Na⁺-Independent Transporters, LAT-2 and b₀,⁺, Exchange L-DOPA with Neutral and Basic Amino Acids in Two Clonal Renal Cell Lines

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Received: 14 May 2001/Revised: 19 November 2001

Abstract. The present study examined the functional characteristics of L-DOPA transporters in two functionally different clonal subpopulations of opossum kidney (OK₁₋₃ and OK₊₃) cells. The uptake of L-DOPA was largely Na⁺-independent, though in OK₊₃ cells a minor component (~15%) required extracellular Na⁺. At least two Na⁺-independent transporters appear to be involved in L-DOPA uptake. One of these transporters has a broad specificity for small and large neutral amino acids, is stimulated by acidic pH and inhibited by 2-aminobicyclo[2.2.1]-heptane-2-carboxylic acid (BCH; OK₁₋₃, Kᵢ = 291 μM; OK₊₃, Kᵢ = 380 μM). The other Na⁺-independent transporter binds neutral and basic amino acids and also recognizes the di-amino acid cystine. [¹⁴C]-L-DOPA efflux from OK₁₋₃ and OK₊₃ cells over 12 min corresponded to a small amount of intracellular [¹⁴C]-L-DOPA. L-Leucine, nonlabelled L-DOPA, BCH and L-arginine, stimulated the efflux of [¹⁴C]-L-DOPA in a Na⁺-independent manner. It is suggested that L-DOPA uses at least two major transporters, systems LAT-2 and b₀,⁺. The transport of L-DOPA by LAT-2 corresponds to a Na⁺-independent transporter with a broad specificity for small and large neutral amino acids, stimulated by acidic pH and inhibited by BCH. The transport of L-DOPA by system b₀,⁺ is a Na⁺-independent transporter for neutral and basic amino acids that also recognizes cystine. LAT-2 was found equally important at the apical and basolateral membranes, whereas system b₀,⁺ had a predominant distribution in apical membranes.

Key words: L-DOPA — OK cells — LAT-2 — System b₀,⁺ — Sodium — pH dependence

Introduction

The renal dopaminergic system is a local non-neuronal system constituted by epithelial cells of proximal convoluted renal tubules rich in aromatic L-amino acid decarboxylase (AADC) activity and using circulating or filtered L-DOPA as a source for dopamine (Jose et al., 1992; Lee, 1993; Soares-da-Silva, 1994). Because the dopamine produced in this area is in close proximity to renal cells that contain receptors for the amine, it has been hypothesized that the amine may act as a paracrine or autocrine substance (Siragy et al., 1989). In order to overcome technical problems related to the handling of freshly isolated renal tubular epithelial cells, opossum kidney (OK) cells, which express proximal tubule cell-like properties in vitro (Koyama et al., 1978), have been used to study dopamine receptors and the renal actions of the amine. These cells have been also shown to take up (Vieira-Coelho & Soares-da-Silva, 1997) and convert intracellular L-DOPA to dopamine in a saturable manner (Soares-da-Silva, Vieira-Coelho & Serrão, 1997). Newly-formed dopamine also stimulated cAMP accumulation in OK cells (Cheng et al., 1990) and inhibited Na⁺-phosphate cotransport, both of which were attenuated by carbidopa or benserazide and blocked by D₁-like receptor antagonists (Glahn et al., 1993; Perrichot et al., 1995). It appears, therefore, that in OK cells, as in epithelial cells of proximal tubules, locally formed dopamine can act as an autocrine/paracrine substance. Furthermore, the amounts of the enzymes aromatic L-amino acid decarboxylase, catechol-O-methyltransferase and monoamine oxidase found in this cell line are likely to be sufficient to reproduce, under in vitro conditions, the environment in which the renal dopaminergic system normally operates (Guimarães et al., 1997).
Although the kidney is endowed with one of the highest levels of AADC in the body and plasma levels of \( \text{l-DOPA} \) are in the nmol/ml range (Grossman et al., 1992; Soares-da-Silva et al., 1995), the rate-limiting step for the synthesis of dopamine in renal tissues is still a matter of debate. However, since \( K_m \) values for \( \text{l-DOPA} \) uptake are 10 times lower than \( K_m \) values for decarboxylation of \( \text{l-DOPA} \), it could be possible that \( \text{l-DOPA} \) uptake rather than decarboxylation, may limit the rate of formation of dopamine. In a previous report we have concluded that OK cells take up \( \text{l-DOPA} \) through a saturable, stereoselective and temperature-dependent process when applied from the apical and basolateral cell border (Soares-da-Silva et al., 1997; Vieira-Coelho & Soares-da-Silva, 1997), this being similar to that occurring in rat proximal tubules (Pinto-do-O & Soares-da-Silva, 1996; Soares-da-Silva, Fernandes & Pinto-do-O, 1994). However, the transporters involved in uptake of \( \text{l-DOPA} \) by renal epithelial cells have not been identified. At present, candidate transport systems for \( \text{l-DOPA} \) may include the Na\(^+\)-dependent systems B, B\(^{0,+}\) and \( y^\text{+L} \), and the Na\(^+\)-independent systems L (LAT-1 and LAT-2) and B\(^{0,+}\). Recently, both B\(^{0,+}\) and LAT-1 were found to transport \( \text{l-DOPA} \), the former in \textit{Xenopus laevis} oocytes injected with poly A\(^+\) RNA prepared from rabbit intestinal epithelium (Ishi et al., 2000) and the latter in mouse brain capillary endothelial cells (Kageyama et al., 2000). The major involvement of Na\(^+\)-independent systems LAT-1 and B\(^{0,+}\) contrast with that expected to occur at the kidney level. In fact, Na\(^+\) is a powerful stimulus for the production of renal dopamine (Lee, 1993) and \( \text{l-DOPA} \) uptake in human and rat kidney slices is a Na\(^+\)-dependent and ouabain-sensitive process (Soares-da-Silva & Fernandes, 1992; Soares-da-Silva, Pestana & Fernandes, 1993).

The present study examined the functional characteristics and regulation of the \( \text{l-DOPA} \) transport in two functionally different clonal subpopulations of opossum kidney (OK) cells, OK\(_{LC}\) and OK\(_{HC}\) cells. These cells derive from the same original batch obtained from the American Type Culture Collection (F-12476) and are morphologically identical, but differ markedly in their ability to transport Na\(^+\) (Gomes & Soares-da-Silva, 2000). To define the Na\(^+\)-sensitivity of the transporters involved in the uptake of \( \text{l-DOPA} \), the effect of maneuvers that affect cellular Na\(^+\) and H\(^+\) gradients and the sensitivity to inhibitors of amino acid transport and Na\(^+\) were examined. Thereafter, in order to have an insight on the molecular mechanisms governing \( \text{l-DOPA} \) uptake, the result of maneuvers that interfere with protein kinase A (PKA), protein kinase G (PKG), protein kinase C (PKC), protein tyrosine kinase (PTK) and Ca\(^{2+}\)-calmodulin mediated pathways were evaluated.

**Materials and Methods**

**CELL CULTURE**

OK cells (ATCC 1840-HTB) were obtained from the American Type Culture Collection (Rockville, MD) and maintained in a humidified atmosphere of 5% CO\(_2\)-95% air at 37°C. OK\(_{LC}\) (passages 49 to 81) and OK\(_{HC}\) (passages 62 to 95) cells were grown in Minimum Essential Medium (Sigma, St. Louis, MO), supplemented with 100 U/ml penicillin G, 0.25 μg/ml amphotericin B, 100 μg/ml streptomycin (Sigma), 10% foetal bovine serum (Sigma) and 25 mM N-2-hydroxyethylpiperazine-N\(^{-}\)-2-ethanesulfonic acid (HEPES; Sigma).

For subculturing, the cells were dissociated with 0.05% trypsin-EDTA, split 1:5 and subcultured in Costar flasks with 75- or 162-cm\(^2\) growth areas (Costar, Badhoevedorp, The Netherlands). For uptake studies, the cells were seeded in collagen-treated 24-well plastic culture clusters (internal diameter 16 mm, Costar) at a density of 40,000 cells per well or onto collagen treated 0.2 μm polycarbonate filter supports (internal diameter 12 mm, Transwell, Costar) at a density of 13,000 cells per well. The cell medium was changed every 2 days, and the cells reached confluence after 3–5 days of incubation. For 24 hours prior to each experiment, the cell medium was free of fetal bovine serum. Experiments were generally performed 2–3 days after cells reached confluence and 6–8 days after the initial seeding and each cm\(^2\) contained about 80–100 μg of cell protein.

**INFUX OF \( \text{l-DOPA} \)**

On the day of the experiment, the growth medium was aspirated and the cells were washed with Hanks’ medium; thereafter, the cell monolayers were preincubated for 15 or 30 min in Hanks’ medium (2 ml) at 37°C. The Hanks’ medium had the following composition (mm): NaCl 137, KCl 5, MgSO\(_4\) 0.8, Na\(_2\)HPO\(_4\) 0.33, KH\(_2\)PO\(_4\) 0.44, CaCl\(_2\) 0.25, MgCl\(_2\) 1.0, Tris HCl 0.15 and sodium butyrate 1.0, pH = 7.4. The incubation medium also contained benserazide (50 μM) and tolcapone (1 μM) in order to inhibit the enzymes AADC and catechol-O-methyltransferase, respectively. Time-course studies were performed in experiments in which cells were incubated with 1 μM substrate for 1, 3, 6, 12, 30 and 60 min. Saturation experiments were performed in cells preincubated for 15 min and then incubated for 6 min with increasing concentrations of \( \text{l-DOPA} \) (10 to 1000 μM). In experiments performed in the presence of different concentrations of Na\(^+\), NaCl was replaced by an equimolar concentration of choline chloride. Test substances were applied from the apical side only. Competing amino acids were present during the incubation (6 min) with \( \text{l-DOPA} \). Modulators of PKA, PKC, PKG, PTK and Ca\(^{2+}\)-calmodulin were present during the preincubation (30 min) and incubation (6 min) periods. During preincubation and incubation, the cells were continuously shaken and maintained at 37°C. Apical uptake was initiated by the addition of 2 ml Hanks’ medium with a given concentration of the substrate. Uptake was terminated by the rapid removal of uptake solution by means of a vacuum pump connected to a Pasteur pipette followed by a rapid wash with cold Hanks’ medium and the addition of 250 μl of 0.2 mM perchloric acid. The acidified samples were stored at 4°C before injection into the high pressure liquid chromatograph for the assay of \( \text{l-DOPA} \).

**ASSAY OF \( \text{l-DOPA} \)**

\( \text{l-DOPA} \) was quantified by means of high pressure liquid chromatography with electrochemical detection, as previously reported.