**Med. 6-6**
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**ISSN 0025-7680**

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MEDICINA (Buenos Aires) 2009; 69: 00-00

# PATTERNS OF RENAL DOPAMINE RELEASE TO REGULATE DIURESIS AND NATRIURESIS DURING VOLUME EXPANSION. ROLE OF RENAL MONOAMINE-OXIDASE

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**Abstract** Diuretic and natriuretic effects of renal dopamine (DA) are well established. However, in volume expansion the pattern of renal DA release into urine ($U_{DA}V$) and the role of enzymes involved in DA synthesis/degradation have not yet been defined. The objective is to determine the pattern of $U_{DA}V$ during volume expansion and to characterize the involvement of monoamine-oxidase (MAO) and aromatic amino-acid decarboxylase (AADC) in this response. In this study male Wistar rats were expanded with NaCl 0.9% at a rate of 5% BWt per hour. At the beginning of expansion three groups received a single drug injection as follows: C (vehicle, Control), IMAO (MAO inhibitor Pargyline, 20 mg/kg BWt, i.v.) and BNZ (AADC inhibitor Benserazide, 25 mg/kg BWt, i.v.). Results revealed that in C rats $U_{DA}V$ (ng/30 min/100g BWt) increased in the first 30 min expansion from 11.5 ± 1.20 to 21.8 ± 3.10 ($p < 0.05$) and decreased thereafter. IMAO showed a similar pattern but significantly higher than C at 30 min expansion (32.5 ± 2.20, $p < 0.05$). IMAO greatly reduced MAO activity from 8.29 ± 0.35 to 1.1 ± 0.03 nmol/mg tissue/hour and significantly increased diuresis and natriuresis over controls. BNZ abolished the early $U_{DA}V$ peak to 3.2±0.72 ($p < 0.01$) and though, $U_{DA}V$ increased over C after 60 min expansion, natriuresis and diuresis were diminished by BNZ treatment. Results indicate that an increment in renal DA release into urine occurs early in expansion and in a peak-shaped way. In this response MAO plays a predominant role.

**Key words:** dopamine, MAO, diuresis, natriuresis

**Resumen** ***Perfiles de secreción de dopamina renal en la expansión de volumen para regular diuresis y natriuresis***. Rol de la monoaminoxidasa renal. La dopamina (DA) intrarrenal ejerce efectos diuréticos y natriuréticos. Sin embargo, en los estado de expansión de volumen aún no está bien definido el patrón de liberación de dopamina renal hacia la orina y si cumplen un rol las enzimas involucradas en la síntesis o degradación de la amina. El objetivo del presente trabajo es determinar el patrón de excreción urinaria de DA ($U_{DA}V$) durante la expansión de volumen, caracterizando la participación de las enzimas monoaminooxidasa (MAO) y decarboxilasa de aminoácidos aromáticos (AADC) en esta respuesta. Para ello ratas Wistar macho fueron expandidas de volumen con NaCl 0.9% al 5% del peso corporal por hora durante dos horas y divididas en tres grupos, los que al comienzo de la expansión recibieron: C (vehículo, Control), IMAO (Pargilina, inhibidor de MAO, 20 mg/kg PC, i.v.) y BNZ (Benserazida, inhibidor de AADC, 25 mg/kg PC, i.v.). Se observó que en C la $U_{DA}V$ (ng/30min/100gPC) aumentó durante los primeros 30 minutos de expansión de 11.5 ± 1.20 a 21.8 ± 3.10 ($p < 0.05$), disminuyendo posteriormente. IMAO mostró un patrón de liberación similar pero significativamente mayor que C a los 30 min de expansión (32.5 ± 2.20, $p < 0.05$). En este grupo la actividad de MAO disminuyó de 8.29 ± 0.35 a 1.1 ± 0.03 nmol/mg tejido/hora y aumentaron la diuresis y natriuresis por sobre los controles. En BNZ, el pico de $U_{DA}V$ observado a los 30 min de la expansión disminuyó a 3.2 ± 0.72 ($p < 0.01$), aunque luego de 60 minutos fue mayor que en C. BNZ disminuyó tanto la diuresis como la natriuresis. Podemos concluir que al comienzo de la expansión de volumen se produce un pico de excreción de dopamina renal hacia la orina. La enzima MAO juega un rol fundamental en esta respuesta.

**Palabras clave**: dopamina, MAO, diuresis, natriuresis

Renal dopamine (DA), a vasodilatory and natriuretic hormone, is synthesized in the cells of the proximal tubule from its precursor L-dihydroxyphenylalanine (L-DOPA). L-DOPA ultrafiltered from plasma is taken up by proximal cells and decarboxylated to DA by the enzyme aromatic amino-acid decarboxylase (AADC). The major renal actions of this amine are achieved by activation of $D_1$-like and $D_2$-like type receptors. The natriuretic effect reflects the reduction in sodium transport due to the inhibition of several transporters, mainly the $Na^+$, $K^+$-ATPase and the $Na^+/H^+$ exchanger[1].

Recibido: ??? Aceptado: ???

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Recent studies have shown that the effect of DA can be enhanced by inhibiting enzymes involved in its breakdown. In renal microdialysis studies it has been shown that COMT inhibition increases natriuresis and urinary DA while MAO inhibition does not[2]. We have reported, instead, that in conscious rats on a normal salt diet, MAO inhibition over 24 hours caused an almost two-fold increase in urinary DA, while COMT inhibition only produced a slight increment of DA in urine[3].

In clinical practice, exogenous DA has been used to improve renal performance and, so far, the concept is that DA has failed to do so[4]. However, infused DA does not entirely resemble the effect of renal DA. Then, to study the role of renal DA under volume expansion could give some help to solve this controversial point.

Thus, the aim of the present work was to investigate the rate of renal DA release into urine under a moderate isotonic extracellular volume expansion and to evaluate the relative contribution of MAO and AADC on renal DA release and hydroelectrolyte excretion during volume expansion.

Male Wistar rats (n = 20) with a body weight of 250-350 g were used for this study. Rats were anesthetized with sodium pentobarbital (50 mg/kg ip) and prepared for experimental procedures. After a suitable basal period rats were moderately expanded through the jugular vein with normal saline (NaCl 0.9%) at a rate of 5% body weight per hour for two hours. Glomerular filtration rate (GFR) was measured by inulin clearance during basal and volume expansion periods. Urine and blood samples were collected every 30 min during basal period and during volume expansion to determine natriuresis, diuresis and urinary dopamine excretion. Dopamine was determined by HPLC with electrochemical detection.

At the end of the basal period and immediately before volume expansion, each group received drugs or vehicle in a single dose as follows: C control, vehicle, 200 µl of normal saline; IMAO, pargyline (MAO inhibitor), 20 mg/kg BWt iv in 200 µl of normal saline and BNZ, benserazide (AADC inhibitor), 25 mg/kg BWt iv in 200 µl of normal saline.

Mean arterial pressure (MAP) was recorded, at the beginning and at the end of the study, in the carotid artery by means of a pressure transducer device. Once the experiment was concluded, portions of renal cortex were separated to determine the activity of monoamine-oxidase (MAO). When indicated, 3 µg iv SCH 23390 (a specific $D_1$-like type receptor antagonist) was used to test DA effect.

Results (mean ± SEM) were analyzed by paired or unpaired *t-test* when two groups were compared. When more than two groups were tested, ANOVA for repeated measurements followed by *post hoc* Tuckey test was used. A *p* value less than 0.05 were considered as significant.

Control rats progressively increased diuresis (expressed as ml/30 min/100g BWt) from a basal rate of 0.08 ± 0.009 to 1.52 ± 0.140 at 120 min of volume expansion ($p<0.01$). Natriuresis ($U_{Na^+}V$) (expressed as mmol/30 min/100g BWt) increased in C rats from 0.016 ± 0.004 to 0.234 ± 0.023 at 120 min of expansion ($p < 0.01$). Urinary dopamine ($U_{DA}V$, ng/30 min/100g BWt) increased from a basal level of 11.5 ± 1.20 to 21.8 ± 3.10 during the first 30 min of volume expansion ($p < 0.01$), (Fig. 1). Thereafter, $U_{DA}V$ returned to basal values.

In IMAO group, pargyline administration increased urinary volume over C after 90 and 120 min volume expansion: 1.04 ± 0.14 vs 1.58 ± 0.280 and 1.52 ± 0.14 vs 2.44±0.530, respectively ($p <0.05$, both), (Fig. 1A). An increment in $U_{Na^+}V$ was also observed at 120 min IMAO treatment from 0.234 ± 0.023 to 0.335 ± 0.020 ($p < 0.05$ vs C), (Fig. 1B). In IMAO group, $U_{DA}V$ increased to 32.5 ± 2.20 and to 22.9 ± 1.40 after 30 and 60 min of volume expansion, ($p < 0.05$ and $p < 0.01$ vs C, respectively). Thereafter, up to 120 min, $U_{DA}V$ in IMAO group was similar to control values (Fig. 1C).

In BNZ group, diuresis was significantly reduced at 60 and 120 min of expansion as compared to C (0.54 ± 0.020 vs 0.79 ± 0.09 and 1.04 ± 0.040 vs 1.52 ± 0.14 respectively, $p < 0.05$, both, (Fig 1A); BNZ treatment also diminished $U_{Na^+}V$ at 120 min expansion from 0.23 ± 0.02 to 0.17 ± 0.014 ($p < 0.05$ vs C rats), (Fig 1B). Regarding urinary DA, the initial and transient increment of $U_{DA}V$ found in control rats after the first 30 min volume expansion was abolished by BNZ to 3.2 ± 0.72 ($p < 0.01$ vs C). Then, at 90 and 120 min expansion $U_{DA}V$ values in BNZ group were significantly higher than in C and IMAO groups ($p < 0.01$, both. Fig 1C).

Renal and systemic hemodynamic parameters are shown in Table 1. Expansion alone (C group) increased GFR. IMAO or BNZ did not alter this pattern of GFR response. Mean arterial pressure did not show variations among groups.

Basal l-dopa filtered load (FL = [Plasma l-dopa] * GFR) was 331 ± 14 pg/min and slightly increased during expansion to 390 ± 65, 384 ± 20 and 395 ± 18 in C, IMAO and BNZ groups, respectively.

Basal MAO activity (nmol/mg tissue/hour) was 8.29 ± 0.35 and decreased with expansion in C rats to 5.56 ± 0.95 ($p < 0.05$). MAO activity was almost completely inhibited by IMAO when measured 2 hours after treatment or 15 min post-treatment as well (1.1 ± 0.03 nmol/mg tissue/hour, either).

SCH 23390 treatment (a specific D1-like type receptor antagonist), reduced diuresis and natriuresis by about 50% in each group.

Our results show how the inhibition of enzymes involved in renal DA synthesis and degradation affects urinary DA, $Na^+$ and water excretion in a model of moderate saline expansion. The increase in $U_{DA}V$, which means DA

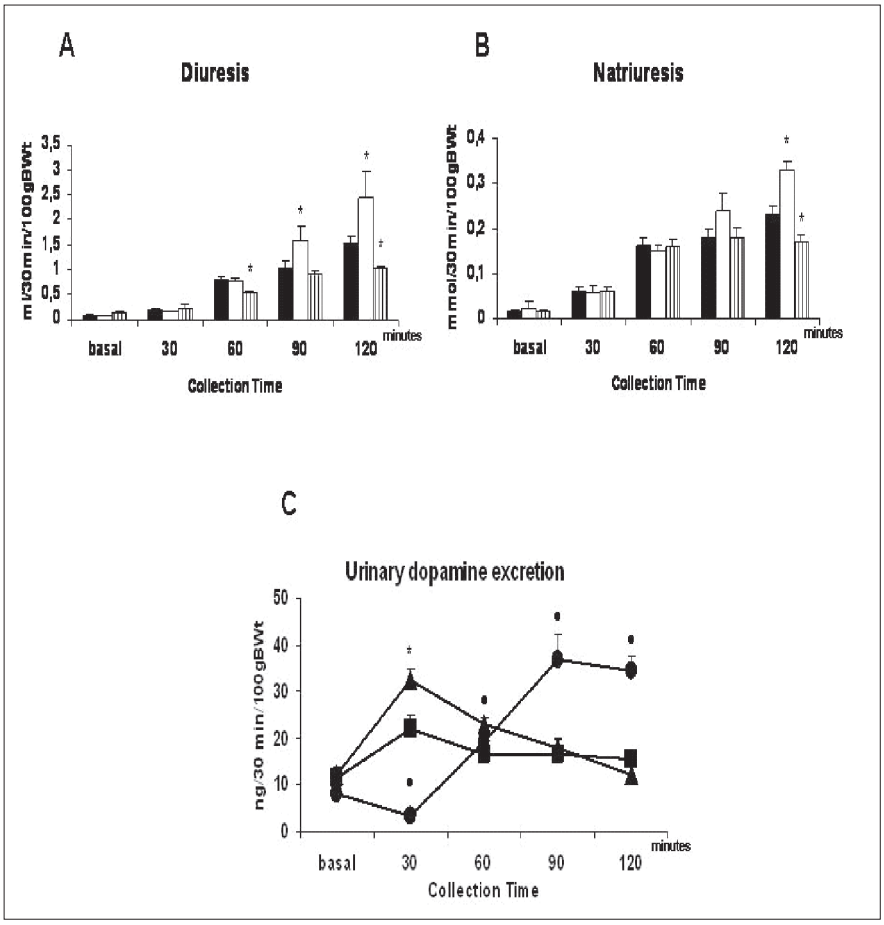


Fig. 1.– A) Diuresis (ml/30min/100gBWt), B) Natriuresis (mmol/30min/100gBWt) and C) Renal dopaminergic response (Urinary DA excretion, ng/30 min/100gBWt) during two hours of a moderate isotonic volume expansion in control rats and in rats treated with different enzyme inhibitors. Urinary collection periods were set at 30 min each. A) and B): Black columns control rats; white columns IMAO, pargyline treated rats and striped columns BNZ, benserazide treated rats. C): squares control rats; triangles IMAO, pargyline treated rats and circles BNZ, benserazide treated rats. Values are shown as mean ± SEM. Symbols denote: *p < 0.05 and • p < 0.01 vs respective control periods.

TABLE 1.– *Systemic and renal hemodynamic parameters in volume expanded rats. Effect of MAO and AADC inhibition*

| | Basal | Control | IMAO | BNZ |
|---|---|---|---|---|
| MAP *(mm Hg)* | 126.8 ± 1.82 | 126.3 ± 5.36 | 111.0 ± 14.22 | 109 ± 7.70 |
| GFR *(ml/min/100gBWt)* | 0.60 ± 0.05 | 0.77* ± 0.05 | 0.78* ± 0.03 | 0.81* ± 0.06 |

*MAP, mean arterial pressure; GFR, glomerular filtration rate; IMAO, pargyline treated rats; BNZ, benserazide treated rats.*p < 0.05 vs basal.*

released from renal tubules into urine, displayed a particular pattern[3, 5, 6]. In controls, after a stable baseline period, an early and transient increase in $U_{DA}V$ was observed during the first 30 min of urine collection under volume expansion. Dopamine, then, returned to basal levels. Together with greater levels of $U_{DA}V$, an increase in GFR and a decrease in MAO activity were also found during expansion. The increase in diuresis was due, at least in part, to DA effect since a specific $D_1$-like type receptor antagonist reduced to a half the diuretic and natriuretic response, a finding consistent with previous works[7, 8].

Thereafter we tested whether the activity of renal MAO could change the response to volume expansion since this enzyme participates in renal DA inactivation[3, 9]. So,

MAO was inhibited at the beginning of volume expansion.

Rats treated with MAO inhibitor pargyline exhibited a significantly higher diuresis and natriuresis than control rats, while neither GFR nor MAP were altered by pargyline when compared to controls. Under MAO inhibition, instead, the amount of DA released into urine in the early peak was significantly greater than in controls. In this regard, two factors may contribute to the early and transient increment of $U_{DA}V$ observed in both, control and IMAO groups. One of them could be an increase in l-dopa decarboxylation secondary to a higher l-dopa filtered load in volume expansion. The increment in GFR, however, was similar in control and IMAO rats. So, l-dopa filtered load was also similar in both groups. The other factor is MAO inhibition which renders less amount of inactivated DA. In this setting, MAO activity was moderately decreased by expansion alone in controls but almost abolished by pargyline. Thus, taken together, results suggest that $U_{DA}V$ peak found in controls and the highest peak observed in IMAO group should be attributed to MAO inhibition. Results indicate that under a moderate isotonic volume expansion the early release of renal DA into urine is modulated by MAO activity.

In BNZ group, immediately before volume expansion started, rats were treated with Benserazide, and because of this, the early and transient increment in $U_{DA}V$ disappeared. This was accompanied by a decrease in diuresis and natriuresis. The amount of $U_{DA}V$ at the end of the experiment was significantly greater than in control or IMAO group but, diuresis and natriuresis remained lower than in C rats. Benserazide did not modify GFR and MAP as compared to C rats. This pattern of response to Benserazide could be due to a short action effect of the drug and also to a possible excess of l-dopa accumulation in proximal tubule cells. The available l-dopa excess was then converted to DA when BNZ effect ended. Similar patterns of BNZ effect on DA release have also been observed in central nervous system studies[10].

It is to remark that GFR did not show variations among C, IMAO and BNZ rats despite that $U_{DA}V$ and sodium and water excretion did. Thus, it suggests that under volume expansion conditions renal DA does not seem to play a role in GFR regulation, and that the effects on hydrolectrolyte excretion are dependent upon tubular effects of the amine.

In this model of moderate volume expansion when the early $U_{DA}V$ peak was either increased by IMAO or decreased by BNZ the effect on $Na^+$ and water excretion became evident in the second hour of urine collection[11]. Thus, changes in DA release preceded changes in hydroelectrolyte excretion. This apparent lag in the response to intrarenal DA during volume expansion could be due to other mediators which oppose to DA effect. Renal denervated rats show a faster and higher diuresis and natriuresis than innervated animals[12]. It has also been demonstrated that DA, together with other neurohormonal systems, bidirectionaly regulates the activity of $Na^+$ transporters like as $Na^+$, $K^+$-ATPase or $Na^+/H^+$ exchanger[13, 14, 15].

In conclusion, 1) During volume expansion the release of renal DA increases early and in a transient peak-shaped way, 2) The diuretic response to volume expansion can be modified either by enhancing the early peak via MAO inhibition or by reducing it via AADC inhibition, 3) A late increment in urinary DA, as it is observed with BNZ treatment, does not increase diuresis in this model of two hours volume expansion.

The results obtained in this experimental model allowed us to define the role of different components involved in intra-renal DA metabolism and actions that may be linked to extracellular volume status and sodium and water regulation. Particularly, the importance of the early and transient peak of DA released into urine and, also, the predominant role of MAO activity in this response. These findings might predict that any maneuver which alters renal DA release at the beginning of volume expansion will modify hydroelectrolyte excretion.

They also give experimental support to test therapeutic strategies such as MAO inhibition together with Henle's loop diuretics to enhance the response to volume expansion or to treat pathophysiological conditions such as sodium retaining states.

**Acknowledgements:** This work was supported by PICT 5-25428 from Agencia de Promoción Científica y Técnica and grants from CONICET and Universidad de Buenos Aires (grant ME067).

**Conflicts of interest**: None.

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