Human organic anion transporter OAT1 is not responsible for glutathione transport but mediates transport of glutamate derivatives

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Submitted 23 July 2012; accepted in final form 12 December 2012

Hagos Y, Burchhardt G, Burchhardt BC. Human organic anion transporter OAT1 is not responsible for glutathione transport but mediates transport of glutamate derivatives. Am J Physiol Renal Physiol 304: F403–F409, 2013. First published December 19, 2012; doi:10.1152/ajprenal.00412.2012.—Due to their clearance function, the kidneys are exposed to high concentrations of oxidants and potentially toxic substances. To maintain cellular integrity, renal cells have to be protected by sufficient concentrations of the antioxidant glutathione (GSH). We tested whether GSH or its precursors are taken up by human organic anion transporters 1 (OAT1) and 3 (OAT3) stably expressed in HEK293 cells. GSH did not inhibit uptake of $p$-aminohippurate (PAH) or of estrone sulfate (ES) in OAT3-transfected HEK293 cells. In OAT1-transfected cells, GSH reduced the uptake of PAH marginally. Among the GSH constituent amino acids, glutamate, cysteine, and glycine, only glutamate inhibited OAT1, but labeled glutamate was not taken up by a probenecid-inhibitable transport system. Thus OAT1 binds glutamate but is unable to translocate it. The GSH precursor dipeptide, cysteylinyl glycine (cysgly), and the glutamate derivative N-acetyl glutamate (NAG), inhibited uptake of PAH when present in the medium and trans-stimulated uptake of PAH from the intracellular side, indicating that they are hitherto unrecognized transported substrates of OAT1. N-acetyl aspartate weakly interacted with OAT1, but aspartate did not. NAG inhibited also OAT3, albeit with much lower affinity compared with OAT1, and glutamate did not interact with OAT3 at all. Taken together, human OAT3 and OAT1 cannot be involved in renal GSH extraction from the blood. However, OAT1 could support intracellular GSH synthesis by taking up cysteinyl glycine.

IN ITS REDUCED FORM, GLUTATHIONE [l-$\gamma$-glutamyl-l-cysteinylglycine (GSH)] is the most abundant nonprotein thiol in mammalian cells. GSH not only acts as a major antioxidant maintaining a tight control of the redox status but also as a mediator of many other important physiological functions. These include cell cycle regulation, proliferation and apoptosis, metabolism of xenobiotics including GSH conjugation to facilitate detoxification and excretion, and thiol disulfide exchange reactions. GSH serves also as an important reservoir of cysteine (20). As a result, disturbances in GSH homeostasis are implicated in the etiology and/or progression of a number of human disorders, including cancer, cystic fibrosis, and cardiovascular, inflammatory, immune, metabolic, and neurodegenerative diseases such as Parkinson’s and Alzheimer’s disease (2, 3, 9). A final proof for an essential role of GSH was provided by mouse models of GSH deficiency. Depending on the gene disrupted, the knockout mice present all phenotypes from early embryonic death to reduced drug excretion (7). Patients exhibiting inborn errors in the metabolism of glutathione showed hemolytic anemia, metabolic acidosis, 5-oxoprolinuria, central nervous system alterations, and recurrent bacterial infections (26).

The tripeptide GSH is comprised of glutamate, cysteine, and glycine (Fig. 1). Synthesis and catabolism of GSH and its adducts occur by a regulated series of enzymatic steps referred to as the $\gamma$-glutamyl cycle (20). The first and rate-limiting step in GSH synthesis is the formation of the dipeptide $\gamma$-glutamyl cysteine (gluclys) mediated by the $\gamma$-glutamyl cysteine synthetase. Gluclys reacts with glycine catalyzed by the GSH synthetase to yield GSH. Glutathione is freely distributed in the cytosol, although it can be compartmentalized in organelles including mitochondria, peroxisomes, nuclear matrix, and endoplasmic reticulum (10, 20). Within the cell, glutathione exists mainly ($>98\%$) as a monomer in the thiol-reduced form (GSH), and as thioester, mercaptide, or other thioester forms (glutathione S-conjugates; Ref. 29).

Due to their clearance function and high rates of aerobic metabolism, the kidneys are exposed to high concentrations of oxidants and reactive electrophiles. Kidney cells, especially those of the proximal tubules, are equipped with an array of GSH-dependent enzymes involved in detoxification and activation of xenobiotics (19). Therefore, proximal tubule cells need transport systems providing sufficient precursors of GSH or even extract GSH from the blood. Indeed, during a single pass through rat kidneys, $>80\%$ of the plasma GSH is extracted, exceeding by far the amount that could be accounted for by glomerular filtration (24). Transport of GSH and GSH conjugates across the basolateral membrane of proximal tubule cells was shown in studies in perfused rat kidneys, in isolated tubules from rabbit kidney, and in studies using vesicles from the basolateral membrane of the rat kidney and renal cells isolated from the proximal as well as from the distal tubule (13–15, 23–25). Collectively, these studies provided evidence for a sodium-dependent uptake of reduced GSH across the basolateral membrane into proximal but not into distal tubular cells. Because uptake of GSH into proximal tubular cells was inhibited by $p$-aminohippurate (PAH) and probenecid (15), Lash et al. (16, 18) suggested a role of organic anion transporters in GSH uptake.

The basolateral membrane of proximal tubule cells is equipped with organic anion transporters, i.e., Oats in rats and mice and OATs in humans, the most prominent ones being Oat1/OAT1 and Oat3/OAT3 (1, 5, 28). Substrates for these Oats/OATs are chemically diverse, including antineoplastics, antibiotics, antihypertensives, nonsteroidal anti-inflammatory drugs, biogenic amine metabolites, steroid hormones and their metabolites, diuretics, and several toxins. Oat1/OAT1 and Oat3/OAT3 operate as anion exchangers, i.e., they couple the uptake of an organic anion into the cell to the release of another organic anion from the cell (1, 5, 28). The finding that cysteine $S$- and methylmercury conjugates

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did not interact with L-glutamate and only weakly with NAG. NAG and cysgly being most probably transported by OAT1. OAT3
glycine (cysgly) turned out to be strong inhibitors of OAT1 with
MATERIALS AND METHODS
OAT3. the L-glutamate, glutathione appeared to be not transported by human OAT1 and (OAT3) stably expressed in HEK293 cells. As opposed to rat amino acids, and selected cysteinyl and aspartyl peptides (Fig. 1)
in (21) observed no interaction of GSH with murine Oat3 expressed endogenous Oat3 in a rat kidney cell line. However, Ohtsuki et al.
tions were obtained by equimolar substitution of NaCl by tetraethyl-
acetate. Structural formulas are taken either from
Fig. 1. Chemicals structures of compounds tested on human organic anion transporters 1 (OAT1) and 3 (OAT3). Structural formulas are taken either from
the homepages of the suppliers or from Wikipedia and have in part been modified by the authors to show the free acid.
interacted with Oat1 in a kidney cell line (30) suggested that GSH and related compounds may also be substrates of this transporter. Lash et al. (17) presented evidence for the interaction of GSH with glutathione in its reduced form (GSH), glycine, cysteine, glutamate, cysteinyl glycine (cysgly), glycyl glycine (glygly), \( \gamma \)-glutamyl cysteine (glucys), ophthalmic acid, aspartate, N-acetyl aspartate (NAA), N-acetyl glutamate (NAG), and N-acetyl aspartyl glutamate (NAAG). Dithiothreitol (DTT) was used to prevent oxidation of GSH and hydrogen peroxide (H\(_2\)O\(_2\)) to decrease the intracellular GSH concentration, respectively. Chemicals were obtained from Sigma (Deisenhofen, Germany), Bachem (Bubendorf, Switzerland), or Appli-
chym (Darmstadt, Germany) and were of analytical grade. With the exception of GSH, which was always prepared freshly, compounds were stored in stock solutions either in MRI or in MRI supplemented with 1% DMSO (ophthalmic acid).

Cell culture and tracer uptake experiments. HEK293 cells were obtained, transfected, and cultured as described by Kaufhold et al. (11). HEK293-OAT1-, -OAT3-, and vector-transfected cells were plated into 24-well plastic dishes (Sarstedt, Nümbrecht, Germany) at a density of 2 \( \times \) 10\(^5\) cells/well. The protein amount was determined by removal of the radioactive medium and immediate 3 \( \times \) ml washes performed. Under all experimental conditions, uptake was terminated by 10.22 \pm 0.33.5 on June 21, 2017 http://ajprenal.physiology.org/ Downloaded from

Statistics. Uptake experiments were performed in at least in triplicate from consecutive cell culture passages. The data are expressed as means \( \pm \) SE. Statistical significance in unpaired Student’s \( t \)-tests was set at \( P < 0.01 \). IC\(_{50}\) values for the inhibition of PAH uptake by GSH and NAG were calculated using the SigmaPlot 10 software (Systat, Point Richmond, CA). Determination of \( K_i \) values for the uptake of PAH by glutamate and cysgly were obtained by Dixon plot analysis.

RESULTS
To exclude a degradation of GSH that may lead to an under-
estimation of GSH concentration, the \( \gamma \)-GT activity of HEK293 cells was evaluated compared with that measured in BBMVs from pig kidney. Whereas BBMVs showed high rates of \( \gamma \)-GT activity, the \( \gamma \)-GT activity evaluated in HEK293 cells was negligible (2,259 arbitrary units/mg protein in BBMVs vs. 83 arbitrary units/mg protein in HEK293 cells). Therefore, all further experiments were performed in the absence of a \( \gamma \)-GT inhibitor.
In previous experiments on OAT3- and OAT1-expressing HEK293 cells (13), the uptake of ES and PAH by OAT3 and OAT1, respectively, was linear within the first 5 min. This time period was chosen also for the experiments described here. Uptake of ES and PAH under control conditions, i.e., in the absence of an inhibitor, was set to 100% to allow for comparison of results obtained with different cell preparations.

Uptake of \(^{3}H\)PAH was slightly, but significantly, higher in OAT3-transfected cells than in vector-transfected HEK293 cells, revealing a small, OAT3-dependent PAH transport (Fig. 2A, control). In the presence of 0.1 mM unlabeled PAH, OAT3-dependent \(^{3}H\)PAH uptake vanished (Fig. 2A, 0.1 mM PAH). In the presence of 5 mM GSH, uptake of PAH in vector-transfected cells was insensitive towards unlabeled PAH and to GSH. Simultaneous application of GSH (5 mM) and DTT (2 mM) to avoid formation of oxidized glutathione (GSSG) and trials to reduce the intracellular GSH concentration by preincubation with 2 mM H\(_2\)O\(_2\) dissolved in MRi for 20 min did not lead to a larger inhibition of OAT1-dependent PAH uptake by 5 mM GSH (data not shown). Whereas small doses (<0.5 mM) of GSH slightly stimulated uptake of PAH, higher GSH concentrations decreased uptake in OAT1-transfected HEK293 cells to 73.4 ± 6.5% of its value in the absence of GSH (Fig. 3B, grey circles). GSH had no effect on vector-transfected HEK293 cells (Fig. 3B, black circles). From these experiments, an IC\(_{50}\) value for the GSH-sensitive part of PAH uptake of 1.78 ± 0.80 mM was calculated (4 independent cell preparations).

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ES uptake into vector-transfected cells. Hence, ES uptake by OAT3 was not inhibited by GSH.

A comparable experiment was performed with OAT1-expressing cells. Under control conditions, \(^{3}H\)PAH uptake into OAT1-expressing cells clearly exceeded that into vector-transfected cells (Fig. 3A). OAT1-dependent uptake was largely inhibited in the presence of 0.5 mM unlabeled PAH. Uptake of PAH by OAT1 was partially, but significantly, inhibited in the presence of 5 mM GSH. Uptake of PAH in vector-transfected cells was insensitive towards unlabeled PAH and to GSH. Simultaneous application of GSH (5 mM) and DTT (2 mM) to avoid formation of oxidized glutathione (GSSG) and trials to reduce the intracellular GSH concentration by preincubation with 2 mM H\(_2\)O\(_2\) dissolved in MRi for 20 min did not lead to a larger inhibition of OAT1-dependent PAH uptake by 5 mM GSH (data not shown). Whereas small doses (<0.5 mM) of GSH slightly stimulated uptake of PAH, higher GSH concentrations decreased uptake in OAT1-transfected HEK293 cells to 73.4 ± 6.5% of its value in the absence of GSH (Fig. 3B, grey circles). GSH had no effect on vector-transfected HEK293 cells (Fig. 3B, black circles). From these experiments, an IC\(_{50}\) value for the GSH-sensitive part of PAH uptake of 1.78 ± 0.80 mM was calculated (4 independent cell preparations).
GSH is composed of glutamate, cysteine, and glycine and can also be synthesized from γ-glutamyl peptides such as glutamyl cysteine (gluyl) and cysteinyl glycine (cysgly). At a concentration of 1 mM, glutamate and cysgly inhibited OAT1-dependent PAH uptake strongly, and glucys weakly. Glycine, cysteine, and glycyl glycine (glygly) were without significant inhibitory effect on PAH uptake (Fig. 4A, grey columns). The effect of these compounds on vector-transfected HEK293 cells was negligible. The PAH uptake pooled for all experimental conditions was 7.6 ± 0.3% of that measured for OAT1-transfected HEK cells under control conditions (Fig. 4A, black column). Increasing concentrations of either cysgly or glutamate inhibited uptake of PAH to ~10% of its initial value (data not shown). A Dixon analysis revealed a Ki of 2.93 ± 0.53 mM for cysgly (Fig. 4B) and of 1.09 ± 0.17 mM for glutamate (Fig. 4C), respectively.

Because glutamate inhibited PAH uptake in OAT1-transfected HEK293 cells, glutamate derivatives and related compounds (all at a concentration of 1 mM) were also tested on the uptake of PAH (Fig. 5A). Uptake of PAH was reduced by 91.5 ± 0.4% by NAG, by 47.0 ± 2.4% by glutamate, by 23.7 ± 2.1% by NAA, by 22.8 ± 1.9% by NAAG, and by 5.7 ± 6.4% by aspartate, respectively. Uptake in vector-transfected cells was small and not affected by these compounds (6.9 ± 0.4% of PAH uptake in OAT1-transfected HEK293 cells in the absence of any compound). The concentration dependence of the strongest inhibitor, NAG, is shown in Fig. 5B. The inhibition was not complete, leaving residual 23.2 ± 1.0% of PAH uptake at the highest tested NAG concentration. With the use of the NAG-sensitive part of PAH uptake, an IC50 of 0.026 ± 0.003 mM was calculated (Fig. 5B, 3 cell preparations).

NAG, cysgly, and glutamate preloaded into the cells increased PAH uptake significantly in OAT1-transfected (grey columns) but not in vector-transfected HEK293 cells (Fig. 6, black column). This trans-stimulation was highest for NAG and suggests that this compound efficiently exchanges against extracellular PAH at OAT1.

To further evaluate the effect of glutamate on OAT1, uptake of radiolabeled PAH and glutamate was examined simultaneously on the same cell preparations (Fig. 7). Because HEK293 cells may contain endogenous amino acid transporters facilitating sodium-dependent uptake of glutamate, experiments were performed under nominal sodium-free conditions. In the presence and absence of sodium, PAH uptake by OAT1 was equal and inhibited to the same extent by 1 mM probenecid (Fig. 7A). In contrast, uptake of radiolabeled glutamate was not inhibited by 1 mM probenecid and was significantly reduced when sodium was removed (Fig. 7B). Similar effects were observed in vector-transfected HEK293 cells (Fig. 7B, black columns), indicating uptake of glutamate by transporter(s) other than OAT1.

Finally, glutamate, NAG, and NAAG were tested also with OAT3. NAG, but not glutamate and NAAG, inhibited uptake of ES in OAT3-transfected HEK293 cells (Fig. 8A). The NAG-evoked inhibition was partial at >5 mM, and the IC50 obtained from these measurements was 1.99 ± 0.45 mM (Fig. 8B).

DISCUSSION

Renal proximal tubule cells possess high activities of enzymes to synthesize GSH and, possibly, also membrane transport proteins for uptake of GSH or its precursors into the proximal tubule cell. There exist conflicting results with regard to the transport of GSH across the basolateral membrane of proximal tubule cells. Rankin and colleagues (24, 25) observed that, during a single pass through rat kidneys, >80% of the plasma GSH is extracted. Such an extraction exceeds by fourfold the filtered amount of GSH and strongly suggests uptake of GSH from the peritubular blood across the basolateral membrane into proximal tubule cells. Investigation of the transport pathways across the basolateral membrane of proximal tubule cells in the Wistar rat kidney in situ showed no impact of GSH on uptake of PAH (27). In contrast, Lash and Jones (14) described several components contributing to GSH uptake into renal basolateral membrane vesicles prepared from Sprague-Dawley rats: an electrogenic sodium-dependent uptake and a sodium- and membrane potential-independent process. The sodium-dependent uptake of GSH was sensitive to probenecid, an inhibitor of organic anion exchange, and to glutamate, glycine, and cysteine, as well as to ophthalmic acid. Since NRK-52E cells derived from rat kidneys showed an endogenous Oat3 expression and transported PAH and GSH,
Lash (17) concluded that GSH was an Oat3 substrate. In contrast, the uptake of radiolabeled benzylpenicillin by murine Oat3 expressed in *Xenopus laevis* oocytes was not inhibited by GSH (21). In our experiments, transport of radiolabeled PAH or ES by human OAT3 was not affected by GSH. Thus at least the human OAT3 is most probably not involved in the uptake of GSH from the blood. Whether species differences account for the divergent results needs to be clarified.

As a corollary, we found that human OAT3 transports ES with a much higher efficiency than PAH. Whereas ES uptake into OAT3-expressing cells was 14.3 ± 5.3 times above background (vector-transfected cells), PAH uptake exceeded background by a factor of only 1.3 ± 0.3, confirming that ES is the preferred substrate of OAT3 (6). In humans, renal PAH secretion should therefore be mainly accomplished by OAT1. In renal slices obtained from Oat1 knockout mice, PAH accumulation was diminished although not completely abolished (8). Therefore, also in mice, renal PAH secretion is mainly due to the operation of Oat1 with small contributions by Oat3.

Testing human OAT1, we did find an inhibition of PAH uptake by GSH. However, this inhibition was marginal: at saturating GSH concentrations, a maximal inhibition of 18.4% was reached. The reason for the partial inhibition is not clear. A complete removal of PAH by competition for a common binding and transport site can be ruled out. However, GSH may bind to OAT1 and slow down its PAH transport rate by forming an inhibitor-substrate-OAT1 complex. Given the low affinity of OAT1 for GSH (IC50 1.8 mM) and a GSH plasma concentration (< 20 μM; Refs. 2, 3), human OAT1 is most probably not responsible for GSH uptake from blood.

Glutamate, a constituent amino acid of GSH, inhibited OAT1-mediated PAH uptake with a *K*ₐ of ~1 mM. This finding is surprising, because glutamate carries a positively charged amino group in addition to the two negatively carboxyl groups. It appears that glutamate acts as a nontransported inhibitor, because uptake of labeled glutamate into OAT1-expressing cells was not inhibited by probenecid and was not detectable in the absence of sodium. In contrast, PAH uptake into the same cells was abolished by probenecid and was sodium insensitive. The observed small trans-stimulation of OAT1-mediated PAH uptake by intracellularly preloaded glutamate is most probably due to a desamination, leading to the appearance of α-ketoglutarate, an excellent counter anion for PAH uptake.

The other two constituent amino acids of GSH, glycine and cysteine, had no impact on PAH uptake by OAT1. The dipeptide precursor cysteinyl glycine (cysgly) inhibited PAH uptake whereas glycyglycine (glygly) and ophthalmic acid, a GSH analog where cysteine is substituted by aminobutyrate (Fig. 1), did not inhibit uptake of PAH by OAT1. Only glutamyl cysteine (glucys) exhibited a small effect on OAT1-mediated PAH uptake. The dipeptide glucys not only inhibited PAH uptake but, after preloading into cells, trans-stimulated it. Therefore, glucys may be a so far unrecognized transported substrate of human OAT1.

In contrast to the five carbon amino acid glutamate, the four carbon amino acid aspartate did not inhibit OAT1. This result...
is in accordance with our previous finding (11) that C4 dicarboxylates (e.g., succinate) have a much lower affinity towards OAT1 than do C5 dicarboxylates (e.g., glutarate). A substitution at the amino group in aspartate, yielding NAA without a positive charge, led to a slightly inhibitory compound. NAG showed a strong inhibitory potency with an IC₅₀ of 26 μM. After preloading into cells, NAG markedly trans-stimulated PAH uptake by OAT1. Thus there is little doubt that NAG is a transported substrate. When the results of glutamate and NAG are compared, it becomes evident that binding to OAT1 requires two negative charges at the end of an aliphatic five-carbon backbone. Translocation, however, is only possible in the absence of an additional positive charge that is present in glutamate, but masked in NAG.

NAG also inhibited OAT3-mediated ES uptake, although with a much higher IC₅₀ compared with OAT1. Glutamate did not inhibit OAT3 as opposed to OAT1. Thus OAT1 and OAT3 exhibit differences in their interaction with glutamate and glutamate derivatives.

From the experiments presented here, two main conclusions can be drawn. First, GSH is not a substrate of human OAT3 and OAT1. If GSH is taken up across the basolateral membrane, an unknown transport system is involved in this process. Second, cysgly and NAG have been identified as hitherto unknown OAT1 substrates. The uptake of cysgly by OAT1 may support intracellular GSH synthesis.

ACKNOWLEDGMENTS

We thank S. Petzke for excellent technical assistance.

GRANTS

The study was supported by a grant from the German Research Council (BU998/5-1; to B. C. Burckhardt).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: Y.H. and B.C.B. performed experiments; Y.H., G.B., and B.C.B. interpreted results of experiments; Y.H., G.B., and B.C.B. edited and revised manuscript; Y.H., G.B., and B.C.B. approved final version of manuscript; G.B. and B.C.B. analyzed data; B.C.B. conception and design of research; B.C.B. prepared figures; B.C.B. drafted manuscript.

Fig. 7. Determination of glutamate uptake by OAT1- (grey columns) and vector-transfected HEK293 cells (black columns). A: 5-min uptake of 0.5 μM [3H]PAH was assayed in the absence (control) and presence of probenecid (1 mM) and sodium. Sodium with was replaced by equimolar concentrations of tetraethylammonium (sodium-free). B: 5-min uptake of 0.5 μM [3H]glutamate was investigated in the presence of sodium (control), in the presence of sodium and probenecid (1 mM), in the absence of sodium and probenecid (control), and in the absence of sodium plus 1 mM probenecid (sodium-free, probenecid). Data presented in A and B were performed in parallel on the same cell preparations for direct comparison of the data. All experiments were done in triplicate. *Statistical significance was set to 0.01.

Fig. 8. Interaction of glutamate, NAG, and NAAG with OAT3. A: 5-min uptake of 10 nM [3H]ES in the absence (control) and presence of 1 mM of either unlabeled ES, glutamate, NAG, and NAAG. Determinations were obtained in three consecutive cell preparations. Uptake of ES was significantly inhibited by ES and NAG at the *P < 0.01 level. B: NAG concentration-dependent inhibition of OAT3-mediated ES uptake. At a constant [3H]ES concentration (10 nM), the concentration of NAG was varied between 0.75 and 5.25 mM. ES uptake was inhibited with an IC₅₀ of 1.99 ± 0.45 mM (means ± SE of 3 cell preparations).
REFERENCES