สกว

รายงานวิจัยฉบับสมบูรณ์

โครงการ

การปฏิสัมพันธ์ของพรอสทาแกลนดินกับตัวขนถ่ายออร์กานิกแอนไอออน 1 ที่ท่อไตของหนูขาว

Interaction of Prostaglandins with rat Renal Organic Anion Transporter 1

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ผศ. ดร. สุรวัฒน์ จริยาวัฒน์

ภาควิชาสรีรวิทยา คณะวิทยาศาสตร์

มหาวิทยาลัยมหิดล

(1 กรกฎาคม 2545- 30 มิถุนายน 2546)

สัญญาเลขที่ TRG 4580004

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สนับสนุนโดย

ทุนส่งเสริมนักวิจัยรุ่นใหม่

สำนักงานกองทุนสนับสนุนการวิจัย

กิตติกรรมประกาศ

งานวิจัยครั้งนี้ได้รับการสนับสนุนจากบุคคลและหน่วยงานที่สำคัญดังนี้

1. ศ.เกียรติคุณ พรชัย มาตังคสมบัติ อดีตคณบดีคณะวิทยาศาสตร์ มหาวิทยาลัยมหิดลที่ได้สนับสนุนในเรื่องครุภัณฑ์ในการเลี้ยงกบ *Xenopus laevis*
2. Professor Dr. Hitoshi Endou แห่ง Department of Pharmacology and Toxicology, Kyorin University School of Medicine, Tokyo, JAPAN ที่ได้ให้ *Xenopus laevis* รวมทั้งอุปกรณ์เครื่องมือบางอย่างที่จำเป็นในการวิจัย
3. รศ.ดร. สมัยศึก โสภาสรรค์ แห่งภาควิชาสรีรวิทยา คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล ที่เป็นนักวิจัยที่ปรึกษาได้ให้คำแนะนำตลอดการวิจัยนี้
4. สำนักงานกองทุนสนับสนุนการวิจัยที่ได้สนับสนุนการวิจัยตลอดระยะเวลา 1 ปี
5. หน่วยงานต้นสังกัดคือ ภาควิชาสรีรวิทยา คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล ที่ได้ให้การสนับสนุนการวิจัยนี้มาตลอด

ผศ.สุรวัฒน์ จริยาวัฒน์

บทคัดย่อ

รหัสโครงการ: TRG4580004

โครงการ: การปฏิสัมพันธ์ของพรอสทาแกลนดินกับตัวขนถ่ายออร์กานิกแอนไอออน 1 ที่ท่อไตของหนูขาว

นักวิจัย: ผศ. ดร. สุรวัฒน์ จริยาวัฒน์

ภาควิชาสรีรวิทยา คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล

E-mail Address: scsjr@mahidol.ac.th

ระยะเวลาโครงการ: 1 กรกฎาคม 2545 – 30 มิถุนายน 2546

การกำจัดสารเป็นหน้าที่ที่สำคัญอันหนึ่งของไต มีรายงานว่าพรอสทาแกลนดินจะถูกกำจัดทิ้งที่ไต เนื่องจากลักษณะโครงสร้างของพรอสทาแกลนดินเป็น organic acid ดังนั้นการขับทิ้งของพรอสทาแกลนดินจะถูกขับออกโดยใช้โปรตีนที่ทำหน้าที่ขนส่งสารอินทรีย์ประจุลบ (organic anion transporter , OAT) จากการศึกษาก่อนหน้านี้พบว่า พรอสทาแกลนดิน อีทู (PGE_2) ถูกขนส่งโดยโปรตีนที่ทำหน้าที่ขนส่งสารอินทรีย์ประจุลบ (hOAT)และโปรตีนที่ทำหน้าที่ขนส่งสารอินทรีย์ประจุบวกที่โคลนได้จากไตคน (hOCT) ซึ่งนำเอาโปรตีนนี้ไปแสดงออก (express) ในเซลล์ของท่อไตส่วนต้น

ในการศึกษาครั้งนี้ได้นำเอา OAT1 ที่โคลนได้จากไตหนูนำไปแสดงออกในไข่กบซีโนปุส เลวิส (*Xenopus laevis*) ศึกษาการปฏิสัมพันธ์ของสารพรอสทาแกลนดินพบว่า สารพรอสทาแกลนดิน ดีทู (PGD_2), อีวัน (PGE_1), อีทู (PGE_2), 6-คีโต-พรอสทาแกลนดินเอฟวันแอลฟา (6-keto-$PGF_{1\alpha}$) และทรอมบ๊อกซานบีทูสามารถยับยั้งการขนส่งของ [^{14}C]*p*-aminohippurate (PAH) เข้าไปในเซลล์ไข่กบ ซึ่งกลไกการยับยั้งนี้เป็นแบบ competitive และได้ค่า inhibition constant (Ki) ของ PGD_2, PGE_1, PGE_2, 6-keto-$PGF_{1\alpha}$ และ TXB_2 ตามลำดับดังนี้ 51, 54, 36, 251, 235 μM นอกจากนี้ rOAT1 ยังสามารถขนส่งพรอสทาแกลดินอีทูที่ติดฉลากสารรังสีได้ ([^{3}H]PGE_2)โดยได้ค่า K_m 37 μM และ V_{max} 16 pmol/h/oocyte ในการทดลอง efflux พบว่าพรอสทาแกลดินอีทูที่ความเข้มข้น 300, 100, 50 และ 10 μM สามารถกระตุ้น efflux ของ [^{14}C] PAH ได้ ในขณะที่พรอสทาแกลนดินอีทูที่ความเข้มข้นสูง 1 mM ไปยับยั้งการ efflux นี้ การศึกษาครั้งนี้สรุปได้ว่า rOAT1 สามารถจับกับพรอสทาแกลนดินที่ทดสอบได้และสามารถขนส่ง PGE_2 ได้ ดังนั้น rOAT1 ซึ่งน่าจะเป็นโปรตีนตัวหนึ่งของไตที่มีบทบาทสำคัญในการกำจัดสารพรอสทาแกลนดินออกจากร่างกายได้

คำหลัก: พรอสทาแกลนดิน, ตัวขนถ่ายออร์กานิกแอนไอออน 1, ไข่กบซีโนปุส เลวิส

ABSTRACT

Project Code: TRG4580004

Project Title: The interaction of prostaglandins with rat renal organic anion transporter 1

Investigator: Assistant Prof. Dr. Surawat Jariyawat

Department of Physiology, Faculty of Science, Mahidol University.

E-mail address: scsjr@mahidol .ac.th

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Renal excretion is known to be an important pathway for elimination of prostaglandins (PGs). Being organic acid, PGs are handed by renal organic anion transporters (OAT). PGE_2 has been shown to be transported by human organic anion transporter (hOAT) and organic cation transporter (hOCT) expressed in the proximal tubular cell. In the present study using *Xenopus laevis* oocytes, the interaction of PGD_2, PGE_1, PGE_2, 6-keto-$PGF_{1\alpha}$ and Thromboxane (TX) B_2 with rat OAT1 was investigated. These PGs inhibited markedly and competitively the uptake of [^{14}C]*p*-aminohippurate (PAH) via rOAT1. The inhibition constant (K_i) for PGD_2, PGE_1, PGE_2, 6-keto-$PGF_{1\alpha}$ and TXB_2 were 51, 54, 36, 251, 235 μM, respectively. [^{3}H]PGE_2 was shown to be transported by rOAT1 in a dose dependent manner with a K_m of 37 μM and a V_{max} of 16 pmol/h/oocyte. PGE_2, at 300, 100, 50, and 10 μM stimulated [^{14}C]PAH efflux, however at higher concentration of 1 mM, the efflux is inhibited. These data indicate that rOAT1 may contribute to prostaglandin uptake at the basolateral membrane of proximal tubular cells.

Keywords: prostaglandins, rat renal organic anion transporter 1, *Xenopus laevis* oocyte,

บทนำ

From several reports demonstrated that prostaglandins (PGs) and related compounds are excreted by the kidneys (Samuelsson B, 1964). PGs secretion were demonstrated as carrier-mediated (Bito, 1976) because PGs predominate as the charged organic anion at physiological pH.

Urinary excretion of PGs were suggested to be mediated by organic anion system. In vivo experiment, PGs were transported by active uphill transport across the basolateral membrane of the renal tubular cell and this transport was inhibited by probenecid, an inhibitor of organic anion transporter (Rennick, 1977). Study in isolated perfused rabbit proximal tubules showed net secretion of PGE_2 in S_2 segment of proximal tubule and was inhibited by probenecid and other organic anion (Irish, 1979). Likewise, in vivo stop-flow peritubular capillary microperfusion technique, demonstrated that various PGs inhibit *p*- aminohippurate (PAH) uptake in the proximal tubules: in addition radiolabelled uptake was also illustrated (Ullrich, 1991). These results suggest that PGs is secreted into urine by PAH transport system, a classical organic anion transporter, of proximal tubule.

Recently, several cDNA encoding organic anion transporter (OAT) family have been successively cloned including rOAT1, rOAT2, rOAT3 (Sekine et. al., 1997, 1998; Sweet et al., 1997; Kusuhara et. al., 1999). Although they transport a typical organic anion, PAH, and have broad substrate specificities, only rOAT1 has been characterized as the predominant PAH transporter corresponding to that across basolateral membrane (PAH/α-ketoglutarate exchanger).

Although, PGE_2 and PGF_{2a} were shown to be mediated by human organic anion transporter (hOAT1-4) and human organic cation transporter (hOCT1-2) expressing in proximal tubular cells (Kimura et al., 2002), the other prostanoid should be identified in different system and different species. Species difference in substrate selectivity should be studied to predict in vivo kinetic profile of PGs from in vitro data.

In the present study, we investigated the interaction of various prostanoids and the transport of PGE_2 in rOAT1 expressed in *Xenopus laevis* oocytes. Furthermore, we studied the transport properties of PGE_2 and rOAT1 as an exchanger.

วัตถุประสงค์

1. To investigate whether prostaglandin could interact with OAT1 as with the PAH transport system in the proximal tubule.
2. To investigate the kinetic of interaction between OAT1 and prostaglandin to better understand the nature of this interaction.
3. To investigate whether prostaglandin could not only interact with but also be transport by this exchanger transporter.

วิธีการทดลอง

Materials

[^{14}C]*p*-aminohippuric acid (1.50 Gbq/mmol) and [^{3}H]Prostaglandin E_2 (1961 GBq/mmol) were purchased from Du Pont NEN (Boston, MA, USA). Collagenase was purchased from Boerhringer Mannheim (Indianapolis, IN). All other compounds used in the present study were purchased from Sigma Chemical Co., Ltd. (St. Louis, MO, USA).

cRNA synthesis

Capped cRNAs for rOAT1 was synthesized in vitro using T7 RNA polymerase as decribed elsewhere (Sekine et al.,1997).

Oocyte isolation

Xenopus laevis oocytes were digested in OR2 solution containing 82.5 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 5 mM HEPES at pH 7.5, and 1.5 mg/ml collagenase for 30 to 40 min. at room temperature. After digestion, the oocytes were defolliculated and kept in ND96 medium containing 96 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2.2H_2O$, 1 mM $MgCl_2.6H_2O$, 5 mM HEPES at pH 7.4 and room temperature until cRNA injection, which was performed on the same day.

Oocyte injection

The defolliculated oocytes was injected with rOAT1-cRNA and the control oocytes with 50 nl of distilled water. After injection, the oocytes were incubated in ND96 medium supplemented with gentamicin 0.05 mg/ml at 18 ^{O}C for 2-3 days.

Transport and inhibition studies

Two to three days after cRNA injection, transport and inhibition studies were performed. The oocytes were transferred to ND96 solution containing a radiolabeled substrate [^{14}C]*p*-aminohippuric acid or [^{3}H] Prostaglandin E_2 were incubated with or without non-radiolabeled drugs for 1hr. For inhibition studies, PG was first dissolved in 70% ethanol, and diluted to 1 mM in ND96 solution. The incubation was terminated by adding ice-cold ND96 solution and the oocytes were washed 5 times with ice-cold ND96 solution. Each oocyte was transferred to a scintillation vial and solubilized in 0.2 ml of 10% SDS. After the addition 2 ml liquid scintillation, the radioactivity in each oocyte was counted with a liquid scintillation counter.

Kinetic analysis.

The oocytes expressing rOAT1 were incubated for 1 h. in ND96 solution with various concentrations of PAH in the presence or absence of prostanoids. A Lineweaver-Burk plot was used to evaluate the type of inhibition. The Ki value for each PG was calculated from the following equation Ki = concentration of PG/{(Km-PAH with inhibitor/Km-PAH without PG)-1}

Efflux experiment

After preincuabtion with 1 mM glutarate for at least 2 h., oocytes injected with rOAT1 and rNaDC1 cRNA were incubated in 50 μM [^{14}C] PAH for 2 h. Then the oocytes were washed 5 times in ice-cold ND96 solution and transferred to wells containing 300 μl of ND96 solution with or without PGE_2. After 90 min of incubation, 250 μl of the incubation medium was removed from each well and the same volume of 20% SDS was added. These oocytes were also transferred to vial and solubilized with 10%SDS. The efflux of [^{14}C]PAH was expressed as a percentage of totally accumulated [^{14}C]PAH {effluent count/ (effluent + oocyte) count}.

ผลการทดลอง

1. To investigate whether the prostaglandins interact with rOAT1

Figure 1 shows inhibitory effects of prostanoid on rOAT1-mediated [^{14}C]PAH uptake. The results show that 1 mM PGD_2, PGE_1, PGE_2, 6-keto PGF_{1a}, TXB_2, significantly inhibited rOAT1 mediated 2 μM [^{14}C]PAH uptake. The degree of inhibition of various prostanoids are nearly in the same potentcy. PGD_2, PGE_1, PGE_2 had strong inhibitory effect on rOAT1 mediated [^{14}C]PAH transport.

2. To investigate whether some of the above prostaglandins can inhibit PAH uptake competitively.

Kinetic analysis of prostanoid inhibition of [^{14}C]PAH uptake were studied. The double reciprocal plot are shown in figure 2. The inhibition constant (Ki) for PGD_2, PGE_1, PGE_2, 6-keto-$PGF_{1\alpha}$, TXB_2 were 51, 54, 36, 251, 235 μM, respectively.

3. To prove whether the prostaglandins can exchange with rOAT1.

To investigate whether PGE_2 also trans stimulate [^{14}C]PAH efflux, PGE_2 mediated [^{14}C]PAH efflux were studied. [^{14}C]PAH efflux was performed at PGE_2 concentration 10, 50, 100, 300 μM and 1 mM. PGE_2 at 300, 100, 50, and 10 μM stimulated [^{14}C]PAH efflux, however at higher concentration of 1 mM, the efflux is inhibited (figure 3).

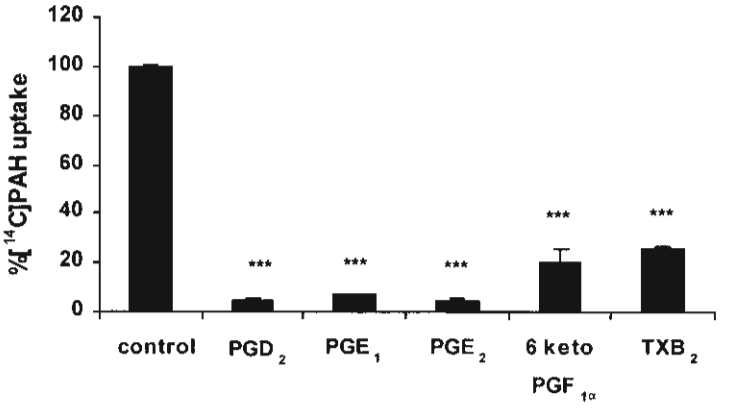
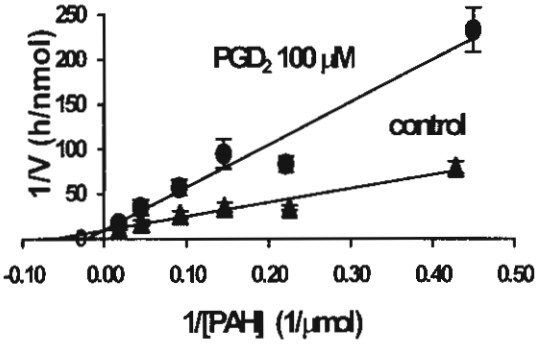
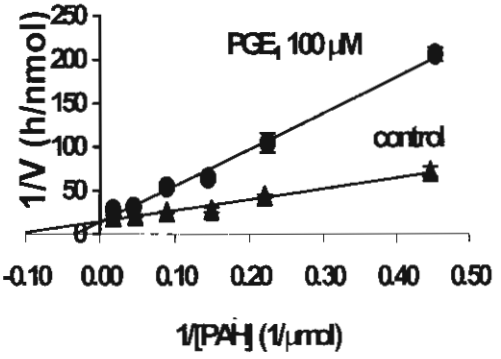
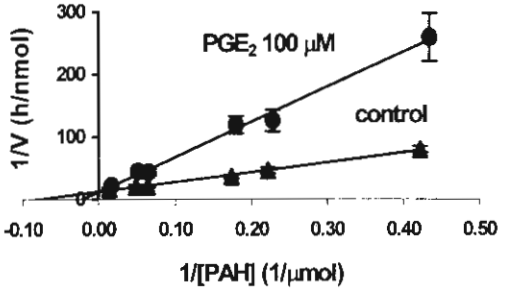
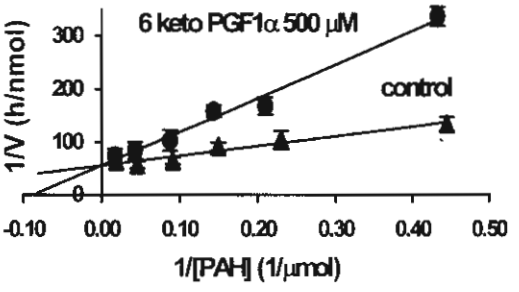


Fig 1. Inhibitory effect of various prostanoids on rOAT1-mediated [^{14}C]PAH uptake. Oocyte were incubated with 2 μM [^{14}C]PAH in the presence of 1 mM of prostanoid for 1 h. Values are expressed as percentage of the PAH uptake by rOAT1-expressing oocytes in the absence of inhibitors (mean ± S.E.M; 3-4 determinations of a representative experiment). ***P < .001 versus without inhibitor.









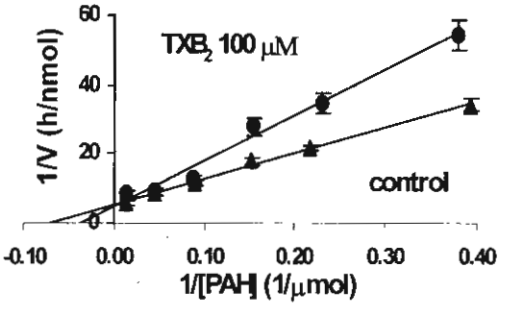


Fig 2. Kinetic analysis of inhibition study by PGD_2, PGE_1, PGE_2, 6-keto-$PGF_{1\alpha}$,TXB_2 of [^{14}C]PAH uptake in rOAT1 cRNA-injected oocytes. Double reciprocal plots of [^{14}C] PAH uptake in the presence or absence of prostaglandins. Oocytes were incubated with various concentration of [^{14}C]PAH for 1 h. Each point represents mean $\pm$ S.E.M.

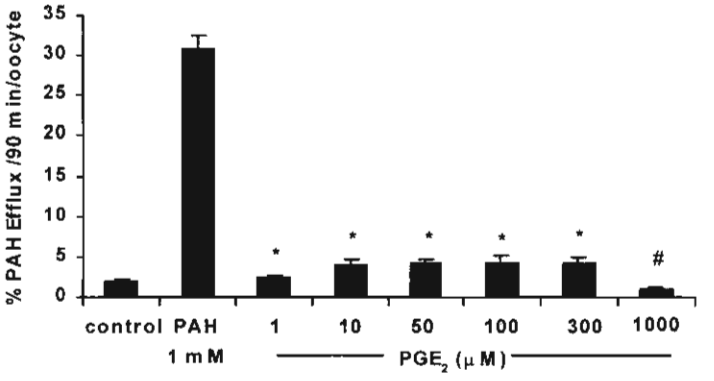


Fig 3. Dose dependents of PGE_2 induced PAH efflux. Oocytes expressing rOAT1 were loaded with 50 μM PAH for 2 h. The efflux of PAH was evaluated in the presence of increasing concentration of PGE_2. * indicates significant efflux above control value at $P < 0.05$. # indicates significant efflux below control value at $P < 0.05$.

บทวิจารณ์

The present study demonstrated that PAH transport via rOAT1 was inhibited by PGD_2, PGE_1, PGE_2, 6-keto-$PGF_{1\alpha}$ and TXB_2. The Ki values of PGD_2, PGE_1, PGE_2, 6-keto-$PGF_{1\alpha}$ and TXB_2 are similar to the results of the stop-flow peritubular capillary microperfusion technique (Ullrich et al., 1991) where Ki values of these prostanoid is 140, 140, 70, 770, and 360 μM, respectively. The comparison of inhibitory kinetics of rOAT1 with those obtained in the previous study on the renal organic anion transport pathway strongly suggest that rOAT1 plays roles for renal uptake of prostanoid at basolateral membrane of proximal tubular cell.

This study demonstrate that rOAT1-mediaed [^{3}H]PGE_2 transport follows Michaelis-Menten kinetics with Km of around 30 μM. The PGE_2 concentration in the arterial plasma of the resting state of the dog was around 0.5 nM (Hohenfellner et. al., 1989) and the value report in rat was around 1.5 nM (Schlondorff et. al., 1987). The affinity of transporter for prostaglandin should correlate well with the concentration in the extracellular fluid. At present prostaglandin transporter (PGT) seems to fit this prospect since the Michaelis-Menten constant (Km) of PGT-mediated PGE_2 transport is around 94 nM and PGT is also expressed in the kidney (Kanai et. al., 1995).

Previous study in vivo microperfusion study (Ullrich et. al., 1991) indicated that Km of [3H]PGE_2 transport in proximal tubule of rat was 610 μM which was between the apparent Ki value of PGE_2 to inhibit PAH and sulfate transport across renal proximal tubular basolateral membrane; 70 and 1100 μM espectively. This indicated that the expression of these two transport system in proximal tubules should be high enough to

mask the transport process of PGT in renal proximal tubules. This idea was supported by the data demonstrating that PGT mRNA expression in the kidney was least in the cortex (Kanai et. al., 1995).

The Ki and Km values of PGE_2 via rOAT1 is close to Ki value of PGE_2 to inhibit PAH transport in rat proximal tubules (Ullrich, 1991). This Km value may be high than PGE_2 concentration in extracellular fluid, high expression of rOAT1 at basolateral membrane of S2 segment of proximal tubule (Tojo et al., 1999) raises up the possibility that rOAT1 may be one of the important transporter for prostaglandin transport at basolateral membrane in renal proximal tubular cells though participation of other transporters can not be neglected.

So far, several OAT isoforms that is, OAT2, OAT3 and OAT4 (Cha et al, 2000) have been reported. HOAT1 and hOAT3 were shown to be transport PGE_2 and PGF_{2a} in the basolateral side of proximal tubule. Km value of PGs in rOAT1 expressing oocyte were higher than Km of that in hOAT1 expressed in S2 cells (Kimura et al., 2002). This discrepancy in Km value may be due to the species difference between rat and humans. The amino acid sequence of hOAT1 exhibited 86% homology compare with rOAT1 (Hosoyamada et al., 1999).

Prostaglandin transport in renal proximal tubular cell has an important function role since prostaglandin degrading enzyme; 15-prostaglandin dehydrogenase (15-PGDH) was shown to be located mainly in renal proximal tubular cell (Uchida et al., 1985). rOAT1 may be responsible for transport of prostaglandin from the blood into intracellular side of proximal tubular cell where 15-PGDH exists.

At luminal side, there are several transporter mediate organic anion transport including OAT-K1 (Saito et al., 1996), OAT-K2 (Masuda et al., 1999), organic anion-transporting peptide 1 (Jacquemin et al., 1994), Mrp2 (Leier et al., 2000) and hOAT4 (Cha et al., 2000). There are two transporter were reported to transport PGs at luminal side. OAT-K2 mRNA, expressed predominantly in the proximal convoluted tubules, proximal straight tubules, and cortical collecting duct, stimulated the uptake of hydrophobic organic anions, such as taurocholate, methotrexate, folate, and prostaglandin E_2. Furthermore, OAT-K2 was suggested to function as bidirection organic anion transporter in the apical membrane. HOAT4 mediatede uptake of PGE_2 and $PGF_{2\alpha}$. Since hOAT4 mediated the efflux of estrone sulfate, it is possible that hOAT4 mediates the bidiractional transport of PG on the apical site of the proximal tubule. However, further study should be performed to elucidate the role of other apical transporter.

The further study is to characterized the transport properties of PGE_2 by rOAT1. The exchange of extracellular PGE_2 with the intracellular [^{14}C] PAH was done in efflux experiment. At lower concentration of PGE_2, [^{14}C]PAH efflux was stimulated with nearly the same degree. At a high concentration of PGE_2 in extracellular medium, [^{14}C]PAH efflux was inhibited. At a high concentration, PGE_2 may simply diffuse into intracellular compartment and compete with [^{14}C]PAH at intracellular binding site of rOAT1, cis-inhibition. This result may indicate the counter effect between intracellular cis-inhibition and extracellular trans-stimulation.

PGE_2 and PGD_2 have been postulated to mediate central sleep-wake cycles (Hayaishi, 1991). PGE_1 and PGE_2 were reported to increase body temperature

(Katzung,). PGE_2 are widely expressed in diverse structures of the rat brain (Matsumura, et. al., 1992). In this regard, rOAT1 was shown to be localized at the brain using northern blot analysis (Sekine et. al., 1997). In the present study, we found that rOAT1 mediated the transport of PGE_2. Thus, rOAT1 may play a role in the release, uptake and/or degradation of this prostanoids in the brain.

In conclusion, the present work suggest that rOAT1 interact with various prostaglandin including thomboxane, thus, rOAT1 may mediated the basolatral uptake of these prostaglandin and thomboxane in the proximal tubule. In addition, there was species difference in the affinity of prostaglandin between rOAT1 and hOAT1. The current results also provide important information for clinical application of prostaglandins. Therefore, concomitant administration of prostaglandin with other drug that share the same transporter for tubular excretion must be play attention.

หนังสืออ้างอิง

1. Bito LZ, Davson H, Salvador RV.. 1976 Inhibition of in vitro concentrative prostaglandin accumulation by prostaglandins, prostaglandin analogues and by some inhibitors of organic anion transport. J Physiol 256, 257-271.
2. Cha SH, Sekine T, Kusuhara H, Yu E, Kim YJ, Kim DK, Sugiyama Y, Kanai Y Endou H.. 2000. Molecular cloning and characterization of multispecific organic anion transport 4 expressed in the placenta. J Biol Chem 275, 4507-4512.
3. Hayaishi, O.. 1991. Molecular mechanisms of sleep-wake regulation: role of prostaglandins D_2 and E_2. FASEB J. 5, 2575-2581.
4. Hohenfellner RM, Rehbock J, Brechtelsbauer H, Klein G, Thurau K.. 1989. PGE_2 concentrations in renal venous plasma and renal lymph during basal and stimulated condition in the dog. Pfluegers Arch. 413, 673-675.
5. Hosoyamada M, Sekine T, Kanai Y, Endou H.. 1999. Molecular cloning and functional expression of a multispecific organic anion transporter from human kidney. Am J Physiol. 276, F122-F128.
6. Irish JM.. 1979. Secretion of prostaglandin E_2 by rabbit proximal tubules. Am J Physiol. 237, F268-F273.
7. Jacquemin E, Hagenbuch B, Stieger B, Wolkoff AW, and Meier PJ.. 1994. Expression cloning of a rat liver Na^+ -independent organic anion transporter. Proc. Natl. Acad Sci USA. 91, 133-137.
8. Kanai N, Lu R, Satriano JA, Bao Y, Wolkoff AW.. 1995. Identification and characterization of a prostaglandin transporter. Science 268, 866-869.

9. Kimura H, Takeda M, Narikawa S, Enomoto A, Ichida K, Endou H.. 2002. J Pharm Exp Ther 301, 293-298.

10. Kusuhara H, Sekine T, Utsunomiya-Tate N, Tsuda M, Kojima R, Cha SH, Sugiyama Y, Kanai Y, Endou H.. 1999. Molecular cloning and characterization of a new multispecific organic anion transporter from rat brain. J Biol Chem. 274, 13675-13680.

11. Leier I, Hummei-Eisenbeiss J, Cui Y and Keppler D.. 2000. ATP-dependnet para-amionohippurate transport by apical multidrug resistance protein MRP2. Kidney Int 57, 1636-1642.

12. Matsumura K, Watanabe Y, Imai-Matsumura K, Connolly M, Koyama Y, Onoe H.. 1992. Mapping of prostaglandin E_2 binding sites in rat brain using quantitative autoradiography. Bbrain Res 581, 292-298

13. Rennick BR.. 1977. Renal tubular transport of prostaglandins: inhibition by probenecid and indomethacin. Am J Physiol. 233, F133-F134.

14. Saito H, Masuda S, and Inui KI.. 1996. Cloning and characterization of anovel rat organic anion tranporter mediating basolateral uptake of methotrexate in the kidney J Biol Chem 272, 18526-18529.

15. Samuelsson B..1964. Prostaglandins and related factors: 27. Synthesis of tritium-labeled prostaglandin E_1 and studies on its distribution and excretion in the rat. J Biol Chem. 239, 4091-4096.

16. Schlondorff D, aynedjian HS, Satriano JA, Bank N.. 1987. In vivo demonstration of glomerular PGE_2 responses to physiological manipulations and experimental agents. Am J Physiol. 252, F717-F723.

17. Sekine T, Watanabe N, Hosoyamada M, Kanai Y, Endou H.. 1997. Expression cloning and characterization of a novel multispecific organic anion transporter. J Biol Chem. 272, 18526-18529.

18. Sekine T, Cha SH, Tsuda M, Apiwattanakul N, Nakajima N, Kanai Y, Endou H.. 1988. Identification of multispecific organic anion transporter 2 expressed predominant in the liver. FEBS *Lett* . 429,179-182.

19. Sweet DH, Wolff NA, Pritchard JB.. 1997. Expression cloning and characterization of ROAT1. The basolateral organic anion transporter in rat kidney. J Biol Chem. 272, 30088-30095.

20. Tojo A, Sekine T, Nakajima N, Hosoyamada M, Kanai Y, Kimura K, Endou H.. 1999. Immunohistochemical localization of multispecific renal organic anion transporter 1 in rat kidney. J Am Soc Nephrol. 10, 464-471.

21. Uchida S, Nonoguchi H, Endou H.. 1985. Localization and properties of NAD^+-dependent 15-hydroxyproataglandin dehydrogenase activity in the rat kidney. Pflugers Arch. 404, 278-284.

22. Ullrich KJ, Rumrich G, Papavassiliou F, Kloess S, Fritzsch G..1991. Contraluminal *p*-aminohippurate transport in the proximal tubule of the rat kidney. VII. Specificity:cyclic nucleotides, eicosanoids. Pfluegers Arch. 418, 360-370.

Output จากโครงการวิจัยที่ได้รับทุนจาก สกว.

1. ผลงานวิจัยนี้กำลังอยู่ในระหว่างเขียน manuscript เพื่อส่งไปตีพิมพ์ในวารสารต่างประเทศ

2. การนำผลงานวิจัยไปใช้ประโยชน์

- เชิงสาธารณะ

งานวิจัยนี้ได้เริ่มมาจากความร่วมมือระหว่างกลุ่มผู้วิจัย ภาควิชาสรีรวิทยา คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล และ Department of Pharmacology and Toxicology, Kyorin University School of Medicine, Tokyo, JAPAN โดยเฉพาะ Professor Dr. Hitoshi Endou ได้ให้ความอนุเคราะห์จัดส่งกบ Xenopus มาให้ จากการวิจัยนี้ได้เพิ่มความสัมพันธ์และความร่วมมือกันระหว่างกลุ่มนักวิจัยดังกล่าวข้างต้น

- เชิงวิชาการ

การวิจัยครั้งนี้มีส่วนช่วยให้นักศึกษาปริญญาโทและเอกของภาควิชาสรีรวิทยา คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล ได้เข้ามาเรียนรู้งานวิจัยด้านนี้ ก่อนที่จะไปเรียนรู้งานวิจัยใหม่ๆ ที่ Department of Pharmacology and Toxicology, Kyorin University School of Medicine, Tokyo, JAPAN.

1. cRNA preparation

The plasmid containing rOAT1 cDNA will be provided from Prof. Hitoshi Endou, our collaborator at Kyorin University School of Medicine, Tokyo, Japan. This clone will be used for plasmid extraction, purification, digestion and will be used as cDNA template for corresponding cRNA synthesis.

After the preparation of cDNA template, the corresponding cRNA will be synthesized. To prevent any contamination of RNase, all vial and micropipette are Rnase free. Procedure will be carried out under stringent condition to avoid contamination, as Rnase is quite stable and difficult to inactivate, whereas RNA is easily destroyed.

In vitro cRNA synthesis will be carried out using Ambion kit.. The synthetic reaction will be performed by adding following chemicals sequentially, 2X Ribonucleotide Mix, 10X Transcription buffer, linearized DNA template and 10X enzyme mix. The reaction will be incubated at 37 $^{\circ}$C for 1hr. Then the cDNA template will be eliminated by adding Rnase-free Dnase and further incubated at 37 $^{\circ}$C. for 15 min. The reaction will be stopped by adding 5M NH_4OAC and cRNA will be isolated by precipitation.

To precipitation cRNA, 100 µl of phenol/chloroform will be added to the reaction mixture. After vigorous mixing, the samples will be centrifuged at 12,000 rpm for 2 min. at room temperature. The supernatant phase (around 95 µl) will be transferred to another vial and 95 µl of chloroform/isoamyl alcohol will be added. The sample will be centrifuged at the same speed and temperature. The supernatant will be

precipitated by adding isopropanol. Precipipation will be performed at -20 °C for at least 24 h. After precipitation, cRNA pellet will be separated from the solution by centrifugation at 15,000 rpm at 4 °C for 20 min. The supernatant will be discarded and 70% ethanol (-20 °C) will be added to wash out the remaining salt. The supernatant will be discarded after centrifugation at 15,000 rpm at 4 °C for 15 min. The pellet will be dried by vacuum pump and dissolved with Rnase-free water 20 µl on ice for at least 10 min with periodic tapping. For determination of cRNA concentration, 1 µl of cRNA will be diluted with 300 µl distilled water. The absorption of diluted cRNA will be recorded by spectrophotometer from the wavelenght of 320 to 220 nm. The peak of absorption from the curve will be used for calculation of cRNA concentration. The disolved cRNA will be kept at -80 °C for oocyte injection. cRNA integrity will be confirmed by RNA gel eletrophoresis. Agarose RNA gel will show one band of cRNA at position size of that cRNA.

2. Microinjection of rOAT1 cRNA into oocyte

2.1 Glass micropipette preparation

Twenty cm long glass pipette will be pulled by micropipette puller yielding 2 glass micropipettes of 10 cm in length. Pulled pipette will be ground by micropipette grinder until the tip is 30-40 µm in diameter for cRNA microinjection and 15-20 µm for [^{14}C]. Ground glass pipettes will be washed using water and acetone. These grounded micropipettes will be made Rnase free by baking at 200 °C for at least 6 hr in the morning of the microinjected experiment.

2.2 rOAT1 cRNA microinjection

A glass micropipette will be back filled with mineral oil using a long flexible needle. During this step, care must be exercise to avoid the formation of air bubbles in the micropipette, as these bubbles will cause inaccuracy in microinjection. The oil filled micropipette will be mounted on to a Drummond microdispensor. Then rOAT1 cRNA will be loaded on to a parafilm and will be sucked into the micropipette by the Drummond microdispensor, which will be mounted on a Narishige micromanipulator.

3. *Xenopus laevis* oocyte preparation

3.1 *Xenopus laevis* operation

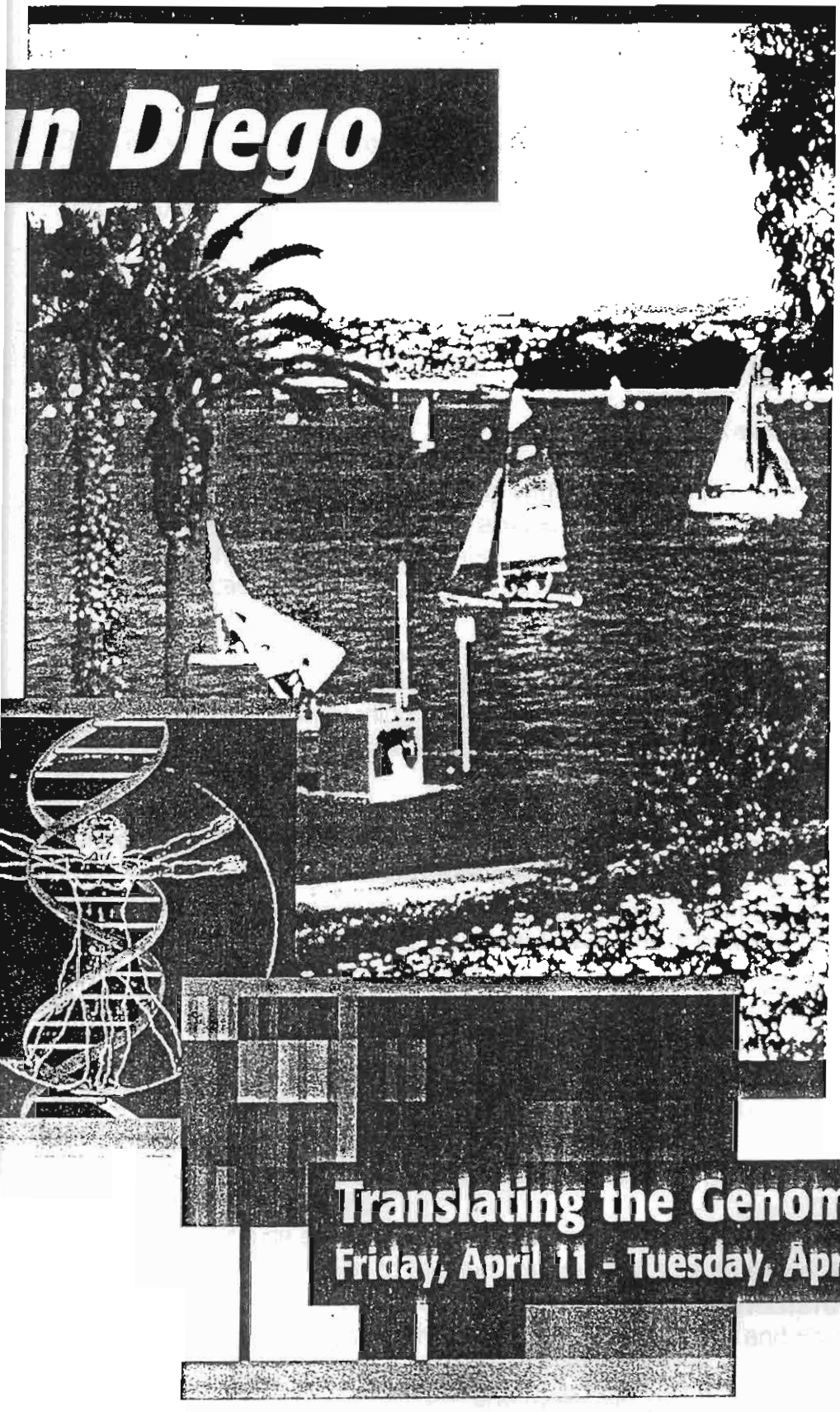
For each set of experiment, a frog will be anesthetized in solution containing 0.1% MS-222 and 0.3% $KHCO_3$ with pH 7.5 for 30-45 min. The frog will be placed on ice for operation. A lateral incision of 0.5-1 cm will be made on one side of the lower belly with the use of a sharp razor on the skin. The underlying muscle will be cut open with a scissors. From the abdomen, 4-5 of lobe or 2/3 of ovary will be harvested by removing with scissors to obtain oocytes. The remaining 1/3 of ovary will be returned to the abdominal cavity for regeneration of new oocytes. Both incisions, muscle and skin will be closed by suture. The operation procedure for frog must be careful and will be carried out under aseptic condition. The operated frog will kept in an isolated container until it completely recover from surgery, as assess by its activity. The frog is then returned to its storage tank. Frogs treated in this way usually recover without subsequent morbidity. In certain frogs, the oocytes can be repeatedly harvested up to 5 times with approximate 1 month interval

3.2 *Xenopus laevis* oocytes defolliculaltion

Harvested oocytes will be kept in ND96 solution, containing NaCl 96 mM, KCl 2 mM, $MgCl_2.H_2O$ 1 mM, HEPES 5 mM, $CaCl_2.2H_2O$ 1.8 mM pH 7.4 Each sag is carefully cut into small clumps, a rather time consuming procedure. This is to ensure the homogeneous enzymatic digestion of follicles. The small clumps will be placed in a vial containing OR2 solution (NaCl 92.5 mM, KCl 2 mM, $MgCl_2.H_2O$ 1 mM, HEPES 5 mM pH 7.5) and oocytes will be washed 5 time with this OR2 solution. Oocytes will be treated with collagenase 2 mg/ml in OR2 solution to partially digest the follicular layer. These oocytes will be mixed by rotating the bottle continuously for 40-50 min. At the end of digestion, oocytes will be washed with OR2 solution 4-5 times. These oocytes will be transferred to petri dish containing ND96 solution. Each oocyte will be manually separated from its surrounding follicle under stereomicroscope. The mature oocytes of stage V-VI about 1-1.2 mm in diameter will be selected and stored in ND96 solution at 18 $^{\circ}C$ till ready for microinjection of capped poly(A^+)RNA.

4. Maintenance of *Xenopus laevis*

The lab-conditional mature female *Xenopus laevis* (South African clawed frogs) will be maintained in glass tanks at 18 $^{\circ}C$. The temperature will be controlled at low temperature by intermittent pumping of chilled water through the lower tank. The frogs will be feed 2 times per week.



Experimental Biology 2003

April 11-15
San Diego, CA

Annual Meetings of:

American Association of Anatomists (AAA)

The American Physiological Society (APS)

American Society for Biochemistry and Molecular Biology (ASBMB)

American Society for Investigative Pathology (ASIP)

American Society for Nutritional Sciences (ASNS)

American Society for Pharmacology and Experiment Therapeutics (ASPET)

Guest Societies

Translating the Genome

Friday, April 11 - Tuesday, April 15

331 Renal Organic Solute Transport

Poster

Sun. 7:30 AM - 5:00 PM--Convention Center, Exhibit Hall

12:45 PM-3:00 PM.

D467 331.1 PKC and ERK1/2 regulate choline transport in cultured renal tubule cells. **A.R. Villalobos, L.K. Kroening and K.M. Kransler** Univ. of Rochester.

D468 331.2 Functional characterisation of endogenous organic cation transporters expressed by HeLa cells. **C.D. Brown and S. Chauhan** Univ. of Newcastle Med. Sch., UK.

D469 331.3 Multispecificity of rbOCT1 and rbOCT2. **S. Kaewmokul, C. Muanprasat and V. Chatsudthipong** Mahidol Univ., Thailand.

D470 331.4 The interaction of steviol with rabbit OCT1 and OCT2. **V. Chatsudthipong, A. Lungkaphin and S. Kaewmokul** Mahidol Univ., Thailand.

D471 331.5 Molecular determinants of substrate/inhibitor binding to the human renal organic cation transport, hOCT2. **W.M. Barendt and S.H. Wright** Univ. of Arizona Col. of Med.

D472 331.6 Multiple pathways for organic anion transport in suspensions of rabbit renal proximal tubules. **C. Groves and S.H. Wright** Univ. of Arizona Col. of Med.

D473 331.7 Molecular cloning and functional characterization of the rabbit organic anion transporter isoform 3. **X. Zhang and S.H. Wright** Univ. of Arizona Col. of Med.

D474 331.8 Human organic anion transporter 3 in human adrenocortical cells transports cortisol. **A.R. Asif, R.W. Grunewald, C. Langenberg, M. Metten, H. Jarry, G.A. M□ller, G. Burckhardt and Y. Hagos** Univ. of G□ttingen, Germany.

D475 331.9 Na^+-dependent, Oat3-mediated organic anion uptake by rat choroid plexus. **S. Lowes, D.B. Sykes, J.B. Pritchard and D.S. Miller** NIEHS, NIH, Research Triangle Park.

D476 331.10 Human organic anion transporter 3 can operate as an exchanger. **N. Bakhiya, A. Bahn, G. Burckhardt and N.A. Wolff** Georg August Univ., Goettingen.

D477 331.11 Organic anion transporter 3 [*Slc22a8*] is a dicarboxylate exchanger indirectly coupled to the Na^+ gradient. **D.H. Sweet, L.M.S. Chan, R. Walden, X-P. Yang, D.S. Miller and J.B. Pritchard** Med. Univ. of South Carolina and NIEHS, NIH, Research Triangle Park.

D478 331.12 Mouse organic anion transporter 3 (Slc22a8) is a dicarboxylate exchanger indirectly coupled to the sodium gradient. **L.M.S. Chan, D.H. Sweet, R. Walden, S.K. Nigam, D.R. Beier, D.S. Miller and J.B. Pritchard** NIEHS, NIH, Research Triangle Park, Med. Univ. of South Carolina, UCSD and Harvard Med. Sch., Brigham and Women's Hosp.

D479 331.13 Functional expression and molecular pharmacology of hNaDC1. **C. Smith, D. McCoy, M. Vaughan, A.M. Pajor, S. Kerner and S. Witherspoon** GlaxoSmithKline,.Vanderbilt Univ. and Univ. of Texas Med. Br..

D480 331.14 Multidrug resistance protein 4 (MRP4/ABCC4) and its functional comparison with MRP2/ABCC2 for transport of renal organic anions. **F.G.M. Russel, P.H.E. Smeets, M. Huls, A.C.M. Mulders, A.C. Wouterse, R. Masereeuw and R.A.M.H. van Aubel** Univ. Med. Ctr. Nijmegen, The Netherlands.

D481 331.15 Short-term gentamicin exposure to renal proximal tubule has long-term intracellular signaling consequences. **R. Masereeuw, S. Notenboom, L.H. Kuik, F.G.M. Russel and D.S. Miller** Univ. Med. Ctr. Nijmegen, The Netherlands and NIEHS, NIH, Research Triangle Park.

D482 331.16 Renal tubular transport of the anionic chelating agent: DMPS (2,3-dimercapto-1-propanesulfonate). **A. Lungkaphin, V. Chatsudthipong, K.K. Evans, S.H. Wright and W.H. Dantzler** Mahidol Univ. Col. of Med., Thailand and Univ. of Arizona.

D483 331.17 The interaction of prostaglandins with rat organic anion transporter 1. **S. Jariyawat, N. Apiwattanakul, T. Sekine, S. Sophasan and H. Endou** Mahidol Univ., Thailand and Kyorin Univ. Sch. of Med., Japan.

D484 331.18 Characterization and regulation of prostaglandin transporter in mouse fibroblast cell line, 3T3. **R. Lu and V. Schuster** Albert Einstein Col. of Med.

D485 331.19 Ascorbic acid-stimulated transport current in avian renal proximal tubule monolayers. **G. Laverty and D. Maurice** Univ. of Delaware and Clemson Univ.

D486 331.20 Structural and functional characteristics of two Na^+-coupled dicarboxylate transporters (ceINDY1 and ceINDY2) from *Caenorhabditis elegans* and their relevance to life span. **Y-J. Fei, K. Inoue and V. Ganapathy** Med. Col. of Georgia.

D487 331.21 The low affinity choline transporter is a member of the organic cation transporter family. **J. Stoll and C. Hoelting** Texas Tech Univ. Hlth. Sci. Ctr., Amarillo.

Program # 331.17

The interaction of prostaglandins with rat organic anion transporter 1

Surawat Jariyawat[1], Nopporn Apiwattanakul[1], Takashi Sekine[2], Samaisukh Sophasan[1], Hitoshi Endou[2]. [1]Department of Physiology, Mahidol University, Rama VI, Rajthavee, Bangkok 10400 Thailand, [2]Department of Pharmacology and Toxicology, Kyorin University School of Medicine, Mitaka, Tokyo Japan

Renal excretion is known to be an important pathway for elimination of prostaglandins (PGs). Being organic acid, PGs are handed by renal organic anion transporters (OAT). PGE_2 has been shown to be transported by human OAT1 expressed in the proximal tubular cell. In the present study using *Xenopus laevis* oocytes, the interaction of PGE_2, PGE_1, PGD_2, 6-keto $PGF_{1\alpha}$ and Thromboxane (TX) B_2 with rat OAT1 was investigated. These PGs inhibited markedly and competitively the uptake of [^{14}C] *p*-aminohippurate (PAH) via rOAT1. The inhibition constant (K_i) for PGE_2, PGE_1, PGD_2, 6-keto $PGF1\alpha$ and TXB_2 were 36, 54, 51, 251, 235 μM, respectively. [^{3}H] PGE_2 was shown to be transported by rOAT1 in a dose dependent manner with a K_m of 37 μM and a V_{max} of 16 pmol/h/oocyte. PGE_2, at 300, 100, 50, and 10 μM stimulated [^{14}C] PAH efflux, however at higher concentration of 1 mM, the efflux is inhibited. These data indicate that rOAT1 may contribute to prostaglandin uptake at the basolateral membrane of proximal tubular cells. *(Supported by Thailand Research Fund)*