

Across all patients the ratio of NE/L-DOPS in the urine averaged 63.2 ± 70.4 times that in the plasma ($p = 0.0009$). There were no difference between the observed and expected urinary excretion rates of L-DOPS, DHPG, NE, DOPA, DOPAC, or DA (Table 2; Fig. 1).

In the seven patients not taking levodopa/carbidopa, the ratio of NE/L-DOPS in urine averaged 89.6 ± 68.7 times that in the plasma ($p < 0.0001$). There was more NE in the urine than expected (56.5 ± 64.8 times expected, $p = 0.00015$). Urinary excretion rates of L-DOPS, DHPG, DOPA, DOPAC, and DA did not differ from expected (Table 3).

In the three patients on levodopa/carbidopa, the mean ratio of NE/L-DOPS in urine did not differ from that in

Table 2 Ratio of the observed vs. expected urinary excretion rates of different catechols across all subjects ($N = 10$)

	observed/expected urinary excretion rate			LOG observed/expected urinary excretion rate		
	Mean	SD	*p*	Mean	SD	*p*
L-DOPS	2.36	3.58	0.26	0.01	0.61	0.88
DHPG	3.15	4.67	0.20	0.25	0.55	0.36
NE	39.72	59.44	0.07	1.05	1.13	0.05*
DOPA	1.00	0.00	1.00	0.00	0.00	0.59
DOPAC	2.04	2.71	0.26	0.02	0.51	0.74
DA	12.24	17.78	0.18	0.52	0.75	0.08

L-DOPS L-threo-3,4-dihydroxyphenylserine, *DHPG* dihydroxyphenylglycol, *NE* norepinephrine, *DOPA* dihydroxyphenylalanine, *DOPAC* dihydroxyphenylacetic acid, *DA* dopamine, *SD* standard deviation, *p* statistical significance

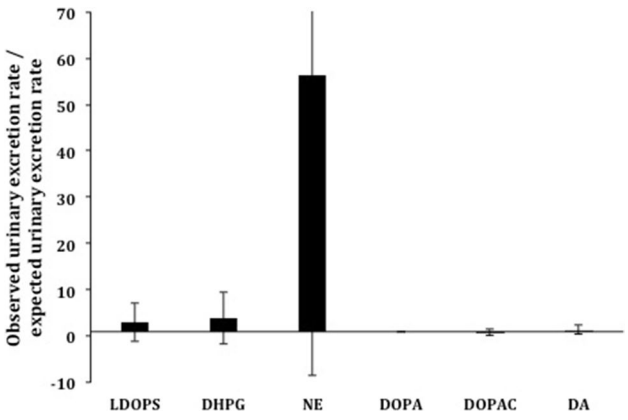

Fig. 1 Observed urinary excretion rate vs. expected urinary excretion rate of different catecholamines in patients taking L-threo-3,4-dihydroxyphenylserine. L-*DOPS* L-threo-3,4-dihydroxyphenylserine, *DHPG* dihydroxyphenylglycol, *NE* norepinephrine, *DOPA* dihydroxyphenylalanine, *DOPAC* dihydroxyphenylacetic acid, *DA* dopamine

plasma, and the mean urine/plasma ratio was 1.5 ± 0.7. There was more urinary excretion of dopamine than expected (mean 1.2 ± 0.5 times expected, $p = 0.04$). urinary excretion rates of L-DOPS, DHPG, NE, DOPA, and DOPAC did not differ from expected (Table 4).

In five patients plasma was obtained before and after L-DOPS treatment. The mean plasma NE level increased from 1.25 ± 1.31 to 1.37 ± 0.70 pmol/mL. In all five patients the increment in plasma NE was less than 100 pg/mL (less than 0.59 pmol/mL).

Discussion

The mechanisms and sites of the pressor effect of L-DOPS are not fully understood. As confirmed here, increments in plasma NE levels after L-DOPS administration are too small to explain the increases in blood pressure. L-DOPS could act via its conversion to NE in non-neuronal cells; the NE so formed would then act on alpha-adrenoceptors to produce vasoconstriction [15]. In line with this view, the present results indicate extensive renal conversion of L-DOPS to NE, at least in patients who are not on levodopa/carbidopa.

Table 3 Ratios of observed vs. expected urinary excretion rates of catechols in patients not taking levodopa/carbidopa (N = 7)

	observed/expected urinary excretion rate			LOG observed/expected urinary excretion rate		
	Mean	SD	*p*	Mean	SD	*p*
L-DOPS	2.9771	4.1705	0.2564	0.0432	0.6555	0.8674
DHPG	4.006	5.6402	0.2486	0.2449	0.6398	0.3914
NE	56.458	64.887	0.0644	1.5274	0.4776	0.0001*
DOPA	1	1E−16	1	0	6E−17	1
DOPAC	0.865	0.6416	0.5978	− 0.1758	0.3443	0.2255
DA	1.4129	0.9044	0.512	0.0635	0.3677	0.793

L-DOPS L-threo-3,4-dihydroxyphenylserine, *DHPG* dihydroxyphenylglycol, *NE* norepinephrine, *DOPA* dihydroxyphenylalanine, *DOPAC* dihydroxyphenylacetic acid, *DA* dopamine, *SD* standard deviation, *p* statistical significance

Table 4 Ratios of observed vs. expected urinary excretion rates of catechols in patients taking levodopa/carbidopa ($N=3$)

	observed/expected urinary excretion rate			LOG observed/expected urinary excretion rate		
	Mean	SD	*p*	Mean	SD	*p*
L-DOPS	0.93	1.00	0.92	− 0.20	0.45	0.53
DHPG	0.87	0.65	0.83	− 0.13	0.36	0.70
NE	0.66	0.99	0.61	− 0.70	0.89	0.31
DOPA	1.00	0.00	1.00	0.00	0.00	0.42
DOPAC	4.77	3.97	0.24	0.59	0.33	0.09
DA	23.06	20.92	0.21	1.22	0.45	0.04*

L-DOPS L-threo-3,4-dihydroxyphenylserine, *DHPG* dihydroxyphenylglycol, *NE* norepinephrine, *DOPA* dihydroxyphenylalanine, *DOPAC* dihydroxyphenylacetic acid, *DA* dopamine, *SD* standard deviation, *p* statistical significance

L-DOPS could also be converted to NE within sympathetic noradrenergic nerves; this mechanism and site of action have been demonstrated in dopamine beta-hydroxylase deficiency [5].

Pharmacokinetic studies of L-DOPS have shown simultaneous attainment of peak plasma levels of L-DOPS and NE, indicating rapid intracellular conversion of L-DOPS to NE and rapid exit of the NE from cells into the extracellular fluid [7]. There is a subsequent slow decline in plasma NE from the peak level, which may reflect ongoing production of NE from L-DOPS in a cellular storage site [7]. We hypothesize that NE built up in the cytoplasm of proximal renal tubular cells may exit the cells via reverse transport through the Uptake-2 carrier. Before reaching the systemic circulation, NE in the interstitium then may act as a paracrine factor, causing vasoconstriction of the afferent renal arterioles and increased proximal tubular sodium reabsorption [12] (Fig. 2). The exact status of the renin–angiotensin–aldosterone system (RAAS) in autonomic failure patients remains incompletely understood and whether there is a downstream effect of L-DOPS on the RAAS via local NE production is unknown. Although plasma renin activity has been shown to be almost undetectable in patients with neurogenic orthostatic hypotension, aldosterone levels are normal, and angiotensin II levels can be seemingly paradoxically increased [2, 4].

There were remarkable differences between the patients on levodopa/carbidopa and those not on levodopa/carbidopa at the time of testing. In patients off levodopa/carbidopa, the data indicated substantial conversion of L-DOPS to NE in the kidneys; however, this was not seen in patients on levodopa/carbidopa. The difference may explain the results of a recent phase 3 study assessing the pressor effect of L-DOPS in patients with neurogenic orthostatic hypotension [14]. In that study, only the group of patients with PAF (i.e., not on levodopa/carbidopa) reported benefit on the Orthostatic Hypotension Questionnaire composite score and had an increase in the standing systolic blood pressure. The two

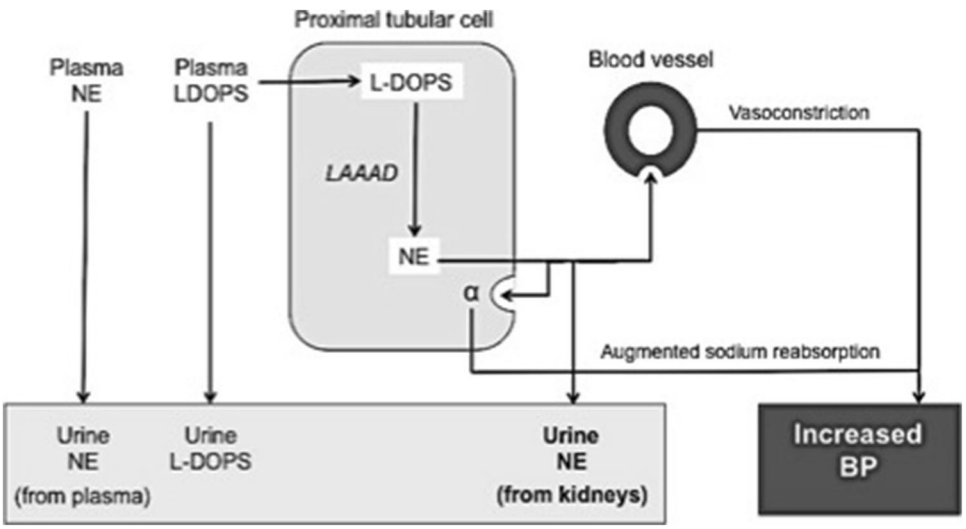

Fig. 2 Model of the renal NE/L-DOPS paracrine system. *NE* norepinephrine, *L-DOPS* L-threo-3,4-dihydroxyphenylserine, *LAAAD* L-aromatic-amino-acid decarboxylase, *BP* blood pressure

primary outcomes did not reach significance in the patients with PD or MSA [14]. High doses of carbidopa are known to inhibit LAAAD and have been shown to abolish the pressor effect of L-DOPS [16]. The dose of carbidopa used in PD is smaller (25 or 50 mg three times/day) and thought to interfere minimally with the pressor effect of L-DOPS. A randomized clinical trial including patients with PD on levodopa/carbidopa showed potential benefit of L-DOPS with a short-term pressor effect and improvement in dizziness/lightheadedness and reduction in falls [9]; however, in this study the difference between the treatment group and placebo group was not significant regarding the primary outcome [9]. A post hoc analysis suggested a smaller pressor effect with carbidopa based on the change in standing systolic BP in patients treated with L-DOPS compared to placebo [3].

L-DOPS has been used successfully to prevent dialysis-associated hypotension in patients with chronic kidney disease [1]. Although commonly disregarded, residual renal function has been shown to be present in hemodialysis patients and might be sufficient to allow effective conversion of L-DOPS to NE in proximal tubular cells [17]. Alternatively, extra-renal sites of conversion of L-DOPS to NE could play a role in dialysis patients. For instance, norepinephrine produced from L-DOPS in the gut might evoke an increase in blood pressure via splanchnic vasoconstriction.

This study had several limitations. First, analyses were done on a small sample of patients in different diagnostic groups that might have different neurochemical and hemodynamic responses to L-DOPS. Second, the role of renal conversion of L-DOPS to NE in the systemic pressor response is a matter for future research, as we did not explore different mechanisms of action and did not correlate any of the neurochemical data with clinical outcomes such as increase in standing blood pressure or responses to treatment. Therefore, the proposed intra-renal paracrine mechanism of the pressor action of L-DOPS is speculative.

Conclusions

There is substantial renal conversion of L-DOPS to NE in patients with neurogenic orthostatic hypotension who are not on levodopa/carbidopa. An intra-renal paracrine mechanism might contribute to the pressor effect of L-DOPS.

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Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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