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Catecholamine Production Along the Nephron

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Key Words
Catecholamines • Nephron • Tyrosine hydroxylase • Renal cells

Abstract
The present work proposes an extra neural site of catecholamine production along the nephron. LLC-PK_1, MDCK, and mIMCD-3 (proximal and distal tubules and inner medullary collecting duct, respectively) presented the following amine concentrations in the cell homogenates: Norepinephrine = 275±34, 56±16 and 255±21; Epinephrine = 161±20, 83±17 and 53±7; and Dopamine = 63±15, 39±6 and 36±7 pg/mg cell protein (Means ± SEM), respectively. The culture medium showed Norepinephrine = 168±25, 22±3 and 135±8; Epinephrine = 32±6, 152±17 and 39±5; and Dopamine = 27±9, 241±34 and 26±5 pg/mg cell protein, respectively. The synthesis enzymes as tyrosine hydroxylase, dopa decarboxylase and dopamine β-hydroxylase were detected by Western blotting. Biopterin, the enzymatic cofactor of tyrosine hydroxylase, was quantified in the intracellular and medium of mIMCD-3 cells (17±4 and 24±3 nmol/mg cell protein, respectively) and in the medium of MDCK cells (19±4 nmol/mg cell protein). The data confirmed that the proximal tubule is an important source of dopa decarboxilase and Dopamine and epithelial cell along the nephron express the biochemical pathway for catecholamine production.

Introduction

Since long time ago, extra neural catecholamine production by rat kidney has been investigated. Silva et al., 1979, studied the excretion and metabolism of labeled epinephrine (EPI) and norepinephrine (NOR) by isolated, perfused rat kidney; secretion of both catecholamine significantly exceeded the amount filtered, thus providing direct evidence of net tubular secretion [1]. Among extra neural tissues, proximal tubular cells are the primary site of dopamine (DA) production after uptake of filtered L-dopa [2], being these non-neural sources of DA production responsible for more than one half of this monoamine which is excreted in the urine [3]. Dawson & Philips, 1990, used the LLC-PK_1 cells to study renal DA synthesis and the mechanisms of DA efflux from these cells [4].

In recent works of our laboratory, the catecholamine production by primary and immortalized mesangial cells was evaluated [5, 6]. Once they presented the complete

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biosynthetic machinery (Figure 1), such as the synthesis enzymes and the cofactor biopterin, these cells were able to store/release significant levels of NOR, EPI and DA.

Besides all these evidences for renal catecholamine production, it is known that the monoamines can affect different nephron segments due to the presence of receptors like DA receptors, α- and β-adrenoceptors [7-9]. In addition, mammalian kidney also possesses monoamine oxidase (MAO) and catechol-O-methyl transferase (COMTE) [10], the enzymes responsible for monoamine degradation. Thus, catecholamine of renal origin can produce significant physiological effects. It is evident that they may act as important intrarenal hormones in the regulation of fluid and electrolyte balance.

As renal cells have a complex metabolic machinery and thus it is interesting to evaluate a potential role of the cells to produce and release catecholamines, the present work proposes an extra neural site of catecholamine production along the nephron. Because there is a need for cells lines as models for the study of particular differentiated functions, epithelial cells lines, LLC-PK_1 and MDCK, and mIMCD-3 were chosen as model lacking neuronal contribution to evaluate for the first time the catecholamine biosynthesis in proximal and distal tubules and inner medullary collecting duct, respectively.

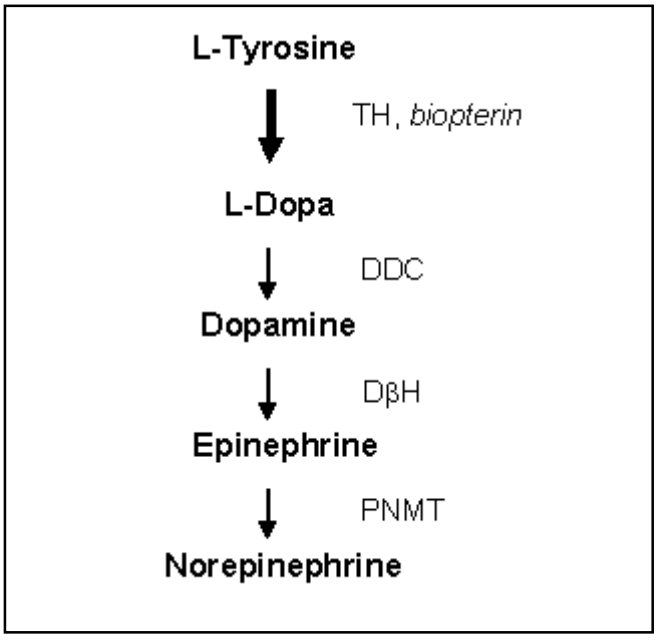


Fig. 1. Catecholamine cascade. (↓) indicates the rate limiting step.

Material and Methods

Cell Culture

LLC-PK_1, MDCK and mIMCD-3 cells obtained from the American Type Culture Collection (ATCC, Rockville, MD) were plated onto DMEM supplemented with 5% fetal bovine serum (FBS), 50 U/mL penicillin and 2.6 mM acid HEPES. The cultures were allowed to develop in a CO_2 incubator (5% CO_2, 95% air) at 37°C. The medium was replaced every 36 hours. At the time of 90% confluence, the cultures were submitted to trypsinization and subcultured in flasks under the same culture conditions. This procedure was repeated up to time of experiments, when the cells were washed twice with 5.0 mL of PBS and then incubated with 2.5 mL of DMEM without FBS for 24 h. This medium was collected and placed on ice or transferred to a freezer for storage at –80°C. The culture flasks (25 cm^2) were rinsed twice with 5 mL of phosphate-buffered saline (PBS) and scraped in the presence of 1 mL of PBS. Cell viability was monitored with Trypan blue.

Chronic adaptation to hyperosmolality

The mIMCD-3 cells were adapted to hyperosmolality by increasing medium osmolality ~150 mOsm every medium replacement until the desired osmolality (910 mOsm) as described by Santos et al.[11]. Osmolality was increased by the addition of urea and NaCl. The cells adapted to hyperosmolality (here called mIMCD(HO)) were gently supplied by Dr. Santos´s laboratory, Nephrology Division, Federal University of São Paulo.

Catecholamine assay

Catecholamines were extracted from the medium and cell lysates using Al_2O_5 (alumina) and DHBA (dihydroxybenzylamine) as internal standard, and quantified by ion-pair reverse phase chromatography coupled with electrochemical detection (0.5 volts) as described by Di Marco et al., 2003 [6].

Biopterin assay

The medium and the lysate were assayed by direct injection of the filtered samples into the HPLC-UV as described by Arita et al., 2002 [5].

Immunocytochemistry

The culture flasks (25 cm^2) of MDCK cells were rinsed twice with 5 mL of phosphate-buffered saline (PBS) and gently scraped in the presence of 1 mL of PBS. MDCK were pelleted and fixed in Bouin solution, embedded in paraplast and sectioned at 5 μm with a rotatory microtome. Prior to use, the thin sections were dewaxed, rehydrated and rinsed in 0.05 mol/L Tris-phosphate-saline buffer (TPS), pH 7.6. The other cells were cultured in cover slips and, at 90% of confluence, they were maintained in medium without FBS for 24 hours and the cells were rinsed twice with 5 mL of PBS and fixed in Bouin solution. Immunostaining and immunocytochemical control were performed by the peroxidase-antiperoxidase (PAP) method as previously done for the localization of these enzymes in mesangial cells [6].

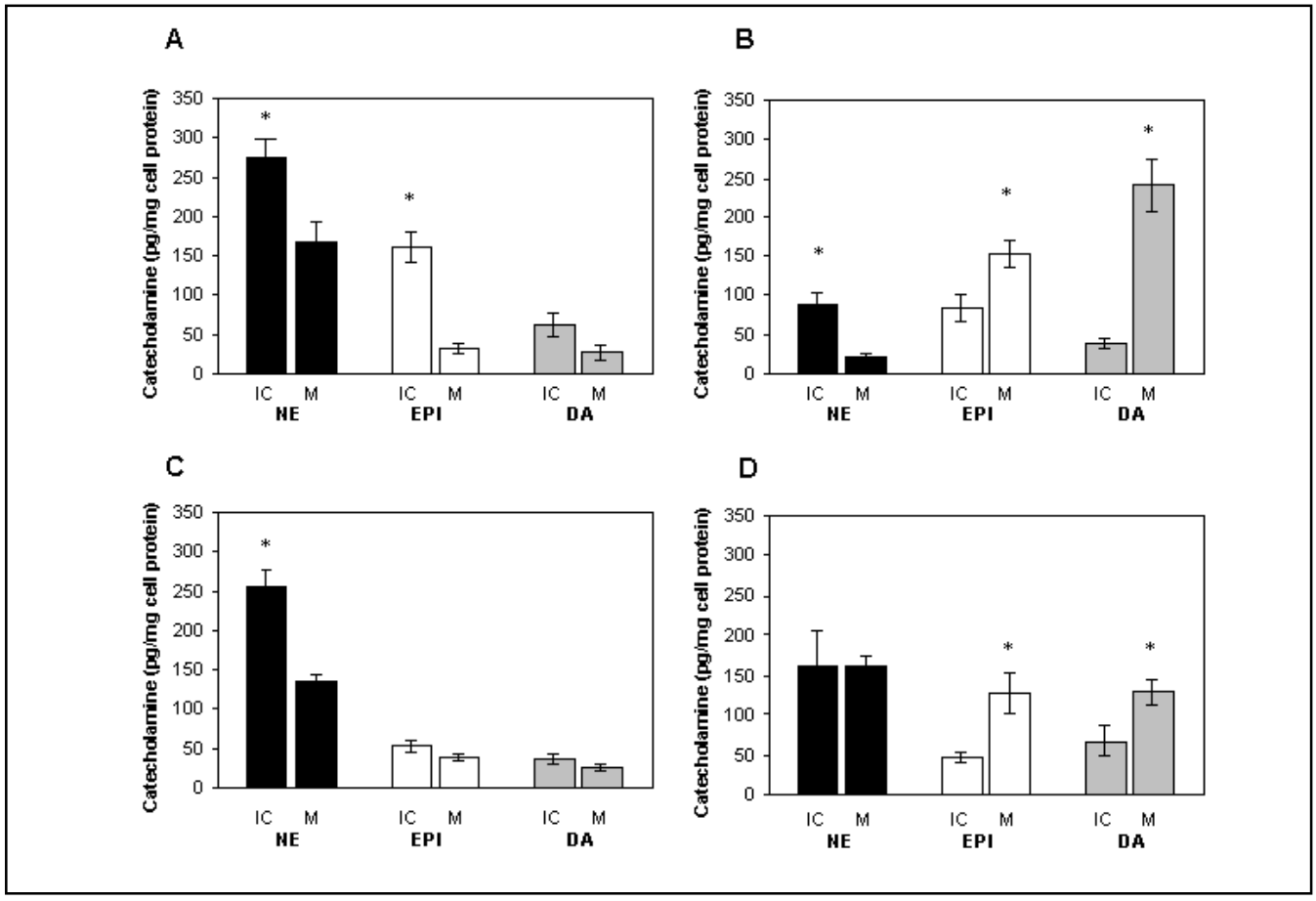


Fig. 2. Distribution of catecholamines in the intracellular compartment (IC) and in the culture medium (M) of LLC-PK$_1$ (1A), MDCK (1B), mIMCD-3 (1C), and mIMCD-3(HO) (1D) cells. All values are means ± SEM, n=10, based on the number of culture dishes. *$P < 0.05$.

Statistical analysis

Results are presented as means ± SEM. Statistical analysis between mean values was performed by the Student *t* test with statistical significance set at $P < 0.05$.

Results

Figure 2 shows the catecholamine levels found in LLC-PK$_1$, MDCK, mIMCD-3, and mIMCD-3(HO) cells. Concerning LLC-PK$_1$ cells (Figure 2A), higher concentrations of NE and EPI were detected in the intracellular (275 ± 34 and 161± 20 pg/mg cell protein, respectively, mean ± SEM) than in the medium (168 ± 25 and 32 ± 6 pg/mg cell protein, respectively) ($p < 0.05$). No statistically significant difference in DA levels was found between the medium and the intracellular compartment (27 ± 9 and 63 ± 15 pg/mg cell protein, respectively). Nevertheless, when LLC-PK$_1$ cells were incubated in the presence of 0.1 mM L-DOPA [4], they are able to increment both the DA production and release in a time-dependent manner. In the kidney, local DA has an effect on sodium and fluid absorption in proximal straight tubules, where these non neural sources of DA production respond for more than half of this catecholamine, which is excreted in the urine [3].

MDCK cells presented higher levels of NE in the cell lysate than in the medium (88 ± 16 x 22 ± 3 pg/mg cell protein, respectively, $p <0.05$), but EPI and DA levels are lower in the intracellular than in the medium (EPI = 83 ± 17 x 152 ± 17 and DA = 39 ± 6 x 241 ± 34 pg/mg cell protein, respectively, $p< 0.05$) (Figure 2B).

A different profile was found in mIMCD-3 cells (Figure 2C). A statistically significant difference between the medium and the cell lysate was found only in NE levels (135 ± 8 x 255 ± 21 pg/mg cell protein, respectively, $p < 0.05$). EPI and DA concentrations in the intracellular and medium were EPI = 53 ± 7 x 39 ± 5 and

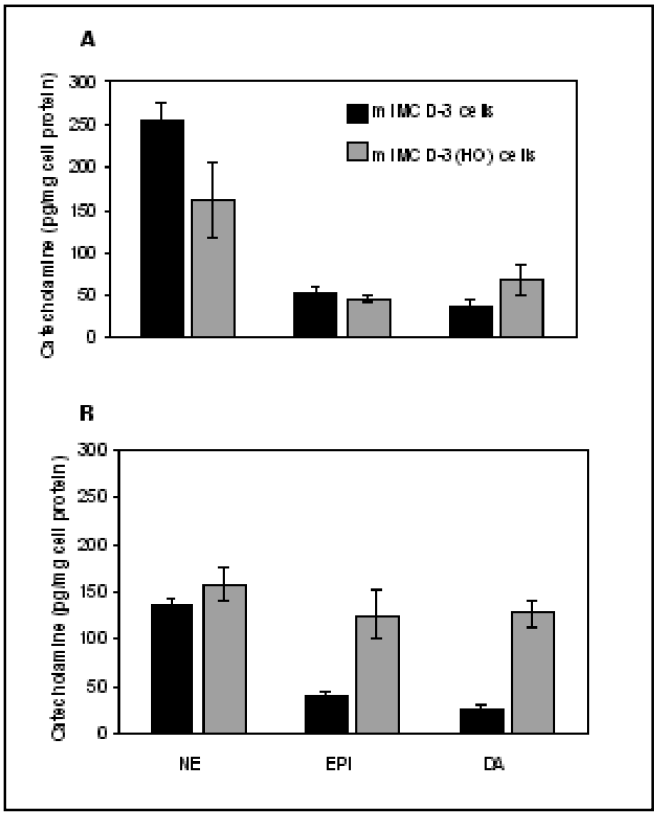
Fig. 3. Comparison between catecholamine levels in the intracellular (A) and culture medium (B) of mIMCD-3 and mIMCD-3(HO) cells. All values are means ± SEM, n=10, based on the number of culture dishes. *$P < 0.05$.

DA = 36 ± 7 x 26 ± 5 pg/mg cell protein, respectively. However, mIMCD-3(HO) cells changed considerably this profile as shown in Figure 2D. The EPI and DA concentrations are increased in the medium when compared to the intracellular compartment (EPI = 126 ± 26 x 46 ± 5 and DA = 128 ± 14 x 67 ± 18 pg/mg cell protein, respectively, $p < 0.05$), and NE values are similar in both intracellular and extracellular compartments (161 ± 45 x 159 ± 17 pg/mg cell protein, respectively).

Comparing adapted and not adapted mIMCD-3 cells, we can observe that the first ones released great amount of EPI and DA to the medium (159 ± 17 x 39 ± 5 and 128 ± 14 x 26 ± 5 pg/mg cell protein, respectively, $p < 0.05$), maintaining the same levels in the intracellular (Figure 3).

Using HPLC determination, the enzymatic cofactor of TH, biopterin, was found in the intracellular and medium of cultured mIMCD-3 cells (17 ± 4 and 24 ± 3 nmol/mg cell protein, respectively) (Figure 4B) and only in the medium of MDCK cells (19 ± 4 nmol/mg cell protein) (Figure 4A). LLC-PK_1 and mIMCD-3(HO) cells were not evaluated.

Western blotting analysis of the cell lysates (data not shown) and immunocytochemistry were employed to identify and locate the enzymes (Figure 5). Immunocytochemistry revealed the expression of the enzymes TH, DDC and DβH using monoclonal anti-TH-1 and anti-DDC-9 antibodies (1:100) and the polyclonal anti-DβH antibody (1:100) in LLC-PK1 (Figure 5A), MDCK (Figure 5B) and mIMCD-3 (Figure 5C cells). High magnification showed heavy staining over the cytoplasm and in the perinuclear area. No staining was observed when the primary antibodies were omitted or when they were replaced with preimmune or nonimmune serum (data not shown).



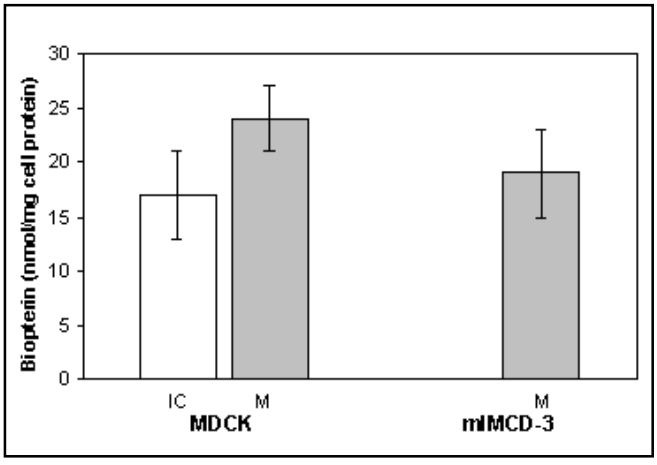


Fig. 4. Distribution of biopterin in intracellular (IC) and medium (M) of MDCK and mIMCD-3 cells. Values are reported as means ± SEM, based on the number of culture dishes. * $P < 0.05$.

Discussion

Previous studies have suggested catecholamine synthesis in the isolated perfused kidney, renal cortical slices and isolated tubules [12-14]; however these preparations are not usually devoid of neural contributions to catecholamine synthesis. The present use of cultured epithelial cells provides a convenient and useful model system for the study of renal catecholamine production without neuronal contribution.

We describe here, for the first time, the pattern of catecholamine production along the nephron segments. Nonetheless, at present, we have not evaluated factors that control the production or release of these hormones by these cells.

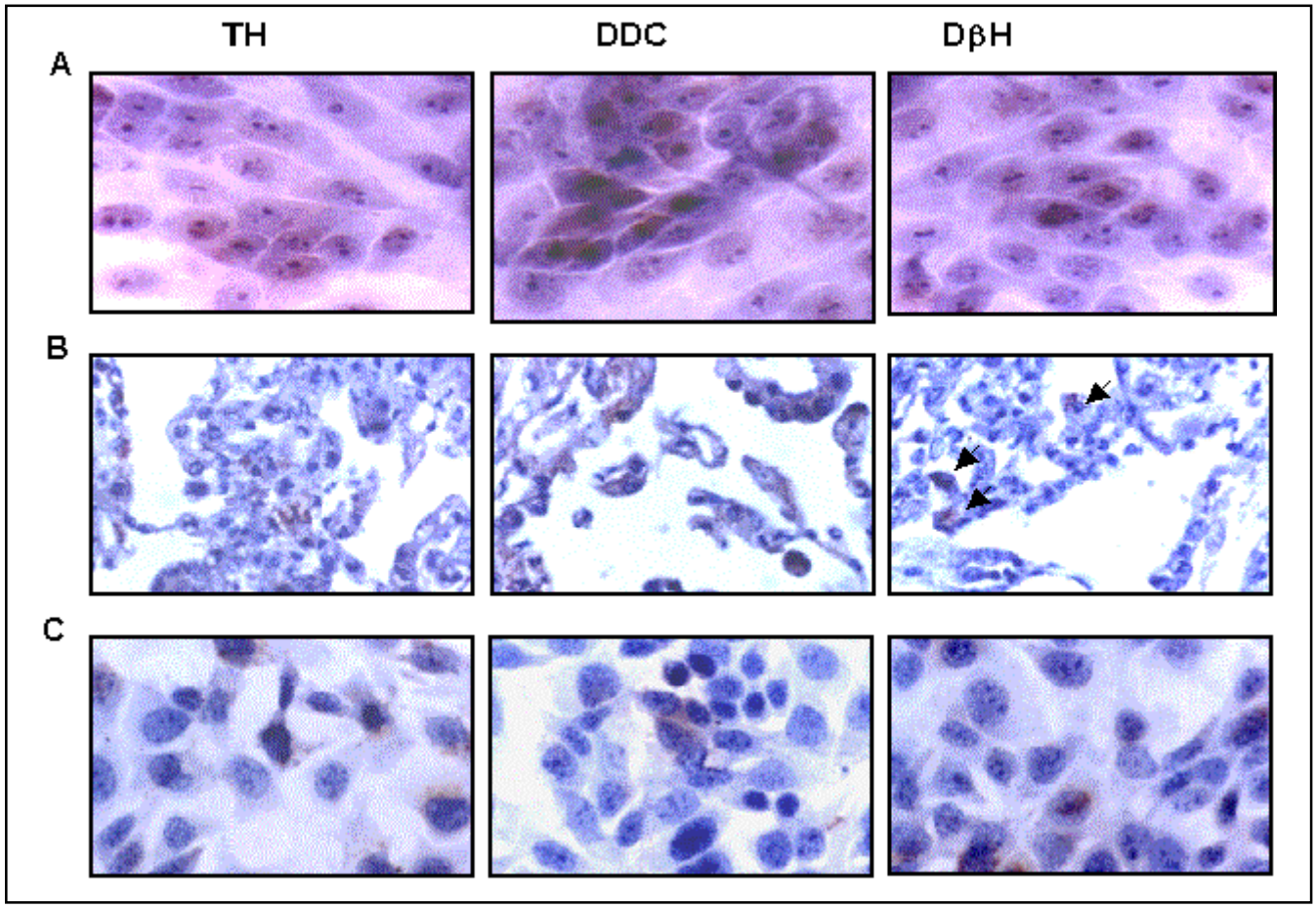


Fig. 5. Photomicrographs showing TH, DDC and DβH immunostaining in LLC-PK$_1$ (Figura 5A), MDCK (Figure 5B) and mIMCD-3 (Figure 5C) cells. These cells were stained for monoclonal TH-2 antibody (1:100), monoclonal DDC-109 antibody (1:100), and polyclonal DβH antibody (1:100).

The findings reported that LLC-PK$_1$, MDCK, mIMCD-3 and mIMCD-3(HO) cells in the basal state produce and store catecholamines in an average order of 10 μM (29 pg/mL), concentration in which catecholamines can exert their functions [15, 16].

The tissue concentration of catecholamines depends, in part, on the presence and activity of the amine-synthesizing or –degrading enzymes. The strongest evidence that these cells are able to produce catecholamine is the presence of TH in the intracellular compartment, as well as the presence of its cofactor, biopterin, indicating that L-dopa could be synthesized locally from the amino acid L-tyrosine, normally presented in the culture medium. Besides the evidences presented in the current study that renal cells express the whole enzymatic machinery to synthesise catecholamines in vitro, there are data from the literature showing that 1) renal cells also express catecholamine receptors, such as dopamine and alpha and beta adrenoreceptors [7, 9, 17-19]; and that 2) the mitochondrial enzyme MAO (A and B), the major metabolic pathways for biogenic amine degradation, and COMTE are widely distributed in renal cortex and medulla [10, 20].

The meanings of different levels of catecholamine production in the different cell lines, as well as in control and mIMCD-3(HO) cells, should be further explored. However, the findings related to adapted and unadapted mIMCD-3 cells are in line with the statement that hyperosmolality alters biosynthesis and protein induction, representing normal adaptive responses of the cells from the inner medullary collecting duct [11].

Catecholamines are involved in various physiological processes in the kidney [4, 7, 8], being factors that impact on renal hemodynamics, and have been reported to be altered in pathological disorders like extracellular volume expansion, hypertension, and diabetes [21-23]. Thus, it is conceivable that these locally produced biogenic amines present a potential contribution to the

intrarenal control of glomerular or tubular function and microcirculation. As we have no direct evidence in favour of this hypothesis, it remains, however, conjectural.

In summary, our efforts showed that glomerular mesangial cells [5, 6] and epithelial cells along the nephron express the biochemical pathway for catecholamine production. Whether this pathway exists in intact organ systems is currently unclear. Further studies are necessary to evaluate the mechanisms that could mediate changes in the regulation, expression and function of the enzymes responsible for the catecholamine production. However, at present, we proposed models without neuronal interferences to evaluate the role of this local catecholamine system in the kidney, as well as to understand its implication in pathophysiological process.

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