Kinetic study of the tubular dopamine outward transporter in the rat and dog kidney

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1 The present study has determined the kinetic characteristics of the outflow of dopamine of renal origin in slices of rat and dog renal cortex loaded with exogenous L-dihydroxyphenylalanine (L-DOPA 5 to 5000 μM).

2 In both dog and rat renal tissues the production of dopamine was found to be dependent on the concentration of L-DOPA used and reached its maximum at 2500 μM L-DOPA. The decarboxylation of L-DOPA in rat cortical slices (16.4 ± 2.6 to 1479.2 ± 85.2 nmol g^{-1}) was 6 fold that in the dog (2.2 ± 0.4 to 252.1 ± 21.2 nmol g^{-1}). In the rat kidney a large amount (approximately 50%) of the dopamine (5.2 ± 0.6 to 743.4 ± 58.3 nmol g^{-1}) was found to escape into the incubation medium, whereas in dog renal slices the amount of newly-formed dopamine escaping into the incubation medium (0.7 ± 0.2 to 46.5 ± 9.3 nmol g^{-1}) was less than 25% of the total amount of the amine formed.

3 The application of the Michaelis-Menten equation to the net transport of newly-formed dopamine has allowed the identification of a saturable (carrier-mediated transfer) and a non-saturable component (diffusion). The V_{max} ($\text{nmol g}^{-1} 15 \text{ min}^{-1}$) and K_m (nM) values for the saturable component were, respectively, 340 ± 41 and 396 ± 45 in the rat kidney and 112 ± 16 and 319 ± 35 in the dog kidney. In both rat and dog renal tissues, the magnitude of the non-saturable component was found to be of minor importance up to a concentration of 250 nmol g^{-1} of dopamine to be transported. At high concentrations of the amine (greater than 250 nmol g^{-1}), only attainable in rat kidney slices, most of the dopamine was found to leave the compartment where the synthesis did occur through a non-saturable transport system.

4 In conclusion, the results presented here show that the outflow of newly-formed dopamine in both dog and rat kidney slices loaded with exogenous L-DOPA follows Michaelis-Menten kinetics with a saturable component and a non-saturable one, the latter assuming particular importance only at higher concentrations of the amine.

Keywords: Dopamine; kidney; cell outward transport

Introduction

The proximal nephron is endowed with a high aromatic L-amino acid decarboxylase (AAAD) activity and epithelial cells of proximal tubules have been demonstrated to synthesize dopamine from 3,4-dihydroxyphenylalanine (DOPA) present in the tubular filtrate (Hayashi *et al.*, 1990; Seri *et al.*, 1990; Soares-da-Silva & Fernandes, 1990a,b). The dopamine formed in tubular epithelial cells has been shown to be of particular importance in the renal handling of water and electrolytes (Jose *et al.*, 1986; Hedge *et al.*, 1989; Siragy *et al.*, 1989); the natriuretic effects of the amine appear to result from the activation of specific receptors located in renal tubules (Jose *et al.*, 1992) and would involve inhibition of both the $\text{Na}^+\text{-H}^+$ exchanger (Felder *et al.*, 1990) and $\text{Na}^+\text{-K}^+$ ATPase (Aperia *et al.*, 1987).

The cellular uptake of DOPA in renal proximal tubules has been shown to occur through an energy-dependent and stereoselective carrier-mediated process (Chan, 1974) and evidence produced subsequently favours the view that sodium may play a part in this process. In fact, sodium loading has been reported to be accompanied by an increased urinary excretion of dopamine in both man and laboratory animals (Alexander *et al.*, 1974; Ball *et al.*, 1978; Bains, 1982; Goldstein *et al.*, 1989; Hayashi *et al.*, 1990) and a low sodium diet results in a decrease in the urinary excretion of dopamine (Bains, 1982; Goldstein *et al.*, 1989; Hayashi *et al.*, 1990). In line with this evidence, it has been reported in both human and rat kidney preparations that the production of dopamine is not only closely dependent on the extracellular sodium, but also appears to be related to the transtubular reabsorption of sodium (Soares-da-Silva & Fernandes, 1991; 1992a,b; Soares-da-Silva *et al.*, 1992; 1993a,c) and on the integrity of the tubular cytoskeleton and the functional integrity of the Na^+ -

K^+ -ATPase (Soares-da-Silva, 1992a). More recently, inhibition or stimulation of the $\text{Na}^+\text{-H}^+$ exchanger has been found to affect the outward transport of newly-formed dopamine in rat kidney slices (Soares-da-Silva, 1992b), thus suggesting that both the cellular uptake of L-DOPA and the outflow of dopamine may directly depend on sodium or may be under the influence of mechanisms which are responsible for the tubular transport of sodium. On the other hand, this may suggest that the tubular cell outward transfer of dopamine would involve the stimulation of an active transport system for the amine. The outflow of newly-formed dopamine in rat kidney slices has been shown to follow a monoexponential pattern of decay with a half-life of about 20 min (Pestana & Soares-da-Silva, 1992).

The aim of the present work was to evaluate the presence of an active transport system for dopamine in slices of rat and dog renal cortex loaded with L-DOPA and to determine the kinetic characteristics of the outflow of dopamine of renal origin.

Methods

The rat and dog renal tissues were obtained from, respectively, male Wistar rats (biotério do Instituto Gulbenkian de Ciência, Oeiras, Portugal) aged 45–60 days and weighing 200 to 280 g and male mongrel dogs of undetermined age and weighing 18 to 25 kg. Rats were kept two per cage under controlled environmental conditions (12 h light/dark cycle room temperature 24°C). Food and tap water were allowed *ad libitum*. The rats were killed by decapitation under ether anaesthesia and both kidneys removed and rinsed free from

blood with saline (0.9% w/v saline solution). Dogs were anaesthetized with sodium pentobarbitone (30 mg kg^{-1}) given i.v. and both kidneys quickly removed through a midline incision of the abdominal wall. The kidneys were then 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 25 mg wet weight were prepared with a scalpel. Thereafter, renal slices were preincubated for 30 min in 2 ml gassed (95% O_2 and 5% CO_2) and warm (37°C) Krebs solution. The Krebs solution had the following composition (mM): NaCl 120, KCl 4.7, CaCl_2 2.4, MgSO_4 1.2, KH_2PO_4 1.2, NaHCO_3 25, EDTA 0.4, ascorbic acid 0.57 and glucose 11; 1- α -methyl-*p*-tyrosine ($50 \mu\text{M}$), pargyline (0.1 mM), tropolone ($50 \mu\text{M}$) and copper sulphate ($10 \mu\text{M}$) were also added to the Krebs solution in order to inhibit the enzymes, tyrosine hydroxylase, monoamine oxidase and catechol-*O*-methyltransferase and inhibit the endogenous inhibitors of dopamine β -hydroxylase, respectively. After preincubation, renal slices were incubated for 15 min in Krebs solution with added L-DOPA. The preincubation and incubation were carried out in glass test tubes, continuously shaken throughout the experiment. After the incubation, renal slices were collected with fine forceps and immediately placed in 2 ml of 0.2 M perchloric acid at 4°C for the next 24 h. In order to allow the determination of the amount of dopamine which had leaked into the incubation medium, 250 μl of 2.0 M perchloric acid was added to the incubation medium after renal tissues had been collected; the samples of the incubation medium were also kept at 4°C for the next 24 h, until quantification of dopamine.

In the experiments performed with the aim of determining the presence and characterize the kinetic parameters of an outward transport mechanism for dopamine in renal slices, tissues were loaded with increasing concentrations (5 to $5000 \mu\text{M}$) of L-DOPA for 15 min and the amount of newly-formed dopamine accumulated in the tissue and that which has escaped into the incubation medium determined; up to a concentration of $5000 \mu\text{M}$ L-DOPA, the pH of the incubation medium remained stable at 7.4. K_m and $V_{\max}$ values for the outward transport of newly-formed dopamine were calculated from linear regression analysis; the substrate transported (nmol of dopamine present in the incubation medium) was plotted against the product of the division between the substrate transported by the amount of the total dopamine formed (obtained from the algebraic sum of the amount accumulated in the tissue plus that present in the incubation medium). In these experimental conditions, the movement of newly-formed dopamine is expected to be mainly from the intracellular to the extracellular medium; though it may be hypothesized that the net transfer of dopamine would involve a carrier-mediated transport, diffusion may also contribute to total amine transfer. The determination of carrier transport and diffusion in this experimental model was performed as defined by Neame & Richards (1972) by use of the expression

$$\frac{V_{\max} [S]/[S] + K_m}{(\text{carrier transport}) + (\text{diffusion})} + (K_d[S])$$

where $[S]$ is the concentration of the substrate to be transported and K_d the diffusion constant. Values for K_m and $V_{\max}$ were obtained by the method described above.

The assay of dopamine in kidney slices and the incubation medium was performed by means of high performance liquid chromatography (h.p.l.c.) with electrochemical detection, as previously described (Soares-da-Silva *et al.*, 1992). In brief, aliquots of 1.5 ml of perchloric acid in which tissues have been kept or 0.5 ml of acidified incubation medium were placed in 5 ml conical-based glass vials with 50 mg alumina and the pH of the samples 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 limit for detection of dopamine was $10 \text{ fmol } \mu\text{l}^{-1}$.

Statistics

Mean values $\pm$ s.e.mean of n experiments are given. Significance of differences between one control and several experimental groups was evaluated by Tukey-Kramer method (Sokal & Rohlf, 1981). A P value less than 0.05 was assumed to denote a significant difference.

Drugs

L-Dihydroxyphenylalanine (L-DOPA), dopamine hydrochloride, 1- α -methyl-*p*-tyrosine pargyline hydrochloride and tropolone hydrochloride were purchased from Sigma Chemical Company (St. Louis, Mo, U.S.A.).

Results

In Figure 1 are shown the levels of total dopamine formed, obtained by the algebraic sum of their respective levels in the tissue and the incubation medium, and the amount of that dopamine which has remained in the tissue, in rat and dog kidney slices incubated with increasing concentrations (5 to $5000 \mu\text{M}$) of L-DOPA. In both dog and rat renal tissues the production of dopamine was found to be dependent on the concentration of L-DOPA used and reached its maximum at $2500 \mu\text{M}$ L-DOPA. The decarboxylation of L-DOPA in rat cortical slices (16.4 ± 2.6 to $1479.2 \pm 85.2 \text{ nmol g}^{-1}$) was 6 fold that in the dog (2.2 ± 0.4 to $252.1 \pm 21.2 \text{ nmol g}^{-1}$); also, in the rat kidney a high proportion (approximately 50%) of dopamine (5.2 ± 0.6 to $743.4 \pm 58.3 \text{ nmol g}^{-1}$) was

Figure 1 Levels of total dopamine formed (open symbols), obtained by the sum of their amounts in the tissue and the incubation medium, and the amount of that dopamine which has remained in the tissue (closed symbols), in (a) dog and (b) rat slices of renal cortex incubated with increasing concentrations (5 to $5000 \mu\text{M}$) of L-DOPA for 15 min. Each point represents the mean with s.e.mean of five to six experiments per group.

found to escape into the incubation medium, whereas in dog renal slices the amount of newly-formed escaping into the incubation medium (0.7 ± 0.2 to 46.5 ± 9.3 nmol g⁻¹) was less than 25% of the total amine formed. The tissue levels of noradrenaline in kidney slices did not change significantly in the course of these experiments even when 5000 μ M L-DOPA was used (data not shown, but see Fernandes & Soares-da-Silva, 1990).

A considerable amount of the total dopamine formed from added L-DOPA in kidney slices, depending on the species used, was found to escape into the incubation medium. The application of the Michaelis-Menten equation to the net transport of newly-formed dopamine has allowed the identification of a saturable (carrier-mediated transfer) and a non-saturable component (diffusion). In Figure 2 are shown the two components of dopamine transport and the total rate of amine transfer, as a plot of the amount of dopamine which has escaped into the incubation medium (rate of transport) against the total amount of dopamine formed (substrate concentration) in both the rat and dog kidney slices. The corresponding Eadie-Hofstee plot of the saturable component is shown in Figure 3. The $V_{\max}$ (nmol g⁻¹ 15 min⁻¹) and K_m (nM) values for the saturable component were, respectively, 340 ± 41 and 396 ± 45 in the rat kidney and 112 ± 16 and 319 ± 35 in the dog kidney. In both rat and dog renal tissues, the magnitude of the non-saturable component was found of minor importance up to a concentration of 250 nmol g⁻¹ of dopamine to be transported (Figure 2). At high concentrations of the amine (greater than 250 nmol g⁻¹), only attainable in rat kidney slices, most of the dopamine was found to leave the compartment where the synthesis did occur through a non-saturable transport system (Figure 2).

Figure 2 Outward transfer of newly-formed dopamine in slices of (a) dog and (b) rat renal cortex incubated with increasing concentrations of L-DOPA (5 to 5000 μ M) for 15 min. The results are on the two components of dopamine transfer, saturable (●) and non-saturable (△), and the total rate of amine transfer (○), as a plot of the amount of dopamine which has escaped into the incubation medium (rate of amine transport; [V], in nmol g⁻¹ 15 min⁻¹) against the total amount of dopamine formed (amine concentration; [S], in nmol g⁻¹) in both the rat and dog kidney slices.

Figure 3 Eadie-Hofstee plot of the saturable component of newly-formed outward transfer in slices of dog (○) and rat (●) renal cortex incubated with increasing concentrations of L-DOPA (5 to 5000 μ M) for 15 min. The $V_{\max}$ (nmol g⁻¹ 15 min⁻¹) and K_m (nM) values for the saturable component were, respectively, 340 ± 41 and 396 ± 45 in the rat kidney and 112 ± 16 and 319 ± 35 in the dog kidney.

Discussion

In order to be active, the dopamine formed in tubular epithelial cells has to leave the compartment where the synthesis has occurred, since the receptors activated by the amine are believed to be located in the external surface of the cell membrane (Jose *et al.*, 1992). The identification and kinetic characterization of an outward transport system for dopamine in renal tissues is, therefore, of particular relevance, considering that the dopamine synthesized in tubular epithelial cells may have important physiological actions in the control of tubular reabsorption of sodium. The results presented here show that the cell outward transfer of dopamine through a carrier-mediated system appears to be an important mechanism for the transport of the amine in tubular epithelial cells, though diffusion may play a role at high concentrations of dopamine.

The data reported here show that the outflow of newly-formed dopamine in kidney slices loaded with exogenous L-DOPA follow Michaelis-Menten kinetics with a saturable component and a non-saturable one, the latter assuming particular importance only at higher concentrations of the amine. Similar K_m values for the saturable component of dopamine outflow in the rat and dog kidney suggests that the renal outward transporter of dopamine in the two species may share some functional properties. In contrast, higher $V_{\max}$ values in the rat may suggest the presence of a high number of outward transporter units in the rat kidney. Another difference between dog and rat renal tissues concerns the greater ability of rat cortical slices to convert L-DOPA into dopamine. This, however, appears to be related to a higher AAAD activity in the rat in comparison with that in the dog, as determined in kidney homogenates (Soares-da-Silva *et al.*, 1993b). Until now, it has not been clarified whether there is a preferential cell outward transport of dopamine at the basolateral or the apical cell borders. Because the apical cell border may not be in operation due to collapse of tubular lumen while using kidney slices (Wedeen & Weiner, 1973), the results reported here on the outflow of dopamine would mainly reflect the outward transfer of dopamine at the basolateral cell border.

The intrinsic mechanisms involved in the modulatory process of the renal outward dopamine transfer have not yet been fully elucidated, but evidence has been presented favouring the involvement of both the organic cation transporter (Rennick & Pryor, 1965; Hakim *et al.*, 1970) and the Na⁺-H⁺ exchanger. The accompanying work (Soares-da-

Silva, 1993) presents evidence suggesting that the outward transport of the amine in kidney slices can be influenced by activators and inhibitors of the $\text{Na}^+\text{-H}^+$ exchanger. Recent studies performed in the proximal nephron indicate that changes in cell volume and intracellular pH observed during the activation of the $\text{Na}^+\text{-H}^+$ exchanger may serve as a signal for generating a biological response (Mahnensmith & Aronson, 1985; Gesek & Schoolwerth, 1989). Thus, it may be possible that modulation of cell outward transport of tubular dopamine by the $\text{Na}^+\text{-H}^+$ exchanger depends upon changes in intracellular pH, Na^+ flux or even changes in the activity

of the organic cation transporter (Ross & Holohan, 1983).

In conclusion, the results presented here show that the outflow of newly-formed dopamine in both dog and rat kidney slices loaded with exogenous L-DOPA follows Michaelis-Menten kinetics with a saturable component and a non-saturable one, the latter assuming particular importance only at higher concentrations of the amine.

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