Intrarenal Dopamine Production and Distribution in the Rat

Physiological Control of Sodium Excretion

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Abstract Dopamine (DA), produced by the renal proximal tubule, has been demonstrated as an intrarenal paracrine hormone mediating diuresis and natriuresis. The precise mechanism by which DA exerts its cell-to-cell action is not fully understood. In the present study, renal interstitial fluid (RIF) DA (by in vivo microdialysis) and urinary DA excretion (U_{DAV}) were compared with anesthetized rats on either normal (0.28% NaCl, NS) or high (4.0% NaCl, HS) sodium balance and in response to acute γ-L-glutamyl-L-dopa (gludopa) administration. Urine flow (UV) and sodium excretion (U_{NaV}) in HS were greater than in NS rats. U_{DAV} was increased in HS compared with NS rats. RIF DA was significantly lower in HS than NS rats. Gludopa at 3.5 and 7.5 nmol/kg (IV bolus) produced a larger increase in U_{DAV} than RIF DA. Only the highest dose of gludopa (7.5 nmol/kg), which resulted in a 7.3-fold increase in U_{DAV} and a 1.7-fold increase in RIF DA, was associated with significant diuresis and natriuresis. Cortical and medullary blood flow remained unchanged after gludopa (7.5 nmol/kg) administration, while angiotensin II (100 ng kg^{-1} min^{-1}) induced significant reduction in cortical and medullary blood flow. Prior bilateral renal denervation did not have a significant effect on basal DA levels (RIF DA and U_{DAV}) or gludopa-induced DA production or natriuresis and diuresis. These data demonstrated that both chronic sodium loading and acute gludopa administration stimulated renal DA production and release predominantly into the tubule lumen, where DA had a direct tubule action in the control of U_{NaV}. Renal DA production and its renal effects were not significantly regulated by renal sympathetic nerve activity. (Hypertension. 1997;30[part 2]:228-234.)

Key Words • dopamine • extracellular space • gludopa • kidney • microdialysis • sodium

The purpose of the present study was to characterize intrarenal DA production and distribution in response to chronic sodium loading and gludopa, a DA produrg, in anesthetized rats with and without renal denervation. We used a novel in situ interstitial microdialysis technique to sample rat RIF DA and to compare the effects of chronic sodium loading and acute gludopa administration on RIF and U_{DAV} in the rat. Responses of renal U_{NaV} and intrarenal blood flow distribution to gludopa were also examined.

Methods

Microdialysis Technique

Microdialysis Probe Construction

For the determination of RIF DA, we constructed a microdialysis probe as previously described. Each end of single 0.5-cm-long hollow fiber dialysis tubing (0.1-mm inner diameter, transmembrane molecular mass cutoff, 5000 D, Hospal) was inserted into a manually dilated end of a 30-cm-long (inflow and outflow) hollow polyethylene tube (0.12-mm inner diameter, 0.65-mm outer diameter, Bioanalytical Systems). The distance between the ends of the polyethylene tubes was 3 mm (dialysis area) and the dialysis fiber was sealed in place within the polyethylene tubes with cyanoacrylate glue. The dead space volume of the dialysis and outflow tubes was 3.6 μL.

In Vitro Relative Recovery of DA

In vitro relative recovery of DA was evaluated by immersing dialysis membranes of individual probes (n=8) in a beaker containing 20 pg/μL DA. The inflow tube of each probe was connected to a gas-tight syringe filled and then perfused with lactated Ringer’s solution at 1, 3, and 5 μL/min (Harvard Apparatus, pump model 22) each for 90 minutes. After 60 minutes of equilibration, the effluent was collected from the outflow tube into a microcentrifuge tube containing 10 μL 4% acetic acid.
In Vivo Renal Microdialysis

Studies were performed on female Sprague-Dawley rats (body weight 400 to 500 g, Harlan Sprague Dawley Inc, Madison, WI). All procedures on animals were approved by the University of Virginia Animal Care Committee. With the animal under general anesthesia (pentobarbital sodium, 30 mg/kg intraperitoneally), the rat was tracheotomized and placed on a heated pad to keep body temperature at 37°C to 38°C. The right jugular vein was cannulated with polyethylene tubing (PE-20), and intravenous infusion of lactated Ringer’s solution was administered at 0.5 mL/h per 100 g body weight throughout the experiment. The left kidney was exposed via a midline abdominal incision. The renal capsule was penetrated with a 31-gauge needle that was tunneled into the outer renal cortex approximately 2 to 3 mm from the outer renal surface for 0.5 cm before it exited by penetrating the capsule again. The tip of the needle was inserted into one end of the dialysis probe, and the needle was pulled together with the dialysis tube until the dialysis fiber was situated in the renal cortex. The inflow tube of the dialysis probe was connected to a gas-tight syringe filled with lactated Ringer’s solution and perfused at 1 μL/min. The effluent was collected from the outflow tube. A 60-minute equilibration period elapsed before the experimental protocol was initiated. In vivo recovery of intravenously infused [3H]mulin in the anesthetized rat has demonstrated that [3H]mulin appearing in the RIF sample is 2% of urinary [3H]mulin, indicating that the dialysate is not significantly contaminated by renal tubule fluid. Moreover, microdialysis probe implantation in the cortex of the rat kidney did not significantly affect renal cortical blood flow in the area directly adjacent to the dialysis probe, or glomerular filtration rate or fractional U_{Na}V.

In Vivo Equilibrium Microdialysis

To estimate the RIF DA level in the anesthetized rat on standard diet (0.28% NaCl, Bioserve) and tap water ad libitum, a gradient dialysis technique was employed, in which exogenous DA was added to the pertussate, as described previously. Rats (n=6) were prepared surgically as described above. After 60 minutes of equilibration, the probe was perfused at 1 μL/mm with different concentrations of DA ranging from 0 to 25 pg/μL. The dialysate fluid was collected during perfusion at each concentration (90 minutes each), and its DA level was determined. A linear regression analysis was performed to determine the relationship between the net loss or gain of DA in the collected dialysate and initial DA concentration in the pertussate. The concentration at which there is no net flux of DA across the dialysis membrane is considered a valid estimate of the RIF DA concentration.

Effect of Chronic Salt Loading on Renal DA Production and Distribution

In this study, rats (n=18, 9 in each group) were given a diet (Bioserve) containing either 0.28% (normal salt, NS) or 4% (high salt, HS) NaCl and tap water ad libitum for 5 consecutive days. Both right and left ureters of the rats, otherwise prepared in an identical manner as described above, were cannulated with polyethylene tubing (PE-10). After 60 minutes of equilibration, RIF and urine samples were collected for 90 minutes and stored at -80°C until assayed.

Effect of Acute Gludopa Administration on Renal DA Production/Distribution, U_{Na}V and Intrarenal Blood Flow

Effect of Gludopa on Renal DA Production/Distribution and U_{Na}V

In this study, rats (n=28) consuming standard diet and tap water ad libitum were divided into four groups (n=7 in each group). Both right and left ureters of the rats, otherwise prepared in an identical manner as described above, were cannulated. A period of 60 minutes was allowed for equilibrium. RIF samples were collected for 90 minutes and urine samples were collected every 15 minutes for 90 minutes before and after IV bolus injection of gludopa (UCB Bioproducts) at 3, 5, or 7.5 nmol/kg in 5% dextrose or vehicle only in the four groups of rats. At the end of the experiments the right kidneys of the rats receiving gludopa at 7.5 nmol/kg or vehicle alone were collected, weighed, and stored at -80°C until assayed for renal tissue NE content.

Effect of Renal Denervation on Gludopa-Induced Renal DA Production/Distribution and U_{Na}V

In this study, female Sprague-Dawley rats (body weight 200 to 250 g, n=10) with prior bilateral renal denervation were purchased from Zivic Miller. During bilateral renal denervation, the renal artery on both sides was stripped of the adventitia and coated with a solution of 10% phenol in absolute alcohol. The rats were placed on standard diet and tap water ad libitum and allowed to recover for 3 days after the surgery. On the experiment day, both right and left ureters of the rats, otherwise prepared in an identical manner as described above, were cannulated. A period of 60 minutes was allowed for equilibrium. The above protocol was repeated with IV bolus injection of gludopa at 7.5 nmol/kg in 5% dextrose or vehicle only in two groups of rats with prior bilateral renal denervation (n=5 in each group). At the end of the experiments the right kidneys were collected, weighed, and stored at -80°C for measurement of kidney NE content to verify the effectiveness of the chronic renal denervation.

Effect of Gludopa and Angiotensin II on Intrarenal Blood Flow Distribution

In this study, rats (n=12) consuming standard diet and tap water ad libitum were divided into two groups (n=6 in each group). Rats were surgically prepared as described above. The left kidney was exposed and laser Doppler probes (Advance Co Ltd) were applied on the ventral surface of the kidney (superficial probe, type C) or inserted into the renal parenchyma at a depth of 4 mm (needle probe, type N). The laser-Doppler probes were connected to a laser-Doppler flowmeter (ALF 21D dual channel flowmeter, Advance Co Ltd) allowing simultaneous measurements of cortical and medullary blood flow as previously described. An equilibrium period of 30 minutes was allowed before the experimental protocol was initiated. Blood flow signals from the renal cortex and medulla were recorded every 10 minutes for a period of 90 minutes before and after the IV bolus injection of gludopa (7.5 nmol/kg in 5% dextrose) or vehicle only in two groups of rats with prior bilateral renal denervation (n=6 in each group). In another group of six rats, blood flow signals from the renal cortex and medulla were recorded every 10 minutes for a period of 30 minutes before and during the IV infusion of angiotensin II at 100 ng kg-1 min-1, followed by a 30-minute recovery period.

Analytical Methods

RIF sample was collected into a microcentrifuge tube containing 10 μL of 4% acetic acid. Urine was collected into a microcentrifuge tube containing 20 μL of 6N HCl. Urine volume was calculated gravimetrically. Urine sodium was measured by flame photometry (IL943, Instrumentation Laboratory). Aliquots of urine were extracted by ion exchange on Bio-Rex 70 resin (50 to 100 mesh, sodium form) and absorption on alumina, followed by
elution with 4% acetic acid. Dihydroxybenzylamine was used as the internal standard. The kidneys were minced and homogenized in 0.1 M perchloric acid. The supernatants obtained by centrifugation (20,000g, 20 minutes) were then extracted with dichloromethane, methanol, and water, respectively. Interassay coefficients of variation were 5.6% and 8.7%, respectively. The detection limit was 2.0 pg per volume assayed. The intrassay and interassay coefficients of variation were 5.6% and 8.7%, respectively.

All data are expressed as mean±SEM. Statistical analysis was performed with a Macintosh StatView program (Abacus Concepts). Comparisons were made with either the t test or analysis of variance, followed by Scheffé's test for multiple comparisons when appropriate. P<0.05 was considered statistically significant.

Results

In Vitro and In Vivo Validation of Renal Interstitial Microdialysis

Studies of in vitro recovery of DA demonstrated that DA concentrations in the dialysate were inversely proportional to the flow rate through the dialysis tubing. The best relative recovery (percentage of DA concentration in dialysate/DA concentration in beaker) was observed with a perfusion rate of 1 µL/min and was 92.7±2.0%, compared with 73.9±4.9% and 44.3±3.3% at 3 and 5 µL/min, respectively (Fig 1A). Using an equilibrium dialysis method, we demonstrated that the theoretically derived point at which there is no net transmembrane flux (the steady state interstitial DA concentration) was similar to the in vitro recovery of DA by individual microdialysis probes (n=8) with 73.9±4.9% and 92.7±2.0%, compared with 73.9±4.9% and 44.3±3.3% at 3 and 5 µL/min, respectively (Fig 1A).

Effect of Chronic Salt Loading on Renal DA Production/Distribution

UV and UN,V in HS (n=9) were greater than in NS (n=9) rats (UV 7.2±0.6 versus 3.8±0.3 µL/min, P<0.01; UN,V 497±66 versus 265±27 nmol/min, P<0.01). U,D,V increased in HS compared with NS rats (601±68 versus 420±37 pg/mm, P<0.05). In contrast, RIF DA was significantly lower in HS than NS rats (1.25±0.36 versus 3.68±0.49 pg/mm, P<0.01; Fig 2).

Effect of Acute Gludopa Administration on Renal DA Production/Distribution and UN,V

Basal U,DA and RIF DA from rats with prior bilateral renal denervation (n=10) were similar to rats with intact renal innervation (n=14) (U,DA 476±30 versus 394±28 pg/min, P>0.05, RIF DA 2.9±0.2 versus 3.4±0.3 µL/mm, P>0.05). UV tended to be higher in rats with prior bilateral renal denervation (4.1±0.2 versus 3.4±0.2 µL/mm, P=0.05), while UN,V was not significantly different between the two groups (286±13 versus 265±16 nmol/min, P>0.05; Fig 3). In rats with intact renal innervation (n=7 in each group), IV injection of gludopa at 3, 5, and 7.5 nmol/kg produced a larger increase in U,DA than RIF DA. Only the highest dose of gludopa (7.5 nmol/kg), which resulted in a 7.3-fold increase in U,DA and 1.7-fold increase in RIF DA, was associated with significant diuresis and natriuresis (UV 3.4±0.4 versus 7.4±0.5 µL/mm, P<0.01; UN,V 265±27 versus 711±120 nmol/min, P<0.01; Fig 4).

In rats with prior bilateral renal denervation (n=5 in each group), gludopa at 7.5 nmol/kg produced significant increase in U,DA (8.3-fold) and RIF DA (1.8-fold), accompanied by significant diuresis and natriuresis (UV 3.9±0.2 versus 8.2±0.5 µL/mm, P<0.01, UN,V 289±778±56 nmol/min, P<0.01) (Fig 5).

Tissue NE content of chronically denervated kidneys (n=5) was significantly lower than intact kidneys (n=6) (13.8±3.6 versus 103.5±10.6 ng/g tissue in vehicletreated rats, and 10.8±2.1 versus 135.5±15.8 ng/g tissue in gludopa-treated rats, both P<0.01).

Effect of Acute Gludopa or Angiotensin II Administration on Intrarenal Blood Flow Distribution

Renal blood flow of the cortex and medulla did not increase in rats receiving gludopa (7.5 nmol/kg) (n=6, Fig 6A), while angiotensin II (100 ng kg⁻¹ mm⁻¹) induced significant reduction in cortical (42.6%) and medullary (28.1%) blood flow, which gradually returned toward preangiotensin levels when the angiotensin infusion was stopped (n=6) (Fig 6B).

Discussion

Using a novel interstitial microdialysis technique, we monitored changes in RIF and urine DA excretion in response to chronic sodium loading and acute gludopa administration in anesthetized rats. It is well documented that the kidney itself is the main source of urinary DA. The...
renal venous concentration of DA has been shown to be significantly higher than in the artery. The concentration of DA in renal lymph, however, is not higher than in the artery. In rats on a normal salt diet in the present study, RIF DA levels were much lower than UDAV, suggesting that intrarenally produced DA is released preferentially into the tubule lumen rather than peritubular space. Cellular mechanisms of renal DA secretion are not understood. DA immunoreactive granules and L-dopa-induced fluorescence are mainly concentrated in the region of the apical membrane of proximal tubule cells. There may be luminal organic cation transporters with high affinity for DA in porcine proximal tubule (LLC-PK1) cells.

Our study confirmed that UDAV increases in response to chronic salt loading, one of the most powerful stimuli known to increase renal DA production. Surprisingly, however, RIF DA levels in rats on high sodium diet were significantly lower than during normal salt balance. The mechanism underlying this reduction with chronic salt loading is not apparent. Acute isotonic saline loading in conscious rabbits has been shown to increase urine DA excretion (threefold) without concurrent increase in renal DA spillover into plasma. The DA prodrug gludopa produced an 800-fold increase in UDAV without significant change in renal venous DA concentration in conscious rabbits. RIF DA may derive from tubular outward transport.
of DA through the basolateral membrane. Histofluorescent and neurochemical findings suggest the presence of dopaminergic neurons in the kidney and adrenergic nerves may also become dopaminergic under certain circumstances.\(^1\) Vagal afferents have been shown to stimulate renal release of DA and produce a neurogenically mediated natriuresis.\(^2\) Our data confirm that the main source of DA in the urine is nonneuronal, and further demonstrate that renal nerve activity does not contribute significantly to either urinary or interstitial fluid DA. During chronic salt loading, intrarenally produced DA is released preferentially into the luminal space where it may act on apical DA receptors as an autocrine or paracrine factor. Therefore, renal DA may act as an intrarenal regulator of kidney function in highly compartmentalized fashion. DA receptors located in the brush border membrane may be exposed to DA originating in the tubule with the physiological consequence of natriuresis and diuresis.\(^6\) On the other hand, DA receptors in the basolateral membrane, renal vasculature, and glomerulus may be exposed to DA released into the renal interstitium and possibly have a compartmentalized role in the control of glomerular filtration rate and/or

![Graphs showing the effects of gludopa (GD; 3, 5, and 7.5 nmol/kg IV) on UV, UoV, UoV, and RIF DA in anesthetized rats with intact renal innervation (n=7 in each group). Control values at -90 and -45 minutes before gludopa administration, experimental values at 45 and 90 minutes after gludopa administration. *P<.05, **P<.01 vs vehicle control.](image1)

![Graphs showing the effects of gludopa (GD, 7.5 nmol/kg IV) on UV, UoV, UoV, and RIF DA in anesthetized rats with prior bilateral renal denervation (n=5 in each group). Control values at -90 and -45 minutes before gludopa administration, experimental values at 45 and 90 minutes after gludopa administration. *P<.05, **P<.01 vs vehicle control.](image2)
pressure remained constant. Our previous study in the isolated perfused rat kidney, DA-m-receptor activation engendered by gludopa resulted in a physiological increase in urinary DA excretion, had a natriuretic effect without any change in blood pressure or glomerular filtration rate in healthy subjects on low sodium diet. Taken together with the present results, these studies suggest that the natriuretic effect of gludopa is secondary to direct tubule inhibition of sodium reabsorption. The functional significance of apical and basolateral DA receptors needs much further investigation.

Gludopa is devoid of pharmacological activity per se, but is converted to L-dopa and then to DA by sequential actions of the brush border enzyme γ-glutamyl transpeptidase and cytosolic amino acid decarboxylase predominantly in the proximal tubule cells, where both enzymes exist in abundance. Significant natriuresis and renal vasodilatation occurs in the whole animal and human after pharmacological increase of UDAV (more than 300-fold) engendered by gludopa. Renal vasodilation is also observed in the isolated perfused rat kidney in response to micromolar range gludopa. In the present study in anesthetized rats, much smaller quantities of gludopa (7.5 nmol/kg) resulted in a physiological increase in UDAV (7.3-fold), accompanied by a slight increase in RIF DA (1.7-fold), and produced significant diuresis and natriuresis without detectable changes in intrarenal blood flow. It is of interest to note that the increase in renal DA production after chronic salt loading was associated with an increase in UDAV but not when the increase was caused by low doses of gludopa. Whether this is as a result of dietary salt loading–induced upregulation of kidney DA receptors or increased efficiency of receptor coupling to signal transduction remains unclear. These results support preferential secretion of DA generated by proximal tubule cells into the tubule lumen. In the isolated perfused rat kidney, DA-induced natriuresis and diuresis was observed even when renal blood flow, glomerular filtration rate, and perfusion pressure remained constant. Our previous study in the

unnephrectomized conscious dog demonstrated that blockade of the renal DA-1 receptor with intrarenal infusion of SCH-23390 produced a 50% decrease in UV and UNaV, but there were no renal hemodynamic changes accompanying the antinatriuresis. Jose et al. recently reported that a 5-fold increase in kidney DA content with gludopa administration was associated with decreased renal cortical brush border Na+/H+ antiporter activity and significant natriuresis (4.9-fold) and diuresis (2.6-fold) without any change in glomerular filtration rate in anesthetized rats. Barendregt et al. reported that low dose L-dopa infusion, which resulted in a fivefold to eightfold increase in urine DA excretion, had a natriuretic effect without any change in blood pressure or glomerular filtration rate in healthy subjects on low sodium diet. Taken together with the present results, these studies suggest that the natriuretic effect of gludopa is secondary to direct tubule inhibition of sodium reabsorption by locally formed DA. These results strongly support the thesis that intrarenal DA plays a role in the control of natriuresis through a tubule mechanism.

The renal sympathetic nerve endings are closely related to the juxtaglomerular apparatus and proximal tubule cells. Whether the sympathetic nervous system influences intrarenal DA generation and regulates renal DA-mediated natriuresis and diuresis is unknown. Basal DA production (as reflected by UDAV and RIF DA) and UNaV and their responses to gludopa were similar between rats with and without chronic renal denervation. The observation is consistent with other studies, which showed that chronic renal denervation did not alter UDAV both basally and in response to increased dietary phosphate intake. Our results further demonstrate that intrarenal production of DA (both UDAV and RIF DA) and its renal effects are not significantly modulated by renal sympathetic nerve activity.

In summary, our data demonstrate renal interstitial microdialysis can be used to monitor DA levels in the RIF in anesthetized rats. According to our results, DA produced in the kidneys is released preferentially into the tubule lumen and exerts a direct tubule effect in the control of UNaV. Renal DA production and its renal effects were not significantly influenced by renal sympathetic nerve activity. The unique compartmentalization of renal DA release warrants further investigation, especially at the cellular level.

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