Monoamine neurotransmitter transport mediated by the polyspecific cation transporter rOCT1

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Received 12 August 1996

Abstract The polyspecific cation transporter rOCT,1 which is localized in the basolateral membrane of rat renal proximal tubules and in sinusoidal membranes of hepatocytes, was analyzed for transport of monoamine neurotransmitters. In voltage-clamp experiments with rOCT1-expressing *Xenopus* oocytes, superfusion with dopamine, serotonin, noradrenaline, histamine and the permanent cation acetylcholine induced saturable inwardly directed currents with apparent K_m values ranging from 20 to 100 μM. Transport of dopamine was also demonstrated by uptake measurements in oocytes and in the mammalian cell line (HEK 293) which was permanently transfected with rOCT1. The high uptake rates measured in rOCT1-expressing oocytes and in transfected HEK 293 cells suggest that rOCT1 is a high capacity transporter which mediates the first step in the excretion of monoamine neurotransmitters.

Key words: Cation transport; Monoamine; Neurotransmitter

1. Introduction

By expression cloning a polyspecific transporter of organic cations (rOCT1) has recently been isolated from rat [1]. rOCT1 belongs to a new transporter family and is mainly expressed in kidney and liver [1,2]. Preliminary immunological data and a functional comparison of rOCT1 expressed transport of tetraethylammonium and N^1-methylnicotinamide with in vivo measurements indicate that rOCT1 is localized in basolateral membranes of renal proximal tubules and in sinusoidal membranes of hepatocytes [2,3]. In these tissues rOCT1 is involved in the transport of organic cations from the blood into epithelial cells, the first step in cation excretion. rOCT1 was expressed in oocytes of *Xenopus laevis* and the induced transport of different organic cations was investigated by tracer flux and electrical measurements. rOCT1 was found to mediate electrogenic NaCl- and pH-independent uptake of various endogenous cations such as choline, spermine, spermidine and of cationic drugs such as quinine, quinidine and d-tubocurarine [4]. Since monoamine neurotransmitters are positively charged at physiological pH and excreted by the kidney [5,6], we investigated whether rOCT1 transports cationic neurotransmitters.

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2. Materials and methods

2.1. Expression of rOCT1 in oocytes of X. laevis and transport measurements

rOCT1 was subcloned in a pRSSP vector (pRSSP vector was a kind gift from Dr. R. Schöpfer, Heidelberg, Germany) and cRNA encoding rOCT1 was synthesized [1]. The *X. laevis* ovaries were dissected and the oocytes were collected as described earlier [7]. The oocytes were stored in 96 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, and 1 mM $MgCl_2$, 5 mM HEPES-NaOH, pH 7.4 (ORi buffer) containing 2.5 mM sodium pyruvate, 1 mM choline chloride, and 50 mg/l gentamicin. 50 nl of water per oocyte without and with 10 ng of rOCT1 cRNA were injected for control and expression experiments, respectively.

For electrophysiological measurements two-electrode voltage-clamp recordings were performed 3–8 days after cRNA injection. The external control solution (superfusate) was ORi buffer. Superfusion was performed at a flow rate of 20 ml/min and the bath solution was completely exchanged within about 10 s. In one set of experiments 96 mM NaCl was substituted with 192 mM D-glucose. The reported currents and depolarizations represent maximal values which were measured during a 30 s period of substrate superfusion. The data were filtered at 10 Hz and recorded as described earlier [7]. The data are represented as arithmetic means ± S.E.M., *n* indicating the number of experiments. In Figs. 1 and 2 sets of experiments are shown which were each performed on the same day. The experiments were repeated with two further batches of oocytes and similar results were obtained.

For influx measurements with [^{3}H]dopamine 3 days after cRNA injection, the oocytes were incubated for 15 min (19°C) with 200 μl ORi buffer with and without 36 μM cyanine 9863, a potent inhibitor of rOCT1-mediated transport [1]. Different amounts of dopamine plus 6 kBq of [^{3}H]dopamine were added and the oocytes were incubated for 60 min at 19°C. During this time period linear uptake rates were observed. Transport was stopped and the oocytes were washed with ice-cold ORi buffer. The oocytes were solubilized with 5% (w/v) SDS and analyzed for radioactivity. The uptake rates in Fig. 3 represent medians of 8–10 oocytes ± S.E.M. The Michaelis Menten equation was fitted to the cyanine 863-inhibitable uptake rates of dopamine.

2.2. Constant expression of rOCT1 in HEK 293 cells and transport measurements

rOCT1 was inserted into the mammalian expression vector pRcCMV (Invitrogen, The Netherlands). The construct pRcCMV-rOCT1 was used to transfect the HEK 293 cells (CRL-1573, American tissue type culture collection) using Lipofectin (Gibco BRL, Eggenstein, Germany) according to the manufacturer's recommendation. The cells were grown as described [8]. Neomycin resistance selection was performed with increasing concentrations of geneticin (200–800 μg/ml). A single clone was isolated, grown and used for transport measurements which were performed in suspension. For transport measurements monolayers of HEK 293 cells were washed twice with 137 mM NaCl, 2.7 mM KCl, 8 mM Na_2HPO_4, 1.6 mM KH_2PO_4, pH 7.4 (PBS) and the cells were suspended in the same buffer by shaking at room temperature. The cells were collected by centrifugation (10 min, 1000 × *g*) and suspended at 37°C in PBS or in 139.7 mM KCl, 8 mM K_2HPO_4, 1.6 mM KH_2PO_4 pH 7.4 (K buffer). For transport measurements at 37°C, the cells were incubated for 0.5, 1 or 2 s with PBS, PBS containing 25 μM cyanine 863, or with K buffer containing

PII S0014-5793(96)01030-7

either 0.1 μM [^{3}H]MPP or 0.1 μM [^{3}H]dopamine. The short term incubations were performed as described earlier [9] and stopped with ice-cold PBS containing 25 μM cyanine 863. The cells were washed twice with this stop solution, solubilized with 4 M guanidine thiocyanate and analyzed for radioactivity.

2.3. Materials

[^{3}H]Dopamine (1.8 Tbq/mmol) was obtained from Amersham Buchler (Braunschweig, Germany) and 1-[^{3}H]methyl-4-phenylpyridinium acetate (3.1 Tbq/mmol) from Du Pont de Nemours (Dreieich, Germany). Tetraethylammonium (TEA), cyanine 863, dopamine, noradrenaline, serotonin, histamine and acetylcholine were purchased from Sigma (Deisenhofen, Germany).

3. Results

In rOCT1 expressing oocytes voltage-clamped at −50 mV, saturating concentrations of TEA (1 mM) induced inward currents between −20 and −120 nA, similar to previously described results [4]. Fig. 1 shows that the monoamines dopamine, noradrenaline, serotonin and histamine and the permanent cation acetylcholine induced inward currents of similar amplitude to those with TEA (n = 4–6 for all substrates). At these concentrations, none of the compounds induced a significant current in non-injected oocytes (n = 6–8 for each compound). The currents induced by the monoamines were saturable and concentration dependent (Fig. 2). Dopamine yielded the highest apparent affinity of all neurotransmitters with an apparent K_m of 19.4 ± 2.9 μM (n = 4). Acetylcholine, serotonin and histamine induced currents with an apparent K_m of 46.8 ± 3.8 μM (n = 4), 37.6 ± 9.1 μM (n = 6) and 98.6 ± 9.1 μM (n = 5), respectively.

Previously, we showed that TEA and choline transport by rOCT1 is independent of extracellular pH and NaCl [4]. Here, in one set of experiments the dopamine (100 μM) induced currents of rOCT1-expressing oocytes were −45.6 ± 4.8 nA (n = 4) and −42.7 ± 5.3 nA (n = 4) in the presence (control solution) and absence of NaCl (96 mM NaCl was substituted by 192 mM D-glucose), respectively. In contrast, the high affinity cocaine-sensitive transporters for monoamines from the central nervous system depend on both Na^+ and Cl^- [10].

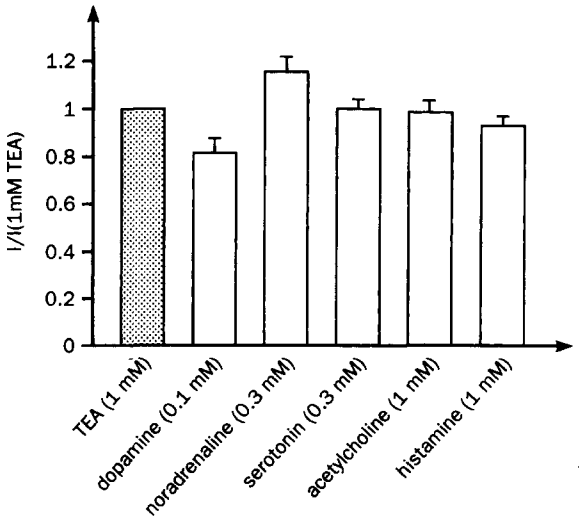

Fig. 1. Neurotransmitter-induced currents in rOCT1 expressing *Xenopus* oocytes. Currents were induced at a holding potential of −50 mV during superfusion with the respective compounds at the indicated concentrations. The currents were individually normalized against the TEA-induced currents at 1 mM concentration. The data represent means (± S.E.M.).

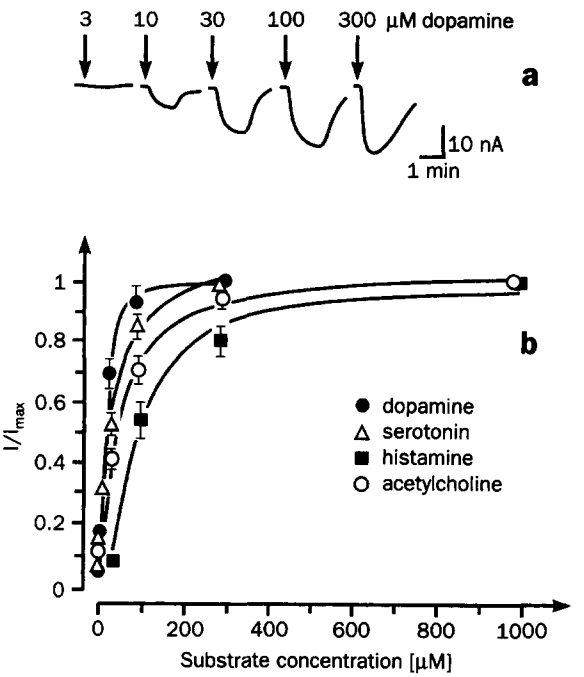

Fig. 2. Concentration dependence of neurotransmitter induced currents in rOCT1 expressing oocytes. (a) An rOCT1 cRNA-injected oocyte was clamped at −50 mV and superfused with the indicated concentrations of dopamine for 30 s. (b) Current-concentration relationship for dopamine, serotonine, histamine and acetylcholine. The currents were normalized against the current induced by 1 mM TEA. The data represent means (± S.E.M.) and were fitted with the Hill equation.

Subsequently, we analyzed the substrate dependence of [^{3}H]dopamine uptake in water-injected and rOCT1-expressing oocytes. In water-injected control oocytes no saturable uptake of dopamine could be detected. However, saturable uptake of dopamine was observed in rOCT1 expressing oocytes (Fig. 3). This uptake could be completely inhibited by 36 μM cyanine 863, a potent inhibitor of rOCT1. An apparent K_m of 51 ± 15 μM was estimated for [^{3}H]dopamine which is compatible with the apparent K_m obtained from the electrical measurements with oocytes clamped to −50 mV (Fig. 2b; n = 8). For tracer influx of dopamine a V_{max} value of 0.60 ± 0.05 μmol oocyte^{-1} h^{-1} was determined. This value is higher than the V_{max} values which were previously determined for the uptake of MPP, TEA or choline [4].

In an attempt to estimate the activity of rOCT1 in eukaryotic cells at 37°C, the renal epithelial cell line HEK 293 was constantly transfected with rOCT1 and initial uptake of MPP and dopamine was determined. In suspended cells uptake of [^{3}H]dopamine and [^{3}H]MPP was only linear for very short time periods (Fig. 4). The uptake rates were significantly reduced when extracellular K^+ was increased from 4.3 mM under control to 149.3 mM. This apparent potential dependence was expected for electrogenic transport by rOCT1. The initial uptake rate of 0.1 μM dopamine is greater than 10 pmol mg protein^{-1} min^{-1}. Considering that 1 mg of protein represents approx. 3.6 × 10^6 cells and assuming an apparent K_m for dopamine transport of 20 μM, a V_{max} of 10^{-15} mol/cell per min can be estimated. In comparison with other monoamine neurotransmitter transporters [11–13], which all

transport in transfected cells with a V_{max} of approx. 10^{-18} mol/cell per min, rOCT1 transport capacity appears very high.

4. Discussion

The present study shows that the polyspecific organic cation transporter rOCT1 mediates electrogenic translocation of the monoamine neurotransmitters dopamine, noradrenaline, serotonin, histamine and the permanent cation acetylcholine. This transporter is mainly expressed in kidney and liver and has been localized to basolateral membranes of renal proximal tubules and to sinusoidal membranes of hepatocytes [1]. There it is responsible for the first step in organic cation secretion [2]. Control of neurotransmitter concentration is very important in the periphery, where neurotransmitters exhibit a variety of physiological functions. Dopamine, for example, has been described as modulator of renal function. It increases glomerular filtration rate and promotes natriuresis and phosphaturia [14]. The concentrations of monoamine neurotransmitters in the blood are determined by: (i) the amount of release from the sites of their synthesis, (ii) their degradation and metabolism, and (iii) their excretion in kidney and liver. Microperfusion experiments in rat proximal tubules suggested that the organic cation transport system in the basolateral membrane also translocates some monoamine neurotransmitters since uptake of N^1-methylnicotinamide could be inhibited by dopamine, noradrenaline and serotonin [15]. The physiological importance of this presumed transport remained questionable, since the apparent K_i values were in the millimolar range (dopamine 4.4 mM, noradrenaline 6.2 mM, and serotonin 1.4 mM) which is 4 orders of magnitude lower than the physiological neurotransmitter concentrations in the blood [15]. In the present study we show that the K_m values of rOCT1-mediated neurotransmitter transport are between 20 and 100 μM. This discrepancy may be explained by technical limitations of the in vivo measurements which do not, for example, allow preincubation of substrates [3].

The substrate affinity of rOCT1 for monoamine neurotransmitters is 20–100-fold lower than that of the Na^+ and Cl^--dependent, cocaine-sensitive monoamine transporters in presynaptic membranes of the brain [11–13]. However, the present study suggests that rOCT1 acts as a neurotransmitter transporter with a high capacity already at concentrations which are physiologically relevant (< 1 μM). In liver and kidney such a system would have the capacity to excrete increased concentrations of neurotransmitters or chemically related drugs. This may become relevant in pathological situations, during medical treatment or during drug abuse. To understand the excretion of neurotransmitters into urine and bile, their transport over the luminal membrane of renal proximal tubules and the biliary membrane of hepatocytes must be investigated. Finally, because rOCT1 can translocate cations in both directions [2], it may also play a role for the cellular release of dopamine which is formed in epithelial cells of renal proximal tubules by decarboxylation of L-dopa [16].

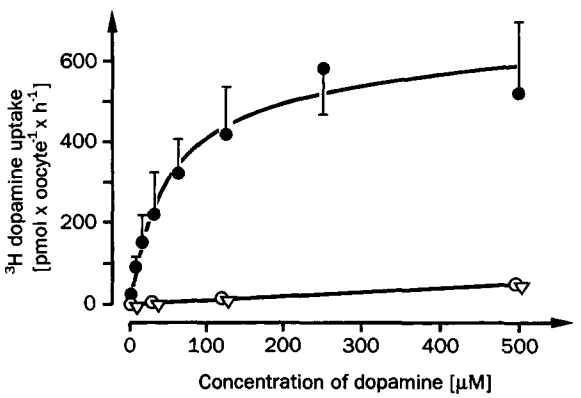

Fig. 3. Substrate dependence of rOCT1-mediated dopamine uptake in oocytes. After injection of rOCT1 cRNA (○,●) or water (▽) into *Xenopus* oocytes the uptake of different [^{3}H]dopamine concentrations was measured in the absence (●,▽) and presence of 36 μM cyanine 863 (○). The medians from 8–10 individual oocytes ± S.E.M. are presented.

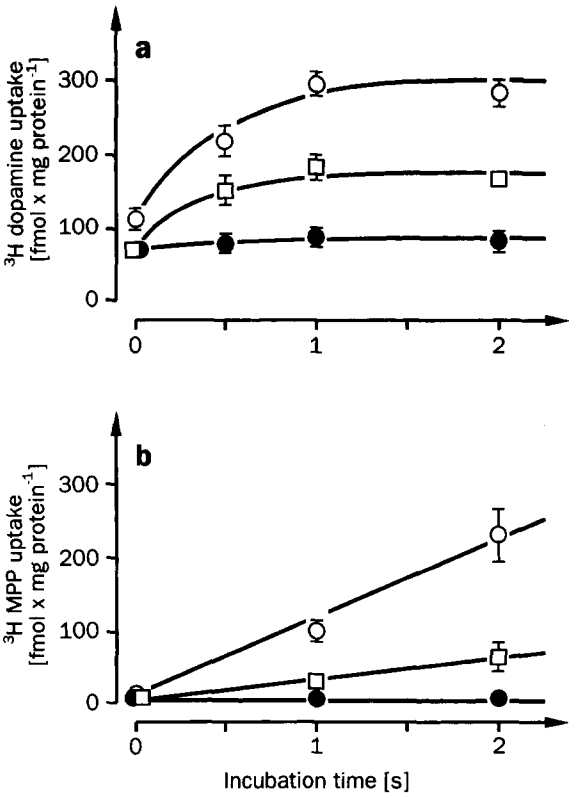

Fig. 4. Uptake of [^{3}H]dopamine and [^{3}H]MPP uptake into rOCT1 expressing HEK 293 cells. The time courses of [^{3}H]dopamine (a) and [^{3}H]MPP uptake (b) were measured in a suspension of HEK 293 cells which were constantly transfected with rOCT1. The uptake measurements were performed with 0.1 μM radiolabeled substrate in the presence (○,●) and absence of Na^+ (□). Some measurements were performed in the presence of 25 μM cyanine 863 (●). Moles of cell-associated substrates per mg of cell protein are presented. Mean values of three independent measurements ± S.D. are indicated.

Acknowledgements: A.E.B. is a Heisenberg Fellow. This work was supported by the Deutsche Forschungsgemeinschaft Grants Ko 872/4-1 (to H.K.) and Bu 704/3-1 (to A.E.B.). The authors are indebted to Dr. G.L. Busch for the discussion of the manuscript. We thank E. Sailer and B. Noll for the preparation and handling of oocytes.

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