Chronic oral L-DOPA increases dopamine and decreases serotonin excretions

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García, Néstor H., Theresa J. Berndt, Gertrude M. Tyce, and Franklyn G. Knox. Chronic oral L-DOPA increases dopamine and decreases serotonin excretions. Am. J. Physiol. 277 (Regulatory Integrative Comp. Physiol. 46): R1476–R1480, 1999.—Given the common pathways for uptake and synthesis for dopamine and serotonin, enhanced renal dopamine synthesis in response to increased substrate 3,4-dihydroxyphenylalanine (L-DOPA) is postulated to decrease renal serotonin synthesis. The present study compared the effects of chronic oral administration of L-DOPA on dopamine and serotonin excretion in vivo, with the effects of enhanced dopamine synthesis per nephron due to adaptation to reduced renal mass (RRM). Four groups of rats were studied: sham-operated rats and rats with RRM in the absence and presence of chronic oral L-DOPA, L-DOPA (2 mg·100 g body wt·day⁻¹) for 6–14 days increased calculated dopamine synthesis per nephron in sham-operated rats from 2.0 ± 0.3 (n = 7) to 13.6 ± 1.8 pg·day⁻¹·nephron⁻¹ (n = 7, P < 0.05) and in rats with RRM from 6.1 ± 1.3 (n = 7) to 39.3 ± 5.2 pg·day⁻¹·nephron⁻¹ (n = 7, P < 0.05). Chronic oral L-DOPA concomitantly decreased serotonin synthesis per nephron in sham-operated rats (1.6 ± 0.1 to 1.0 ± 0.1 pg·day⁻¹·nephron⁻¹, n = 7, P < 0.05) and in rats with RRM (5.6 ± 0.9 to 2.6 ± 0.4 pg·day⁻¹·nephron⁻¹, n = 7, P < 0.05). Both serotonin and dopamine synthesis per nephron were increased in rats with RRM. In conclusion, chronic oral administration of L-DOPA enhances dopamine excretion and decreases serotonin excretion in normal rats and in rats with reduced renal mass. Both dopamine and serotonin excretions per nephron were elevated by renal mass reduction.

SYNTHESIS OF DOPAMINE and serotonin by the kidney from their precursors 3,4-dihydroxyphenylalanine (L-DOPA) and 5-hydroxytryptophan (5-HTP) occurs in the proximal tubules (10, 27). Uptake of L-DOPA and 5-HTP by the proximal tubule cells occurs by the same transporter (33), and both are decarboxylated by the enzyme L-aromatic amino acid decarboxylase (L-AADC) to produce dopamine and serotonin (10, 27). Given the common pathway for uptake and synthesis, stimulation of the dopamine synthesis by increased delivery of substrate would be predicted to result in a decrease in serotonin synthesis. This notion is supported by in vitro studies by Soares-da-Silva and colleagues (25, 26) but not by in vivo studies (15, 20).

Experiments performed in vitro using isolated rat renal proximal tubules demonstrated that L-DOPA interfered with inward transport of 5-HTP and also its decarboxylation by L-AADC in proximal tubule cells (25, 26). Isolated tubules were bathed with increasing concentration of L-DOPA or 5-HTP ranging from 50 to 2,000 µM (25). L-DOPA was found to produce a concentration-dependent inhibition of 5-HTP accumulation. These investigators also examined the interaction at the decarboxylation level of these two precursors. Incubation of proximal tubule cells with L-DOPA or 5-HTP resulted in a concentration-dependent increase in formation of dopamine and serotonin, respectively, but when L-DOPA (5 µM) was added to the bath in the presence of 5-HTP, serotonin synthesis was reduced by 30%. These two effects would lead to increased synthesis of dopamine and decreased serotonin synthesis by the proximal tubule, an effect that would be expected to decrease in vivo serotonin excretion.

In vivo studies by Itskovitz et al. (15) determined the renal effects of individual and simultaneous acute infusions of L-DOPA and 5-HTP. In these studies, serotonin excretion was not affected by L-DOPA infusion. Likewise, infusion of equimolar amounts of the kidney-specific precursors for dopamine and serotonin, γ-L-glutamyl-L-DOPA or γ-L-glutamyl 5-HTP in humans showed no evidence of competitive inhibition of synthesis of either amine (20).

A previous study demonstrated that rats with reduced renal mass (RRM) exhibit increased dopamine synthesis per nephron (12, 13). However, the effect of RRM and the increased dopamine synthesis per nephron on renal serotonin synthesis has not been studied. Thus, in the present study, the effect on serotonin synthesis of increased dopamine synthesis per nephron in response to adaptation to RRM was compared with the effect of increased dopamine synthesis in response to increased substrate.

METHODS

Male Sprague-Dawley rats, weighing 250–350 g, from Harlan Sprague Dawley (Indianapolis, IN) were used in this study.

Surgical procedures were performed as follows. For the reduction of the renal mass, rats were anesthetized with an intramuscular injection (0.1 ml/100 g body wt) of equal volumes of a solution of xylazine (Lloyd Laboratories, 20 mg/ml) and ketamine hydrochloride (Ketalar, 100 mg/ml, Parke-Davis). Under aseptic and antiseptic conditions, a right nephrectomy and surgical ablation of the left renal poles were performed. The adrenal glands were left intact. The amount of renal mass removed (in grams) was calculated by adding the weight of the right kidney to the weight of the
removed left kidney poles. The percentage of residual renal mass was then calculated on the assumption that the right and left kidney were the same weight and that each kidney contained 37,538 nephrons (17). Approximately 45% of the renal mass remained in rats that underwent RRM. This value was used to normalize the data per remaining nephron. Sham surgery was performed in another group of rats and they served as controls.

After recovery from the anesthesia, rats were placed in the animal facility and allowed free access to water and food the same night. Beginning the next day, all animals were pair-fed 12 g/day of a diet containing 1.8% phosphate and 1% sodium. Food intake was monitored to ensure that all food was consumed daily. L-DOPA (2 mg·100 g body wt−1·day−1) was added to the food. This dose of L-DOPA was previously shown not to affect blood pressure (15).

Rats were divided in four groups: group 1, sham-operated rats (n = 7); group 2, rats with RRM (n = 7); group 3, sham-operated rats treated with L-DOPA (n = 7); and group 4, rats with RRM treated with L-DOPA (n = 7). Rats were kept in metabolic cages, and urine was collected on the 6th, 10th, and 14th days after surgery and the initiation of L-DOPA. Twenty-four-hour urine samples were collected in a container in dry ice with the addition of 5 ml of 33% acetic acid and 0.5 ml of 1% cysteine to prevent dopamine and serotonin degradation. We determined dopamine, serotonin, sodium (Na⁺), potassium (K⁺) and phosphate (Pi) excretions.

Assays for urine measurements. Urinary free dopamine and serotonin were measured by the method of Tyce et al. (32). Briefly, 0.1 ml of urine was diluted with 3 ml water, adjusted to pH 6.1, and applied to small columns (diameter 0.4 cm, height 2.3 cm) of Amberlite CG 50 (200–400 mesh). After washing the resin with 3 ml of 2 mM phosphate buffer (pH 6.1) and 3 ml of water, catecholamines were eluted with 5 ml of 0.2 M acetic acid. After the preliminary extractions, compounds were quantitated by HPLC with electrochemical detection. Recoveries of added standards in all extractions averaged 80–95%.

Sodium and potassium were measured with an IL943 Automatic Flame Photometer (Instrumentation Laboratory, Lexington, MA). Urinary phosphate concentration was measured by the Chen method (3).

Statistical analysis. Data are reported as the mean for the 3 days that urine samples were collected. All data are reported as means ± SE. Unpaired two-tailed t-test was used to determine if the changes observed were statistically significant, and P < 0.05 was accepted as a statistically significant difference.

RESULTS

Because there were no significant differences in the results between the collections on days 6, 10, and 14, the data are presented as the average values for the three 24-h urine collections. Absolute dopamine and serotonin excretions in untreated sham-operated or RRM rats and L-DOPA-treated sham-operated and RRM rats are shown in Table 1. Absolute dopamine excretions are similar in the untreated sham-operated rats and RRM rats. Likewise, absolute serotonin excretions are similar in sham-operated rats and rats with RRM. L-DOPA treatment significantly increased dopamine excretion in sham-operated rats and rats with RRM. Absolute serotonin excretion significantly decreased in the L-DOPA-treated sham-operated rats and rats with RRM.

Because rats with RRM had a reduced number of nephrons, Fig. 1 presents dopamine synthesis per nephron. As has been previously reported, rats with RRM have higher dopamine synthesis per nephron than sham-operated rats (6.1 ± 1.3 compared with 2.0 ± 0.3 pg·day−1·nephron−1). In the chronic L-DOPA-treated groups, dopamine synthesis per nephron increased to 13.6 ± 1.8 pg·day−1·nephron−1 in sham-operated rats and to 39.3 ± 5.2 pg·day−1·nephron−1 in RRM rats. Chronic L-DOPA treatment resulted in a sixfold increase in dopamine synthesis in the sham-operated rats as well as rats with RRM. These high levels of dopamine synthesis were sustained throughout the experiment (data not shown).

The effects of L-DOPA administration on serotonin synthesis per nephron in sham-operated rats and rats with RRM are summarized in Fig. 2. As we observed with dopamine excretion, serotonin synthesis per nephron was increased in rats with RRM (5.6 ± 0.9 pg·day−1·nephron−1) compared with sham-operated rats (1.6 ± 0.1 pg·day−1·nephron−1). In the

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Data represent means ± SE average for 3 days in µg/day. L-DOPA, 3,4-dihydroxyphenylalanine; RRM, reduced renal mass. *Significant difference between untreated group and L-DOPA group, unpaired t-test, P < 0.05.
L-DOPA treatment increased dopamine excretion and serotonin synthesis per nephron (13), dopamine synthesis per nephron is also increased in rats with RRM. Consistent with previous studies (13), dopamine synthesis per nephron is significantly higher in rats with RRM. L-DOPA-treated groups, serotonin excretion decreased to 1.0 ± 0.1 pg·day⁻¹·nephron⁻¹ (P < 0.05) in sham-operated rats and to 2.6 ± 0.4 pg·day⁻¹·nephron⁻¹ (P < 0.05) in rats with RRM. L-DOPA treatment decreased serotonin synthesis by 50% in both groups.

Table 2 summarizes electrolyte excretion in sham and rats with RRM rats in the absence and presence of chronic oral L-DOPA. Phosphate excretion per nephron (Uₚ,V/nephron) was twofold higher in the rats with RRM (142.6 ± 7.9 pM·day⁻¹·nephron⁻¹) compared with sham-operated rats (67.5 ± 1.7 pM·day⁻¹·nephron⁻¹). Phosphate excretion was 65.5 ± 2.3 pM·day⁻¹·nephron⁻¹ in sham-operated rats treated with L-DOPA and 136.9 ± 4.8 pM·day⁻¹·nephron⁻¹ in rats with RRM that received L-DOPA. Phosphate excretion did not change with chronic administration of L-DOPA in either group (Table 2). Likewise, sodium (Uₙa,V/nephron) and potassium excretions (Uₖ,V/nephron) were higher in rats with RRM, but chronic L-DOPA treatment did not affect Uₙa,V or Uₖ,V in either group.

**DISCUSSION**

The present study demonstrates that chronic oral L-DOPA treatment increases dopamine excretion and concomitantly decreases serotonin excretion in normal rats and rats with RRM. Additionally, rats with RRM had higher dopamine and serotonin synthesis per nephron than their respective controls.

In the kidney, serotonin is synthesized by the proximal tubule from its substrate 5-HTP (27). Because the rate of synthesis of serotonin is dependent on the availability of dietary tryptophan, which is a precursor for serotonin synthesis (6, 8, 30), all rats in the present study were fed the same amount of food. The enzyme L-AADC is not thought to be a rate-limiting step in the synthesis of either serotonin or dopamine, because their synthesis is markedly increased by infusion of their substrates (15, 29). Although absolute serotonin excretions are not different between the sham-operated and remnant kidney rats, it is important to note that rats with a remnant kidney have a markedly decreased number of total nephrons. When serotonin excretion is expressed on the basis of the number of nephrons, serotonin synthesis per nephron is significantly higher in rats with RRM. Consistent with previous studies (13), dopamine synthesis per nephron is also increased in rats with a remnant kidney. Thus the intrarenal synthesis of serotonin and dopamine parallel other after renal mass reduction.

It is interesting to note that dopamine and serotonin excretions per nephron significantly increase within the first week after renal mass reduction (data not shown), and these levels were sustained throughout the course of the experiment. This early increase of dopamine and serotonin synthesis may accompany other changes such as an increase in growth factors (7), angiotensinogen (22), and other autacoids to achieve balance in this new condition. Thus the parallel increases in dopamine and serotonin synthesis per nephron represent enhanced metabolism by hypertrophied proximal tubule cells.

In agreement with the in vitro studies of Soares da Silva and Pinto-do (25), we found in vivo that chronic L-DOPA treatment decreased serotonin excretion. This effect was observed throughout the course of the experiment. However, the competition between dopamine and serotonin synthesis by increased L-DOPA observed in vivo in vitro (25) and reported here in vivo was not reproduced in previous in vivo studies using rats (15) or in humans (20). The in vivo studies by Itskovitz et al. (15) investigated the renal effects of individual and simultaneous acute infusion of L-DOPA (76–380 nmol/min) and 5-HTP (341 nmol/min) to rats during a period of 120 min. In contrast to our findings, excretion of serotonin was not influenced by the concomitant administration of L-DOPA. The reason for this difference may be related to the fact that they investigated the effect of L-DOPA and 5-HTP for a short period of time (2 h), whereas we collected urine for 24-h periods during L-DOPA treatment.

A lack of interaction between dopamine and serotonin synthesis was also observed by Li Kam Wa et al. (20) investigating the renal effects of the glutamyl derivative of L-DOPA (Glu-Dopa) in humans. In this study, the infusion of 18.7 µg·min⁻¹·kg⁻¹ Glu-Dopa for...
6 h was associated with a marked increase in dopamine excretion, but serotonin excretion did not change. The reason for this difference may be related to the lower concentration of infused L-DOPA compared with the present study. In agreement with our findings, studies performed in patients with Parkinson's disease receiving L-DOPA reported that the urinary concentrations of 5-hydroxyindoleacetic acid, a stable metabolite of serotonin, were decreased (31).

Dopamine excretion increased sixfold after oral L-DOPA administration and serotonin excretion decreased by 50%, whereas sodium, potassium, and phosphate excretions were not increased. This result was unexpected, inasmuch as several reports have demonstrated that the acute intravenous or intrarenal infusion of dopamine or L-DOPA increased sodium (11) and phosphate (4, 5, 14, 18) excretions. However, the effect of chronic oral administration of L-DOPA on phosphate excretion has not been previously investigated. The lack of effect of chronic oral L-DOPA on sodium and phosphate excretions in the present studies may be the result of several mechanisms. First, it is possible that L-DOPA induces phosphaturia and natriuresis only after infusion for a short period of time. This possibility is supported by the observation of Lederer et al. (19) in which dopamine inhibited phosphate transport in opossum kidney cells for only 3 h despite the continued presence of dopamine in the media. Downregulation of dopamine receptors may also explain these results, and this suggestion is supported by the observation of Song et al. (28) in vitro and Katayama et al. (16) in rat kidneys. Both groups found that after 30 days (28) and 15 days (16) of treatment with dopamine, the binding sites for DA-1 receptors decreased, whereas DA-2 receptors were unaffected. However, the possibility that binding changes were followed by functional changes were not investigated in these studies. Decreased intrarenal synthesis of serotonin would be predicted to be natriuretic and phosphaturic, because acute increases in serotonin have been reported to enhance sodium and phosphate reabsorption in cultured proximal tubular cells (6, 8, 15, 29). It is likely that downregulation of serotonin receptors occurs. Studies in opossum kidney cells demonstrated that incubation with serotonin caused a desensitization of the functional response after 3 h followed by downregulation of the receptor (23). Alternatively, it is important to note that the natriuretic effect of dopamine is dependent on the experimental conditions (1, 9, 21, 24), because previous studies demonstrated that the natriuretic effect of dopamine is evident in conditions of extracellular fluid volume expansion but not in sodium-depleted states. It is unlikely that the lack of natriuretic response to L-DOPA is due to sodium depletion, because in the present study all animals consumed a similar normal sodium intake; however, the effect of L-DOPA was not determined in salt-loaded rats. Another less likely mechanism for the lack of effect of increased dopamine synthesis on phosphate and sodium excretions is that dopamine may also bind α- and β-adrenergic receptors, which would increase sodium and phosphate reabsorption (2), thus any natriuretic and phosphaturic effects could be blunted by activation of the α- and β-adrenergic system.

In conclusion, chronic oral administration of L-DOPA enhances dopamine excretion and decreases serotonin excretion in normal rats and in rats with RRM. Both dopamine and serotonin excretions per remaining nephron were elevated by renal mass reduction.

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