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Transport Properties of Nonsteroidal Anti-Inflammatory Drugs by Organic Anion Transporter 1 Expressed in *Xenopus laevis* Oocytes

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ABSTRACT

Organic anion transporter 1 (OAT1) is the *para*-aminohippurate (PAH) transporter in the basolateral membrane of the proximal tubule. The present study investigated whether or not nonsteroidal anti-inflammatory drugs (NSAIDs) are transported by OAT1. All of the NSAIDs tested inhibited [^{14}C]PAH uptake via OAT1 expressed in *Xenopus laevis* oocytes. Ibuprofen, indomethacin, salicylurate, and naproxen showed the strongest potency to inhibit [^{14}C]PAH uptake ($K_i \sim 2$–10 μM); acetylsalicylate, salicylate, and phenacetin exhibited moderate potency ($K_i \sim 300$–400 μM), and acetaminophen (paracetamol) exhibited the weakest inhibitory potency ($K_i \sim 2$ mM). Radiolabeled acetylsalicylate, salicylate, and indomethacin were taken up by OAT1 and the uptake rate of these three NSAIDs was enhanced by the outwardly directed dicarboxylate gradient. The efflux of the preloaded [^{14}C]PAH from the oocytes via OAT1 was *trans*-stimulated by PAH and glutarate added to the media. The addition of salicylate, acetylsalicylate, or salicylurate into the media also *trans*-stimulated the efflux of PAH, whereas indomethacin did not. The present study indicates that OAT1 is responsible for the renal uptake and secretion of NSAIDs.

The kidneys, along with the liver, are the main organs for drug excretion and metabolism. Three processes are involved in the renal handling of drugs: glomerular filtration, tubular reabsorption, and tubular secretion. The tubular secretion of xenobiotics, especially organic anions, has been studied extensively (Sperber, 1959; Weiner and Mudge, 1964; Ullrich and Rumrich, 1988; Pritchard and Miller, 1991), and *para*-aminohippurate (PAH) has been used as a prototypical substrate for the renal organic anion transport pathway(s). Previous studies on renal organic anion transport by micropuncture experiments in vivo and uptake experiments with renal slices, tubule suspensions, isolated tubules, and culture cells have suggested that PAH transporter is responsible for the secretion of a variety of anionic drugs.

In 1997, cDNA encoding a PAH transporter was isolated from rat kidneys and was designated organic anion transporter 1 (OAT1; Sekine et al., 1997). Independently, rat renal organic anion transporter 1 (ROAT1) was isolated, also as a PAH transporter (Sweet et al., 1997); the amino acid sequence of ROAT1 is identical with that of OAT1. As had been predicted, OAT1 is a multispecific organic anion transporter at the basolateral membrane of the middle portion of the proximal tubule S2 (Sekine et al., 1997; Tojo et al., 1999).

Previous studies indicated active accumulation of nonsteroidal anti-inflammatory drugs (NSAIDs) in the renal proximal tubular cells. Accumulation of indomethacin and salicylate has been demonstrated in rat proximal tubular cells (De Zeeuw et al., 1988; Cox et al., 1992). In particular, renal handling of salicylate was studied extensively by micropuncture experiments in vivo (Ferrier et al., 1983), isolated proximal tubules (Schild and Roch-Ramel, 1988; Cox et al., 1992), renal cortical slices (Despopoulos, 1960, Putney and Borzelleca, 1973), and a kidney epithelial cell line (Chatton and Roch-Ramel, 1992). In addition, there are also the reports on the interaction of NSAIDs with other organic anions, such as prostaglandins (Bito et al., 1976) and penicillin (Nierenberg, 1986). The results of these studies suggest that NSAIDs may be transported via the renal organic anion transporter.

We already reported that indomethacin potently inhibited [^{14}C]PAH uptake via OAT1 (Sekine et al., 1997). There are many types of NSAIDs with different chemical properties. In the present study, we aimed to determine whether the various NSAIDs interact with and are transported by OAT1.

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ABBREVIATIONS: OAT1, organic anion transporter 1; rNaDC-1, rat sodium-dependent dicarboxylate transporter; PAH, *para*-aminohippurate; NSAID, nonsteroidal anti-inflammatory drug.

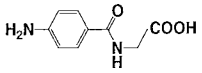
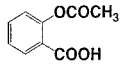
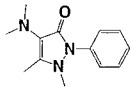
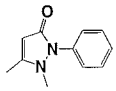
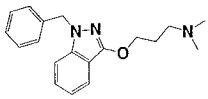
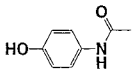
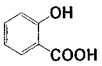
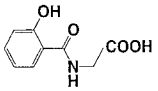
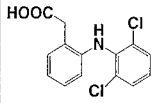
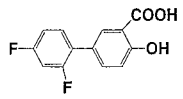
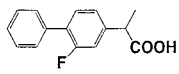
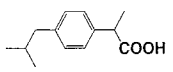
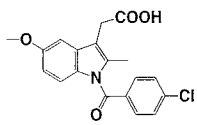
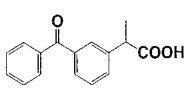
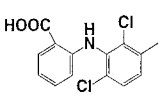
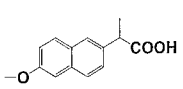
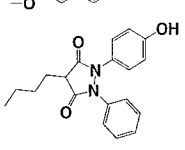
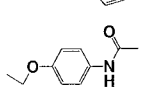
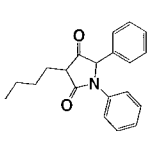
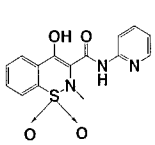
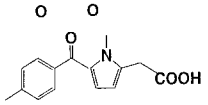
Chemical structure	Names	M.W.	Log P	%[^{14}C]PAH inhibition at 1 mM
	Para amino hippurate	194.2	-2.79[a]	98.5 ± 0.3
Hydrophilic NSAIDs				
	Acetylsalicylate	180.2	1.23[b]	63.5 ± 4.2
	Aminopyrine	231.3		23.8 ± 11.6
	Antipyrine	188.2		37.8 ± 6.8
	Benzydamine	345.9		60.0 ± 5.2
	Paracetamol	151.2		35.9 ± 5.7
	Salicylate	138.2	2.25[b]	64.6 ± 4.4
	Salicylurate	195.2	1.13[b]	98.9 ± 0.4

Fig. 1. Summary of the *cis*-inhibition of OAT1-mediated PAH uptake by NSAIDs. One-hour uptakes of 2 μM [^{14}C]PAH in oocytes injected with OAT1 cRNA were measured in the absence (control) or presence of NSAIDs (1 mM). Data are mean ± S.E.M. of the percentage of inhibition of [^{14}C]PAH uptake (each group consists of 5–8 oocytes). Chemical structures, molecular weights, and log *P* values (log octanol/water coefficient) of NSAIDs are also depicted.

Chemical structure	Names	M.W.	Log P	%[^{14}C]PAH inhibition at 1 mM
Hydrophobic NSAIDs				
	Diclofenac	318.1	4.31[b]	100 ± 0.5
	Diflunisal	250.2		100 ± 0.2
	Flurbiprofen	244.3	3.81[b]	100 ± 0.3
	Ibuprofen	206.3	3.51[b]	98.9 ± 0.7
	Indomethacin	357.8	4.42[b]	99.9 ± 0.5
	Ketoprofen	254.3	2.94[b]	100 ± 0.3
	Meclofenamate	296.2	5.75[c]	99.8 ± 0.4
	Naproxen	230.3	3.18[b]	100 ± 0.1
	Oxyphenbutazone	324.4		93.9 ± 0.7
	Phenacetin	179.2		58.4 ± 6.0
	Phenylbutazone	308.4	3.24[d]	98.8 ± 0.3
	Piroxicam	331.3		100 ± 0.6
	Tolmetin	315.3		99.5 ± 0.6

[a]Matsumoto and Ohsako, 1990; [b]Yano et al., 1986; [c]Chalmers et al., 1993; [d]Di Francesco and Bickel, 1985.

Furthermore, we investigated the transport properties of OAT1 as an exchanger.

Experimental Procedures

Materials. [^{14}C]Salicylate (2.05 GBq/mmol), [^{14}C]PAH (2.0 GBq/mmol), and [^{14}C]indomethacin (0.825 GBq/mmol) were purchased from Du Pont/New England Nuclear (Boston, MA). [^{14}C]Acetylsalicylate (2.0 GBq/mmol) and [^{14}C]glutarate (2.035 GBq/mmol) were purchased from American Radiolabeled Chemicals, Inc. (St. Louis, MO). The other NSAIDs were from Sigma Chemical Co. (St. Louis, MO).

cRNA Synthesis and Oocyte Injection. Capped cRNAs for OAT1 and rat sodium-dependent dicarboxylate transporter (rNaDC-1; Sekine et al., 1998) were synthesized in vitro by T7 RNA polymerase, as described elsewhere (Sekine et al., 1997). Defolliculated oocytes were injected with 10 ng of OAT1 cRNA. For coexpression experiments, both OAT1 cRNA (7.5 ng) and rNaDC-1 cRNA (2.5 ng) were injected into the oocytes. After injection, the oocytes were incubated for 2 to 3 days in modified Barth's solution containing gentamicin (88 mM NaCl, 1 mM KCl, 0.33 mM $Ca(NO_3)_2{\cdot}4H_2O$, 0.4 mM $CaCl_2{\cdot}2H_2O$, 0.8 mM $MgSO_4{\cdot}7H_2O$, 2.4 mM $NaHCO_3$, 10 mM HEPES, and 150 mg/ml gentamicin; pH 7.4) at 18°C.

Transport Assays. Two to 3 days after cRNA injection, oocytes were preincubated in ND96 solution (96 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, and 5 mM HEPES; pH 7.4) containing 1 mM glutarate for 2 h to generate an outwardly directed glutarate gradient. This preincubation with glutarate was performed routinely for all of the following experiments. After washing with ND96, 450 μl of ND96 solution with radiolabeled substrates was added, and the oocytes were incubated for 1 h at 25°C unless otherwise indicated. In the experiment shown in Fig. 4B, control oocytes and oocytes expressing OAT1 were injected with 100 nl of water or 100 nl of 10 mM glutarate 30 min before the uptake experiment with [^{14}C]salicylate. Transport assay was stopped by adding ice-cold ND96 solution to the well and the oocytes were washed five times with the same solution. Radioactivity was counted after solubilizing oocytes with 250 μl of 10% SDS.

Inhibition Study. For inhibition experiments, oocytes expressing only OAT1 were incubated for 1 h in ND96 solution containing 2 μM [^{14}C]PAH in the absence or presence of inhibitors (1 or 5 mM). Acetylsalicylate, salicylate and salicylurate (a metabolite of salicylate), acetaminophen (paracetamol), benzydamine, aminopyrine, and antipyrine were directly dissolved in ND96 solution. Substrates that were hard to dissolve in ND96 solution (meclofenamate, ibuprofen, phenylbutazone, oxyphenbutazone, flurbiprofen, diclofenac, ketoprofen, indomethacin, phenaceten, diflunisal, naproxen, and tolmetin) were first dissolved in ethanol and diluted to 1 mM with ND96 solution. Piroxicam was first dissolved in dimethylsulfoxide and diluted to 1 mM with ND96 solution. The final concentrations of ethanol and dimethylsulfoxide were adjusted to 0.7% and 1%, respectively, for all of these compounds.

Kinetic Analysis. After preincubation with glutarate, oocytes expressing only OAT1 were incubated for 1 h in ND96 solution containing different concentrations of PAH in the absence or presence of inhibitors, and double reciprocal plot analyses were performed. K_i values were calculated based on the following equation, when the inhibition was revealed to be competitive: K_i = concentration of inhibitor/[(K_m PAH with inhibitor/K_m PAH without inhibitor)−1].

Efflux Experiment. Oocytes injected with both OAT1 and rNaDC-1 cRNA or with only OAT1 cRNA were incubated in 50 μM [^{14}C]PAH or 50 μM [^{14}C]glutarate for 2 h. When [^{14}C]PAH was used as a tracer, oocytes were preincubated with 1 mM glutarate for 2 h before the experiments. The oocytes were washed 5 times in ice-cold ND96 solution and transferred to wells containing 300 μl of ND96 solution with or without 1 mM test substrates. After 90 min of incubation, the radioactivities of the incubation medium and the corresponding oocyte were counted.

Statistical Analyses. The values represent the mean ± S.E.M. The statistical differences were analyzed by unpaired Student's t test.

Results

Figure 1 summarizes the *cis*-inhibitory effect of various NSAIDs on PAH uptake via OAT1. Two μM [^{14}C]PAH uptake via OAT1 was inhibited by all NSAIDs (1 mM) that tested significantly ($P < .05$), except aminopyrine. A high concentration of aminopyrine (5 mM) also inhibited the OAT1-mediated uptake of PAH (83.6 ± 3.4% inhibition). The degree of inhibition was relatively low among hydrophilic NSAIDs. However, all compounds with higher log P values strongly inhibited [^{14}C]PAH uptake via OAT1 (>95%), except phenacetin (60% inhibition). To characterize the interaction of NSAIDs with OAT1, inhibitory kinetics of acetylsalicylate, salicylate, salicylurate, paracetamol, naproxen, oxyphenbutazone, piroxicam, ibuprofen, indomethacin, and phenacetin were analyzed. The uptake of different concentrations of PAH via OAT1 was determined in the absence and presence of inhibitors (concentrations of inhibitors are depicted in Table 1). As shown in Fig. 2, Lineweaver-Burk plot analysis of salicylurate and ibuprofen demonstrated that these two compounds inhibited OAT1-mediated PAH uptake in a competitive manner. The calculated K_i values for salicylurate and ibuprofen were 11 μM and 3.5 μM, respectively (Table 1). The inhibitions of the other drugs were also revealed to be competitive (data not shown). The K_i values for each compound are shown in Table 1. On the basis of the calculated K_i values, the drugs were placed in the following order in the affinity to OAT1: naproxen > ibuprofen > indomethacin = salicylurate > oxyphenbutazone > piroxicam ≫ salicylate = acetylsalicylate = phenacetin ≫ paracetamol.

Figure 3 shows the uptake of 30 μM [^{14}C]salicylate, 30 μM [^{14}C]acetylsalicylate, and 5 μM [^{14}C]indomethacin in control oocytes, oocytes expressing rNaDC-1 or OAT1, and oocytes expressing both rNaDC-1 and OAT1(coexpression). Figure 4A schematically represents the coexpression system in *Xenopus* oocytes, which generates the steep, outwardly directed gradient of dicarboxylate. Figure 4B shows the membrane localization of OAT1 and rNaDC-1 in renal proximal tubule cells (Sekine et al., 1998; Tojo et al., 1999). The uptake rates of these three NSAIDs by oocytes expressing rNaDC-1 are all the same as those by control oocytes. By contrast, oocytes expressing OAT1 showed significantly higher uptake of salicylate, acetylsalicylate, and indomethacin. Furthermore, oocytes expressing both OAT1 and rNaDC-1 took up these compounds more than those expressing only OAT1.

When we did not preincubate the oocytes coexpressing OAT1 and rNaDC-1 with glutarate, the cell-associated count of [^{14}C]salicylate was lower than that of the coexpressing oocytes with glutarate preload (Fig. 5A). To directly exclude the effect of the coexpression, we performed microinjection of glutarate into oocytes that express only OAT1. The injection of 100 nl of 10 mM glutarate before the uptake experiment increased the cell-associated count of [^{14}C]salicylate (Fig. 5B).

Next, we investigated the transport properties of OAT1 as an exchanger (Fig. 6). Oocytes that coexpress OAT1 and rNaDC-1 were preloaded with 50 μM [^{14}C]glutarate for 2 h, and after being washed, they were transferred to ND96 so-

lution with or without 1 mM nonradioactive PAH. The left half of Fig. 6A shows that the efflux of intracellularly accumulated [^{14}C]glutarate was enhanced by the addition of 1 mM PAH into the medium (open columns, control versus PAH). Because the efflux of [^{14}C]glutarate from oocytes expressing only rNaDC-1 was not influenced by 1 mM PAH (data not shown), the efflux of [^{14}C]glutarate is considered to be mediated by OAT1. We also performed efflux experiments with oocytes expressing only OAT1 (Fig. 6A, right). The efflux of [^{14}C]glutarate was also stimulated by 1 mM PAH (open columns, control versus PAH), although the accumulated glutarate was much smaller (closed columns, coexpression versus OAT1).

We also used [^{14}C]PAH as a tracer for the efflux experiment (Fig. 6B). The accumulated [^{14}C]PAH was higher in oocytes coexpressing rNaDC-1 and OAT1 than in those expressing only OAT1 (closed columns, coexpression versus OAT1). The efflux of [^{14}C]PAH via OAT1 was stimulated by the addition of 1 mM PAH, both in oocytes coexpressing rNaDC-1 and OAT1 and in those expressing only OAT1 (Fig. 6B). The efflux of [^{14}C]PAH stimulated by extracellular PAH increased linearly up to 90 min of incubation (Fig. 6B, inset).

We investigated whether or not the NSAIDs *trans*-stimulated OAT1-mediated organic anion transport (Fig. 7). Because oocytes coexpressing rNaDC-1 and OAT1 took up a larger amount of PAH, and the efflux rate of PAH is higher than those expressing only OAT1, we used the coexpression system for this purpose. Three NSAIDs (acetylsalicylate, sa-

TABLE 1
K_i values of NSAIDs to inhibit [^{14}C]PAH uptake via OAT1

Drugs	Concentration Tested	K_i
	μM	
Naproxen	10	2
Ibuprofen	10	3.5
Indomethacin	5	10
Salicylurate	25	11
Oxyphenbutazone	150	32
Piroxicam	30	52
Salicylate	500	341
Acetylsalicylate	250	428
Phenacetin	1000	488
Paracetamol	2500	2099

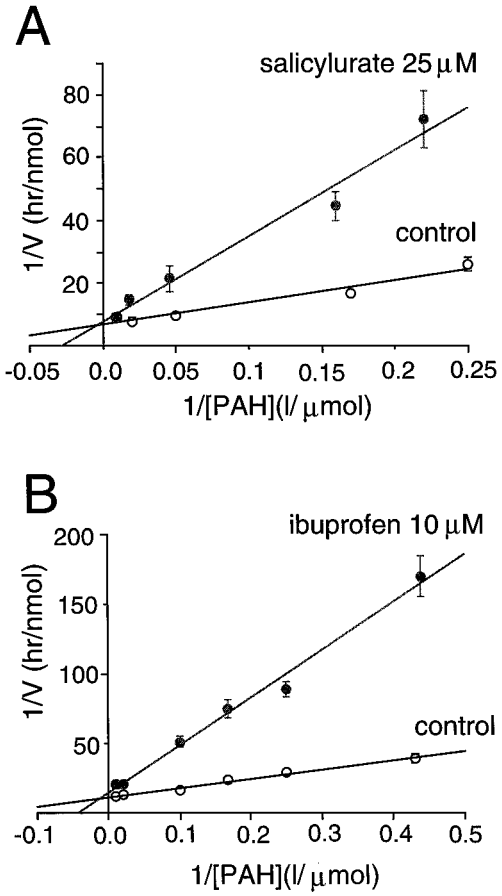


Fig. 2. Inhibitory effect of salicyluric acid and ibuprofen on OAT1-mediated PAH uptake. OAT1-mediated uptake of PAH of several concentrations were measured in the presence (●) or absence (○) of salicyluric acid or ibuprofen and Lineweaver-Burk plot analyses were performed. The concentration of salicyluric acid was 25 μM (A) and that of ibuprofen was 10 μM (B).

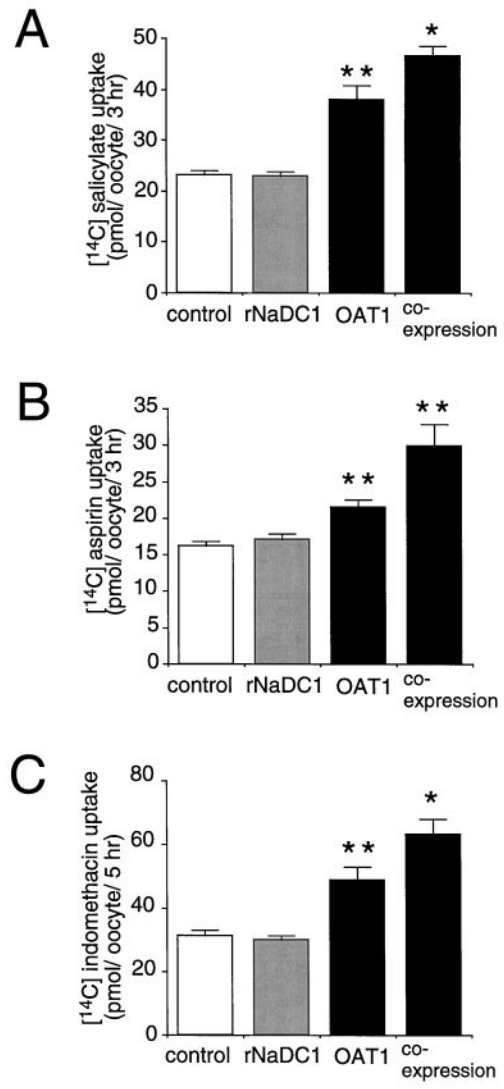


Fig. 3. [^{14}C]Salicylate, [^{14}C]acetylsalicylate, and [^{14}C]indomethacin uptake in noninjected oocytes (control), oocytes expressed with rNaDC-1 (rNaDC1), oocytes expressed with OAT1 (OAT1), and oocytes coexpressed with both rNaDC-1 and OAT1 (coexpression). Uptake of 30 μM [^{14}C]salicylate (A), 30 μM [^{14}C]acetylsalicylate (B), and 5 μM [^{14}C]indomethacin (C) was determined in each group consisting of 8 to 10 oocytes. Statistical differences (OAT1 versus control and coexpression versus OAT1) were calculated by unpaired Student's t test. *$P < .05$, **$P < .01$.

licylate, and indomethacin) and one metabolite of salicylate (salicylurate) were examined. As shown in Fig. 7A, acetylsalicylate, salicylate, and salicylurate significantly promoted [^{14}C]PAH efflux. Salicylurate was almost as effective as PAH in the stimulation of [^{14}C]PAH efflux via OAT1. In contrast, indomethacin did not promote [^{14}C]PAH efflux; in fact, it significantly depressed the efflux.

As for indomethacin, we examined the time course of its effect on PAH efflux. As shown in Fig. 7B, indomethacin revealed the inhibitory effect on the PAH efflux at 90 min of incubation. However, at 15 and 30 min of incubation, neither a *trans*-stimulation nor a *trans*-inhibition effect was observed.

Discussion

The present study demonstrated that PAH transport via OAT1 was inhibited by NSAIDs possessing different chemical structures. The results of this study and previous reports (Sekine et al., 1997) indicate that OAT1 is a multispecific organic anion transporter. From kinetic analyses, at least 10 tested drugs showed a competitive inhibitory effect on the PAH uptake via OAT1, suggesting that these drugs all bound to the binding site of OAT1. Presently, we have no definite explanations for this multispecific nature of OAT1. Moller and Sheikh (1983) suggested that the binding of organic anions with the renal organic anion transporter depends mainly on hydrophobic, hydrogen bonding and electrostatic interactions between the substrate and the carrier. Later, Ullrich and Rumrich (1988) proposed that PAH transporter interacts with the substrates that contain hydrophobic cores with negative charges or negative partial charges. Our results revealed that all hydrophobic NSAIDs potently inhibited PAH uptake (except phenacetin), whereas hydrophilic NSAIDs inhibited PAH uptake to lesser degrees (except salicyluric acid and PAH). Phenacetin, which possesses the least affinity among the hydrophobic NSAIDs tested, contains smaller hydrophobic side chains (Table 1). The different affinity of NSAIDs to OAT1 may signify the importance of hydrophobic interaction between substrates and OAT1. Large hydrophobic side chains in the molecules could well stabilize the molecules with the binding site of OAT1.

Among hydrophobic NSAIDs, phenacetin, piroxicam, oxyphenbutazone, and phenylbutazone possess no carboxylic groups. Phenacetin, piroxicam, and oxyphenbutazone have higher K_i values than ibuprofen and indomethacin, which possess carboxylic groups. This indicates that the negative charge of the substrates increases their affinity to OAT1. The importance of a negative charge is also demonstrated when

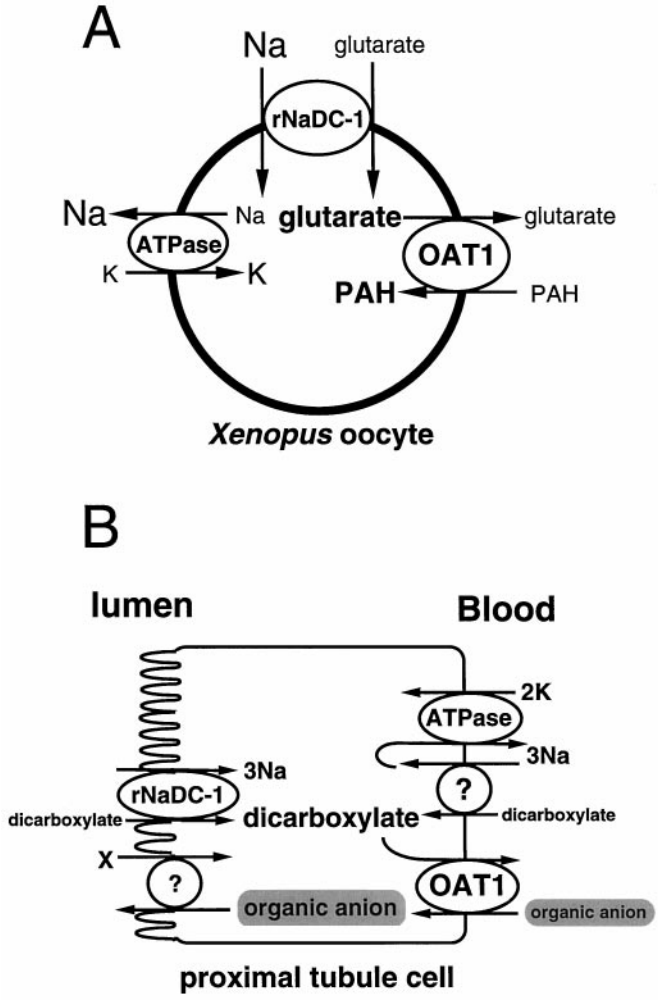


Fig. 4. A, schematic representation of coexpression system in *Xenopus laevis* oocytes. B, membrane localization of rNaDC-1 and OAT1 in the renal proximal tubule .

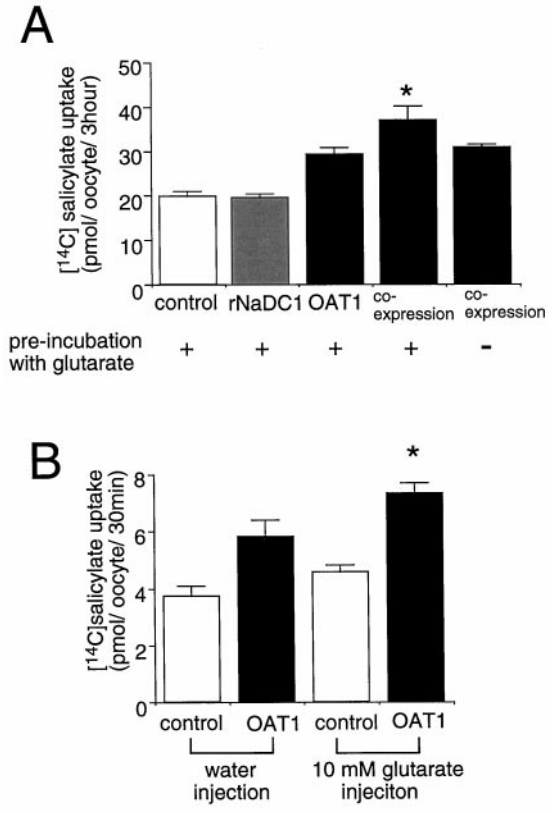


Fig. 5. [^{14}C]Salicylate uptake in oocytes under various conditions. A, uptake rate of [^{14}C]salicylate was determined in the coexpression oocytes with or without glutarate preincubation. The uptake of control oocytes and oocytes expressing OAT1 or rNaDC-1 was also determined. *$P < .05$ (coexpression oocytes with glutarate preincubation versus coexpression oocytes without glutarate preincubation) B, control oocytes and oocytes expressed with only OAT1 were injected with 100 nl of water or 100 nl of 10 mM glutarate 30 min before uptake experiments. *$P < .05$ (OAT1 with glutarate injection versus OAT1 with water injection)

the affinity among hydrophilic NSAIDs is considered. Small hydrophilic compounds that lack carboxylic groups (paracetamol, aminopyrine, antipyrine) show lower degrees of inhibition to PAH uptake.

Salicylate can be actively accumulated and secreted by proximal renal tubules (Putney and Borzelleca, 1973; Weiner, 1973; Roch-Ramel et al., 1978; Ferrier et al., 1983; Schild and Roch-Ramel, 1988). The secretory mechanism of salicylate was suggested to be the same as that for PAH (Ferrier et al., 1983). Acetylsalicylate was shown to be accumulated in renal tissues, although its mechanism is not clearly understood (Gaspari et al., 1989). Active accumulation of indomethacin in renal slices (Cox et al., 1992) and isolated perfused proximal straight tubules (De Zeeuw et al., 1988) was also reported. Salicylate and indomethacin inhibited PAH uptake by rabbit renal slices with a K_i of 310 μM and 60 μM, respectively (Nierenberg, 1986). In rat cortical renal slices (Melendez and Reyes, 1982), the K_i of indomethacin for PAH uptake was 11 μM. Some of these K_i values are slightly lower than those reported from micropuncture experiments (Ullrich et al., 1990); however, the inhibition potencies tend to be similar. It should be noted that these K_i values of NSAIDs are comparable to those obtained in the present study for OAT1.

In the results shown in Fig. 3, we demonstrated the significant uptake of salicylate, acetylsalicylate, and indomethacin by OAT1. The uptake of these three NSAIDs via OAT1 was enhanced by the steep, outwardly directed glutarate gradient generated by rNaDC-1. This *trans*-stimulatory effect of glutarate on the uptake of salicylate, acetylsalicylate, and indomethacin via OAT1 indicates that at least these three NSAIDs not only bind to, but are really translocated by OAT1, because the *trans*-stimulation occurs only along with the translocation process. The results obtained in the experiments shown in Fig. 5 reinforced the conclusion that salicylate is a transportable substrate of OAT1. These results, together with the fact that the affinity of NSAIDs for OAT1 is similar to those for the renal organic anion transporter, strongly suggest that OAT1 is the major organic anion transporter in the kidney.

The transport rates of salicylate, acetylsalicylate, and indomethacin via OAT1 are low compared with that of PAH. In particular, the transport of indomethacin, despite its high

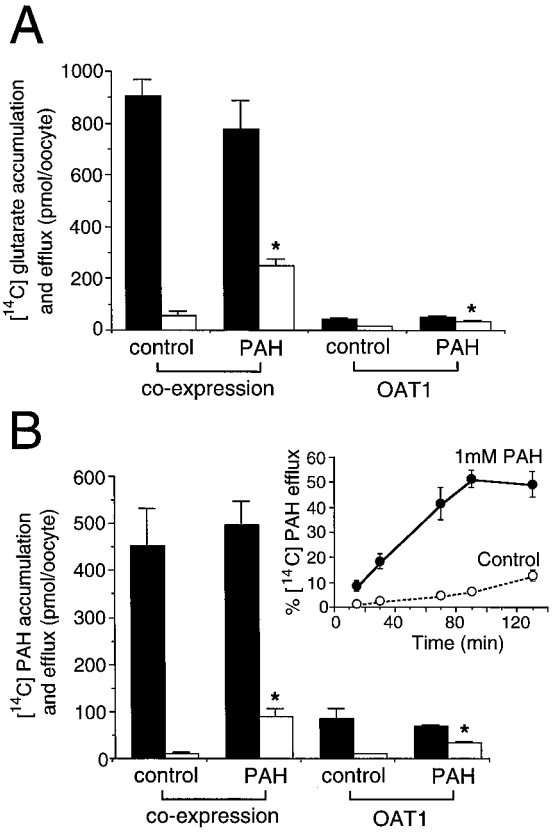


Fig. 6. Efflux experiment with [^{14}C]glutarate (A) or [^{14}C]PAH (B) as tracer. Oocytes expressed with both OAT1 and rNaDC-1 (coexpression) and those expressed with only OAT1 were used. After being preloaded with 50 μM [^{14}C]PAH or 50 μM [^{14}C]glutarate, oocytes were washed by ND96 solution and transferred to 48 wells containing 300 μl of ND96 solution in the absence (control) or presence (PAH) of 1 mM nonradiolabeled PAH. After 90 min, the radioactivity in the medium and oocytes was determined, respectively. Closed columns depict the sum of the labeled compounds in oocytes and medium, which represents [^{14}C]PAH or [^{14}C]glutarate accumulated in oocytes before the efflux experiment. Open columns depict the amount of [^{14}C]PAH or [^{14}C]glutarate in the media, which represents the amount of effluxed PAH or glutarate from the oocytes. Statistical significance was determined by the unpaired Student's t test. *$P < .01$ (effluxed radioactivity in the control media versus those in the media containing 1 mM PAH). Inset, time-dependent [^{14}C]PAH efflux from oocytes coexpressing both rNaDC-1 and OAT1.

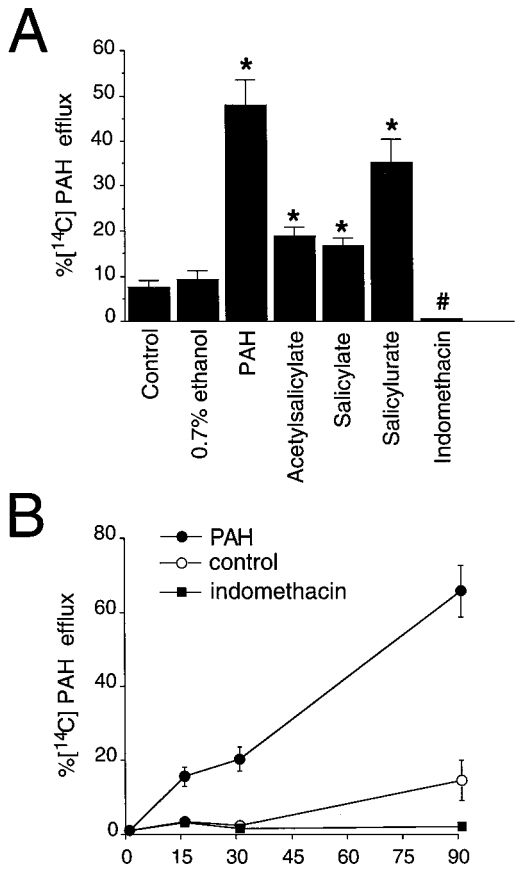


Fig. 7. A, efflux experiment with NSAIDs. Coexpression oocytes were preloaded with [^{14}C]PAH and transferred in ND96 solution in the absence (control) or presence of 1 mM PAH or NSAIDs. The y-axis represents the effluxed [^{14}C]PAH expressed as a percentage of the total count of radioactivity accumulated in oocytes. Statistical differences were measured with the unpaired Student's t test. *$P < .05$ (versus control), $^{\#}P < .05$ (versus control with 0.7% ethanol). B, time course (15, 30, and 90 min) of the PAH efflux was determined in the absence (control) or presence of PAH or indomethacin.

affinity to OAT1 (10 μM), is very small. This result is consistent with the in vivo kinetics: a low rate of administered indomethacin (15%) is excreted into the urine in its original form. The present study indicates that at the molecular level, salicylate, acetylsalicylate, and indomethacin are transportable substrates of OAT1. Nonetheless, NSAIDs, especially the hydrophobic ones, may act rather as inhibitors for the organic anion transporter in the kidney. Although OAT1 accepts a number of compounds, their chemical structures are considerably different. The transport of substrates by carrier proteins consists of three processes: substrate binding, translocation, and dissociation. Among the chemically heterogeneous substances, not only the binding, but also the translocation and dissociation processes, are presumed to be different. Thus, it is no wonder that differences exist in the efficiency of substrates transport by a multispecific transporter like OAT1.

In previous studies, it was proposed that the basolateral uptake of organic anions at the middle portion of the proximal tubule (S2 segment) is mediated by a dicarboxylate/organic anion exchanger (Shimada et al., 1987; Pritchard and Miller, 1991) and we also suggested that OAT1 is an organic anion/dicarboxylate exchanger (Sekine et al., 1997). In the present study, we analyzed the efflux of substrates via OAT1 to confirm this exchange model. When oocytes expressing OAT1 were preloaded with [^{14}C]PAH or [^{14}C]glutarate and were transferred to the incubation medium containing PAH, the efflux of [^{14}C]PAH or [^{14}C]glutarate was stimulated. When we considered this result in conjunction with the previous observation that preloading the oocytes expressing OAT1 with glutarate stimulated the uptake of [^{14}C]PAH via OAT1, it is clear that OAT1 is, indeed, an exchanger. Furthermore, the fact that not only dicarboxylate (glutarate), but also PAH itself, is effluxed from the oocytes expressing OAT1, suggests that the intracellular binding site of OAT1 is also multispecific. OAT1 can act as both a heteroexchanger (dicarboxylate/PAH exchange) and homoexchanger (PAH/PAH exchange) and there seems to be no strict rectification in the transmembrane transport via OAT1. Our results, that the addition of PAH to the extracellular compartment could stimulate the efflux of preloaded PAH, are consistent with the in vitro experiment with isolated S2 segments of proximal tubules (Chatsudthipong and Dantzler, 1992).

Three of the NSAIDs tested and salicylurate showed different actions on [^{14}C]PAH efflux. Salicylate, acetylsalicylate, and salicylurate clearly induced the efflux of PAH. This result suggests that these three compounds are actually transported via OAT1 by the exchange mechanism. This was supported by the result of the uptake experiment with radiolabeled acetylsalicylate and salicylate (Fig. 3). On the contrary, indomethacin depressed [^{14}C]PAH efflux during 90 min of incubation. Short-time incubation with indomethacin did not show significant depression. There are at least two possibilities for the suppression of PAH efflux by indomethacin. One possibility is that these compounds simply diffuse into the cells because of their high hydrophobicity and compete with [^{14}C]PAH at the intracellular binding site of OAT1, as suggested by Huang and Lin (1965). The fact that only the long-time incubation revealed depressed efflux may support this possibility. The other possible mechanism is that hydrophobic NSAIDs may slow down the translocation process and/or dissociation of the transporter after binding to OAT1. This possibility has already been considered in the experiment with probenecid (Dantzler et al., 1995) and is also supported by the finding that substitution of phenolsulfophthalein dyes by a hydrophobic core inhibits their movement by the organic anion transport system in rabbit (Sheikh, 1976). Although further experiments are needed to substantiate these possibilities, we propose that this efflux system can be used for the determination of transportable substrates. If the tested compounds can induce the efflux of intracellularly accumulated anion, they are considered to be transportable substrates.

OAT1 may also be responsible for drug metabolism and renal toxicity. With regard to drug metabolism, there is evidence that salicylate can be converted to salicylurate (Bekersky et al., 1980; Laznicek and Laznickova, 1994) and acetylsalicylate to salicylate in kidneys (Gaspari et al., 1989). There has been a report that hydrolases responsible for converting aspirin to salicylate were found in kidneys of various species, including humans and rats (Eyring and Ford, 1972). Furthermore, proximal tubules, especially the S2 segments, are very rich in drug-metabolizing enzymes, i.e., cytochrome P-450-dependent mixed function oxidase (Endou et al., 1982). This coincides with the high expression of OAT1 in S2 segments (Tojo et al., 1999). OAT1 may be responsible for the transport of organic anions into the proximal tubular cells where the drug-metabolizing enzymes exist. In regard to drug-induced nephrotoxicity, there are reports showing that acetylsalicylate could induce renal papillary necrosis (Axelsen, 1976; Molland, 1976) and proximal tubular cell damage (Molland, 1976). Salicylate was also reported to cause necrosis of renal papilla (Fellers et al., 1965). Uptake of acetylsalicylate via OAT1 may be responsible for acetylsalicylate accumulation in the cells or it could be further metabolized to salicylate. Subsequent secretion of these drugs into the lumen may lead to high concentrations of the drugs in papillary tips, causing renal papillary necrosis. Moreover, it was found that cortical tubular necrosis induced by either acetylsalicylate or oxyphenbutazone was reduced when probenecid, a potent inhibitor of organic anion transporters, was administered concomitantly (Arnold et al., 1976). Thus, OAT1 may be one of the factors responsible for drug-induced nephrotoxicity.

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