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Characterization of prostaglandin E_1 transport by rat renal cortical slices

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Abstract

The purpose of this study was to examine prostaglandin E_1 (PGE_1) transport in rat kidney. [^{3}H]PGE_1 administered intravenously was accumulated most abundantly in the renal cortex. Infusion of probenecid and bromcresol green (BCG) decreased [^{3}H]PGE_1 accumulation in the renal cortex after injection of [^{3}H]PGE_1. To further investigate PGE_1 transport in the kidney, [^{3}H]PGE_1 uptake by renal cortical slices was examined. Probenecid and BCG inhibited [^{3}H]PGE_1 uptake by the slices. Unlabeled PGE_1 decreased [^{3}H]PGE_1 uptake by renal cortical slices in a concentration-dependent manner. The inhibitory effect of various dicarboxylates with different carbon atoms on [^{3}H]PGE_1 uptake was maximal at 6 carbon atoms. Preloading cortical slices with glutarate significantly increased [^{3}H]PGE_1 uptake. [^{3}H]PGE_1 uptake was inhibited by various eicosanoids and compounds with other structures (*p*-aminohippurate, benzylpenicillin, estrone-3-sulfate, etc.). These findings suggest that PGE_1 uptake by renal cortical slices may be mediated by the members of the organic anion transporter family.

Keywords: Prostaglandin; Organic anion transporter; Renal cortical slice; Renal proximal tubule

1. Introduction

Earlier studies showed that prostaglandins are efficiently secreted into the urine by the organic anion transport system in renal proximal tubules (Bito, 1976; Rosenblatt et al., 1978; Bito and Baroody, 1978; Irish, 1979). Bito and Baroody (1978) showed that *p*-aminohippurate inhibits the basolateral transport of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) into the proximal tubule in a concentration-dependent manner. The rate of net secretion of prostaglandin E_2 (PGE_2) is significantly higher in the segment 2 of the proximal straight tubule than segment 1 or 3, whereas the net secretion of PGE_2 in the descending limb of Henle's loop is significantly lower than that observed in any segments of the proximal tubule (Irish, 1979). This profile of the transport activity of PGE_2 along the renal tubule was very similar to that of *p*-aminohippurate (Woodhall et al., 1978). In addition, PGE_2 transport in renal basolateral membrane vesicles was inhibited by probenecid, a classical inhibitor of organic anion transport, in a concentration-dependent manner (Boumendil-Podevin and Podevin, 1985). Ullrich et al. (1991) showed that various prostanoids including PGE_1, PGE_2 and $PGF_{2\alpha}$ inhibit the basolateral transport of *p*-aminohippurate in the proximal tubule, using the stop-flow peritubular capillary microperfusion method. Thus, transport of prostaglandins across the basolateral membrane in renal proximal tubule may be similar to that of the prototypical organic anion *p*-aminohippurate.

Using basolateral membrane vesicles from rat renal cortex, Shimada et al. (1987) found that uptake of *p*-aminohippurate at the basolateral membrane is stimulated in the presence of inward gradients of sodium and α-ketoglutarate. This finding suggests that the basolateral transport of *p*-aminohippurate is coupled indirectly to the sodium gradient at the membrane through the following two processes: entry of α-ketoglutarate via Na^+/dicarboxylate cotransporter and subsequent uptake of *p*-aminohippurate via *p*-aminohippurate/dicarboxylate exchanger. In addition, several studies with isolated renal proximal tubules and renal cortical slices showed that uptake of *p*-aminohippurate was increased by preloading with α-ketoglutarate or glutarate (Chatsudthipong and Dantzler, 1992; Pritchard, 1990). Using the OK kidney epithelial cell line, it was observed that intracellular α-ketoglutarate is effluxed to

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the basolateral side with application of *p*-aminohippurate to the basolateral side of the cells, showing *p*-aminohippurate/dicarboxylate exchange is involved in the basolateral uptake of *p*-aminohippurate (Nagai et al., 1998).

In 1997, the *p*-aminohippurate/dicarboxylate exchanger protein was isolated by expression cloning with *Xenopus* oocytes (Sekine et al., 1997; Sweet et al., 1997) and designated organic anion transporter 1 (OAT1). Rat (r) OAT1 mRNA is expressed predominantly in the kidney and very weakly in the brain. Immunohistochemical analysis revealed that rOAT1 is localized in the basolateral membrane of the middle portion of the proximal tubules (segment 2) (Tojo et al., 1999). rOAT1 as well as human (h) OAT1 displays a remarkably wide substrate specificity. Those transporters have been reported to interact with a variety of organic anion drugs such as β-lactam antibiotics, nonsteroidal anti-inflammatory drugs, diuretics and anti-diabetic drugs in addition to endogenous compounds such as cyclic nucleotides, uric acid, α-ketoglutarate and PGE_2 (Sekine et al., 1997; Sweet et al., 1997; Uwai et al., 1998, 2000). In addition, rOAT1-mediated *p*-aminohippurate uptake was increased by the outwardly directed dicarboxylate gradient, indicating that rOAT1 is an organic anion/dicarboxylate exchanger.

Subsequently, Kusuhara et al. (1999) isolated another member of the OAT family, rOAT3, from rat brain. Expression of rOAT3 mRNA was detected in the liver, brain, kidney and eye. When expressed in oocytes, rOAT3 transported organic anions such as *p*-aminohippurate, estrone-3-sulfate and organic cations such as cimetidine. Recently, it was reported that PGE_2 and $PGF_{2\alpha}$ are transported not only by OATs (hOAT1, hOAT2, hOAT3, hOAT4) but also by organic cation transporters (hOCT1 and hOCT2) (Kimura et al., 2002). Furthermore, mouse (m) OAT3 has been reported to mediate the uptake of prostaglandins such as PGE_2 and $PGF_{2\alpha}$ in addition to *p*-aminohippurate and estrone-3-sulfate (Kobayashi et al., 2004). However, it has not been fully clarified whether the members of the OAT and OCT family are involved in the transport of prostaglandins across the basolateral membrane in the renal proximal tubule.

In the present study, we investigated the transport of [^{3}H]PGE_1 by renal cortical slices to explore the mechanism underlying the basolateral uptake of PGE_1 in the renal proximal tubule. Since the cortical slices include large parts of the proximal tubules and the lumens of the proximal tubules are substantially collapsed (Wedeen and Weiner, 1973; Pritchard, 1990), uptake of [^{3}H]PGE_1 by the renal cortical slices reflects the basolateral transport of PGE_1 in the renal proximal tubule.

2. Materials and methods

2.1. Materials

[5,6(*n*)-^{3}H]PGE_1 (2.22 TBq/mmol) was purchased from NEN Life Science Products Inc. (Boston, MA, USA). PGE_2, $PGF_{2\alpha}$, thromboxane B_2 (TXB_2) and 15-keto PGE_1 were obtained from Cyman Chemical Company (Ann Arbor, Ml, USA). Probenecid, indomethacin, methotrexate and sulfobromophthalein were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Benzylpenicillin, cimetidine, taurocholate, chlorpropamide, tolbutamide, and glibenclamide were from Wako Pure Chemical Industries (Osaka, Japan). Bromcresol green (BCG), 2,4-dinitrophenol, *p*-aminohippurate, benzylpenicillin, tetraethylammonium and salicylate were obtained from Nacalai Tesque (Kyoto, Japan). Unlabeled PGE_1 was the generous gift of Ono Pharmaceutical Company (Osaka, Japan). All other chemicals used for the experiments were of the highest purity available.

2.2. In vivo uptake study

Experiments with animals were performed in accordance with the Guide for Animal Experimentation, Hiroshima University, and the Committee of Research Facilities for Laboratory Animal Sciences, Graduate School of Biomedical Sciences, Hiroshima University. Male Wistar albino rats (250–300 g) were anesthetized with pentobarbital sodium (30 mg/kg, i.p.), and the femoral artery and vein were cannulated with polyethylene tubing for blood sampling and drug administration, respectively. At 60 s after an intravenous bolus administration of [^{3}H]PGE_1 (37.6 pmol/kg), blood sample was withdrawn and each tissue was excised. The tissues were weighed and then homogenized with 10 ml of ethanol. After centrifugation at 1600 ×*g* for 10 min, an aliquot of the homogenates (200 μl) or the plasma (50 μl) was mixed with 3 ml of ACSII (Amersham Pharmacia Biotech, Buckinghamshire, UK) and the radioactivity was measured by liquid scintillation counting. Coadministration of probenecid and BCG was performed by constant-rate intravenous infusion of each compound (52.5 μmol/h/kg for probenecid, 96.0 μmol/h/kg for BCG) at a rate of 2 ml/h, after the loading dose (56.0 μmol/kg for probenecid, 29.7 μmol/kg for BCG) was introduced by intravenous bolus injection 60 min before [^{3}H]PGE_1 administration.

2.3. Uptake by renal slices

Male Wistar rats were anesthetized with pentobarbital sodium (30 mg/kg, i.p.) and the kidneys were removed rapidly and decapsulated. Slices from the renal cortex and medulla, each weighing 15–25 mg, were prepared using a Stadie–Riggs microtome and were stored in ice-cold oxygenated incubation buffer composed of 110 mM NaCl, 5 mM KCl, 25 mM $NaHCO_3$, 1 mM $CaCl_2$, 1.2 mM NaH_2PO_4, 1.2 mM $MgSO_4$, 10 mM CH_3COONa, 7 mM glucose, pH 7.4). Tissue slices were incubated in the oxygenated incubation buffer containing [^{3}H]PGE_1. An incubation temperature of 37 °C was used in this study. After the incubation, tissue slices were removed from the incubation buffer, washed with the ice-cold buffer and blotted on filter paper. Tissue slices were weighed and solubilized with 500 μl NCSII (Amersham Pharmacia Biotech). Subsequently, an aliquot of the solubilized slices (150 μl) and the incubation buffer containing [^{3}H]PGE_1 (50 μl) was mixed with 3 ml ACSII and the radioactivity was measured by liquid scintillation counting. Uptake of [^{3}H]PGE_1 by tissue

slices was expressed as the tissue-to-medium (T/M) concentration ratio, where T is dpm of [^{3}H]PGE$_1$ per milligram of tissue slice (wet weight) and M is dpm of [^{3}H]PGE$_1$ per microliter of the incubation medium.

2.4. Analytical methods

Statistical analysis was performed by Student's *t*-test or by the one- way analysis of variance with the Dunnet test for post hoc analysis. A difference of $P<0.05$ was considered statistically significant.

3. Results

3.1. Tissue distribution after an intravenous bolus administration of [^{3}H]PGE$_1$

Tissue distribution of [^{3}H]PGE$_1$, expressed as the value of tissue-to-plasma concentration ratio, was evaluated in various tissues 60 s after an intravenous bolus administration of [^{3}H]PGE$_1$ (Fig. 1). The rank order of tissue uptake of [^{3}H]PGE$_1$ in control rats was renal cortex>renal medulla, liver, lung>renal papilla, intestine, heart>stomach, brain, testis, eye, skeletal muscle. When coadministration of probenecid and BCG was performed by a constant-rate intravenous infusion, the tissue uptake of [^{3}H]PGE$_1$ was significantly decreased in the renal cortex (Fig. 1).

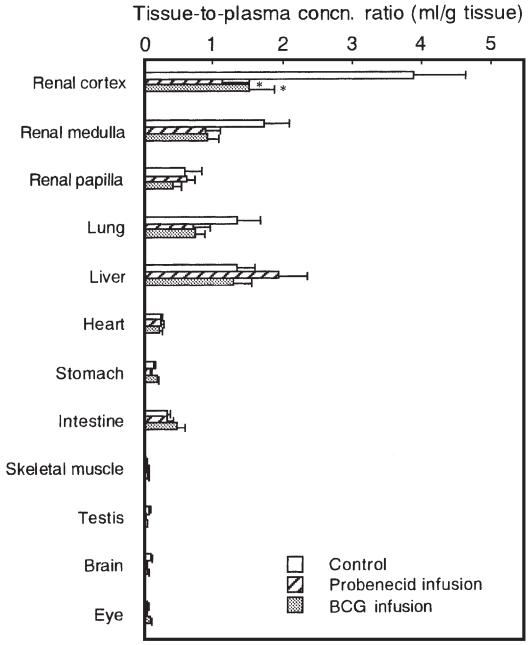

Fig. 1. Tissue distribution of [^{3}H]prostaglandin E$_1$ ([^{3}H]PGE$_1$) after the intravenous bolus administration to rats. At 60 s after an injection of [^{3}H]PGE$_1$ (37.6 pmol/kg), plasma was collected and each tissue was isolated. Administration of probenecid and bromcresol green (BCG) was performed by constant-rate intravenous infusion of each compound (52.5 μmol/h/kg for probenecid, 96.0 μmol/h/kg for BCG) at a rate of 2 ml/h, after the loading dose (56.0 μmol/kg for probenecid, 29.7 μmol/kg for BCG) was introduced by the intravenous bolus injection 60 min before [^{3}H]PGE$_1$ administration. Control rats received the infusion of saline, following the intravenous bolus injection of saline. The radioactivity in the plasma and tissue was measured as described in Materials and methods. Tissue distribution of [^{3}H]PGE$_1$ was expressed as the value of tissue-to-plasma concentration ratio. Each column represents the mean±S.E.M. of results from three to four rats. *$P<0.05$, significantly different from the value for control rats.

3.2. Uptake of [^{3}H]PGE$_1$ by slices from the kidney

To further investigate PGE$_1$ transport in the kidney, in vitro tissue slice uptake of [^{3}H]PGE$_1$, expressed as tissue slice-to-medium concentration ratio, was performed. When tissue slices from the renal cortex and medulla were incubated with the medium including 1 nM [^{3}H]PGE$_1$ for 60 min at 37 °C, the accumulation of [^{3}H]PGE$_1$ by renal cortex was higher than that by renal medulla (Fig. 2). In addition, probenecid and BCG significantly inhibited [^{3}H]PGE$_1$ accumulation by renal cortical slices, but not by renal medullary slices (Fig. 2).

3.3. Effects of metabolic inhibitors and eicosanoids on [^{3}H]PGE$_1$ uptake by renal cortical slices

Effects of metabolic inhibitors on [^{3}H]PGE$_1$ accumulation by renal cortical slices were examined. 2,4-Dinitrophenol as well as sodium azide in combination with 2-deoxy-D-glucose significantly reduced the accumulation of [^{3}H]PGE$_1$ by renal cortical slices, showing that [^{3}H]PGE$_1$ uptake by rat renal cortical slices is energy-dependent (Fig. 3). As shown in Fig. 4, not only unlabeled PGE$_1$ but also PGE$_2$, PGF$_{2\alpha}$, TXB$_2$ and 15-keto PGE$_1$ significantly decreased the uptake of [^{3}H]PGE$_1$ by the renal cortex.

3.4. Concentration dependence of [^{3}H]PGE$_1$ uptake by renal cortical slices

The concentration dependence of PGE$_1$ uptake by renal cortical slices was examined at 20 min after incubation with [^{3}H]PGE$_1$. The uptake of [^{3}H]PGE$_1$ (5 nM) by renal cortical slices was inhibited by unlabeled PGE$_1$ in a concentration-dependent manner (Fig. 5), reflecting competition of [^{3}H]PGE$_1$ and unlabeled PGE$_1$ for binding to the transporter(s). On the other hand, the transport of [^{3}H]PGE$_1$ was not completely inhibited even by a large excess of unlabeled PGE$_1$ (1 mM), which may result from a nonspecific uptake and/or binding of [^{3}H]PGE$_1$ to the renal cortical slices.

3.5. Uptake of [^{3}H]PGE$_1$ by glutarate-preloaded renal cortical slices

Next, PGE$_1$ transport by renal cortical slices preloaded with glutarate was compared to that without glutarate (Fig. 6). The uptake of [^{3}H]PGE$_1$ by renal cortical slices without glutarate (control) reached a steady state within 30 min after incubation with [^{3}H]PGE$_1$. In contrast, [^{3}H]PGE$_1$ accumulation by slices preloaded with 1 mM glutarate reached a maximum at 30 min and then showed a decline. There was a significant increase in [^{3}H]PGE$_1$ uptake by renal cortical slices preloaded with glutarate, compared to that without glutarate, suggesting that

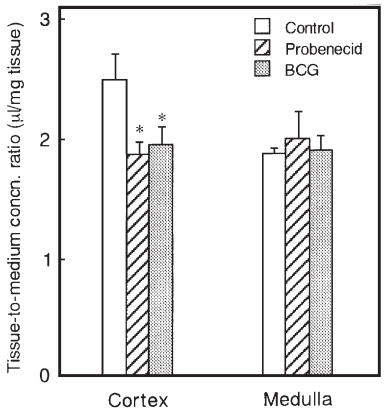

Fig. 2. Uptake of [^{3}H]PGE_1 by slices from rat renal cortex and medulla. Renal cortical and medullary slices were incubated with the medium including [^{3}H]PGE_1 (1 nM) in the absence (control) or presence of probenecid or BCG at a concentration of 1 mM for 60 min at 37 °C. Each column represents the mean ±S.E.M. of five to six determinations. *P<0.05, significantly different from the value for control.

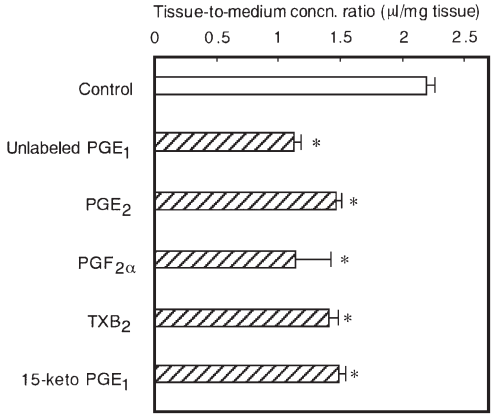

Fig. 4. Effects of various eicosanoids on [^{3}H]PGE_1 uptake by renal cortical slices from rats. Renal cortical slices were incubated with the medium including [^{3}H]PGE_1 (1 nM) in the absence (control) or presence of the indicated eicosanoids (100 μM) for 60 min at 37 °C. Each column represents the mean± S.E.M. of three determinations. *P<0.05, significantly different from the value of control.

PGE_1/glutarate exchanger is involved in the uptake of [^{3}H]PGE_1 by renal cortical slices.

3.6. Effects of various dicarboxylates on [^{3}H]PGE_1 uptake by renal cortical slices

We next studied the effects of aliphatic dicarboxylates with different carbon atoms (3–10 carbon atoms) on [^{3}H]PGE_1 uptake by renal cortical slices (Fig. 7). For dicarboxylates with 3–6 carbon atoms, inhibitory effects on [^{3}H]PGE_1 uptake by renal cortical slices became more potent as the number of carbon atoms increased, reaching a maximum when adipate (6 carbon atoms) was used. For molecules with more than 6 carbon atoms, the inhibition grew weaker as the number of carbon atoms increased. However, the inhibitory effect on [^{3}H]PGE_1 uptake by sebacate (10 carbon atoms) tended to be stronger than by azelate (9 carbon atoms).

3.7. Effects of various compounds on [^{3}H]PGE_1 uptake by renal cortical slices

The effects of various compounds on the uptake of [^{3}H]PGE_1 were also examined (Fig. 8). Substrates and/or inhibitors for OAT1 such as indomethacin, glibenclamide, tolbutamide and chlorpropamide significantly decreased [^{3}H]PGE_1 uptake by renal cortical slices. In addition, substrates and/or inhibitors of OAT3 such as cimetidine, benzylpenicillin, estrone-3-sulfate, taurocholate, cholate and sulfobromophthalein also significantly inhibited the transport of [^{3}H]PGE_1 by renal cortical slices. Tetraethylammonium, a substrate of OCT1 and OCT2,

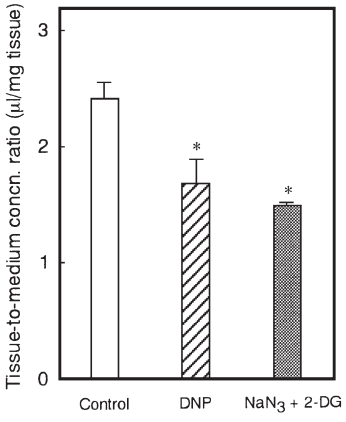

Fig. 3. Effects of metabolic inhibitors on [^{3}H]PGE_1 uptake by renal cortical slices from rats. After preincubation of renal cortical slices for 10 min with the medium in the absence (control) or presence of 1 mM 2,4-dinitrophenol (DNP) or 10 mM sodium azide (NaN_3) in combination with 20 mM 2-deoxy-D-glucose (2-DG), the slices were incubated with the medium including [^{3}H]PGE_1 (1 nM) without or with metabolic inhibitors for 60 min at 37 °C. Each column represents the mean±S.E.M. of three determinations. *P<0.05, significantly different from the value of control.

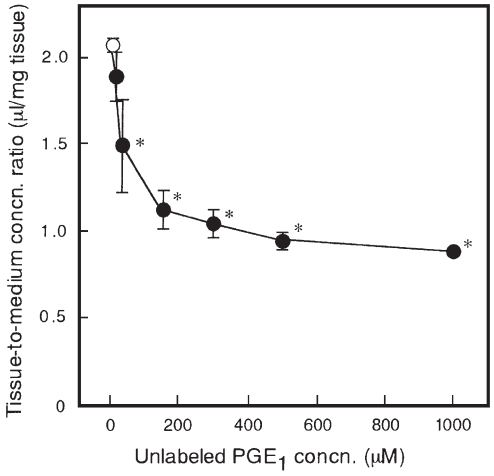

Fig. 5. Concentration-dependence of [^{3}H]PGE_1 uptake by renal cortical slices from rats. Renal cortical slices were incubated with the medium including [^{3}H]PGE_1 (5 nM) in the absence or presence of various concentrations of unlabeled PGE_1 (10, 30, 150, 300, 500 and 1000 μM) for 20 min at 37 °C. Each symbol represents the mean±S.E.M. of three determinations. *P<0.05, significantly different from the value of control.

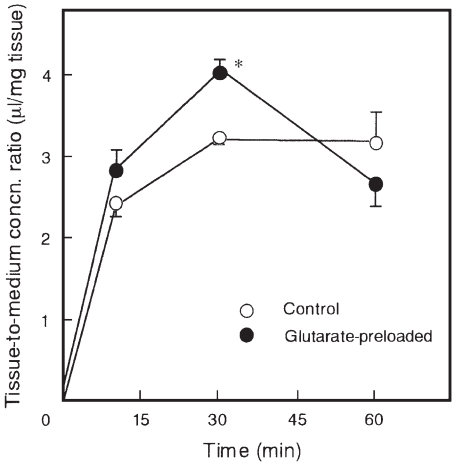

Fig. 6. Uptake of [^{3}H]PGE$_1$ by glutarate-preloaded slices from rat renal cortex. After preincubation of renal cortical slices with the medium without (control) or with 1 mM glutarate for 30 min, the slices were incubated with the medium including [^{3}H]PGE$_1$ (1 nM) for 10, 30 and 60 min at 37 °C. Each symbol represents the mean±S.E.M. of three determinations. *P<0.05, significantly different from the value of control at the same incubation period.

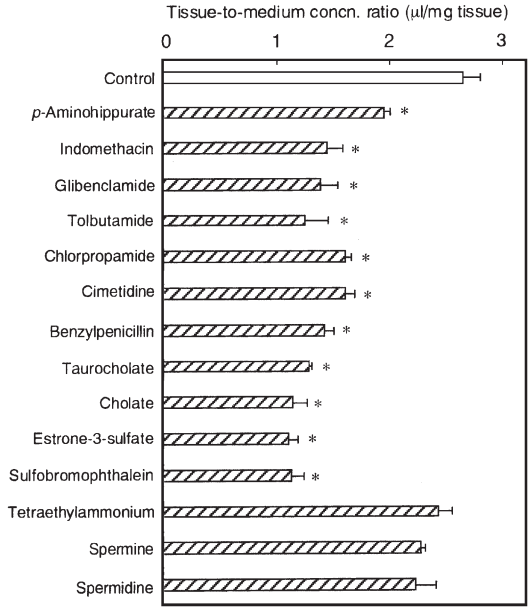

Fig. 8. Effects of various compounds on [^{3}H]PGE$_1$ uptake by renal cortical slices from rats. Renal cortical slices were incubated with the medium including [^{3}H]PGE$_1$ (1 nM) in the absence (control) or presence of 1 mM *p*-aminohippurate, indomethacin, glibenclamide, tolbutamide, chlorpropamide, cimetidine, benzylpenicillin, taurocholate, cholate, estrone-3-sulfate, sulfobromophthalein, tetraethylammonium, spermine and spermidine for 60 min at 37 °C. Each column represents the mean±S.E.M. of three to six determinations. *P<0.05, significantly different from the value of control.

as well as polyamines such as spermine and spermidine did not inhibit [^{3}H]PGE$_1$ transport by renal cortical slices.

Next, we attempted to estimate the inhibitory effects of relatively specific inhibitors for rOAT1 (indomethacin) and rOAT3 (benzylpenicillin and taurocholate) on the specific PGE$_1$ uptake by rat renal cortical slices. The specific PGE$_1$ uptake was evaluated by subtracting the tissue-to-medium concentration ratio in the presence of unlabeled 1 mM PGE$_1$, which would reflect a non-specific uptake and/or binding of [^{3}H]PGE$_1$ to the renal cortical slices (Fig. 5), from that in the absence of unlabeled 1 mM PGE$_1$. Indomethacin (1 mM) inhibited the specific uptake of PGE$_1$ by 84.3%, while 1 mM benzylpenicillin and taurocholate inhibited the specific uptake of PGE$_1$ by 80.3% and 90.5%, respectively (Fig. 9). We also examined the effect of salicylate, a substrate for rOAT2 (Sekine et al., 1998), on the specific uptake of PGE$_1$. Unexpectedly, salicylate inhibited the specific uptake of PGE$_1$ by 55.4% (Fig. 9).

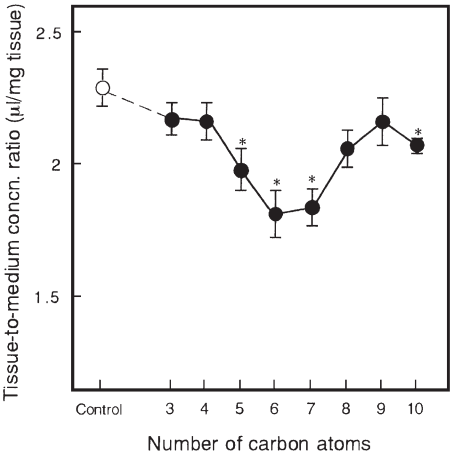

Fig. 7. Effects of various dicarboxylates on [^{3}H]PGE$_1$ uptake by renal cortical slices from rats. Renal cortical slices were incubated with the medium including [^{3}H]PGE$_1$ (1 nM) in the absence (control) or presence of 1 mM dicarboxylate (HOOC–(CH$_2$)$_x$–COOH) with 3 (malonate), 4 (succinate), 5 (glutarate), 6 (adipate), 7 (pimelate), 8 (suberate), 9 (azelate) and 10 (sebacate) carbon atoms for 60 min at 37 °C. Each symbol represents the mean±S.E.M. of five to nine determinations, *P<0.05, significantly different from the value of control.

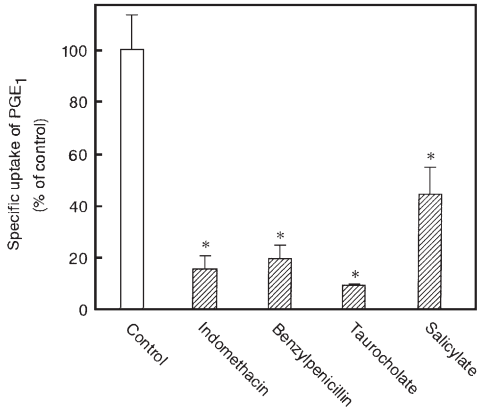

Fig. 9. Effects of indomethacin, benzylpenicillin, taurocholate and salicylate on the specific uptake of PGE$_1$ by renal cortical slices from rats. The specific PGE$_1$ uptake for 60 min at 37 °C was evaluated by subtracting the tissue-to-medium concentration ratio in the presence of unlabeled 1 mM PGE$_1$ from that in the absence (control) or presence of indomethacin, benzylpenicillin, taurocholate and salicylate at a concentration of 1 mM. Each column represents the mean±S.E.M. of three determinations. *P<0.05, significantly different from the value of control.

4. Discussion

In the present study, we investigated the transport of PGE_1 by slices from renal cortex, where the accumulation of [^{3}H]PGE_1 60 s after an intravenous bolus injection was greatest among various tissues examined. Consistent with a decrease in accumulation of [^{3}H]PGE_1 in renal cortex by probenecid and BCG under in vivo conditions, in vitro renal cortical slice uptake of [^{3}H]PGE_1 was also significantly inhibited by those compounds. In addition, metabolic inhibitors such as 2,4-dinitrophenol and sodium azide in combination with 2-deoxyglucose had an inhibitory effect on [^{3}H]PGE_1 uptake by renal cortical slices. These observations suggest that the renal cortical accumulation of [^{3}H]PGE_1 is mediated by a transporter system expressing in the renal proximal tubules.

Recently, a variety of cloned transporters have been shown to transport eicosanoids (Schuster, 1998; Masuda et al., 1999; Inui et al., 2000; Cattori et al., 2001). Prostaglandin transporter (PGT) is the first cloned transporter found to transport prostaglandins, including PGE_1, PGE_2, $PGF_{2\alpha}$, and TXB_2 (Kanai et al., 1995). PGT mRNA in rat, human and mouse is broadly expressed, suggesting that PGT is involved in the release of newly synthesized prostaglandins and/or the uptake of the released prostaglandins (Kanai et al., 1995). In the kidney, PGT mRNA is most abundant in the renal papilla (Kanai et al., 1995). In addition, immunoblot and immunohistochemical analyses of PGT revealed that the rank order of the zonal distribution is renal cortex < renal medulla ≈ renal papilla and that expression of PGT in the renal proximal tubules is negative (Bao et al., 2002). In contrast, the uptake of [^{3}H]PGE_1 was higher in the renal cortex than in the renal medulla, as shown in Fig. 2. Like the tubular transport of prostaglandins in the perfused kidney (Rennick, 1977; Bito and Baroody, 1978), the transport of PGE_1 by renal cortical slices was inhibited by *p*-aminohippurate, indomethacin and probenecid, which had no effect on PGT-mediated $PGF_{2\alpha}$ uptake into HeLa cells (Kanai et al., 1995). Therefore, it is unlikely that PGT plays an important role in the basolateral uptake of prostaglandins in the renal proximal tubules.

Irish (1979) showed that secretion of PGE_2 is higher in the S_2 segment of the proximal straight tubule than the S_1 or S_3 segment. Thus, the transport activity of PGE_2 was very similar to that of *p*-aminohippurate along the renal tubule (Woodhall et al., 1978). In 1997, the *p*-aminohippurate transporter from rat kidney (rOAT1), predominantly expressed in the kidney, was independently isolated by two groups (Sekine et al., 1997; Sweet et al., 1997). Subsequently, Tojo et al. (1999) showed that rOAT1 is exclusively localized to the basolateral membrane of S_2 segment of rat renal proximal tubules. Functional studies using rOAT1-expressing oocytes revealed that rOAT1 is a *p*-aminohippurate/dicarboxylate exchanger and transports various compounds such as methotrexate, cAMP, urate and PGE_2.

Therefore, we looked for involvement of rOAT1 in [^{3}H]PGE_1 transport by renal cortical slices. First, [^{3}H]PGE_1 uptake by renal cortical slices preloaded with glutarate was compared to that without glutarate. As shown in Fig. 6, preloading renal cortical slices with 1 mM glutarate significantly increased [^{3}H]PGE_1 uptake by the slices. This was consistent with previous studies showing that glutarate stimulated the renal cortical accumulation of *p*-[^{14}C]aminohippurate (Pritchard, 1990). This finding suggests that PGE_1 uptake by renal cortical slices is, at least in part, mediated by PGE_1/dicarboxylate exchange like *p*-aminohippurate/dicarboxylate exchange.

The effects of various aliphatic dicarboxylates with various carbon chain lengths on [^{3}H]PGE_1 uptake by renal cortical slices were examined. It has been reported that the inhibitory effects of various dicarboxylates with 3–10 carbon atoms on the transport of *p*-aminohippurate show a similar and characteristic pattern in rat renal proximal tubules (Fritzsch et al., 1989), OK kidney epithelial cell line (Nagai et al., 1995) and rOAT1-expressing *Xenopus* oocytes (Uwai et al., 1998). Briefly, the *p*-aminohippurate uptake is not substantially inhibited by dicarboxylates with 3 (malonate) or 4 (succinate) carbon atoms, but is strongly inhibited by those with 5 (glutarate) or 6 (adipate) carbon atoms. The inhibition became weaker with 7 carbon atoms (pimelate) and stronger again with increasing number of carbon atoms. In this study, the inhibition pattern of [^{3}H]PGE_1 uptake by renal cortical slices was basically similar to the above-mentioned pattern of *p*-aminohippurate though it was slightly shifted to the right compared to the pattern of *p*-aminohippurate. Thus, these observations may also suggest that the members of the OAT family is, at least in part, involved in [^{3}H]PGE_1 uptake by renal cortical slices.

Distinct differences in substrate recognition among members of the OAT family have been reported. For example, indomethacin is a potent inhibitor of rOAT1, whereas the inhibitory effect on rOAT3-mediated uptake is very weak (Sekine et al., 1997; Kusuhara et al., 1999). In contrast, the affinity of benzylpenicillin for rOAT1 is much lower than that for rOAT3 (Sekine et al., 1997; Kusuhara et al., 1999). In addition, taurocholate inhibits rOAT3-mediated uptake of [^{3}H]estrone-3-sulfate but not rOAT1-mediated uptake of *p*-[^{14}C]aminohippurate (Uwai et al., 1998; Kusuhara et al., 1999). In this study, indomethacin, benzylpenicillin and taurocholate markedly decreased [^{3}H]PGE_1 uptake by renal cortical slices, indicating that both rOAT1 and rOAT3 may be involved in the uptake of [^{3}H]PGE_1 by renal cortical slices. However, we found it difficult to estimate the relative contribution of OAT1 and OAT3 to the uptake of PGE_1 by employing these inhibitors, because each inhibitor for OAT1 and OAT3 alone inhibited the specific uptake of PGE_1 by more than 80%. Recently, Sweet et al. (2003) demonstrated that not only rOAT1 but also rOAT3 is an organic anion/dicarboxylate exchanger that is indirectly coupled to the sodium gradient through Na^+/dicarboxylate cotransporter. Further experiments employing more specific inhibitors for these transporters are needed to determine the relative contribution of OAT1 and OAT3 to the basolateral PGE_1 transport. Unexpectedly, salicylate, a substrate for rOAT2, inhibited the specific uptake of PGE_1 by 55.4%. Since OAT2 mRNA is detected in rat kidney (Sekine et al., 1998), the possible involvement of rOAT2, at least in part, cannot be ruled out either.

In conclusion, [^{3}H]PGE_1 was taken up by renal cortical slices in an energy dependent process, The uptake of [^{3}H]PGE_1 was stimulated by preloading the renal cortical slices with glutarate. Various compounds that inhibit rOAT-mediated transport decreased [^{3}H]PGE_1 uptake by renal cortical slices. These observations suggest that the basolateral transport of PGE_1 in rat renal proximal tubules is mediated by the members of the OAT family.

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