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Relative contribution of OAT1 and OAT3 transport activities in isolated perfused rabbit renal proximal tubules

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Abstract

The expression of both OAT1 and OAT3 along the isolated rabbit renal proximal tubule (RPT) was determined using RT-PCR. They were found to be very strong in S2 segment and weak in S1 and S3 segments. We further examined the relative transport activity of these transporters in isolated perfused rabbit RPT using [^{3}H]*para*-aminohippurate ([^{3}H]PAH), and estrone sulfate ([^{3}H]ES) as specific substrates for rbOAT1 and rbOAT3, respectively. The transport activity of OAT1 was in the order S2 > S1 = S3 segments and that of OAT3 was in the order S1 = S2 >> S3 segments. The addition of α-ketoglutarate (100 μM) in the bathing medium increased both OAT1 and OAT3 transport activities in all segments of proximal tubule. The kinetics of [^{3}H]succinic acid transport, used to measure the activity of sodium dicarboxylate transporter 3 (NaDC3), were examined. The J_{max} for succinic acid was in the order S2 > S3 and unmeasurable in the S1 segment. Our data indicate that both OAT1 and OAT3 play quantitatively significant roles in the renal transport of organic anions along the proximal tubule but predominately in S2 segment. The relative contribution of both transporters depends on their relative expression levels and may possibly be affected by the activity of NaDC3 in RPT.

Keywords: Organic anion transporter 1; Organic anion transporter 3; Renal proximal tubule

1. Introduction

The kidney plays an important role in clearing the body of a large and diverse number of compounds. These include many potentially toxic endogenous and exogenous organic substances that exist as anions at physiological pH. Organic anion (OA) transporters (OATs) located in the basolateral membrane of the renal proximal tubule make a major contribution to actively eliminating these substances and their metabolites from the body. OAs are transported from the blood across the basolateral membrane into the tubule cell against their electrochemical gradient via a tertiary transport system, the terminal step in which involves exchange for α-ketoglutarate (αKG) moving out of the cells down its electrochemical gradient. This tertiary active transport process is the rate-limiting step in tubule secretion of these compounds [1,2]. The outwardly directed gradient of αKG is maintained by the combination of intracellular metabolic generation of αKG and the active uptake of αKG into the cells across both luminal and basolateral membranes via Na-dicarboxylate cotransporters (NaDC1 and NaDC3). The inwardly directed gradient for sodium is in turn established by Na/K-ATPase, the primary energy requiring step in the tertiary process [3–5].

At present, several renal organic anion transporters (OATs) have been found, including OAT1, OAT2, OAT3, OAT4, OAT-P1, OAT-K1 and OAT-K2 [6]. Among these, only OAT1 and OAT3 have been shown to play a major role in the basolateral uptake of OAs into renal proximal tubule cells via exchange for αKG [7,8]. They are highly expressed in the basolateral membrane of the proximal tubule of the kidney [9]. However, the expression and distribution of OAT1 and OAT3 are reported to be different in different regions of the proximal tubule. In a previous study on rat kidneys, OAT1 was reported to be located only in the S2 segment

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[10] but not in the S1 and S3 segments. A recent study in human kidneys found that the expression of OAT1 was not restricted to the S2 segment only but was found in other segments as well [9]. A subsequent study in rat kidneys also found that OAT1 was expressed in all segments of the renal proximal tubule but with its highest expression in the S2 segment [11]. Therefore, the proximal tubular distribution of OAT1 is still controversial and may differ among species. High expression of OAT3 in the kidney has also been reported. In human kidneys, OAT3 is highly expressed in the basolateral membrane of the proximal tubule and apparently has the highest level of expression of any member of the organic anion transporter family [9]. A study in rat renal proximal tubule cells also indicates that OAT3 is localized in the basolateral membrane in all proximal tubule segments [11]. Taken together, these data indicate that OAT1 and OAT3 are co-localized in the basolateral membrane of the renal proximal tubule, suggesting that they both contribute significantly to renal organic anion secretion. However, differences in the level of expression of each of these transporters along the proximal tubules could play an important role in determining which segments are normally most important in secretion of a given OA and which are most likely to be affected adversely by a given nephrotoxic xenobiotic OA.

At present, the relative contributions of OAT1 and OAT3 to OA secretion is not clear and no direct quantitative information on their functional distribution along the proximal tubule is available. However, we recently reported that the activity of OAT1 and OAT3 transporters in rabbit renal tubules can be distinguished [12] using transporter-specific substrates. Basolateral estrone sulfate (ES) transport is effectively restricted to OAT3, whereas *para*-aminohippurate (PAH) transport is effectively restricted to OAT1 in isolated nonperfused rabbit renal proximal tubules. In the present study, we used this information as a tool to characterize the relative activities and functions of OAT1 and OAT3 along rabbit renal proximal tubules.

OAT1 and OAT3 share a common energetic mechanism (OA/αKG exchange) [8,13,14]. However, the transport of organic anions into proximal tubule cells is affected not only by the level of expression of OATs, but also by the level of expression of NaDC3 in the basolateral membrane, which helps to maintain the outwardly directed gradient for αKG. At present, the functional distribution of NaDC3 along the renal proximal tubules is in question. We postulate that differences in the pattern of expression of OAT1, OAT3, and NaDC3 along the length of renal proximal tubules affect the observed transport activity of OAT1 and OAT3 in each segment of renal proximal tubule. Thus, our study aims to determine and compare the functional activities of OAT1, OAT3, and NaDC3 in each segment of the rabbit renal proximal tubules. We chose to use both isolated nonperfused rabbit renal proximal tubules as well as isolated perfused tubules, preparations that have proven to be powerful tools to study physiological function of tubules. In the present study, we confirmed that rbOAT3, like rbOAT1, operates as an OA/αKG exchanger in native tissue. Our results also indicate that OAT1, OAT3, and NaDC3 play their most quantitatively significant roles in the renal transport of OAs in the S2 segment of the proximal tubule, whereas slightly lower activities of both OAT1 and OAT3 are found in the S1 segment. OAT1 is more predominant than OAT3 in the S3 segment.

2. Methods

2.1. Materials

[^{3}H]PAH (4.54 Ci/mmol), [^{14}C]PAH (40.6 mCi/mmol), [^{3}H]estrone sulfate (ES) (43.5 Ci/mmol) and [^{3}H] succinic acid (40 Ci/mmol) were purchased from Perkin Elmer Life Science Products (Boston, MA, USA). RT-PCR reagents were purchased from Roche Molecular System (Alameda, CA, USA). All other chemicals were purchased from standard sources and were generally the highest purity available.

2.2. Preparation of isolated tubules

New Zealand white rabbits [1.5–2.0 kg, National Laboratory Animal Center (NLAC), Bangkok, Thailand] were killed by intravenous injection of pentobarbital sodium, and all protocols employing rabbits were conducted in accordance with principles and guidelines of the Laboratory Animal Ethical Committee of Mahidol University, Bangkok, Thailand. The kidneys were flushed via the renal artery with an ice-chilled HEPES-sucrose buffer containing 250 mM sucrose and 10 mM N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid (HEPES), adjusted to pH 7.4 with Tris (hydroxy)methylaminomethane base, bubbled with 100% O_2 before use. They were then gently removed and sliced transversely using a single-edge razor. A kidney slice was transferred to the lid of a plastic petri dish on ice, which contained the standard solution used for dissecting and bathing tubules (in mM: 110 NaCl, 25 $NaHCO_3$, 5 KCl, 2 NaH_2PO_4, 1 $MgSO_4$, 1.8 $CaCl_2$, 10 Na-acetate, 8.3 D-glucose, 5 L-alanine, 0.9 glycine, 1.5 lactate, 1 malate, and 1 sodium citrate). This standard solution was aerated continuously with 95%O_2 and 5% CO_2 to maintain the pH at 7.4. The osmolality of the solutions averaged 290 mOsmol/kg H_2O.

Segments of proximal tubules were dissected individually under a stereomicroscope without the aid of enzymatic agents in a chilled medium (on ice) according to the modified method of Burg et al. [15]. The S1, S2 or S3 segment of the proximal tubule can be differentiated from each other under a microscope. The preparation was continuously gassed with a 5% CO_2 and 95% O_2 mixture for the entire duration of dissection. All dissections were performed at 4 °C, but all experiments were performed at 37 °C. S1, S2 and S3 segments were used in the perfusion and nonperfusion experiments to study the functional characteristics of OAT1 and OAT3 along the renal proximal tubules.

2.3. Measurement of transport rate in single nonperfused isolated RPT

These nonperfused experiments were performed in a manner similar to that used previously [16,17]. Briefly, tubules were maintained oxygenated (95% O_2 and 5% CO_2), transferred to oil-covered wells in a temperature-controlled chamber containing bicarbonate-buffered solution at 4 °C to prevent evaporation until the start of each experiment, and photographed through a dissecting microscope equipped with a video camera and a digital image capture system (Snappy Play, Inc.). Five minutes prior to the experiment, the bathing medium was warmed to 37 °C. The tubules were then individually transferred to the oil-covered incubation medium at 37 °C containing labeled substrate and appropriate test agents. After a 15-s exposure to labeled substrate, uptake was stopped by transferring each tubule into 1 N NaOH for extraction. Accumulated labeled substrate was determined by liquid scintillation counting (Liquid Scintillation Counter Model 1214 Rackbeta). Control and experimental uptakes were determined alternately and sequentially in tubules from the same kidney. Transport rates were normalized to tubule surface area based upon tubule lengths and average diameters as determined from photomicrographs using Snappy taken of each tubule prior to the experiment.

2.4. Determination of transport kinetics

The kinetics of peritubular succinic acid uptake in single, nonperfused RPT S1, S2 and S3 segments was examined to evaluate the physiological characteristics of the transport of succinic acid, presumably by NaDC3. The kinetics of succinic acid uptakes were described by the following form of the Michaelis–Menten equation [18]:

$$J = \frac{J_{max}[T^*]}{K_t + [T^*] + [S]} + C[T^*]$$

where J is the rate of [^{3}H] succinic acid (labeled substrate) transport at concentration equal to [$*T$]; J_{max} is the maximum rate of NaDC3 mediated succinic acid transport,

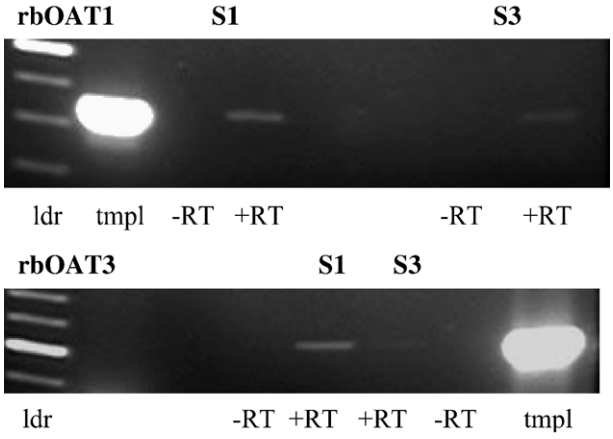

Fig. 1. Detection of rbOAT1 and rbOAT3 mRNA by RT-PCR in isolated single S1 and S3 segments of rabbit RPT. mRNA prepared from 18 to 20 tubule segments for S1 and S3 segments was used for 1st-strand cDNA synthesis. The reverse transcriptase (RT) reaction steps were performed in the presence of RT (+RT) or in the absence of RT (−RT). Subsequent PCR amplification was performed with rbOAT1- or rbOAT3-specific primers using the indicated amount of RT product. Negative controls consisted of product obtained from tubule reactions run without added reverse transcriptase (to test for the influence of genomic DNA). PCR products for the negative controls (−RT), and for rbOAT1 and rbOAT3 (RT), were loaded on gels and visualized with ethidium bromide. The lanes also show the base pair ladder (ldr) and the amplified products obtained when the cDNA-containing plasmid for each transporter was used as a template (tmpl).

respectively; K_t is the PAH, ES or succinate concentration that results in half-maximal transport (Michaelis constant); [*S*] is the concentration of unlabeled succinic acid in the transport reaction; *C* is a first-order constant reflecting the combined influence of diffusive flux, non-specific binding and/or incomplete rinsing of the tubules that resulted in a component of total uptake of labeled substrate that was not saturated over the range of substrate concentrations tested.

2.5. Perfusion of isolated tubules

The in vitro perfusion technique of isolated proximal tubule segment used in the present studies is the same as that first described by Burg and coworkers [15] and modified by Dantzler [19]. Briefly, each dissected tubule chosen for perfusion was transferred into a special temperature-controlled lucite bathing chamber on the stereomicroscope stage connected to a circulating water bath (Julabo F10). The bathing medium was a bicarbonate-buffered solution containing 3 g/100 ml dextran (MW 40,000±3000) to approximate plasma protein concentration. The buffered medium was continuously gassed with 95% O_2 and 5% CO_2 to maintain its pH at 7.4.

The lengths of the tubule segments used in the perfusion experiments for S1, S2 and S3 were about 0.3–0.5, 1.0–1.5 and 0.3–0.6 mm, respectively. Both ends of the tubule were held between the two sets of glass micropipettes. The left-hand set was used to perfuse the suspended tubule, whereas the right-hand set was used to collect the perfusion fluid. The perfusion rate was set at 10–15 nl/min and maintained by the regulating perfusion pressure system. Samples from the collecting pipettes were collected every 5 min intervals with calibrated volumetric glass capillary tube. All of the experiments were carried out at 37 °C.

2.6. Transepithelial PAH and ES flux

To measure transepithelial PAH or ES flux (J_{PAH} or J_{ES}), [^{14}C]PAH or [^{3}H]ES was added to the bathing medium at a concentration of 25 μM or 0.05 μM, respectively. These concentrations do not saturate the transport of PAH [20,21] or ES. No PAH or ES was present in the initial perfusate or the tubular lumen. Net transepithelial secretion of PAH (J_{PAH}) or ES (J_{ES}) was determined from the amount of [^{14}C]PAH or [^{3}H]ES appearing in the perfusate on the collection side, and was expressed per unit time (duration between each collection), using the following relationship: J_{PAH} or $J_{ES}=(V_C C_C)/(X_b L)$. In this equation, V_C is the fluid collection rate (in nl/min) measured directly by collecting the luminal fluid at the end of each period. C_C is the concentration of [^{14}C]PAH or [^{3}H]ES in the collected luminal fluid (in dpm/nl), X_b is the specific activity of [^{14}C]PAH or [^{3}H]ES in the bathing medium, and *L* is the length of the perfused tubule (in mm), measured by ocular micrometry. J_{PAH} or J_{ES} was expressed as fmol/min/mm of tubule length.

For all transepithelial flux experiments, three collections were made with the appropriate control solution containing [^{14}C]PAH or [^{3}H]ES in the bathing medium prior to any treatment. At the end of these control collections, the bathing medium was changed to one containing the appropriate substrate, and three more collections were then made. At the end of those collections, the bathing medium was changed back to the control solution for final control collections. A 10-min equilibration period was allowed between each change of bathing medium. Each collection period was 5 min in duration.

2.7. RT-PCR protocol using grouped renal segments

The RT-PCR technique of isolated proximal tubule segment used in the present studies is the same as that described in our previous study [12]. Briefly, the kidney was perfused via the renal artery with cold sterile HEPES-sucrose buffer, pH 7.4. The kidney slice was gently removed and dissected in HEPES-sucrose buffer on ice using sterile conditions. Twelve to fifteen S2 segments or fifteen to twenty segments for S1 and S3 were used for each reverse transcriptase (RT) reaction. A 2 μl aliquot of tubules suspended in HEPES-sucrose buffer was transferred into a microfuge tube containing 4 μl of 2% Triton mix (composed of 44.5 μl sterile water, 2 μl RNase inhibitor, 2.5 μl 0.1 M DTT and 1.2 μl sterile Triton X-100). The digested tubules were kept at room temperature for 5 min and then quick-frozen in liquid nitrogen.

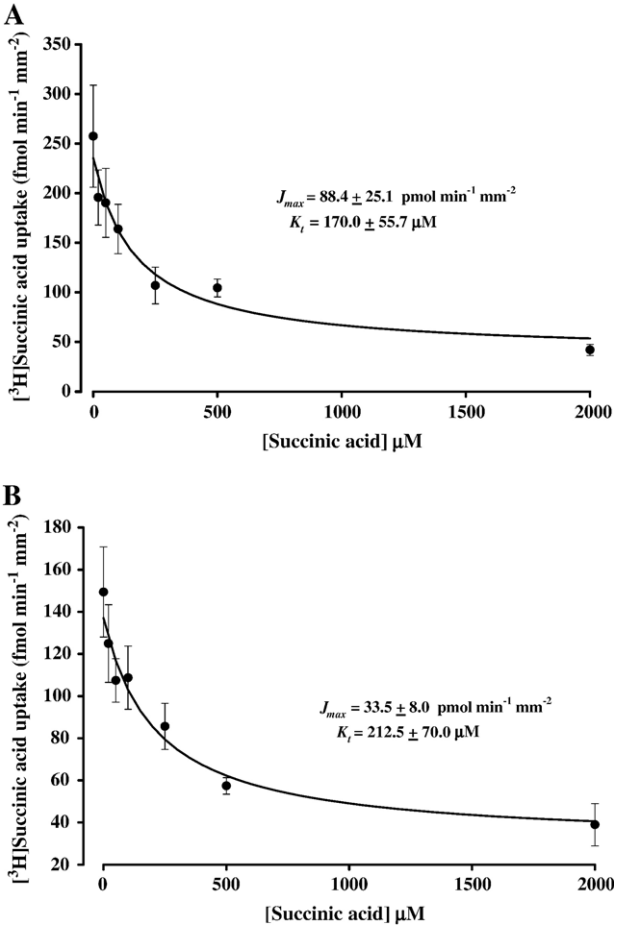

Fig. 2. A. Kinetics of [^{3}H]succinic acid uptake across basolateral membrane in nonperfused S2 segments of rabbit RPT ($n=5$). B. Kinetics of [^{3}H]succinic acid uptake across basolateral membrane in nonperfused S3 segments of rabbit RPT ($n=3$).

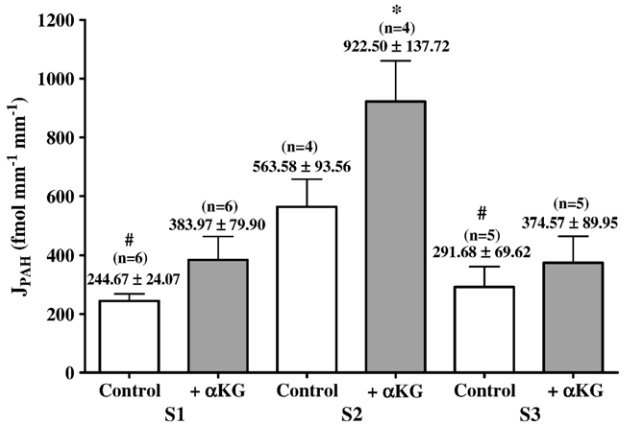

Fig. 3. Effect of 100 μM αKG on J_{PAH} in S1, S2 and S3 segments of rabbit RPT perfused and bathed with the bicarbonate buffer medium. Values for J_{PAH} are shown as means±SE; n=number of tubules. *Significantly different from its control, $P<0.05$. #Significantly different from control of S2, $P<0.05$.

2.7.1. Reverse transcription (RT)

Oligo (dT) 12–18 primers, random primer (3 μg/ml), 10 mM dNTP mix and sterile water were added to the digested tubules. After heating at 65 °C for 5 min, (5X) 1st strand buffer, 0.1 M DTT and RNase inhibitor were added to each tube (RT and negative RT samples), and incubated at 42 °C for 2 min. Superscript II (SSII) (for RT) or sterile water (for negative RT) was mixed in each tube and incubated at 42 °C for 50 min. The reaction was inactivated by heating at 70 °C for 15 min. RNase H was added to each tube and the samples were incubated at 37 °C for 20 min to remove RNA complementary to the cDNA. The resulting cDNA was used as a template for amplification via polymerase chain reaction (PCR).

2.7.2. Polymerase chain reaction (PCR)

cDNA from RT product or negative RT product was added to sterile water, (10×) PCR buffer, 50 mM $MgCl_2$, 10 mM dNTP mix and *Taq* DNA Polymerase. The forward and reverse primers for OAT1 and OAT3 were added to each tube for the RT or negative RT products used for amplification of cDNA. Plasmid cDNA for OAT1 and OAT3 were also run for PCR to use as positive controls for OAT1 and OAT3 amplification. Each tube was run on the thermal cycler by using a standard PCR program (94 °C for 3 min followed by 34 cycles of 94 °C for 30 s, 55 °C for 30 s, 72 °C for 3 min; then final elongation for 10 min at 72 °C and held at 4 °C). Amplified products were visualized with ethidium bromide on agarose gels.

2.8. Statistical analysis

Data are summarized as means±SE. Tubules from different rabbits were used for each experiment. Multiple means were compared using Fisher's protected least significant difference post hoc test and were assumed to be significantly different when $P<0.05$.

In the perfusion experiments, the mean value for the three control periods was compared with the value for each experimental period in the same tubule (a single tubule from a single animal was used for each experiment) using one-way analysis of variance, and the significance of the difference between these values was determined with Fisher's protected least significant difference post hoc test. Values were assumed to be significantly different when $P<0.05$.

3. Results

3.1. Expression of OAT1 and OAT3 mRNA in S1, S2 and S3 segments of rabbit RPT

Our previously published paper had already demonstrated the message for OAT1 and OAT3 in the S2 segment [12]. In this study, the expression of OAT1 and OAT3 mRNA in S1 and S3 segments of rabbit renal proximal tubule was examined. RT-PCR was used to amplify RNA sequences specific for the rabbit orthologs of OAT1 and OAT3 mRNA obtained from groups of isolated S1, and S3 segments of rabbit renal proximal tubules. The specific forward and reverse primers for both OAT1 and OAT3 were used in the PCR step. The representative gels of amplified OAT1 and OAT3 products showed that the message for both orthologs was routinely found in isolated S1 and S3 segments (Fig. 1). Amplified fragments were excised from a representative gel and sequenced to confirm the identity of the products. The PCR product bands in Fig. 1 and in our previous study [12] indicate that mRNA for OAT1 and OAT3 is present in all S1, S2, and S3 segments of rabbit renal proximal tubules. Therefore, in the present study, we further investigated the functional activities of these transporters in each segment of the proximal tubule.

3.2. Kinetics of succinic acid uptake across the basolateral membrane of single, nonperfused S2 and S3 segments of rabbit renal proximal tubules

In addition to the number of OATs, the Na^+/dicarboxylate cotransporter 3 (NaDC3) which is expressed in the basolateral membrane of renal proximal tubules also affects the transport activity of the OATs. Indeed, it supplies renal proximal tubule cells with dicarboxylates from the blood side that maintains the driving force for an exchange of organic anions via OATs. Thus, we examined the transport activity of NaDC3 in S1, S2 and S3 segments of rabbit RPT. For this purpose, the kinetics of succinic acid uptake (a specific substrate for NaDC3) across the basolateral membrane in nonperfused rabbit RPT was performed.

The K_t for succinic acid uptake in S2 segments was 170.0±55.7 μM and the J_{max} was 88.4±25.1 pmol min^{-1} mm^{-2} (n=5) (Fig. 2A). In S3 segments, the K_t for succinic acid uptake was 212.5±70.0 μM with the J_{max} of 33.5±8.0 pmol min^{-1} mm^{-2} (Fig. 2B). These data indicate that the affinity of this transporter (NaDC3) for succinic acid in S2 and S3 segments was similar. The uptake of succinic acid in S1 segments was very low although we

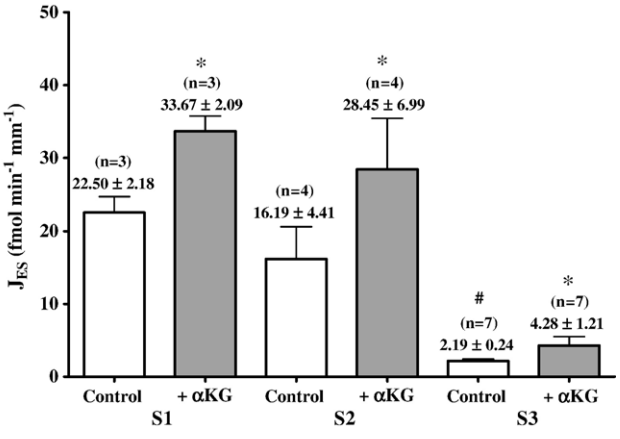

Fig. 4. Effect of 100 μM αKG on J_{ES} in S1, S2 and S3 segments of rabbit RPT perfused and bathed with the bicarbonate buffer medium. Values for J_{ES} are shown as means±SE; n=number of tubules. *Significantly different from its control, $P<0.05$. #Significantly different from control of S2, $P<0.05$.

increased the concentration of [^{3}H] succinic acid in the bathing medium by 2 fold. Therefore, reliable counts for this segment could not be obtained to determine kinetic parameters. The results suggest that the amount of NaDC3 is lowest in S1 segment of rabbit RPT.

3.3. Effects of α-ketoglutarate (αKG) on transepithelial transport of PAH (J_{PAH}) and ES (J_{ES}) in isolated perfused S1, S2 and S3 segments of rabbit renal proximal tubules

Our previous [12] and present results from RT-PCR revealed that both OAT1 and OAT3 are co-expressed in all segments of rabbit renal proximal tubules (Fig. 1). These experiments were, therefore, designed to examine and compare the transepithelial secretion of PAH (J_{PAH}), presumably via OAT1, and ES (J_{ES}), presumably via OAT3, in S1, S2 and S3 segments of rabbit renal proximal tubules. The basal J_{PAH} was highest in S2 segment (563.58±93.56 fmol min^{-1} mm^{-1}) (n=4) when compared to that of S1 (244.67±24.07 fmol min^{-1} mm^{-1}) (n=6) and S3 (291.68 ±69.62 fmol min^{-1} mm^{-1}) (n=5) segments (Fig. 3). The basal transepithelial transport rate of ES (J_{ES}) was relatively high and similar in S1 (22.50±2.18 fmol min^{-1} mm^{-1})(n=3) and S2 (16.19±4.41 fmol min^{-1} mm^{-1}) (n=4) segments whereas it was significantly lower in S3 segments (n=7) (Fig. 4). These data also confirmed that OAT1 and OAT3 were co-expressed and functioned in all segments of rabbit renal proximal tubules. However, the transport activity of OAT1 was much higher than that of OAT3 and both of them have different activities along the segments of proximal tubule. The transport of PAH was highest in S2 but significantly lower in S1 and S3 segments. Major transport of ES was found to be about an equal extent in both S1 and S2 segments whereas very low transport was found in the S3 segment. The profiles of PAH and ES secretion along the length of the rabbit renal proximal tubule probably reflect the functional distribution of the OAT1 and OAT3 transporters, respectively.

As α-ketoglutarate (αKG) is a dicarboxylate that can be exchanged for other organic anions by both OAT1 and OAT3, we examined the effects of this dicarboxylate on transepithelial secretion of PAH and ES along the length of isolated, perfused rabbit renal proximal tubules. In previous studies with perfused rabbit renal tubule, the addition of αKG into the bathing medium at concentration below 200 μM significantly stimulated organic anion transport [22]. These findings support the idea that exogenous αKG added to the bathing medium is actively transported into the cell across the basolateral membrane via the Na-dicarboxylate cotransporter and subsequently exchange for peritubular OAs. The concentration of exogenous αKG used in this study was 100 μM, the concentration used in previous studies on mammalian proximal tubules to obtain maximum stimulation of organic anion transport [22].

Stimulation of transepithelial secretion of both PAH and ES from the bath to lumen appeared to occur when exogenous αKG was added (100 μM) to the bathing medium in all segments of proximal tubules (Figs. 3 and 4). However, the stimulation of J_{PAH} was statistically significant only in the S2 segment (Fig. 3) whereas stimulation of J_{ES} was statistically significant in all three segments.

4. Discussion

The contribution of OAT1 and OAT3 transport functions along the proximal tubule is far from clear. In rats, distribution of OAT1 along the tubule is largely limited to the S2 segment [10,11], whereas OAT3 shows immunoreactivity in all segments (S1> S2=S3) [11]. However, in human renal tissue, OAT1 expression is not restricted to the S2 segment. Instead, it appears to occur in all segments of the proximal tubule [9]. Taken together, the data indicate that mRNA for OAT1 and OAT3 is expressed along the proximal tubule in human and rat kidneys but their contributions to the transport activity are not clear [9,23]. Indeed, the functional activity of the OAT1 and OAT3 in the basolateral uptake of OAs is extremely important in the kidney's ability to excrete specific xenobiotic agents under normal or pathological conditions. Their presence and function could also play a role in determining which segments are affected most by a given nephrotoxic xenobiotic agent. Although, as noted above, co-expression of OAT1 and OAT3 mRNA apparently occurs in mammalian renal tubules, there is no direct quantitative information on the functional contribution/distribution of OAT1 and OAT3 in each segment of the proximal tubule. Thus, more thorough investigations of OAT1 and OAT3 mediated transport are clearly required to determine functional distribution.

In the present study, we investigated the tubular distribution and relative functional significance of OAT1 and OAT3 along the length of the rabbit renal proximal tubule. We used RT-PCR and transport measurements in isolated rabbit proximal tubule segments to determine the distribution and physiological function of OAT1 and OAT3 within their native epithelium. The activity of rabbit OAT1 (rbOAT1) and rabbit OAT3 (rbOAT3) can be distinguished using selective substrates estrone sulfate (ES) and *para*-aminohippurate (PAH): whereas basolateral ES transport is effectively restricted to OAT3, PAH transport is effectively restricted to OAT1 [12]. Since both OAT1 and OAT3 are claimed to operate as OA/αKG exchangers [7], the effect of exogenous αKG on their transport activities in various segments was also examined.

Our RT-PCR data on rabbit RPT show the coexistence of both OAT1 and OAT3 in all segments (Fig. 1 and previous study [12]). These findings are in close agreement with the studies of Kojima et al. [11] and Motohashi et al. [9]. Using the perfusion technique, we found, as others have [24,25], that transepithelial transport of PAH was highest in S2 segments and significantly lower in S1 and S3 segments (Fig. 3). This observation was also in agreement with the immunolocalization study of Kojima et al. [11], which showed that in rats the expression levels of OAT1 in S1 and S3 segments were much lower than the expression level in the S2 segment.

The RT-PCR data from this study also showed the expression of OAT3 mRNA in all segments of rabbit RPT (Fig. 1). We further examined the transport activity of OAT3 in various segments of the proximal tubule using the perfusion technique. The net secretion of ES was high in S1 and S2 segments and low in the S3 segment (Fig. 4). This finding is in agreement with a previous study demonstrating high expression of OAT3 in S1 and low expression in S3 segments of the human kidney [9,26].

The renal basolateral uptake of OA's via organic anion transporters is a tertiary active transport process, the final step of which involves exchange for α-ketoglutarate (αKG) [27,28]. The outwardly directed gradient for αKG is maintained in turn by metabolism (~40%) and by transport into the cell across the basolateral and luminal membranes by the activity of Na^+-dicarboxylate (NaDC3) cotransporters (~60%) [5,29]. Recently, OAT3 has been reported to operate via a similar mechanism as OAT1. The manipulation of intracellular dicarboxylate concentrations resulted in changes in basolateral (peritubular) PAH transport [30]. The inhibition of basolateral Na^+-αKG cotransporter has been shown to eliminate the stimulation of OA uptake by the stimulatory influence of exogenous αKG [31]. Thus, we anticipated that enhancing transport activity of NaDC3 would affect the activity of both OAT1 and OAT3. To test this hypothesis, the effect of exogenously added αKG on transepithelial transport of PAH was determined. Addition of exogenous αKG to the bathing medium was found to enhance the transepithelial transport of PAH via OAT1 in all segments of the proximal tubule, although the stimulation was only statistically significant in the S2 segment (Fig. 4). These findings are consistent with previous studies, which showed the trans-stimulation of fluorescein (FL) uptake by αKG in isolated nonperfused S2 segments [5] and the stimulation of the net secretion of FL in isolated perfused S2 segments of rabbit RPT in the presence of αKG in the bathing medium [22]. The stimulation of PAH uptake has also been demonstrated in the S3 segment of the rabbit RPT after preloading with exogenous αKG [32]. However, no report of this effect in the S1 segment is available. Together with the net transepithelial secretion of PAH mentioned above, the stimulation by αKG indicates that the activities of both OAT1 and NaDC3 are high in S2 segment.

The results from perfused tubules also indicated a high transepithelial secretion of ES in S1 and S2 segments and very low secretion in the S3 segment (Fig. 4). We further determined the characteristics of OAT3 transport in the isolated perfused tubules. It has been reported from previous studies in heterologous expression systems and in rat renal cortical slices that OAT3, similar to OAT1, operates in exchange with a dicarboxylate [7]. An increase in ES uptake was shown after preloading with αKG or glutarate in rOAT3-expressing oocytes and in rat renal cortical slices. Recently, a study using hOAT3-expressing oocytes confirmed that OAT3 has the ability to mediate organic anion/dicarboxylate countertransport [13]. The present study showed that transepithelial secretion of ES was significantly increased in all segments of rabbit renal proximal tubules when αKG was added to the bathing medium (Fig. 4). These findings demonstrate that OAT3, indeed, operates by OA/αKG exchange in intact RPT. They are in close agreement with the recent data which showed that preloading with glutarate led to a doubling of the rate of ES uptake in both CHO cells expressing rbOAT3 (Zhang, et al., unpublished data) and in rabbit proximal tubules (Groves et al., unpublished data). Taken together, our results suggest that although the transport activities of OAT1 and OAT3 are different in each segment, the mechanistic characteristics of these transporters are similar along the length of proximal tubule.

The transport of a given substrate in any tubule segment depends on substrate-transporter affinity and the transporter turnover rate, as well as the number of transporters in the particular tubule segment. Also, as the basolateral transport of αKG via NaDC3 plays a major role in maintaining an outward gradient of αKG in exchange for organic anions [5,29] the difference in the amount of NaDC3 expression in each segment may also affect the transport activity of OAT1 and OAT3 among S1, S2 and S3 segments. Therefore, the difference in the transport activity of OAT1 or OAT3 in each segment might be due to the differences in the number of those transporters as well as the amount of NaDC3 expression. The kinetics of succinic acid transport in this study suggest that the number of NaDC3 transporters is higher in S2 (J_{max} 88.4 pmol min^{-1} mm^{-2}) than in S3 segments (J_{max} 33.5 pmol min^{-1} mm^{-2}). This apparent higher number of NaDC3 transporters in the S2 segment may have played some role in the apparent high level of transport of both OAT1 and OAT3 in the S2 segment. The data on succinic acid transport also suggest that NaDC3 activity is very low in the S1 segment. This might have played some role in the observed low transport by OAT1 in the S1 segment. However, the functional activity of OAT3 (as judged by ES transport) appeared to be similar in both the S1 and S2 segments. In general, cells of the S1 segment have high rates of solute transport and abundant mitochondria as compared to the other two segments, and the active metabolism in this segment could provide sufficient αKG inside the cells to maintain the outwardly directed gradient for αKG exchange with OAs. Therefore, we propose that high NaDC3 activity in the S1 segment is not necessary to maintain the outwardly directed αKG gradient for OA uptake.

In conclusion, this study indicates that OAT1 and OAT3 are co-expressed and play quantitatively significant roles in transport of organic anions along the length of intact rabbit RPT but predominantly in S2 segment. The different activities of OAT1 and OAT3 in each segment could be extremely important in the kidney's ability to excrete specific xenobiotic agents under pathological conditions.

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