British Journal of Pharmacology (1998) 125, 218–224
http://www.stockton-press.co.uk/bjp

Catecholamine transport by the organic cation transporter type 1 (OCT1)

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1 Liver and kidney extract adrenaline and noradrenaline from the circulation by a mechanism which does not seem to be one of the classical catecholamine transporters. The hypothesis that OCT1 is involved – the organic cation transporter type 1 which exists in rat kidney and liver – was tested.

2 Based on human embryonic kidney cells (293), we constructed a cell line which stably expresses OCT1r (293_{OCT1r} cells). Transfection with OCT1 resulted in a transport activity not only for prototypical known substrates of OCT1 such as ^{3}H-1-methyl-4-phenylpyridinium and ^{14}C-tetraethylammonium but also for the catecholamines ^{3}H-adrenaline, ^{3}H-noradrenaline (^{3}H-NA) and ^{3}H-dopamine (^{3}H-DA), the indoleamine ^{3}H-5-hydroxytryptamine (^{3}H-5HT) as well as the indirect sympathomimetic ^{14}C-tyramine.

3 For ^{3}H-DA, ^{3}H-5HT and ^{3}H-NA, at non-saturating concentrations, the rate constants for inwardly directed substrate flux (k_{in}) were 6.9 ± 0.8, 3.1 ± 0.2, and 1.2 ± 0.1 μl min^{-1} mg protein^{-1}. In wild type cells (293_{WT}) the corresponding k_{in}'s were considerably lower, being 0.94 ± 0.40, 0.47 ± 0.08 and 0.23 ± 0.05 μl min^{-1} mg protein^{-1} ($n = 12$). The indirectly determined half-saturating concentrations of DA, 5HT, and NA were 1.1 (95% c.i.: 0.8, 1.4), 0.65 (0.49, 0.86), and 2.8 (2.1, 3.7) mmol l^{-1} ($n = 3$).

4 Specific ^{3}H-DA uptake in 293_{OCT1r} cells was resistant to cocaine (1 μmol l^{-1}), ^{3}H-5HT uptake was resistant to citalopram (300 nmol l^{-1}) and ^{3}H-NA uptake was resistant to desipramine (100 nmoll^{-1}), corticosterone (1 μmol l^{-1}), and reserpine (10 nmoll^{-1}) which rules out the involvement of classical transporters for biogenic amines.

5 The findings demonstrate that OCT1 efficiently transports catecholamines and other biogenic amines and support the hypothesis that OCT1 is responsible for hepatic and renal inactivation of circulating catecholamines.

Keywords: Catecholamine uptake; organic cation transport; OCT1; norepinephrine; epinephrine

Introduction

The liver and the kidney play a pivotal role in the removal of circulating endogenous and exogenous organic cations. Organic cations include a variety of chemical structures which bear one or more positive charges at physiological pH, such as biogenic amines, alkaloids, and the majority of clinically employed drugs. In the past, hepatic and renal transport of organic cations have widely been investigated by functional methods (Meijer *et al*., 1990; Oude Elferink *et al*., 1995; Rennick, 1981; Weiner, 1985). With the organic cation transporter type 1 (OCT1r), the primary structure of a transport system for organic cations was elucidated which has originally been cloned from a rat kidney cDNA library but also exists in the liver (Gründemann *et al*., 1994). In the kidney, OCT1 presumably mediates the uptake of organic cations through the basolateral membrane into kidney tubule cells. In the liver, OCT1 is responsible for the uptake of small type I organic cations (Martel *et al*., 1996).

Various physiological functions of mesenteric organs are closely controlled by the sympathetic innervation (Gardemann *et al*., 1992; Sjövall *et al*., 1987). Because of the relative inaccessibility of the mesenteric organs to physiological investigation, the contribution to total body catecholamine spillover and turnover was realized only recently. With an elegant clinical approach, Aneman *et al*. (1996) demonstrated that in humans 42% of total body noradrenaline spillover into the circulation is caused by the hepatomesenteric organs. However, this major contribution to catecholamine turnover is obscured by efficient hepatic extraction of noradrenaline which amounts to about 90%. In other words, considerable amounts of catecholamines are inactivated by the liver before entering the systemic circulation. Surprisingly, only a small portion of hepatic catecholamine inactivation is due to the classical transport system for catecholamines such as the despiramine-sensitive neuronal noradrenaline transporter or the corticosterone-sensitive extraneuronal monoamine transporter (Aneman *et al*., 1995; Martel *et al*., 1994).

Renal handling of catecholamines is also not fully explained with the classical catecholamine transporters. Already in 1969 Quebbeman & Rennick (1969) and later Lappe *et al*. (1980) observed that the kidney excretes catecholamines by a transport mechanism with properties similar to the renal organic cation transport system. From the results of Szabo *et al*. (1992) and Friedgen *et al*. (1994), it is possible to estimate the renal clearance of circulating adrenaline and noradrenaline, being about 20% and 15%, respectively, of the total body clearance of these amines.

At physiological pH, catecholamines are predominantly protonated, i.e. belong to the group of organic cations (Mack & Bönisch, 1979), which raises the hypothesis of OCT1 being involved in hepatic and renal extraction of monoamine transmitters. Until now, however, unambiguous experimental evidence that OCT1 accepts monoamine transmitters as substrates is lacking. A preliminary account of the results was communicated at the 8th International Catecholamine Symposium 1996 (Gründemann *et al*., 1998) and to the German Society for Experimental and Clinical Pharmacology and Toxicology (Breidert *et al*., 1997).

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Methods

Construction of a cell line that stably expresses the organic cation transporter OCT1r

OCT1r cDNA was donated by Prof Koepsell (Würzburg). For the construction of pcDNA3OCT1r, the OCT1r cDNA has been released from pBluescript II OCT1r by digestion with *Not*I and *Xho*I and inserted into the polylinker site of pcDNA3 (Invitrogen, San Diego, CA, U.S.A.). Human embryonic kidney (HEK) 293 cells (ATCC CRL-1573) (Graham *et al*., 1977) were transfected by a cationic liposome technique with the Lipofectin reagent (Life Technologies, Eggenstein, Germany) according to the recommendations of the manufacturer. Selection was carried out with the aminoglycoside Geneticin (Boehringer Mannhein, Mannheim, Germany) resulting in stably transfected 293_{OCT1r} cells. Success of stable transfection was demonstrated by RT–PCR and functional testing (see Results). 293_{WT} refers to wild-type 293 cells.

RT–PCR

Total RNA was extracted from 293_{WT} and 293_{OCT1r} cells by the method of Chomczynski & Sacchi (1987). Remaining DNA was degraded by 1 U/10 μg RNA RQ1-DNase (Boehringer Ingelheim Bioproducts, Heidelberg, Germany). After phenol-chloroform extraction, ethanol precipitation, and first strand cDNA synthesis with Superscript II RT (Life Technologies, Eggenstein, Germany), PCR (30 cycles of 30 s 94°C, 60 s 55°C, and 60 s 72°C, followed by a final elongation cycle with 10 min at 72°C) was carried out essentially as described elsewhere (Gründemann *et al*., 1997) with the following primers: 5′-AATTCGATTTAGGTGACACTATAGAATAG-3′ and 5′-GCCCGGCACAGTGTAGTT-3′. Additional experiments with a different set of primers for OCT1r gave virtually identical results.

Transport assays

The cells were grown in surface culture on standard tissue culture plastic materials. After a preincubation period of 30 min at 37°C with buffer A (in mmol l^{-1}) NaCl 125, KCl 4.8, $CaCl_2$ 1.2, KH_2PO_4 1.2, $MgSO_4$ 1.2, HEPES NaOH 25, pH 7.4; D(+)-glucose 5.6, the cells were incubated with 100 nmol l^{-1} of ^{3}H- or ^{14}C-labeled substrates in buffer A. Where appropriate, transport inhibitors were present during both the preincubation and incubation periods. Incubation was stopped by rinsing the cells four times with 3 ml ice-cold buffer A. Subsequently, the cells were solubilized with 0.1% v/v Triton X-100 (dissolved in 5 mmol l^{-1} Tris HCl, pH 7.4), and radioactivity was measured by liquid scintillation counting. L(+)-ascorbic acid (1 mmol l^{-1}) was present in all experiments with tyramine, dopamine, noradrenaline, adrenaline, and 5-hydroxytryptamine. Intracellular metabolism *via* monoamineoxidase (MAO) and/or catechol-O-methyltransferase (COMT) was blocked by 10 μmol l^{-1} pargyline alone (in the case of 5-hydroxytryptamine) or by 10 μmol l^{-1} pargyline and 10 μg ml l^{-1} U-0521 (in the case of dopamine, noradrenaline, adrenaline).

Protein determination

Protein was determined by the method of Lowry (1951).

Calculations and statistics

Analysis of the time course of substrate accumulation was based on a one-compartment model as described earlier (Russ *et al*., 1992). For the calculation of IC_{50}'s, the data were fitted to the Hill-equation for multisite inhibition (Segel, 1975) by a non-linear computer-assisted quasi Newton method (Wilkinson, 1989). The IC_{50}'s are identical with K_i-values, since non-saturating substrate concentrations were used (Cheng & Prusoff, 1973). Geometric means are given with 95% confidence limits and arithmetic means are given with s.e.mean.

Drugs used

(−)-N-[methyl-^{3}H]-adrenaline (2 kBq pmol^{-1}), [^{3}H]-MPP$^+$ (N-[methyl-^{3}H]-4-phenyl-pyridinium acetate) (2.9 kBq pmol^{-1}), (−)-[7-^{3}H]-noradrenaline (381 Bq pmol^{-1}), [^{14}C]-tetraethylammonium chloride (124 Bq pmol^{-1}), and [1-^{14}C]-tyramine hydrochloride (1.6 Bq pmol^{-1}) (DuPont/NEN, Dreieich, Germany). [7,8-^{3}H]-dopamine (1.7 kBq pmol^{-1}), 5-hydroxy-[^{3}H]-tryptamine trifluoracetate (4.5 kBq pmol^{-1}) (Amersham Life Science, Braunschweig, Germany); cyanine 863 (1-ethyl-2-([1,4-dimethyl-2-phenyl-6-pyridmidinylidene] methyl) quinolinium chloride), dopamine hydrochloride, 5-hydroxytryptamine creatinine sulfate, corticosterone, MPP$^+$ (1-methyl-4-phenyl-pyridinium iodide), (−)-noradrenaline hydrochloride, and reserpine (Sigma, München, Germany); tetraethylammonium bromide (Aldrich, München, Germany); cocaine hydrochloride (Merck, Darmstadt); and tetramethylammonium chloride (ACROS, Neuss Germany); O-methylisoprenaline hydrochloride (Boehringer, Ingelheim, Germany); U-0251 (Upjohn, U.S.A.); desipramine hydrochloride (Ciba-Geigy, Basel, Switzerland); citalopram hydrobromide (Lundbeck, Kopenhagen, Denmark); all other chemicals were either purchased from Sigma (München; Germany) or Roth (Karlsruhe, Germany).

Results

Test for stable transfection of 293_{OCT1r} cells

Success of stable transfection was tested for by RT–PCR. Four weeks after transfection with pcDNA3OCT1r, total RNA was prepared from the Geneticin-resistant 293_{OCT1r} cells and, for control purposes, from wild-type 293_{WT} cells. On 293_{OCT1r} RNA, RT–PCR with OCT1r primers resulted in the amplification of the expected 295 bp PCR product (Figure 1). On 293_{WT} RNA, RT–PCR failed to produce an amplification product.

Expression of OCT1r was also confirmed by functional testing. 293_{OCT1r} cells were incubated with 100 nmol l^{-1} ^{3}H-MPP$^+$ for 1 min in the absence and presence of various concentrations of cyanine863, quinine, procainamide, O-methylisoprenaline, and tetramethylammonium (Figure 2, Table 1). The resulting pharmacological profile was virtually identical with the profile obtained with OCT1r cRNA-injected *Xenopus laevis* oocytes (Gründemann *et al*., 1994).

Uptake of monoamine transmitters in 293_{OCT1r} cells

293_{OCT1r} and for control purposes 293_{WT} cells were tested for uptake of various monoamines. The cells were incubated for 4 min at 37°C with 100 nmol l^{-1} of the labelled compounds. Only ^{14}C-tetraethylammonium was used at 2 μmol l^{-1}. Specific uptake was defined as that fraction of total uptake which was sensitive to 10 μmol l^{-1} cyanine863–the most potent inhibitor of OCT1r known so far. Expressed transport

activity was defined as the difference between specific uptake in 293_{OCT1r} and in 293_{WT} cells.

Stable expression of OCT1r in 293 cells induced specific uptake not only of the known prototypical organic cations such as ^{14}C-TEA and ^{3}H-MPP^{+} but also of ^{3}H-dopamine, ^{3}H-5-hydroxytryptamine, ^{3}H-noradrenaline, ^{3}H-adrenaline and ^{14}C-tyramine (Figure 3).

Upon incubation with 100 nmol l^{-1} ^{3}H-dopamine, ^{3}H-5-hydroxytryptamine, and ^{3}H-noradrenaline the intracellular accumulation of monoamines in 293_{OCT1r} cells increased almost linearly with time for at least 4 min. Monoamine uptake in 293_{OCT1r} cells markedly exceeded uptake in 293_{WT} cells as well as non-specific uptake which was determined in the presence of 10 μmol l^{-1} cyanine863 (Figure 4). The analysis of the time courses of ^{3}H-dopamine, ^{3}H-5-hydroxytryptamine, and ^{3}H-noradrenaline accumulation in 293_{OCT1r} cells revealed k_{in}-values of 6.9 ± 0.8, 3.1 ± 0.2, and 1.2 ± 0.1 μl min^{-1} mg protein^{-1} ($n=12$). The k_{out}-values were 0.05, 0.1, and 0.04 min^{-1}, respectively. In other words, an amount of 293_{OCT1r} cells which corresponds to 1 mg of cell protein clears per minute 6.9 μl of incubation medium from ^{3}H-dopamine. Concurrently, 5% of intracellular ^{3}H-dopamine left the 293_{OCT1r} cells. At equilibrium, the intracellular concentration of ^{3}H-dopamine, ^{3}H-5-hydroxytryptamine, and ^{3}H-noradrenaline amounted to 14.4 ± 1.8, 3.0 ± 0.1 and 3.3 ± 0.3 pmol mg^{-1} protein^{-1} ($n=12$). Based on the water space of 293 cells of 6.7 μl mg protein^{-1} (Martel *et al.*, 1996) the corresponding factors of intracellular accumulation at equilibrium were 21.5, 4.5 and 4.9. In wild type cells (293_{WT}) the corresponding k_{in}'s for ^{3}H-dopamine, ^{3}H-5-hydroxytryptamine, and ^{3}H-noradrenaline were 0.94 ± 0.40, 0.47 ± 0.08, and 0.23 ± 0.05 μl min^{-1} mg protein^{-1} ($n=12$).

Transport of ^{3}H-noradrenaline, ^{3}H-dopamine and ^{3}H-hydroxytryptamine into 293_{OCT1r} cells was sensitive to cyanine863, the IC_{50}'s for the inhibition of initial rates of uptake being 0.14 (0.10; 0.18), 0.25 (0.22, 0.29), and 0.27 (0.21, 0.35) μmol l^{-1} ($n=3$), respectively.

A brief pharmacological characterization was carried out to distinguish OCT1r-mediated monoamine uptake from known classical neuronal, extraneuronal and vesicular monoamine transport systems (Figure 5). 293_{OCT1r} and 293_{WT} cells were incubated for 2 min at 37°C with 100 nmol l^{-1} ^{3}H-dopamine in the absence and presence of 1 μmol l^{-1} cocaine. Cocaine inhibits both the sodium-dependent neuronal dopamine and noradrenaline transporter with K_i's markedly below 1 μmol l^{-1} (Giros *et al.*, 1992; Pacholczyk *et al.*, 1991). ^{3}H-5-hydroxytryptamine (100 nmol l^{-1}) uptake into 293_{OCT1r} cells was measured in the absence and presence of 0.3 μmol l^{-1} citalopram, a known inhibitor of the sodium-dependent neuronal 5-hydroxytryptamine transporter with a K_i in the low nanomolar range (Blakely *et al.*, 1991; Hyttel, 1982). ^{3}H-noradrenaline (100 nmol l^{-1}) uptake into 293_{OCT1r} cells was determined in the absence and presence of 0.1 μmol l^{-1} desipramine, 1 μmol l^{-1} corticosterone, and 0.01 μmol l^{-1}

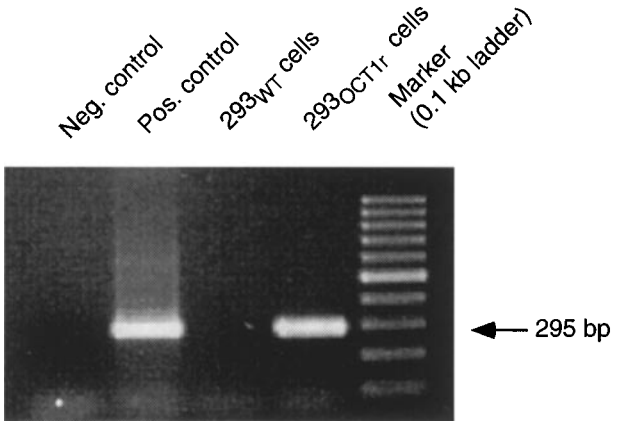

Figure 1 RT–PCR analysis of stably transfected 293 cells. Total RNA was prepared from 293_{OCT1r} and 293_{WT} cells and used for RT–PCR analysis with primers for the pcDNA3OCT1 sequence. For the negative and the positive controls the PCR was carried out without template (Neg. Control) or with 1 ng of purified pcDNA3OCT1 (Pos. control), respectively.

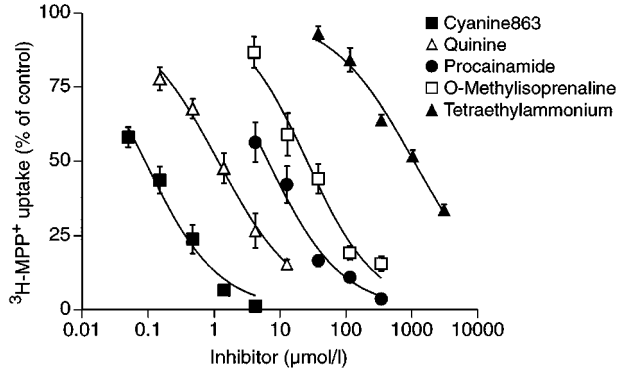

Figure 2 Inhibition of specific ^{3}H-MPP^{+} uptake in 293_{OCT1r} cells. Initial rates of transport were determined in the presence or absence of various inhibitors. Shown are means$\pm$s.e.mean ($n=3$) in the presence of inhibitor relative to the control.

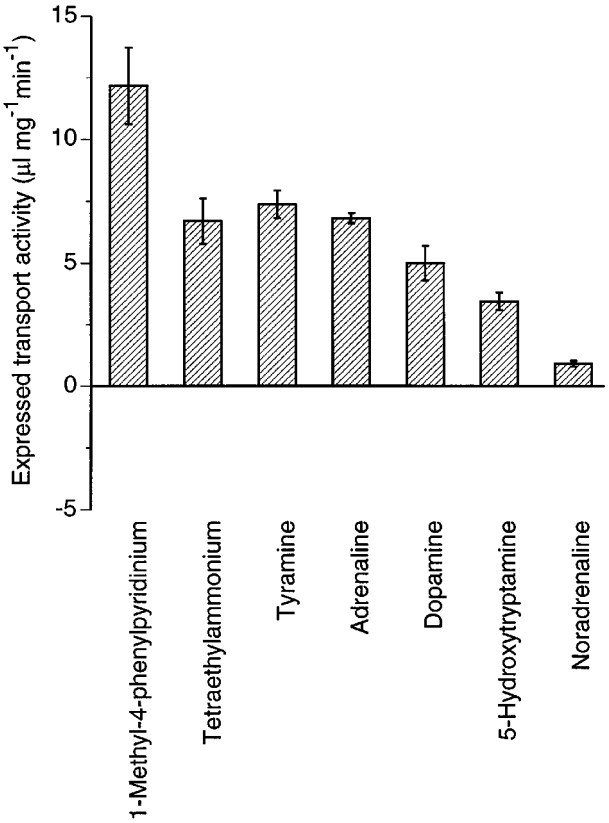

Figure 3 Expressed transport activities in 293_{OCT1r} cells. 293_{OCT1r} and 293_{WT} cells were incubated for 4 min with non-saturating concentrations of various labelled compounds. Expressed transport activity is defined as the difference in the specific uptake in 293_{OCT1r} cells and 293_{WT} wild-type cells. Transport activities are given as clearances. Shown are means$\pm$s.e.mean ($n=3$).

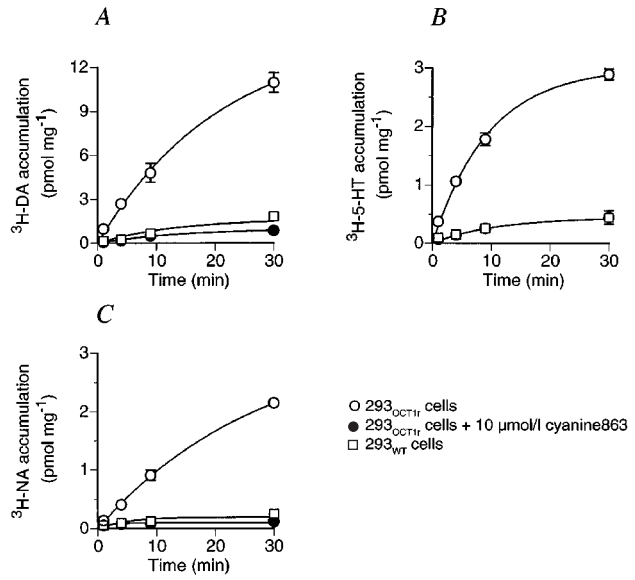

Figure 4 Time courses of ^{3}H-dopamine (A), ^{3}H-5-hydroxytryptamine (B) and ^{3}H-noradrenaline (C) accumulation. 293_{WT} cells (□) and 293_{OCT1r} (○,●) cells were incubated with 0.1 µmol l^{-1} tritiated monoamine in the absence (○, □) and presence of 10 µmol l^{-1} cyanine863 (●). Shown are the means ± s.e.mean ($n = 3$).

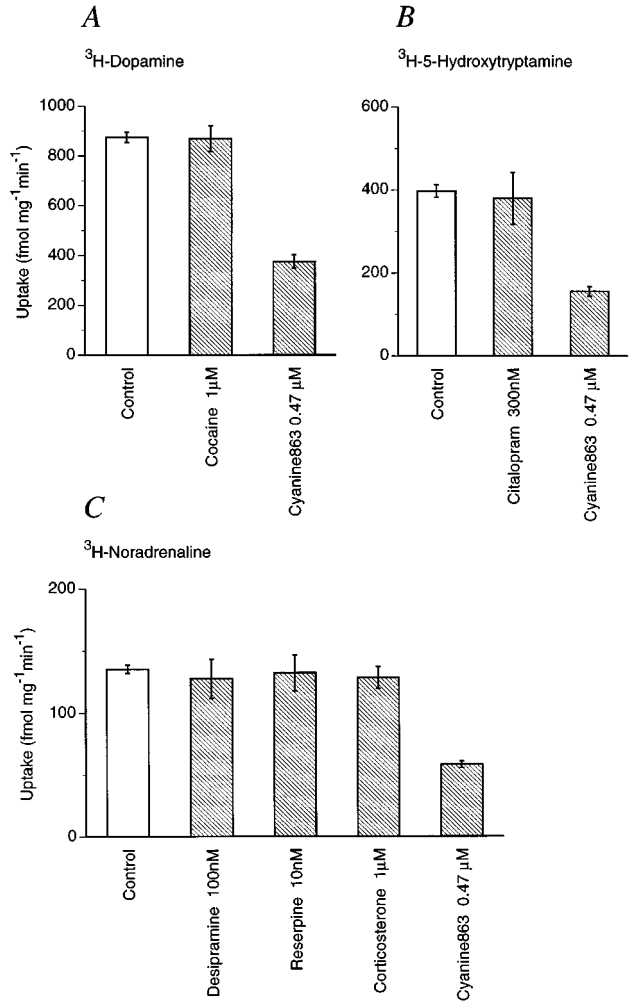

Figure 5 Effect of various inhibitors of classical monoamine transport systems on initial rates of biogenic amine transport in 293_{OCT1r} cells. 293_{OCT1r} cells were incubated for 2 min with 100 nmol l^{-1} of the labeled amine in the absence and presence of various inhibitors. Shown are the means ± s.e.mean ($n = 3$).

reserpine. Desipramine, corticosterone and reserpine inhibit the neuronal noradrenaline transporter, the extraneuronal monoamine transporter and the vesicular monoamine transporter with K_i's of 5, 140 and 1 nmol l^{-1}, respectively (Parti *et al*., 1987; Schömig & Bönisch, 1986; Schömig & Schönfeld, 1990). Monoamine transport in 293_{OCT1r} cells was resistant to all these known monoamine transport inhibitors but sensitive to the OCT1 inhibitor cyanine863 (Figure 5).

The half-saturating concentrations of 5-hydroxytryptamine, dopamine, and noradrenaline were estimated by competition experiments. 293_{OCT1r} cells were incubated for 2 min at 37°C with 100 nmol l^{-1} ^{3}H-MPP$^+$ in the absence and presence of various concentrations of 5-hydroxytryptamine, dopamine, and noradrenaline. The K_i values for the inhibition of initial rates of specific ^{3}H-MPP$^+$ transport directly reflect the half-saturating concentrations for 5-hydroxytryptamine, dopamine, and noradrenaline, being 0.65 (95% c.i. 0.49, 0.86), 1.1. (0.86, 1.4), and 2.8 (2.1, 3.7) mmol l^{-1} ($n = 3$), respectively (Figure 6).

Discussion

It is well known that the liver and the kidney remove considerable amounts of catecholamines from the circulation (Aneman *et al*., 1996; Silva *et al*., 1979). The nature of the underlying transporter or transporters has not yet been unambiguously clarified. Based on *in vivo* experiments, a contribution of a transport system for organic cations to renal catecholamine extraction has been proposed (Lappe *et al*., 1980; Quebbemann & Rennick, 1969). Interestingly, the basolateral organic cation transporter type 1 (OCT1) which was originally cloned from rat kidney (Gründemann *et al*., 1994) is functionally expressed also in the liver, where it is responsible for hepatic uptake of type I organic cations (Martel *et al*., 1996).

By stop-flow microperfusion experiments in the rat kidney, it was demonstrated that catecholamines display affinity for the basolateral tubular transport system for organic cations. Unlabelled catecholamines interact with the removal of the labeled organic cation N^1-methylnicotinamide from the peritubular fluid, the estimated IC_{50} values being in the low millimolar range (Ullrich *et al*., 1992). These results, however, do not prove that OCT1 transports catecholamines, since non-

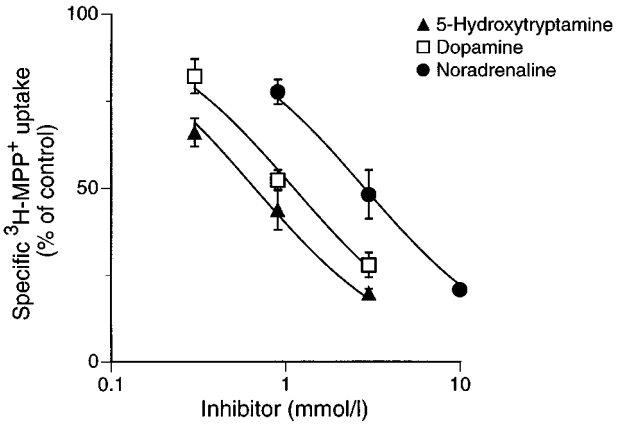

Figure 6 Inhibition of specific ^{3}H-MPP$^+$ transport in 293_{OCT1r} cells by dopamine, 5-hydroxytryptamine and noradrenaline. Specific transport was defined as the cyanine863-sensitive fraction of total transport. Shown are initial rates of specific ^{3}H-MPP$^+$ transport relative to controls in the absence of an inhibitor. Shown are means ± s.e.mean ($n = 3$).

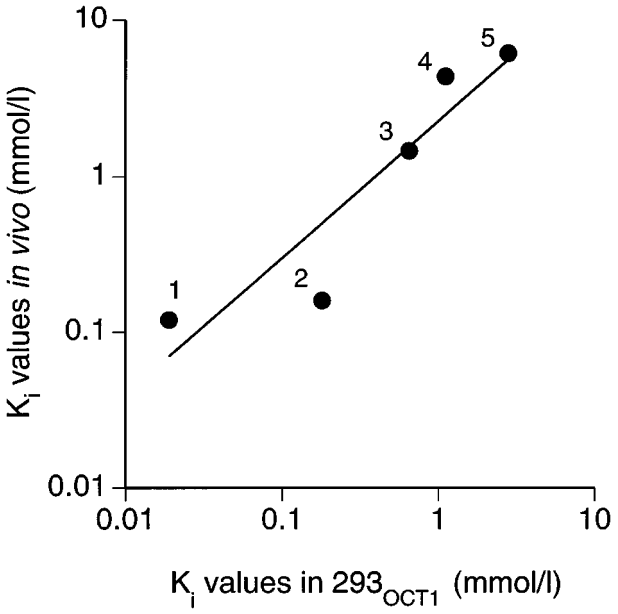

Figure 7 Correlation between the K_i's for the inhibition of ^{3}H-MPP^+ uptake in 293_{OCT1r} cells and N^1-methylnicotinamide uptake *in vivo* in the isolated rat kidney. The *in vivo* data were measured by microperfusion techniques and taken from David *et al.* (1995). Each symbol represents one of the tested compounds: MPP^+ (1), TEA (2), 5-HT (3), DA (4) and NA (5). Note that the K_m of MPP^+ is included instead of the respective K_i value for uptake in 293_{OCT1r} cells. Slope $= 0.88 \pm 0.21$, $r = 0.93$, $P < 0.05$.

transported inhibitors would also interfere with the transport of N^1-methylnicotinamide. Thus, it is necessary to address the question more directly whether OCT1 accepts catecholamines as substrates.

A recent study – based mainly on electrophysiology with cRNA-injected *Xenopus laevis* oocytes – seemingly supported the concept that OCT1 accepts catecholamines as substrates (Busch *et al.*, 1996). The kinetic parameters for catecholamine transport reported in this study, however, do not fit in with the results obtained *in vivo* in the kidney by microperfusion techniques (David *et al.*, 1995; Ullrich *et al.*, 1992). The reported half-saturating concentrations for biogenic amines were between 30 and 100 times lower than the concentrations measured *in vivo* for half-maximal inhibition of N^1-methylnicotinamide transport. Michaelis-Menten Kinetics, however, predict that the inhibition constant (K_i) of a transported substrate equals its half-saturating concentration (K_m). Meanwhile, the authors of this early study on cRNA-injected oocytes concede that their strategy based on membrane potential measurements suffers from a false assumption and, hence, is inadequate to measure transport (Nagel *et al.*, 1997).

It was the goal of the present study to establish a tissue culture system for the investigation of OCT1 and to use this model in order to answer directly, by radiotracer flux experiments, the question whether OCT1 accepts catecholamines as substrates. The report describes the successful construction of a cell line (293_{OCT1r} cells) which at a high level stably expresses the basolateral renal transport system for organic cations (OCT1r). Meanwhile, the mouse and human orthologues of OCT1 have been cloned (Schweifer & Barlow, 1996; Zhang *et al.*, 1997) as well as a variety of related transport proteins which are characterized by a similar membrane topology and common sequence motifs (Gorboulev *et al.*, 1997; Gründemann *et al.*, 1997; Lopez-Nieto *et al.*, 1997; Okuda *et al.*, 1996; Sekine *et al.*, 1997; Simonson *et al.*, 1994; Tamai *et al.*, 1997). OCT1 and the related transport systems constitute a new family of amphiphilic solute facilitators (ASF family) (Schömig *et al.*, 1998) which by itself is a member of the major facilitator superfamily as defined by Marger & Saier (1993).

Stable transfection of HEK 293 cells with OCT1r was achieved by a cationic liposome technique and success of transfection was tested for by RT – PCR and functional testing with the prototypical organic cation 1-methyl-4-phenylpyridinium (MPP^+). The pharmacological profile of organic cation (MPP^+) transport in 293_{OCT1r} cells is identical with the profile of TEA transport into cRNA-injected *Xenopus laevis* oocytes (Gründemann *et al.*, 1994), measured by tracer flux experiments.

Our results demonstrate that, beside the well known prototypical substrates, OCT1 accepts in decreasing rank-order of transport efficiency tyramine, adrenaline, dopamine, 5-hydroxytryptamine, and noradrenaline as substrates. Transport efficiency was measured as the amount of incubation medium which – at non-saturating substrate concentrations – was cleared by a given amount of 293_{OCT1r} cells. Transport of monoamine transmitters into 293_{OCT1r} cells was sensitive to cyanine863, a known inhibitor of OCT1 but resistant to various inhibitors of the classical transport systems for monoamine transmitters such as the neuronal transporters for noradrenaline, dopamine and 5-hydroxytryptamine, the extraneuronal monoamine transporter and the vesicular monoamine carrier.

Figure 7 demonstrated that the K_m-values of monoamine transport in 293_{OCT1r} cells fit in with the K_i's reported for the basolateral transport of organic cations in the rat kidney *in vivo*, measured by stop-flow microperfusion (Ullrich *et al.*, 1992). In both cases, the half-saturating concentrations were in the low millimolar range.

There is an earlier report about ^{3}H-dopamine uptake in OCT1r-transfected 293 cells (Busch *et al.*, 1996) which unfortunately lacks necessary controls, such as the uptake of tritiated dopamine in wild-type 293 cells. In addition, the extremely rapid approach of steady-state reported in this earlier study is reminiscent of a binding phenomenon rather than cellular transport. Under experimental conditions which were almost identical with those described in the account at hand - i.e. after incubation of the cells with 0.1 μmol l^{-1} tritiated dopamine, the maximal intracellular concentration in the cells prepared in the earlier study (Busch *et al.*, 1996) reaches only one third of the extracellular ^{3}H-dopamine concentration. This degree of accumulation is by a factor of about 60 lower than the equilibrium accumulation we report for 293_{OCT1r} cells and is even lower than the ^{3}H-dopamine accumulation we found in non-transfected 293_{WT} wild-type cells. It is speculated that this discrepancy is due to differences in the experimental protocol. We measure radiotracer uptake on cells in surface culture, i.e. under standard tissue culture conditions. Busch *et al.* (1996) in this earlier study tried to determine intracellular accumulation in suspension after the cells were detached from the substrate and mechanically dissociated. This maneuvre might disrupt the integrity of the plasma membranes.

In the peripheral nervous system, inactivation of released catecholamines is not only due to the classical monoamine transport systems – e.g. desipramine-sensitive neuronal and disprocyniuim24-sensitive extraneuronal transport. For the first time, the study at hand provides direct and consistent evidence that the catecholamines adrenaline, noradrenaline and dopamine, the indoleamine 5-hydroxytryptamine as well as the indirect sympathomimetic tyramine are transported

substrates of OCT1. Together with the *in vivo* data about catecholamine uptake and the pattern of OCT1 distribution, the present findings open the possibility that OCT1 plays an important role in hepatic and renal inactivation of circulating catecholamines.

We are grateful to Mrs Anke Ripperger and Mrs Barbara Wallenwein for skillful technical assistance. The study was supported by the Deutsche Forschungsgemeinschaft (Scho373/1-2, Un34/19-1/B2 and SFB601/A4).

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(Received March 19, 1998
Revised June 8, 1998
Accepted June 17, 1998)