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# Actin cytoskeleton, tubular sodium and the renal synthesis of dopamine

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**Abstract**—The present study has examined the effect of colchicine and cytochalasin B, two cytoskeleton disrupter compounds, on the formation of dopamine in slices of rat renal cortex loaded with exogenous L-3,4-dihydroxyphenylalanine (L-DOPA); the deamination of newly formed dopamine into 3,4-dihydroxyphenylacetic acid (DOPAC) was also examined. The accumulation of newly formed dopamine and DOPAC in kidney slices loaded with L-DOPA (10–100 μM) was found to be dependent on the concentration of L-DOPA, being similar in control conditions and in preparations treated with increasing concentrations of colchicine (5, 10 and 50 μM). By contrast, cytochalasin B (5, 10 and 50 μM) was found to produce a concentration-dependent reduction in the formation of dopamine and of its deaminated metabolite DOPAC in kidney slices loaded with L-DOPA (10–100 μM). The inhibitory effect of cytochalasin B on the formation of dopamine was found to be completely abolished in kidney slices pretreated with ouabain (500 μM) or when sodium concentration in the incubation was reduced from 120 to 20 mM. On its own, ouabain (500 μM) was found to reduce the formation of dopamine by 55%; the effect of reducing sodium concentration in the incubation medium to 20 mM was also a significant reduction (53% decrease) in the formation of dopamine. The accumulation of DOPAC did always parallel that of its parent amine. It is concluded that the renal formation of dopamine is dependent on the concentration of sodium in the medium and the integrity of the tubular transport of sodium, namely on the association between actin cytoskeleton and $Na^+,K^+$-ATPase, appears to be determinant.

In kidney, the activation of type $D_1$ dopamine receptors located in tubular epithelial cells leads to an increased natriuresis, mainly as a result of a decreased tubular reabsorption of sodium [1, 2]. There is evidence to suggest that the dopamine responsible for this natriuretic effect is of renal origin [1, 3, 4]. In fact, tubular epithelial cells, namely those of proximal convoluted tubules, are endowed with a high aromatic L-amino acid decarboxylase (AAAD*; EC 4.1.1.28) activity and circulating 3,4-dihydroxyphenylalanine (DOPA) is converted to dopamine after being taken up into this cellular compartment [5–7]. The formation of dopamine in tubular epithelial cells has, however, been shown to be dependent on the concentration of sodium in the extracellular medium and sensitive to inhibition of $Na^+,K^+$-ATPase (EC 3.6.1.—) [8]. Tubular epithelial cells are polarized structures and the ultimate mechanism intervening in the process of sodium transport across these cells is that governed by $Na^+,K^+$-ATPase [9]. This enzyme is localized at the basolateral membrane of tubular epithelial cells and the association between the actin cytoskeleton and $Na^+,K^+$-ATPase is determinant for the vectorial transport of sodium [10]. Damage of actin cytoskeleton in tubular epithelial cells leads to a modification in the localization of $Na^+,K^+$-ATPase in the cell wall which ultimately results in a decrease in the tubular reabsorption of sodium [10, 11]. The present work reports on the effects of inhibition of actin cytoskeleton and on the association between actin cytoskeleton and tubular sodium transport in the formation of dopamine in rat kidney slices loaded with L-DOPA. A preliminary account of these findings has been reported previously [12].

## *Materials and Methods*

Male Wistar rats (Biotério do Instituto Gulbenkian de Ciência, Oerias, Portugal) aged 45–60 days and weighing 200–280 g were used in the experiments. Animals were kept two per cage under controlled environmental conditions (12 hr light/dark cycle and room temperature 24°). Food and tap water were allowed *ad lib.* The experiments were all carried out during day time. The rats were killed by decapitation under ether anaesthesia and both kidneys removed and rinsed free from blood with saline (0.9% NaCl). The kidneys were placed on an ice-cold glass plate, the kidney poles removed and slices of the renal cortex approximately 0.5 mm thick and weighing about 30 mg wet weight were prepared with a scalpel. Thereafter, renal slices were preincubated for 30 min in 2 mL warmed (37°) and gassed (95% $O_2$ and 5% $CO_2$) Krebs' solution. The Krebs' solution had the following composition (mM): NaCl 120, KCl 4.7, $CaCl_2$ 2.4, $MgSO_4$ 1.2, $NaHCO_3$ 25, $KH_2PO_4$ 1.2, EDTA 0.4, ascorbic acid 0.57, glucose 10 and sodium butyrate 1; 1-α-methyl-*p*-tyrosine (50 μM), tropolone (50 μM) and copper sulphate (10 μM) were also added to the Krebs' solution in order to inhibit the enzymes tyrosine hydroxylase and catechol-*O*-methyltransferase and inhibit the endogenous inhibitors of dopamine β-hydroxylase, respectively. After pre-incubation, renal slices were incubated for 15 min in Krebs' solution with increasing concentrations of L-DOPA (10–100 μM). The preincubation and incubation were carried out in glass test tubes, continuously shaken throughout the experiment. In experiments in which the effects of colchicine (5, 10 and 50 μM) or cytochalasin B (5, 10 and 50 μM) on the renal production of dopamine were tested, the compounds were present during the preincubation and incubation periods. In some of the experiments in which the effect of cytochalasin B was tested, the concentration of sodium chloride in the incubation medium was reduced to 20 mM; the osmolarity of the incubation medium was kept constant by the addition of choline chloride. The other constituents in the Krebs' solution were the same as described above. In another series of experiments, the effect of cytochalasin B was tested in conditions of $Na^+,K^+$-ATPase inhibition; ouabain (500 μM), the $Na^+,K^+$-ATPase inhibitor, was present during the preincubation and incubation periods. At the end of incubation, the reaction was stopped by the addition of 250 μL 2 M perchloric acid and samples were stored at 4° until the quantification of catecholamines within the next 24 hr.

* Abbreviations: AAAD, aromatic L-amino acid decarboxylase; DOPAC, 3,4-dihydroxyphenylacetic acid; DOPA, 3,4-dihydroxyphenylalanine.

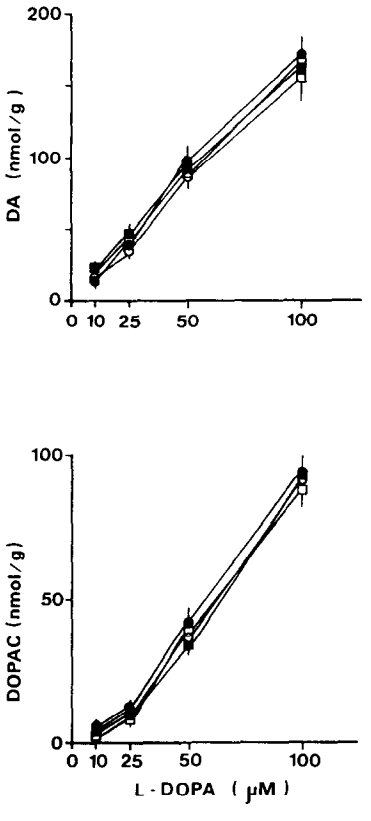


Fig. 1. Effect of increasing concentrations of colchicine (5, 10 and 50 μM) on the concentration-dependent accumulation of dopamine (DA) and DOPAC (in nmol/g) in rat kidney slices incubated with exogenous L-DOPA (10–100 μM) for 15 min. Each point represents the mean of five experiments per group; vertical lines show SEM. (●) Control; (○) 5 μM colchicine; (■) 10 μM colchicine; (□) 50 μM colchicine.

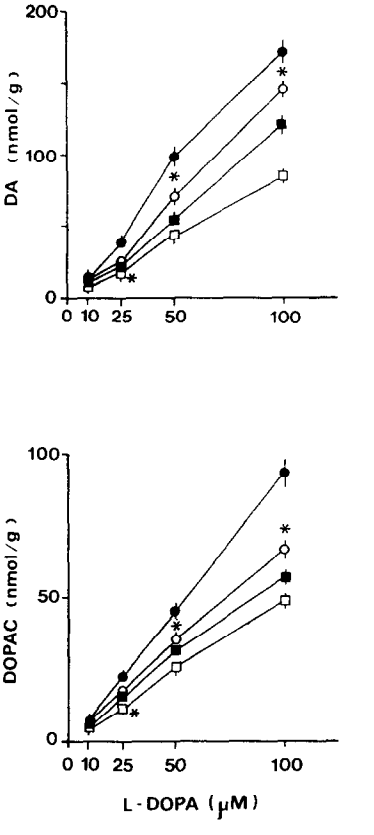


Fig. 2. Effect of increasing concentrations of cytochalasin B (5, 10 and 50 μM) on the concentration-dependent accumulation of dopamine (DA) and DOPAC (in nmol/g) in rat kidney slices incubated with exogenous L-DOPA (10–100 μM) for 15 min. Each point represents the mean of five experiments per group; vertical lines show SEM. Significantly different from corresponding values of control using the Tuckey–Kramer method (*$P < 0.01$). (●) Control; (○) 5 μM cytochalasin B; (■) 10 μM cytochalasin B; (□) 50 μM cytochalasin B.

The assay of L-DOPA, dopamine and 3,4-dihydroxyphenylacetic acid (DOPAC) in renal tissues and kidney homogenates was performed by means of HPLC with electrochemical detection, as described previously [8]. In brief, aliquots of 1.0 mL of the acidified incubation medium (in which the kidney slices were kept for 24 hr) were placed in 5 mL conical-based glass vials with 50 mg alumina and the pH of the samples immediately adjusted to pH 8.6 by the addition of Tris buffer. The adsorbed catecholamines were then eluted from the alumina with 200 μL of 0.2 M perchloric acid on Millipore microfilters (MF1); 50 μL of the eluate was injected into a high pressure liquid chromatograph (Gilson Medical Electronics, Villiers le Bel, France). The lower limits for detection of L-DOPA, dopamine, noradrenaline and DOPAC were 1.0, 1.4, 0.9 and 2.5 pmol/g, respectively.

Mean values ± SEM of N experiments are given. Significance of differences between one control and several experimental groups was evaluated by the Tuckey–Kramer method [13]. A P value less than 0.05 was assumed to denote a significant difference.

Colchicine, cytochalasin B, DOPAC, L-DOPA, dopamine hydrochloride, 1-α-methyl-*p*-tyrosine, ouabain and tropolone hydrochloride were purchased from the Sigma Chemical Co. (St Louis, MO, U.S.A.).

*Results*

Incubation of slices of rat renal cortex with increasing concentrations of L-DOPA (10–100 μM) for 15 min resulted in a concentration-dependent accumulation of newly formed dopamine and of its deaminated metabolite, DOPAC (Fig. 1). The addition of increasing concentrations of colchicine (5, 10 and 50 μM) was found to affect neither the decarboxylation of L-DOPA into dopamine nor the formation of DOPAC in kidney slices loaded with increasing concentrations of L-DOPA (10–100 μM) (Fig. 1). By contrast, incubation of kidney slices with increasing concentrations of cytochalasin B (5, 10 and 50 μM) resulted in a concentration-dependent decrease in the accumulation of newly formed dopamine and of its deaminated metabolite, DOPAC (Fig. 2). The magnitude of the inhibitory effect of cytochalasin B on the formation of dopamine was found not to depend on the concentration of L-DOPA used, i.e. similar per cent inhibitory figures were found to occur while using 10, 25, 50 and 100 μM L-DOPA.

As has been presented previously [8], the formation of dopamine and DOPAC in kidney slices incubated in the presence of ouabain (500 μM) or low sodium (20 mM) in

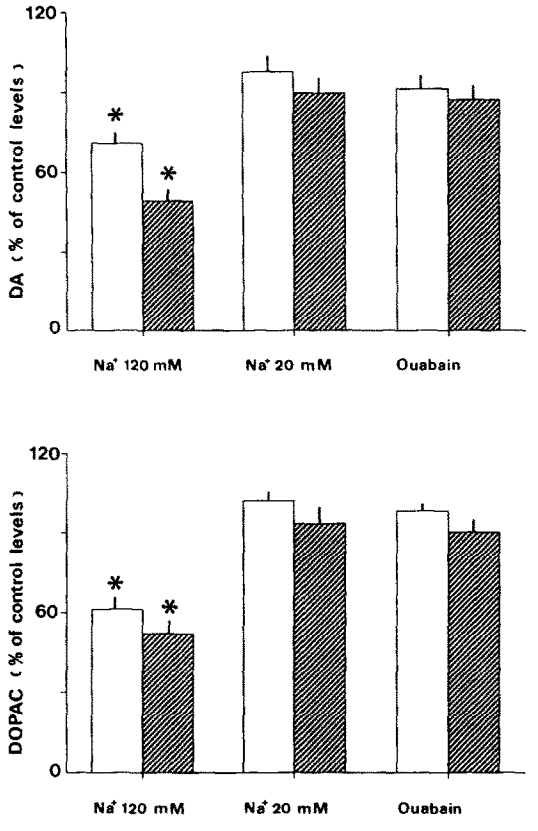


Fig. 3. Effect of cytochalasin B (10 and 50 $\mu$M) on the formation of dopamine (DA) and DOPAC (in nmol/g) in rat kidney slices loaded with exogenous L-DOPA (100 $\mu$M) for 15 min in the presence of 120 mM (control conditions) or 20 mM sodium in the incubation medium and in the presence of ouabain (500 $\mu$M). Data are per cent dopamine and DOPAC levels of corresponding control levels; control levels of dopamine and DOPAC for the experiments performed in the presence of ouabain and 20 or 120 mM sodium in the incubation medium are shown. Each column represents the mean of five experiments per group; vertical lines show SEM. Significantly different from corresponding values of control using the Tuckey–Kramer method (*$P < 0.01$). (□) 10 $\mu$M cytochalasin B; (▨) 50 $\mu$M cytochalasin B.

the incubation medium was reduced markedly (51 to 53% decrease) when compared with that observed in control conditions (120 mM sodium in the incubation and without ouabain; data not shown). In Fig. 3, are shown the results of experiments in which the effect of 10 and 50 $\mu$M cytochalasin B were tested on the formation of dopamine in kidney slices loaded with L-DOPA and incubated either in the presence of ouabain (500 $\mu$M) in a low (20 mM) sodium-containing medium or in control conditions (without ouabain and in the presence of 120 mM sodium). As shown in this figure, 10 and 50 $\mu$M cytochalasin B in control conditions were found to reduce the formation of dopamine in kidney slices loaded with 100 $\mu$M L-DOPA, respectively, by 29% and 51%. In the presence of ouabain (500 $\mu$M) or in the presence of low sodium (20 mM) in the medium cytochalasin (5 and 10 $\mu$M) was found not to significantly affect the formation of dopamine. In these experiments, the formation of DOPAC, the deaminated metabolite of dopamine, did always parallel that of the parent amine.

*Discussion*

The results presented here are in agreement with those reported in previous studies showing that the renal formation of dopamine in kidney slices loaded with L-DOPA is a sodium-dependent process [8] and sensitive to mechanisms interfering with the tubular transport of sodium [14, 15]; it is further suggested that the sodium dependency of the renal formation of dopamine is not only related to the extracellular concentrations of sodium but also appears to depend on the integrity of the tubular transport system for sodium, namely on the association between actin cytoskeleton and $Na^+,K^+$-ATPase.

The suggestion that the inhibitory effect of cytochalasin B in the formation of dopamine may be related to inhibition of the tubular transport of the precursor L-DOPA stands mainly on two sets of findings. Firstly, the formation of dopamine from L-DOPA in kidney slices is clearly a sodium-dependent and ouabain-sensitive process, although some of the amine can still be synthetized in conditions of reduced tubular transport of sodium. Secondly, the inhibitory effect of cytochalasin B was only found to occur in experimental conditions in which the tubular transport of sodium is facilitated, i.e. when the extracellular concentration of sodium is greater than that inside the cell [9] or in the absence of ouabain. On the other hand, the result that cytochalasin B was found not to affect the synthesis of dopamine in ouabain-treated kidney slices or in conditions of low sodium in the extracellular medium suggests that the sodium-independent and ouabain-insensitive component of dopamine synthesis is not sensitive to disruption of the actin cytoskeleton. The results obtained in experiments in which the effect of colchicine was tested suggest that the integrity of the microtubular system may be of minor importance in the transport of tubular sodium and L-DOPA.

The surface of proximal tubular epithelial cells presents distinct structural, biochemical and physiological characteristics at the apical and basolateral membrane domains, the maintenance of which is essential for the vectorial transport of water, ions, amino acids and macromolecules [16]. Some of these different membrane features in the apical and basolateral cell borders are related to differences in the physicochemical properties of the membranes [17–19], whereas others are dependent on the different distribution of membrane components (enzymes and ionic channels) between the two membrane domains [10]. This is largely accomplished by the presence of a cytoskeleton at which some of the polar membrane components are anchored [20]. Tubular sodium enters the epithelial cell down its electrochemical gradient across the apical membrane and is then transported up to its electrochemical gradient by an ATP-driven mechanism at the basolateral membrane. $Na^+,K^+$-ATPase, the enzyme responsible for the vectorial transport of tubular sodium, is specifically distributed at the basolateral membrane of tubular epithelial cells through the indirect anchoring of the enzyme at the basolateral membrane by actin, where it forms a metabolically stable complex with fodrin and ankyrin [20–23]. Disruption of the cytoskeleton of tubular epithelial cells, either by inhibitors of actin cytoskeleton or during renal ischemia, has been shown to result in a modification in the localization of $Na^+,K^+$-ATPase in the cell wall and in a decrease in the tubular reabsorption of sodium [10, 11].

In conclusion, the renal formation of dopamine is dependent on the concentration of sodium in the medium and the integrity of the tubular transport of sodium, namely on the association between actin cytoskeleton and $Na^+,K^+$-ATPase.

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REFERENCES

1. Siragy HM, Felder RA, Howell NL, Chevalier RL, Peach MJ and Carey RM, Evidence that intrarenal dopamine acts as a paracrine substance at the renal tubule. *Am J Physiol* **257**: F469–F477, 1989.
2. Hedge SS, Ricci A, Amenta F and Lokhandwala MF, Evidence from functional and autoradiographic studies for the presence of tubular dopamine-1 receptors and their involvement in the renal effects of fenoldopam. *J Pharmacol Exp Ther* **251**: 1237–1245, 1989.
3. Jose PA, Felder RA, Holloway RR and Eisner GM, Dopamine receptors modulate sodium excretion in denervated kidney. *Am J Physiol* **250**: F1033–F1038, 1986.
4. Carey RM, Siragy HM and Felder RA, Physiological modulation of renal function by the renal dopaminergic system. *J Auton Pharmacol* **10** (Suppl 1): s47–s51, 1990.
5. Hayashi M, Yamaji Y, Kitajima W and Saruta A, Aromatic L-amino acid decarboxylase activity along the rat nephron. *Am J Physiol* **258**: F28–F33, 1990.
6. Soares-da-Silva P and Fernandes MH, Synthesis and metabolism of dopamine in the kidney. Effects of sodium chloride, monoamine oxidase inhibitors and atrial natriuretic peptide. *Am J Hypertens* **3**: 7S–10S, 1990a.
7. Soares-da-Silva P and Fernandes MH, Regulation of dopamine synthesis in the rat kidney. *J Auton Pharmacol* **10** (Suppl 1): s25–s30, 1990b.
8. Soares-da-Silva P and Fernandes MH, Sodium dependence and ouabain sensitivity of the synthesis of dopamine in renal tissues of the rat. *Br J Pharmacol* **105**: 811–816, 1992a.
9. Lang F, Messner G and Rehwald W, Electrophysiology of sodium-coupled transport in proximal renal tubules. *Am J Physiol* **250**: F953–F962, 1986.
10. Molitoris BA, Ischemia-induced loss of epithelial polarity: potential role the actin cytoskeleton. *Am J Physiol* **260**: F769–778, 1991.
11. Kellerman PS, Clark RAF, Hoilien CA, Linas SL and Molitoris BA, Role of microfilaments in the maintenance of proximal tubule structure and functional integrity. *Am J Physiol* **259**: F279–F285, 1990.
12. Soares-da-Silva P, Role of actin cytoskeleton in the regulation of the renal synthesis of dopamine. *Br J Pharmacol* **105**: 1P, 1992.
13. Sokal RR and Rohlf FJ, *Biometry. The Principles and Practice of Statistics in Biological Research.* Freeman and Co., New York, 1981.
14. Soares-da-Silva P and Fernandes MH, Inhibitory effects of guanosine 3′:5′-cyclic monophosphate on the synthesis of dopamine in the rat kidney. *Br J Pharmacol* **103**: 1923–1927, 1991.
15. Soares-da-Silva P and Fernandes MH, Effect of alpha-human atrial natriuretic peptide on the synthesis of dopamine in the rat kidney. *Br J Pharmacol* **105**: 869–874, 1992b.
16. Molitoris BA, Membrane fluidity: measurement and relationship to solute transport. *Semin Nephrol* **7**: 61–67, 1987.
17. Carmel G, Rodriguez F, Carriere S and Le Grimellec C, Composition and physical properties of lipids from plasma membranes of dog kidney. *Biochim Biophys Acta* **818**: 149–157, 1985.
18. Molitoris BA and Hoilien CA, Static and dynamic components of renal cortical brush border and basolateral membrane fluidity: role of cholesterol. *J Membr Biol* **99**: 165–172, 1987.
19. Molitoris BA and Simon FR, Renal cortical brush border and basolateral membranes: cholesterol and phospholipid composition and relative turnover. *J Membr Biol* **83**: 207–215, 1985.
20. Salas PJI, Vega-Salas E, Hochman J, Rodriguez-Boulan E and Edidin M, Selective anchoring in the specific plasma membrane domain. A role in epithelial cell polarity. *J Cell Biol* **107**: 2363–2376, 1988.
21. Koob R, Kraemer D, Trippe G, Aebi U and Drenckhahn O, Association of kidney and parotid $Na^+,K^+$-ATPase microsomes with actin and analogs of spectrin and ankyrin. *Eur J Cell Biol* **53**: 93–100, 1990.
22. Morrow JS, Cianci CD, Ardito T, Mann AS and Kashgarian M, Ankyrin links fodrin to the alpha subunit of Na-K-ATPase in Madin–Darby canine kidney cells and in intact renal tubule cells. *J Cell Biol* **108**: 455–465, 1989.
23. Nelson WJ and Hammerton RW, A membrane cytoskeletal complex containing Na-K-ATPase, ankyrin and fodrin in Marbin–Darby canine kidney (MDCK) cell: implications for the biogenesis of epithelial cell polarity. *J Cell Biol* **108**: 893–902, 1989.

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