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# Urodilatin regulates renal dopamine metabolism

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## Abstract

***Background:*** **Sodium and water transport across renal proximal tubules is regulated by diverse hormones such as dopamine and urodilatin. We have previously reported that urodilatin stimulates extraneuronal dopamine uptake in external renal cortex by activation of the type A natriuretic peptide receptor, coupled to cyclic guanylate monophosphate signaling and protein kinase G. Moreover, urodilatin enhances dopamine-induced inhibition of $Na^+$, $K^+$-ATPase activity in renal tubules. The aim of the present study was to evaluate whether urodilatin could also alter renal dopamine synthesis, release, catabolism and turnover.**

***Methods:*** **The effects of urodilatin on dopamine synthesis, release, catabolism and turnover were measured in samples of renal cortex from Sprague Dawley rats.**

***Results:*** **The results indicate that urodilatin increases L-DOPA decarboxylase activity and decreases catechol-o-methyl transferase and monoamine oxidase activity. Moreover, urodilatin does not affect either dopamine basal secretion or potassium chloride-induced dopamine release in external renal cortex, and reduces amine turnover.**

***Conclusions:*** **Both the present results and previous findings show that urodilatin modifies dopamine metabolism in external renal cortex of rats by enhancing dopamine uptake and synthesis and by decreasing catechol-o-methyl transferase and monoamine oxidase activity and dopamine turnover. Those effects taken together may favor dopamine accumulation in renal cells and increase its endogenous content and availability. This would permit $D_1$ receptor recruitment and stimulation and, in turn, overinhibition of $Na^+$, $K^+$-ATPase activity, which results in decreased sodium reabsorption. Therefore, urodilatin and dopamine enhance natriuresis and diuresis through a common pathway.**

**Key words:** *Catechol-o-methyl transferase, L-DOPA decarboxylase, Dopamine, Kidney, Monoamine oxidase, Urodilatin*

## Introduction

Sodium metabolism is closely linked to blood pressure control, and the kidneys play a fundamental role in this process (1). Diverse endocrine, autocrine and neuronal factors affect renal sodium handling and are involved in blood pressure regulation (2). The natriuretic peptide urodilatin plays a crucial role, modulating renal sodium excretion at different levels of the nephron (3, 4). In contrast with circulating atrial natriuretic peptide (ANP), urodilatin is characterized by higher stability against enzymatic degradation by neutral endoproteases (5). When exogenous infusions of both peptides are compared, the renal effects of urodilatin are more effective than those of ANP (5).

Renal dopamine (DA) content depends on two sources: L-DOPA decarboxylation (after uptake of L-DOPA from the tubular fluid) and DA uptake by tubular cells from the blood (6, 7). The main source of renal DA comes from proximal tubular cells, which have a high concentration of aromatic L-amino acid decarboxylase (AADC), also called L-DOPA decarboxylase (8). DA is catabolized in the kidney, mainly by monoamine oxidase (MAO) and catechol-o-methyl transferase (COMT) (9, 10). The activity of both enzymes in renal tissues is high compared with their activity in other peripheral tissues. COMT is abundantly expressed in proximal tubular cells of the kidney and appears to play an important role in the physiological regulation of renal DA tonus (9).

Intrarenal DA acts together with other natriuretic hormones and antagonizes the effects of antinatriuretic agents (11). It has been reported that part of the inhibitory effects of ANP on sodium and water reabsorption are mediated by dopaminergic mechanisms, given that haloperidol partially blocks the natriuretic and diuretic effects of the peptide (12). This finding allows us to hypothesize that urodilatin might modulate renal DA metabolism, and that both systems would interact together, enhancing the natriuretic and diuretic effects of urodilatin.

Based on previous findings and taking into account that urodilatin regulates DA uptake in renal tissues (13), the aim of the present study was to explore the effects of urodilatin on other aspects of DA metabolism, such as renal DA synthesis, release, catabolism and turnover. Consequently, the effects of urodilatin on renal L-DOPA decarboxylase, COMT and MAO activity, $^{3}$H-DA release and $^{3}$H-DA turnover were studied in samples of renal cortex.

## Subjects and methods

Male Sprague Dawley rats weighing 250-300 g (from the Pathophysiology Department, School of Pharmacy and Biochemistry of University of Buenos Aires) were used following international guiding principles and local regulations concerning the care and use of laboratory animals for biomedical research (ANMAT, 6344/96; Institute of Laboratory Animal Resources, 1996) (14), as well as the "International Ethical Guiding Principles for Biomedical Research on Animals" established by the CIOMS (Council for International Organizations of Medical Sciences) (15). Animals were housed in cages with a 12-hour light/dark cycle under conditions of controlled temperature and humidity. All animals were given free access to water and food (Commercial Rodents Purina Chow; Cooperation SRL, Argentina).

The following drugs were used in the experiments: $^{3}$H-DA (28.0 Ci/mmol specific activity) and $^{3}$H-S-adenosylmethionine ($^{3}$H-SAM) (74.7 Ci/mmol specific activity) (New England Nuclear, Boston, MA, USA), urodilatin (fragment 95-126), nomifensine, 3,4-dihydroxybenzoic acid, SAM, bovine serum albumin Cohn Fraction V 4-aminoantipyrine, vanillic acid, peroxidase type II (4 U/mL), tyramine, clorgyline, pargyline, tropolone, picryl sulfonic acid solution 5% w/v (TNB), pyridoxal 5-phosphate hydrate (PLP), L-DOPA, DA, carbidopa (all from Sigma-Aldrich, St. Louis, MO, USA), benzene, Folin Ciocalteu's phenol reagent (Merck Co., USA), potassium cyanide (Fluka BioChemika, Germany), and EcoLite for liquid scintillation (ICN Pharmaceuticals, CA, USA).

Composition of the standard Krebs bicarbonate (SKB) solution (mmol/L) was 118 NaCl, 4.7 KCl, 1.2 $MgSO_4$, 7 $H_2O$, 1.0 $NaH_2PO_4$, 2.4 $CaCl_2$, 0.004 EDTA, 11.1 glucose, 0.11 ascorbic acid and 26.0 $NaHCO_3$. The depolarizing 25 mmol/L KCl solution had the same composition as the SKB solution, except that the NaCl concentration was decreased to keep the osmolality constant.

### Experimental protocols

Rats were anesthetized with 10% w/v ethyl urethane (1.3 mg/kg, intraperitoneally [i.p.]), both kidneys were removed and slices of renal cortex were cut, minced and weighed (approximately 50 mg).

### Effect of urodilatin on AADC activity

Experiments were performed according to the techniques previously described by Fernández et al (16). Samples of renal cortex were placed in 2.0 mL of SKB medium in a Dubnoff incubator and preincubated at 37°C, pH 7.40, and bubbled with a gaseous mixture of 95% $O_2$ and 5% $CO_2$ for 15 minutes. The number of samples (50 mg of minced slices per sample) used in each group (n) is shown in parentheses. The samples were subsequently transferred to a fresh SKB medium and incubated for 30 minutes in the absence (n = 7; control group) or presence of 200 μM carbidopa (n = 6) or 10 nM urodilatin (n = 7) (experimental groups). After the incubation period, tissues were washed with cold SKB for 5 minutes, blotted, weighed, homogenized in a cool 0.1 M phosphate buffer (1:10 w/v), pH 7.10, and then centrifuged at 21,420 g at 4°C for 15 minutes. The supernatants were centrifuged once more at 24,480 g at 4°C for 30 minutes. L-DOPA decarboxylase activity in the supernatant was determined by the modified spectrophotometric UV method (UV-VIS Beckman DB-GT) of Sherald et al (17), adapted to rat renal cortex samples. Briefly, the reaction was based on the colorimetric complex generated between 2,4,6-trinitrobenzene 1-sulfonic acid (TNB) or picryl sulfonic acid and DA, employing L-DOPA (5 μM/mL) as a substrate and pyridoxal 5-phosphate hydrate (PLP; 1 μM/mL) as a cofactor. To avoid DA degradation by MAO A, MAO B or COMT, the assays were performed in the presence of clorgyline (500 μM), pargyline (500 μM) and tropolone (100 μM), respectively. Dopamine formed by AADC can be detected by its reaction with TNB to form a TNP-DA colored complex, which was measured at its maximum absorbance at 340 nm. Tissue protein concentration was measured according to the method described by Lowry et al (18). Results of L-DOPA decarboxylase activity are expressed as nmol/mg protein per minute.

### Effects of urodilatin on MAO and COMT activity

Samples of renal cortex were preincubated for 15 minutes as described previously, and then incubated for 30 minutes in the absence (control group) or presence of 10 nM urodilatin (experimental group). After the incubation period, tissues were washed with cold SKB for 5 minutes, dried, weighed, homogenized in a cool 0.2 M phosphate buffer (1:10 w/v), pH 7.60, and then centrifuged at 1,000 g at 4°C for 30 minutes. MAO activity in the supernatants (control: n = 6; urodilatin: n = 8) was determined by the modified spectrophotomet-

ric UV method described by Holt et al (19), adapted to rat renal cortex samples. Briefly, 100 µL of tissue supernatant was incubated for 45 minutes with 50 µL chromogenic solution, 200 µL tyramine (500 µM) and 650 µL 0.2 M potassium phosphate buffer (pH 7.60). The chromogenic solution contained 1 mM vanillic acid, 500 µM 4-aminoantipyrine and 4 U/mL peroxidase in potassium phosphate buffer. All reagent concentrations were chosen after a preliminary experiment to obtain optimal assay conditions.

COMT activity in the supernatants (control: n = 7; urodilatin: n = 8) was determined by the radioenzymatic method described by Jarrot (20), modified and adapted to rat renal cortex samples. Briefly, the reaction contained a mixture of 2 mM 3,4-dihydroxybenzoic acid and 250 µM [$^3$H]S-adenosylmethionine (specific activity 74.7 Ci/mmol) as substrate and cofactor, respectively, and 25 µL of tissue homogenate in a final volume of 100 µL. $^3$H-SAM was used as a methyl donor, and tritium activity was measured by the standard liquid scintillation method. Tissue protein concentration was measured according to the method described by Lowry et al (18). Complementary experiments confirmed that 10 nM urodilatin added to the reaction lacked interfering effects on the methods for measuring MAO and COMT activity. Results of COMT and MAO activity are expressed as pmol/mg protein per minute and nmol/mg protein per hour, respectively.

### Effects of urodilatin on $^3$H-dopamine release

Tissue samples of renal cortex were minced and preincubated for 15 minutes as previously described, and then incubated for 30 minutes with 0.625 µCi/mL $^3$H-DA. After labeling, tissue samples were transferred to a fresh SKB medium for 120 minutes, and the drugs under testing were added. The SKB medium was changed every 10 minutes. To examine the effects of urodilatin on $^3$H-DA-induced release, the following groups were studied: (a) control (basal spontaneous $^3$H-DA release, n = 8) which was incubated only with SKB; (b and c) experimental groups, incubated with 10 nM urodilatin (n = 7) or with 10 nM urodilatin plus 25 mM KCl (n = 9), respectively. Medium samples corresponding to the first 3 minutes were collected at 0 (basal), 30, 60, 90 and 120 minutes and stored for measurement of tritium activity by the standard scintillation counting method. Nomifensine was added (50 µM during preincubation and then 17 µM during incubation) to avoid neuronal DA uptake.

Renal $^3$H-DA release was calculated as log disintegrations per minute (dpm)/g fresh tissue ± SEM, and the slope of the regression line of the different groups was used for comparison.

### Effects of urodilatin on $^3$H-dopamine turnover

Animals were treated with carbidopa, a DA synthesis inhibitor (200 µg/kg i.p.), 24 and 2 hours before sacrifice. Tissues from the renal cortex were obtained and preincubated and incubated in the presence of 100 µM carbidopa as described above. Labeling was followed by incubation with either SKB (control group, n = 6) or 10 nM urodilatin (experimental group, n = 8) for measurement at 0 (basal, no urodilatin), 30, 60, 90, 120 and 150 minutes. Nomifensine was used under the conditions described for the DA release experiments. To avoid DA degradation by MAO A, MAO B and COMT, the assay was performed in the presence of clorgyline (500 µM), pargyline (500 µM) and tropolone (100 µM), respectively. Since DA synthesis and catabolism were inhibited, the amount of $^3$H retained reflects DA turnover, which depends only of DA uptake and release. At the end of the above mentioned periods, tissue samples were blotted, weighed, homogenized with 10% trichloroacetic acid and centrifuged at 1,700 g at 4°C for 30 minutes. Tritium activity in the supernatants was determined by standard scintillation counting methods. Results are expressed as dpm/mg fresh tissue, and linear regression analysis and the slopes (k) of control and experimental groups were used for comparison.

### Statistical analysis

All values are expressed as means ± SEM. Data were processed using Graph Pad InStat Software (San Diego, CA, USA). Statistical analysis was performed using Student's *t*-test and 1-way ANOVA. A p values under 0.05 was considered statistically significant.

## Results

First the effects of urodilatin on renal DA synthesis were investigated. L-DOPA decarboxylase is the enzyme responsible for DA synthesis in the renal cortex. Then, L-DOPA decarboxylase-specific activity was measured in the absence and presence of 10 nM urodilatin.

Figure 1 shows that L-DOPA decarboxylase activity increased from 5.00 ± 0.05 (control) to 6.86 ± 0.18 (nmol/mg per minute) in the presence of urodilatin. To ensure that the activity measured did not correspond to decarboxylating enzymes other than L-DOPA decarboxylase, 200 µM of carbidopa, which is a specific inhibitor of L-DOPA decarboxylase, was employed. Carbidopa suppressed approximately 96% of the basal enzymatic activity in renal cortex samples: 0.21 ± 0.04 vs. 5.00 ± 0.05 (control).

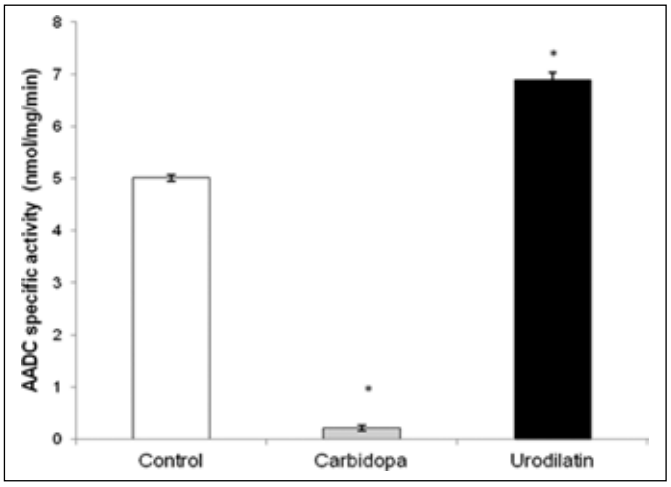


**Fig. 1 - Effects of 10 nM urodilatin on dopamine formed as the product of aromatic L-amino acid decarboxylase (AADC)-specific activity (nmol/mg of protein per minute ± SEM) in renal cortex (n = number of samples); *p<0.005, vs. control. Control group: n = 7; carbidopa group: n = 6; urodilatin group: n = 7.**

DA degradation or catabolism is another process that determines DA cellular levels and DA availability in tubules cells. Hence, whether urodilatin could affect the catabolism of renal DA was investigated by measuring the effects of 10 nM urodilatin on the specific activity of MAO and COMT in the renal cortex. In the presence of urodilatin, MAO-specific activity decreased from 7.4 ± 0.1 nmol/mg per hour $10^2$ (control) to 5.3 ± 0.4 nmol/mg per hour $10^2$ (Fig. 2A), while COMT specific activity decreased from 30.3 ± 1.9 pmol/mg per minute (control) to 21.5 ± 1.2 pmol/mg per minute (Fig. 2B).

Based on our previous findings, urodilatin stimulates renal DA uptake (13). Therefore, whether urodilatin could also regulate the DA release process was investigated in the next group of experiments. The effects of 10 nM urodilatin on $^3$H-DA basal and KCl-induced DA release in renal cortex were studied from 0 to 150 minutes of incubation. The peptide altered neither spontaneous nor KCl-induced $^3$H-DA release, which is represented by the slope of the lineal regression curve: $R^2$ = control 0.982; urodilatin 0.980; urodilatin plus KCl 0.983; k (log dpm/g) = control –0.024 ± 0.004; DA –0.027 ± 0.003; DA plus KCl –0.027 ± 0.002 (Fig. 3). These results suggest that in this experimental condition, urodilatin does not affect either DA basal or KCl-induced release.

Finally, the effects of urodilatin on DA turnover were studied. As shown in Figure 4, 10 nM urodilatin decreased $^3$H-DA turnover within 150 minutes of incubation. In that figure, DA turnover is represented by the slope of the lineal regression curve ($R^2$ = control 0.985; urodilatin 0.996; k = control, –0.50 ± 0.02 dpm/mg $10^{-2}$; urodilatin, –0.31 ± 0.01 dpm/mg $10^{-2}$. It can be observed that from 0 to 150 minutes, the time-dependent decrease in $^3$H-DA content induced by urodilatin was lower than in the control group, indicating that urodilatin decreased DA turnover.

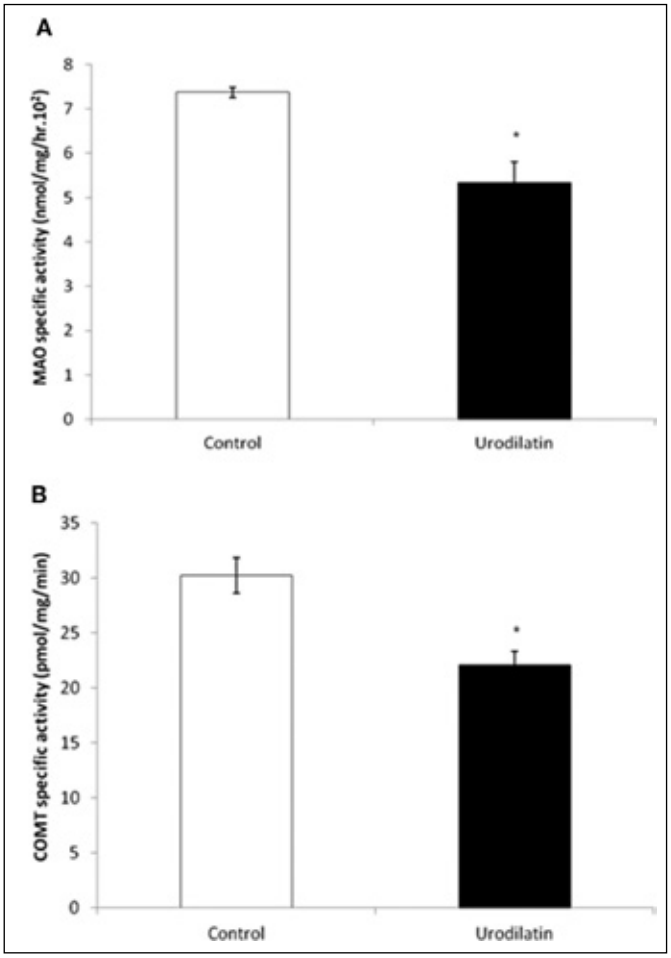


**Fig. 2 - A) Effects of 10 nM urodilatin on monoamine oxidase (MAO)-specific activity (nmol/mg of protein/hour.$10^2$ ± SEM) in renal cortex. *p<0.01, vs. control. MAO activity was calculated as the amount of hydrogen peroxide released in the amine oxidase reaction, which was proportional to the absorbance at 498 nm of the red quinoneimine dye given by 4-aminoantipyrine when it was oxidized and then condensed with vanillic acid. Control group: n = 6; urodilatin group: n = 8. B) Effects of 10 nM urodilatin on catechol-o-methyl transferase (COMT)-specific activity (pmol/mg of protein/min ± SEM) in renal cortex. *p<0.001, vs. control. Control group: n = 7; urodilatin group; n = 8. COMT activity was calculated as the amount of tritium activity from 3-methoxy($^3$H)-4-hydroxybenzoic acid formed by the enzyme.**

## Discussion

DA, locally produced and independent of innervation, is an important hormone as a paracrine/autocrine regulator of

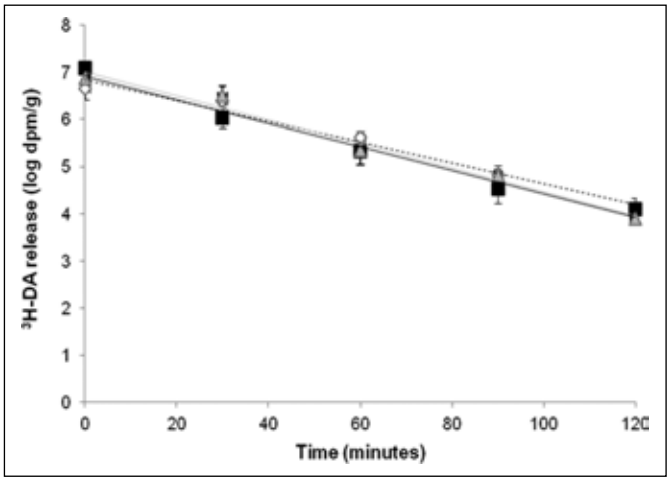


**Fig. 3 - Effects of 10 nM urodilatin on spontaneous and induced (25 mM KCl) release of $^3$H-dopamine ($^3$H-DA; log dpm/g ± SEM) in renal cortex. Dotted line and open diamonds = control; black line and solid squares = 10 nM urodilatin; gray line and gray triangles = 10 nM urodilatin plus 25 mM KCl (n = number of samples). The slope of the linear regression curve represents dopamine release. Control group: n = 8; urodilatin group: n = 7; urodilatin plus KCl group: n = 9.**

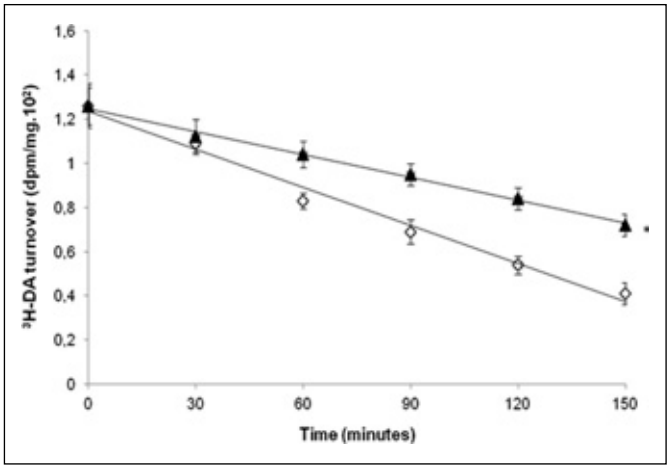


**Fig. 4 - Effects of 10 nM urodilatin on $^3$H-dopamine ($^3$H-DA) turnover in renal cortex (dpm/mg.10$^2$ ± SEM). Open diamonds = Control; solid triangles = urodilatin (n = number of samples). The slope of the linear regression curve represents dopamine turnover; *p<0.01, vs. with control. Control group: n = 6; urodilatin group: n = 8.**

renal tubular sodium transport (2, 21). Natriuretic peptides may alter DA intrarenal levels through modulation of its synthesis and catabolism, and in consequence, they may control renal sodium handling and blood pressure. The present results confirm and complement previous results of our laboratory (13) showing that urodilatin promotes the increase in intrarenal DA content in the renal cortex tubules, regulating various steps of DA metabolism in the kidney.

Renal production of DA is dependent on the substrate availability, on the uptake of L-DOPA into tubular epithelial cells, and on the activity of L-DOPA decarboxylase, which is the key enzyme responsible for conversion of L-DOPA into DA (6). Changes in L-DOPA decarboxylase activity could affect intrarenal DA content and, consequently, renal DA effects. Zhang et al have demonstrated that, for the AADC gene in the kidney proximal tubules, knockout mice showed selective decreases in kidney and urinary DA with simultaneous increased expression of nephron sodium transporters, decreased natriuresis and diuresis in response to L-dihydroxyphenylalanine, and decreased medullary COX-2 expression and urinary prostaglandin E2 excretion (22). Moreover, they had unbuffered responses to angiotensin II and results in the development of hypertension and a dramatic decrease in longevity (22). Consequently, whether urodilatin is capable of regulating the activity of the key enzyme in renal DA synthesis was investigated. Our results have demonstrated that urodilatin stimulates L-DOPA decarboxylase specific activity by approximately 37%. The increase elicited by urodilatin could indirectly enhance the tubular effects of the peptide by promoting renal DA synthesis. Our results suggest that, although endogenous urodilatin is synthesized in distal tubules, the exogenous administration of urodilatin is able to stimulate NPR-A receptors located in the proximal tubules, which are the main source of L-DOPA decarboxylase.

DA enzymatic catabolism is another pathway that may influence tubular DA levels. We therefore explored whether urodilatin modulates DA catabolism in the kidney. MAO and COMT activities are high in renal tissues compared with those in other peripheral tissues (23). Renal COMT appears to play a key role in the physiological regulation of renal DA levels, suggesting that the kidney is the methylation site for inactivation of catecholamines (24). COMT is abundantly expressed in proximal tubular cells, and its inhibition significantly increases DA content in both renal tubules and interstitial fluids and urine (9, 23, 25). Experiments conducted in the presence of the MAO inhibitor phenelzine have demonstrated that MAO is less important than COMT for regulation of DA-mediated natriuresis in rat kidney (26). The present results indicate that urodilatin diminishes the specific activity of both DA catabolic enzymes COMT and MAO by approximately 29% and 28%, respectively. Accordingly, urodilatin inhibition of COMT and MAO activity, together with DA synthesis stimulation, may contribute to increased intracellular DA availability in renal tubules.

We have previously reported that urodilatin stimulates DA uptake in the kidney through NPR-A receptors coupled to

guanylate cyclase and cyclic guanosine monophosphate (cGMP) as second messenger (13, 27). In the present study, the possibility that urodilatin could also alter the renal DA release process, in addition to its effect on DA uptake, was also explored. The present results show that urodilatin affects neither the basal secretion of the amine by the epithelial tubular cells in external renal cortex nor its KCl-induced release. It must first be considered that DA is not stored in vesicles in renal tissues, like norepinephrine in neurons, and secondly, that renal DA release is not an exocytotic process as in the case of neurons. The present experiments, in accordance with previously published results (13, 27), showed that urodilatin may contribute to increased tubular DA availability by stimulating its uptake but not by regulating its renal release. Taking these results together, and considering that DA synthesis and catabolism were inhibited in the turnover assay, we can conclude that urodilatin-decreased DA turnover is the balance between DA uptake and release.

The present findings, as well as previous reports of our laboratory, show that urodilatin increases endogenous DA content in renal external cortex. This experimental observation is based on the effects evoked by urodilatin: increase in DA uptake and synthesis, decrease in COMT and MAO activity and unaltered DA secretion. These effects may produce DA accumulation in tubular renal cells, resulting in an increase in DA availability. These mechanisms would permit recruitment and stimulation of $D_1$ receptors, which would result in overinhibition of $Na^+$, $K^+$-ATPase activity, decreasing sodium reabsorption and increasing natriuresis (2). This interaction would enable both renal hormones (urodilatin and DA) to act via a common intracellular pathway to enhance natriuresis and diuresis. There are recent experimental models that definitely demonstrate that abnormalities in intrarenal DA content can lead to salt-sensitive hypertension and a dysregulated renin-angiotensin system (28).

Finally, and as a limitation of this study, further *in vivo* experiments should be carried out to provide a physiological demonstration and corroboration of the results obtained *in vitro* and the corresponding impact on urinary and hemodynamic parameters.

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Conflict of interest: No conflict of interest.

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