6. H^+/有機カチオンアンチポータ（MATE/SLC47A）

京都大学医学部附属病院薬剤部　寺田智祐
同 教授　乾　賢一

key words　H^+/organic cation antiporter, renal secretion, brush-border membranes

動　向

腎臓における排泄は，糸球体濾過と尿細管分泌より構成されるが，薬物や代謝産物などの有機イオンは主に近位尿細管での分泌によって尿中に排泄される．近位尿細管での分泌過程は経上皮細胞的に進行し，血管側側底膜を介する細胞内への取り込みと，管腔側刷子縁膜を介する細胞内から管腔への放出という二段階の膜輸送によって支配されている[1,2]．たとえば，有機カチオンは側底膜の膜電位依存性有機カチオントランスポータによって細胞内に運ばれ，さらに刷子縁膜のH^+/有機カチオンアンチポータを介して能動的に管腔内へ分泌されることが知られている[3]．これまで，主に側底膜に発現する膜電位依存性の有機カチオントランスポータ（OCT1-3/SLC22A1-3）の構造，機能，発現に関する*in vitro*研究と，それらの分子情報を基盤とした臨床研究が進展してきた[4-8]．しかし，H^+/有機カチオンアンチポータの分子実体については未解明のまま残されており，その同定が待ち望まれていた．2005年に岡山大学の森山らのグループによって，大腸菌の多剤耐性に関与するmultidrug and toxin extrusion（MATE）の哺乳類ホモログとして，ヒト（h）およびマウス（m）のMATE1ならびにMATE2が同定された[9]．その後我々は，ラット(r)MATE1，hMATE1，hMATE2-KのcDNAを単離し[10,11]，その分子特性や薬物動態学的役割を明らかにしてきた．本稿では，MATEファミリーの遺伝子同定に至るまでの経緯と，両トランスポータの特徴について紹介する．

A. MATEがクローニングされるまで

1980年代前半より，腎膜小胞系や培養腎上皮細胞系などを用いて，側底膜ならびに刷子縁膜における有機カチオンの輸送機構が，生理あるいは薬物動態学的観点から解析されてきた．我々は，H^+/有機カチオンアンチポータが，逆向きのH^+勾配を駆動力とすること[12]，シメチジン（H_2ブロッカー）などのカチオン性薬物を基質にすること[13]などを実証し，その機能的特徴を明らかにしてきた．その後，1994年に，Gründemannら[14]が側底膜の有機カチオントランスポータOCT1を，また我々もそのアイソフォームであるOCT2を同定し[15]，腎臓における有機カチオン輸送の分子的解析はOCTを中心に進展してきた．しかし，その間も多くの研究者が，H^+/有機カチオンアンチポータの分子同定を試みてきたが，いずれも決定的な証拠を示すものではなかった．

最初の候補としては，ブタ由来の培養腎上皮細

胞LLC-PK_1から単離されたOCT2pがあげられる[16]．報告によると，基質特異性や阻害剤の感受性を基に，OCT2pがapical typeの有機カチオントランスポータであることが結論されている．しかし，その後の様々な種のOCT2の基質特異性や膜局在性などの解析によって，OCT2pはapical typeの有機カチオントランスポータではないと考えられている．

次に，有機イオントランスポータファミリーに属するOCTN1（SLC22A4）が，H^+/有機カチオンアンチポータとして機能していることが示唆された[17]．これは，OCTN1が腎尿細管刷子縁膜に局在することや，典型的な有機カチオンであるtetraethylammonium（TEA）をpH依存的に輸送したデータに基づいている．しかし，1) OCTN1を介したTEA輸送はelectrogenicであること[18]（古典的なH^+/有機カチオンアンチポータはelectroneutral），2) OCTN1 mRNAのヒト腎臓における発現はきわめて低いこと[19]，3) OCTN1の特異的基質として抗酸化物質であるergothioneineが見出され，その輸送活性はTEAよりもはるかに大きいこと[20]などから，少なくともOCTN1は従来，生理・薬物動態学的に示唆されてきたH^+/有機カチオンアンチポータの分子実体ではないと考えられている．

最後の候補としては，OCTN2（SLC22A5）があげられる．腎尿細管刷子縁膜に局在するOCTN2は，Na^+勾配を駆動力としてカルニチンの再吸収を媒介し，遺伝性全身カルニチン欠乏症の原因遺伝子として同定されている[21,22]．一方で，OCTN2は，Na^+非依存的にTEAを輸送すること[23]や，OCTN2を欠損している*jvs*マウスにおいてTEAの腎分泌クリアランスが低下すること[24]などから，OCTN2は刷子縁膜における有機カチオン輸送系としても機能していることが示唆されていた．しかし，我々は腎刷子縁膜小胞を用いた輸送実験によって，カルニチン輸送はTEA輸送とは独立していることを明らかにし，OCTN2の有機カチオン輸送への関与は小さいことを報告した[25]．したがって，OCTN2がH^+/有機カチオンアンチポータの分子実体であるという証拠は不充分であった．

このように，H^+/有機カチオンアンチポータとしていくつもの分子が候補としてあがってきたが，古典的なH^+/有機カチオンアンチポータの生化学的特性を完全に満たすものはなく，その分子実体は長年ベールに包まれていた．ところが，2005年に岡山大学の森山らのグループによって，大腸菌の多剤耐性に関与するMATEの哺乳類ホモログとして，ヒトおよびマウスのMATE1ならびにMATE2が同定された[9]．MATE1の臓器分布，膜局在ならびに輸送特性などから，本トランスポータが刷子縁膜においてH^+勾配によって駆動される輸送系として機能していることが示された．その後，我々の研究室を含め複数の研究室から，MATEの単離と機能解析が報告され，本トランスポータがH^+/有機カチオンアンチポータとして機能していることが実証されるに至った[10,11,26,27]．また，HUGO Gene Nomenclature Committeeによって，MATEはSLC47Aとして登録され，名実ともに重要なトランスポータとして位置付けられることになった．図1には，ヒト近位尿細管における有機カチオン輸送の模式図を示した．

B. MATE1の構造，臓器分布，膜局在

哺乳類のMATE1は現在，ヒト，マウス，ラットならびにウサギから同定されている[9-11,26,27]．ヒト，ラット，ウサギMATE1は，約570のアミノ酸からなるが，mMATE1は，C末端のアミノ酸配列が他種のMATE1よりも短く，532個のアミノ酸から構成されている[9,28]．ラットやウサギMATE1では13回膜貫通型タンパクであること

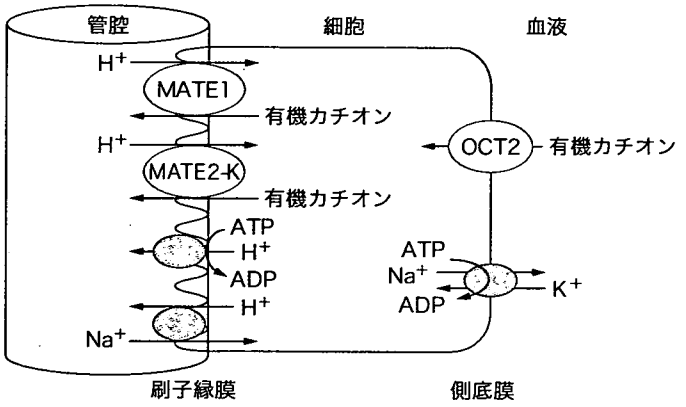

図1　ヒト近位尿細管における有機カチオン輸送

側底膜では，膜電位依存性のhOCT2が血管側から有機カチオンの取り込みを媒介し，刷子縁膜側では逆向きのH^+勾配を駆動力とするhMATE1やhMATE2-Kが細胞内の有機カチオンを管腔側へ排出する．H^+勾配はNa^+/H^+exchangerやH^+ポンプによって形成されている．

が推察されている[26,27]．いずれの種のMATE1も腎臓に発現し，microdissection法によるネフロン内分布の解析の結果，rMATE1 mRNAは近位曲尿細管および直尿細管に限局していることが判明した[11]．またreal-time PCR法によって，組織間の発現量の比較を行ったところ，hMATE1 mRNAは，腎臓，副腎＞＞精巣＞骨格筋，肝臓，子宮[10]に，またrMATE1 mRNAは，腎臓，胎盤＞膵臓＞脾臓[11]の順に発現していることがわかった．rMATE1は，ヒトやマウスで発現の認められた肝臓では発現していない．また特異抗体を用いた解析によって，MATE1は近位尿細管上皮細胞の刷子縁膜に局在していることが示されている[9,10,28,29]．

C. MATE2-Kの構造，臓器分布，膜局在

Otsukaらは，hMATE1 cDNAクローニング時に，hMATE2ならびにmMATE2 cDNAの報告を併せて行った[9]．我々は，hMATE2 cDNAの塩基配列を基にその遺伝子の増幅を試みたが，hMATE2 cDNAは単離されず，代わりにhMATE2-K cDNAを同定した[10]．*hMATE2-K*遺伝子は*hMATE2*遺伝子のエクソン7が108塩基短く，566個のアミノ酸をコードしている．hMATE1とのアミノ酸相同性は52%である．その後，Wrightらのグループも，ウサギ（rb）腎よりrbMATE2ではなく，rbMATE2-Kを同定し[27]，Otsukaらの報告したMATE2の機能や発現については現在のところ明らかにされていない．hMATE2-Kは腎特異的に発現し，近位尿細管上皮細胞の刷子縁膜に局在している．両トランスポータの特徴を表1にまとめた．

一方，mMATE2は，腎臓には発現しておらず，主に精巣に発現している[28]．また，森山らのグループは，mMATE2がMATE3のファミリーに分類されることをアミノ酸配列の相同性から提唱している[30]．アイソフォームとファミリーの名称が異なることは混乱をもたらす原因と考えられ，今後名称の統一が必要と考えられる．

表1 hMATE1, hMATE2およびhMATE2-Kの比較

	アミノ酸数	輸送活性	発現臓器*	膜局在	他の種でのアイソフォーム
hMATE1[9]	570	有	腎臓，肝臓など	刷子縁膜	マウス[9]，ラット[11,26]，ウサギ[27]
hMATE2[9]	602	?	腎臓のみ	?	?
hMATE2-K[10]	566	有	腎臓のみ	刷子縁膜	ウサギ[27]

*Otsukaらの論文では，Northern blottingによりhMATE2の腎臓での発現が示されているが，hMATE2-Kを検出している可能性も考えられる．またPCR産物長によりhMATE2とhMATE2-Kを区別するPCRを行ったところ，腎臓ではMATE2-Kのみ発現していた[10]．

D. MATEの薬物輸送特性

hMATE1ならびにhMATE2-Kは，尿細管分泌を受けるカチオン性薬物：メトホルミン（糖尿病治療薬），シメチジン（H_2ブロッカー），プロカインアミド（抗不整脈薬）などを顕著に輸送し，類似の薬物認識特性を示す[31]．これらhMATEsの基質認識特性および腎臓における発現分布は側底膜側hOCT2と類似しており，hOCT2を介して取り込まれた薬物はhMATE1やhMATE2-Kを介して尿細管管腔へ分泌されると考えられる．実際我々は，有機カチオン輸送系を有していないMDCK細胞をホストとしてhMATE1/hOCT2の共発現系を作製し，上述した薬物が尿細管分泌に対応した方向選択的な輸送を受けることを確認している．一方で，hMATE1は両性イオン型薬物であるセファレキシンを，またhMATE2-Kは白金系抗がん剤のオキサリプラチンをそれぞれ特異的に輸送し，両者の基質特異性に関する差異も見受けられた[31-33]．白金系抗がん剤の基質認識の異同については，腎毒性発現機構の観点から後述する．

E. MATEの駆動力と必須アミノ酸残基

これまで，腎刷子縁膜小胞を用いた輸送実験によって，H^+/有機カチオンアンチポータの駆動力は，逆向きのH^+勾配であることが示されていた[12,13]．実際，MATEを介したTEA取り込みは，細胞外pHがアルカリ側において高く，NH_4Cl処理による細胞内酸性化によって顕著に促進された[9-11]．しかし，いずれも間接的な証明であり，正確な輸送機序の解明には膜小胞を用いた解析が必須と考えられた．そこで，MATE1安定発現細胞からnitrogen cavitation法によって膜小胞を調製し，駆動力の直接的な証明を試みた[34]．その結果，TEAの取り込みは，逆向きのH^+勾配が存在するときのみ濃度勾配に逆らった上り坂輸送，すなわちオーバーシュート現象が観察された．さらにそのオーバーシュートは，プロトノフォアであるFCCP共存によって消失したことから，MATE1は逆向きのH^+勾配を駆動力とすることが判明した．またMATE1を介したTEA取り込みは膜電位の影響を受けなかったことから，H^+とTEAの化学量論比は1：1であることが推察された．

また，刷子縁膜小胞を用いた輸送解析より，H^+/有機カチオンアンチポータに存在するシステイン（Cys）やヒスチジン（His）残基が輸送機能に必須であることが示されていた[35,36]．またrMATE1の輸送活性は，Cys残基修飾試薬によって顕著に低下することが報告された[26]．そこで，rMATE1に存在するこれら必須アミノ酸残基の同定を試みたところ，MATE1の保存性Cysのうち，第1ならびに第3番目の膜貫通領域に存在す

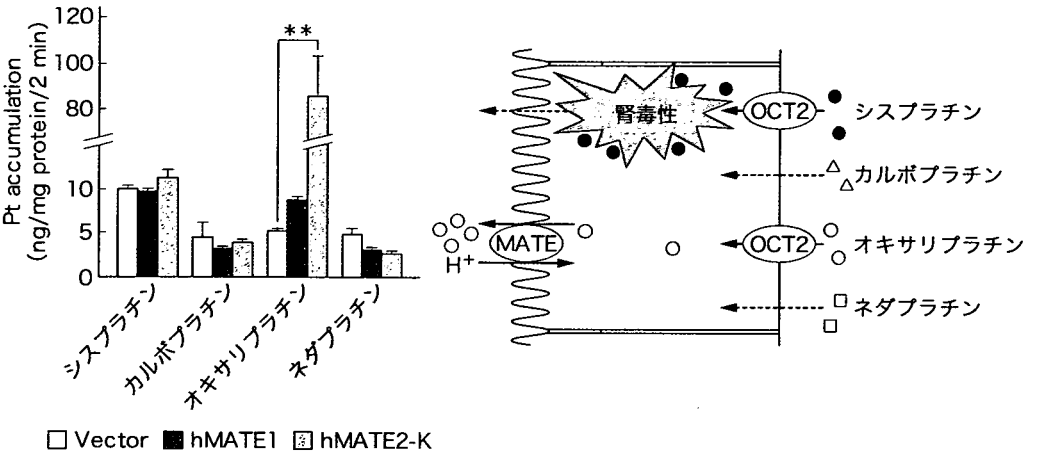

図2 MATEによる白金系抗がん剤の輸送特性と腎毒性発現機構
オキサリプラチンはOCT2によって腎尿細管に取り込まれるものの，MATEsによって効率的に尿中へ分泌されるため，腎蓄積性・腎毒性を示さない．一方，シスプラチンでは，MATEsによる分泌を受けないため腎臓に蓄積し，腎毒性を惹起する．カルボプラチンとネダプラチンは，OCT2やMATEsによって輸送されない．

るCys変異体，ならびに第10番目の膜貫通領域のHis変異体で輸送活性が低下した[37]．また，CysやHisのアミノ酸修飾試薬処理に及ぼす基質の保護効果について調べたところ，Cys残基は基質の結合部位として機能していることが示唆された．また7番目の膜貫通領域に存在するグルタミン酸も，輸送機能に重要な役割を果たしていることが示されている[9]．

F. hMATE1ならびにhMATE2-Kと腎毒性との関係

最後にhMATE1およびhMATE2-Kの機能と，白金系抗がん剤の腎毒性との関連について紹介する．本邦では現在，4種類の白金系抗がん剤（シスプラチン，オキサリプラチン，カルボプラチン，ネダプラチン）が承認されており，シスプラチンのみ高い腎蓄積と強い腎毒性を有することが知られている．その機序の一つとして，トランスポータによる白金系抗がん剤の腎動態制御が関与しているのではないかと考え検討を加えた．その結果，シスプラチンと腎毒性をほとんど示さないオキサリプラチンが，側底膜に発現するhOCT2によって有意に輸送されたが，カルボプラチンやネダプラチンの輸送は認められなかった[32]．一方，刷子縁膜側の輸送では，オキサリプラチンは両トランスポータにより顕著に輸送されたが(輸送活性：hMATE2-K＞hMATE1)，シスプラチンはいずれのトランスポータによっても輸送されなかった[32,33]．したがって，オキサリプラチンはOCT2によって腎尿細管に取り込まれるものの，MATEsによって効率的に尿中へ分泌されるため，腎蓄積性・腎毒性を示さないことがわかった（図2）．一方，シスプラチンでは，MATEsによる分泌を受けないため腎臓に蓄積し，その結果腎毒性を惹起することが示唆された（図2）．

むすび

長年，その分子実体が不明であった，H^+/有機カチオンアンチポータ（MATE/SLC47A）のcDNAがクローニングされ，これまで生理的に示されていたカチオン性薬物の腎排泄機構の全貌

が，ようやく分子的に理解されるようになった．事実，hMATE1/hOCT2共発現系の構築により，これまで適切な評価系のなかったヒトにおけるカチオン性薬物の尿細管分泌，薬物間相互作用そして腎毒性発現機構を，*in vitro*で解析可能になることは，医薬品開発や臨床の現場に多大なる有益な情報をもたらすものと考えられる．今後，MATEの調節機構や遺伝子多型に関する研究が進展することによって，本トランスポータの生理・薬物動態学的意義の解明がより一層進むことが期待される．

文献

1) Pritchard JB, Miller DS. Mechanisms mediating renal secretion of organic anions and cations. Physiol Rev. 1993; 73: 765-96.
2) Inui K, Okuda M. Cellular and molecular mechanisms of renal tubular secretion of organic anions and cations. Clin Exp Nephrol. 1998; 2: 100-8.
3) Inui K, Takano M, Hori R. Organic cation transport in the renal brush-border and basolateral membranes. In: Hatano M. editors. Nephrology. Tokyo: Springer-Verlag; 1991. p. 1391-8.
4) Inui K, Masuda S, Saito H. Cellular and molecular aspects of drug transport in the kidney. Kidney Int. 2000; 58: 944-58.
5) Burckhardt G, Wolff NA. Structure of renal organic anion and cation transporters. Am J Physiol Renal Physiol. 2000; 278: F853-66.
6) Jonker JW, Schinkel AH. Pharmacological and physiological functions of the polyspecific organic cation transporters: OCT1, 2, and 3 (SLC22A1-3). J Pharmacol Exp Ther. 2004; 308: 2-9.
7) Wright SH. Role of organic cation transporters in the renal handling of therapeutic agents and xenobiotics. Toxicol Appl Pharmacol. 2005; 204: 309-19.
8) Koepsell H, Lips K, Volk C. Polyspecific organic cation transporters: structure, function, physiological roles, and biopharmaceutical implications. Pharm Res. 2007; 24: 1227-51.
9) Otsuka M, Matsumoto T, Morimoto R, et al. A human transporter protein that mediates the final excretion step for toxic organic cations. Proc Natl Acad Sci USA. 2005; 102: 17923-8.
10) Masuda S, Terada T, Yonezawa A, et al. Identification and functional characterization of a new human kidney-specific H^+/organic cation antiporter, kidney-specific multidrug and toxin extrusion 2. J Am Soc Nephrol. 2006; 17: 2127-35.
11) Terada T, Masuda S, Asaka J, et al. Molecular cloning, functional characterization and tissue distribution of rat H^+/organic cation antiporter MATE1. Pharm Res. 2006; 23: 1696-701.
12) Takano M, Inui K, Okano T, et al. Carrier-mediated transport systems of tetraethylammonium in rat renal brush-border and basolateral membrane vesicles. Biochim Biophys Acta. 1984; 773: 113-24.
13) Takano M, Inui K, Okano T, et al. Cimetidine transport in rat renal brush border and basolateral membrane vesicles. Life Sci. 1985; 37: 1579-85.
14) Gründemann D, Gorboulev V, Gambaryan S, et al. Drug excretion mediated by a new prototype of polyspecific transporter. Nature. 1994; 372: 549-52.
15) Okuda M, Saito H, Urakami Y, et al. cDNA cloning and functional expression of a novel rat kidney organic cation transporter, OCT2. Biochem Biophys Res Commun. 1996; 224: 500-7.
16) Gründemann D, Babin-Ebell J, Martel F, et al. Primary structure and functional expression of the apical organic cation transporter from kidney epithelial LLC-PK_1 cells. J Biol Chem. 1997; 272: 10408-13.
17) Tamai I, Yabuuchi H, Nezu J, et al. Cloning and characterization of a novel human pH-dependent organic cation transporter, OCTN1. FEBS Lett. 1997; 419: 107-11.
18) Tamai I, Nakanishi T, Kobayashi D, et al. Involvement of OCTN1 (SLC22A4) in pH-dependent transport of organic cations. Mol Pharm. 2004; 1: 57-66.
19) Motohashi H, Sakurai Y, Saito H, et al. Gene

expression levels and immunolocalization of organic ion transporters in the human kidney. J Am Soc Nephrol. 2002; 13: 866-74.
20) Gründemann D, Harlfinger S, Golz S, et al. Discovery of the ergothioneine transporter. Proc Natl Acad Sci USA. 2005; 102: 5256-61.
21) Tamai I, Ohashi R, Nezu J, et al. Molecular and functional identification of sodium ion-dependent, high affinity human carnitine transporter OCTN2. J Biol Chem. 1998; 273: 20378-82.
22) Nezu J, Tamai I, Oku A, et al. Primary systemic carnitine deficiency is caused by mutations in a gene encoding sodium ion-dependent carnitine transporter. Nature Genet. 1999; 21: 91-4.
23) Ohashi R, Tamai I, Yabuuchi H, et al. Na^+-dependent carnitine transport by organic cation transporter (OCTN2): its pharmacological and toxicological relevance. J Pharmacol Exp Ther. 1999; 291: 778-84.
24) Ohashi R, Tamai I, Nezu J, et al. Molecular and physiological evidence for multifunctionality of carnitine/organic cation transporter OCTN2. Mol Pharmacol. 2001; 59: 358-66.
25) Ohnishi S, Saito H, Fukada A, et al. Distinct transport activity of tetraethylammonium from L-carnitine in rat renal brush-border membranes. Biochim Biophys Acta. 2003; 1609: 218-24.
26) Ohta K, Inoue K, Hayashi Y, et al. Molecular identification and functional characterization of rat multidrug and toxin extrusion type transporter 1 as an organic cation/H^+ antiporter in the kidney. Drug Metab Dispos. 2006; 34: 1868-74.
27) Zhang X, Cherrington NJ, Wright SH. Molecular identification and functional characterization of rabbit MATE1 and MATE2-K. Am J Physiol Renal Physiol. 2007; 293: F360-70.
28) Hiasa M, Matsumoto T, Komatsu T, et al. Wide variety of locations for rodent MATE1, a transporter protein that mediates the final excretion step for toxic organic cations. Am J Physiol Cell Physiol. 2006; 291: C678-86.
29) Nishihara K, Masuda S, Ji L, et al. Pharmacokinetic significance of luminal multidrug and toxin extrusion 1 in chronic renal failure rats. Biochem Pharmacol. 2007; 73: 1482-90.
30) Omote H, Hiasa M, Matsumoto T, et al. The MATE proteins as fundamental transporters of metabolic and xenobiotic organic cations. Trends Pharmacol Sci. 2006; 27: 587-93.
31) Tanihara Y, Masuda S, Sato T, et al. Substrate specificity of MATE1 and MATE2-K, human multidrug and toxin extrusions/H^+-organic cation antiporters. Biochem Pharmacol. 2007; 74: 359-71.
32) Yonezawa A, Masuda S, Yokoo S, et al. Cisplatin and oxaliplatin, but not carboplatin and nedaplatin, are substrates for human organic cation transporters (SLC22A1-3 and multidrug and toxin extrusion family). J Pharmacol Exp Ther. 2006; 319: 879-86.
33) Yokoo S, Yonezawa A, Masuda S, et al. Differential contribution of organic cation transporters, OCT2 and MATE1, in platinum agent-induced nephrotoxicity. Biochem Pharmacol. 2007; 74: 477-87.
34) Tsuda M, Terada T, Asaka J, et al. Oppositely directed H^+ gradient functions as a driving force of rat H^+/organic cation antiporter MATE1. Am J Physiol Renal Physiol. 2007; 292: F593-8.
35) Hori R, Maegawa H, Kato M, et al. Inhibitory effect of diethyl pyrocarbonate on the H^+/organic cation antiport system in rat renal brush-border membranes. J Biol Chem. 1989; 264: 12232-7.
36) Hori R, Maegawa H, Okano T, et al. Effect of sulfhydryl reagents on tetraethylammonium transport in rat renal brush border membranes. J Pharmacol Exp Ther. 1987; 241: 1010-6.
37) Asaka J, Terada T, Tsuda M, et al. Identification of essential histidine and cysteine residues of the H^+/organic cation antiporter multidrug and toxin extrusion (MATE). Mol Pharmacol. 2007; 71: 1487-93.
38) Yonezawa A, Masuda S, Nishihara K, et al. Association between tubular toxicity of cisplatin and expression of organic cation transporter rOCT2 (Slc22a2) in the rat. Biochem Pharmacol. 2005; 70: 1823-31.

Pharmaceutical Research, Vol. 22, No. 2, February 2005 (© 2005)
DOI: 10.1007/s11095-004-1193-3

Research Paper

Metformin Transport by Renal Basolateral Organic Cation Transporter hOCT2

Naoko Kimura,[1] Masahiro Okuda,[1] and Ken-ichi Inui[1,2]

Received September 2, 2004; accepted October 22, 2004

Purpose. Metformin, an antihyperglycemic agent, is eliminated by tubular secretion in addition to glomerular filtration in the human kidney. This study was performed to characterize metformin transport by human organic cation transporter 2 (hOCT2), the most abundant organic cation transporter in the basolateral membranes of the human kidney.
Methods. Accumulation of [^{14}C]metformin was assessed by the tracer experiments in the human embryonic kidney (HEK293) cells expressing hOCT2.
Results. The transport of [^{14}C]metformin was markedly stimulated in hOCT2-expressing cells compared with the vector-transfected cells. The accumulation of [^{14}C]metformin was concentrative and was dependent on the membrane potential, showing consistency with the characteristics of hOCT2. The apparent K_m and V_{max} values of [^{14}C]metformin transport by hOCT2-expressing HEK293 cells were 1.38 ± 0.21 mM and 11.9 ± 1.5 nmol mg protein^{-1} min^{-1}, respectively. The order of the potencies of unlabeled biguanides to inhibit [^{14}C]metformin transport by hOCT2 was phenformin > buformin > metformin. Furthermore, [^{14}C]metformin transport was inhibited slightly or moderately by cationic drugs such as procainamide and quinidine at respective therapeutic concentrations.
Conclusions. Metformin is transported by the basolateral organic cation transporter hOCT2 in the human kidney. hOCT2 could play a role in the drug interactions between metformin and some cationic drugs.

KEY WORDS: hOCT2; human kidney; metformin; organic cation transporter; renal tubular secretion.

INTRODUCTION

Biguanide agents have been used for the treatment of type 2 diabetes mellitus since the late 1950s. These drugs are useful in primary therapy for type 2 diabetes mellitus with obesity or hyperlipidemia and can also be used for add-on therapy in patients with diabetes uncontrolled by sulfonylureas and diet (1–2). The mechanisms of the pharmacological action of metformin involve the decreased hepatic glucose production without an effect on the release of insulin and the increased glycogenesis and lactate production. The most life-threatening adverse effect of biguanides is lactic acidosis, because there is a risk of overproduction of lactate through inhibition of mitochondrial respiration and increased anaerobic glycolysis (1). A risk of decreased use of lactate through inhibition of gluconeogenesis by biguanides has been also documented (1). Although phenformin was removed from the market because of its association with lactic acidosis, the relative risk is much lower for metformin than for phenformin. Moreover, the risk of lactic acidosis caused by metformin is less than the risk of severe hypoglycemia induced by sulfonylurea drugs (3).

Metformin is mainly excreted into urine, almost entirely in an unchanged form. The renal clearance of metformin (440–454 ml/min) is much higher than the glomerular filtration rate in humans (4–5), suggesting a significant contribution of tubular secretion in addition to glomerular filtration. As biguanides consist of two molecules of guanidine linked together by the removal of an ammonia group, they are protonated at physiologic pH. Organic cation transporters have been suggested to mediate tubular secretion of metformin (6–7); however, the molecular mechanisms underlying the renal tubular secretion of biguanides have not been clarified.

Organic cation transporters in the kidney, liver, intestine, brain, and placenta play essential physiologic and pharmacological roles in the handling of cationic drugs and endogenous organic ions. Human organic cation transporter 1 (hOCT1) is expressed primarily in the liver (8–9) and is likely responsible for the hepatic uptake of various cationic drugs. In contrast to hOCT1, we demonstrated that human organic cation transporter 2 (hOCT2) is the most abundant organic cation transporter in the basolateral membranes of human kidney (10). Moreover, hOCT2 as well as its orthologue in the rat (rOCT2) were localized at the basolateral membranes of renal proximal tubules and were suggested to contribute to the secretion of organic cations, such as tetraethylammonium,

[1] Department of Pharmacy, Kyoto University Hospital, Kyoto University, Kyoto 606-8507, Japan.
[2] To whom correspondence should be addressed. (E-mail: inui@kuhp.kyoto-u.ac.jp)

ABBREVIATIONS: hOCT1, human organic cation transporter 1; hOCT2, human organic cation transporter 2; rOCT1, rat organic cation transporter 1; HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid.

1-methyl-4-phenylpyridinium, cimetidine, and guanidine (10–13). Recently, rat (r) OCT1 as well as mouse (m) OCT1 was revealed to transport biguanides (14,15). It was also demonstrated that tissue distribution of metformin was decreased in the liver, duodenum, jejunum, ileum, but not in the kidney and colon, in the mOCT1 gene-knockout mice. Moreover, Dresser *et al.* (16) reported that metformin and phenformin induced currents and *trans*-stimulated [^{3}H]1-methyl-4-phenylpyridinium uptake in *Xenopus* oocytes expressing hOCT1 and hOCT2. However, information has been limited regarding the characteristics of metformin transport by hOCT2 and hOCT2-mediated drug interactions between metformin and concomitantly administered drugs. In the current study, we characterized the transport of [^{14}C]metformin using human embryonic kidney (HEK293) cells stably expressing hOCT2. We also assessed drug interactions between [^{14}C]metformin and various cationic drugs.

MATERIALS AND METHODS

Materials

[Biguanidine-^{14}C]metformin hydrochloride (26 mCi/mmol) was purchased from Moravek Biochemicals, Inc. (Brea, CA, USA). [Ethyl-1-^{14}C]tetraethylammonium bromide (55 mCi/mmol) was purchased from American Radiolabeled Chemicals (St. Louis, MO, USA). Metformin, phenformin, and 1-methyl-4-phenylpyridinium iodide were obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). Buformin was purchased from Wako Pure Chemicals (Osaka, Japan). Tetraethylammonium bromide and cimetidine were obtained from Nacalai Tesque Inc. (Kyoto, Japan). All other compounds used were of the highest purity available.

Cell Culture and Transfection

To construct the transfectant stably expressing hOCT2, HEK 293 cells (ATCC CRL-1573, American Type Culture Collection, Manassas, VA, USA), a transformed cell line derived from human embryonic kidney, were transfected with 0.8 μg of total plasmid DNA (pCMV-XL4:pBK-CMV vector = 2:1) per well. At 24 h after transfection, the cells split between 1:15 and 1:30 were cultured in complete medium consisting of Dulbecco's modified Eagle's medium with 10% fetal bovine serum in an atmosphere of 5% CO_2/95% air at 37°C containing G418 (0.5 mg/ml) (Wako Pure Chemical Industries, Osaka, Japan). Then, 14 to 21 days after transfection, single colonies were selected. G418-resistant colonies were analyzed by RT-PCR for expression of hOCT2 mRNA (17).

For uptake experiments, the cells were seeded onto poly-D-lysine–coated 24-well plates at a density of 2.0×10^5 cells per well. The cell monolayers were used at day 3 of culture for uptake experiments. In this study, HEK293 cells between passages 70 and 83 were used.

Uptake Experiments

Cellular uptake of cationic compounds was measured with monolayer cultures of HEK293 cells grown on poly-D-lysine–coated 24-well plates (17). The incubation medium for uptake experiments contained 145 mM NaCl, 3 mM KCl, 1 mM $CaCl_2$, 0.5 mM $MgCl_2$, 5 mM D-glucose, and 5 mM HEPES (pH 7.4). The composition of the high K^+ incubation medium was 3 mM NaCl, 145 mM KCl, 1 mM $CaCl_2$, 0.5 mM $MgCl_2$, 5 mM D-glucose, and 5 mM HEPES (pH 7.4). When indicated, 9.2 mM $BaCl_2$ was added to the incubation medium. The pH of the medium was adjusted with NaOH or HCl. The cells were preincubated with 0.2 ml of incubation medium for 10 min at 37°C. The medium was then removed, and 0.2 ml of incubation medium containing [^{14}C]metformin or [^{14}C]tetraethylammonium was added. The medium was aspirated off at the end of the incubation, and the monolayers were rapidly rinsed twice with 1 ml of ice-cold incubation medium. The cells were solubilized in 0.5 ml of 0.5 N NaOH, and then the radioactivity in aliquots was determined by liquid scintillation counting. The protein content of the solubilized cells was determined by the method of Bradford (18), using a Bio-Rad Protein Assay Kit (Bio-Rad Laboratories, Hercules, CA, USA) with bovine γ-globulin as a standard. The concentration dependence of metformin transport by hOCT2 was analyzed using the Michaelis-Menten equation; $V = V_{max} \cdot [S]/(K_m + [S]) + K_d \cdot [S]$, where V is the transport rate, V_{max} is the maximum transport rate, [S] is the concentration of metformin, K_m is the Michaelis constant, and K_d is a diffusion constant. For the *cis*-inhibition study, the uptake of [^{14}C]metformin was achieved by adding various concentrations of unlabeled inhibitors to the incubation medium. The IC_{50} values were calculated from the inhibition plots based on the equation, $V = V_0/[1 + (I/IC_{50})^n]$ by nonlinear least square regression analysis with Kaleidagraph Version 3.5 (Synergy Software, Reading, PA, USA) (12). V and V_0 are the uptake rates of [^{14}C]metformin in the presence and absence of inhibitor, respectively. I is the concentration of inhibitor, and n is the Hill coefficient.

Statistical Analyses

Data were analyzed statistically by one-way analysis of variance followed by Dunnett's test. p values of less than 0.05 were considered to be significant.

RESULTS

Concentration Dependence of [^{14}C]Metformin Transport by hOCT2

To examine whether metformin is transported by hOCT2, we evaluated the uptake of [^{14}C]metformin by HEK293 cells stably expressing hOCT2. Figure 1 illustrates the time-course of [^{14}C]metformin uptake by HEK 293 cells transfected with hOCT2 and empty vector (Fig. 1). The uptake of [^{14}C]metformin increased time-dependently, and was linear for up to 2 min. Figure 2 shows the concentration dependence of [^{14}C]metformin uptake by hOCT2-expressing cells. The uptake of [^{14}C]metformin by these cells was saturated at high concentrations. The apparent Michaelis-Menten constant (K_m) value of [^{14}C]metformin uptake by hOCT2-transfected cells, estimated by subtracting the nonsaturable component of [^{14}C]metformin transport in the presence of 1-methyl-4-phenylpyridinium (5 mM) was 1.38 ± 0.21 mM. The maximal uptake rate (V_{max}) value of the [^{14}C]metformin uptake by hOCT2-transfected cells was 11.9 ± 1.5 nmol mg protein^{-1} min^{-1} (mean ± SE of three separate experiments using three monolayers).

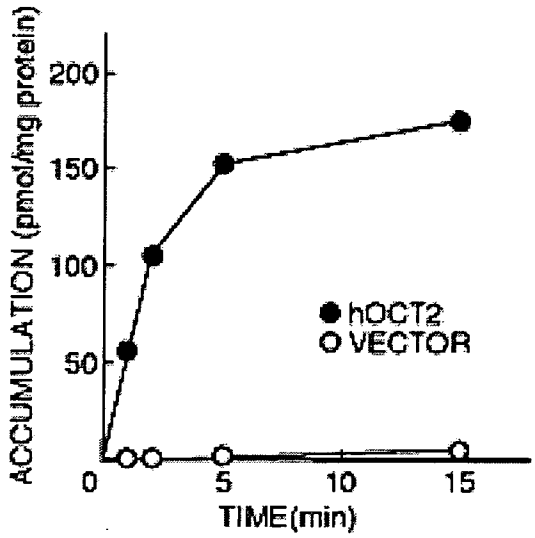

Fig. 1. Time course of [^{14}C]metformin uptake by HEK293 cells stably expressing hOCT2. HEK 293 cells transfected with hOCT2 (●) or pCMV6-XL4 vector (○) were incubated for the specified periods (1, 2, 5, and 15 min) at 37°C with 0.2 ml of 10 μM [^{14}C]metformin (pH 7.4). Each point represents the mean ± SE of three monolayers from a typical experiment.

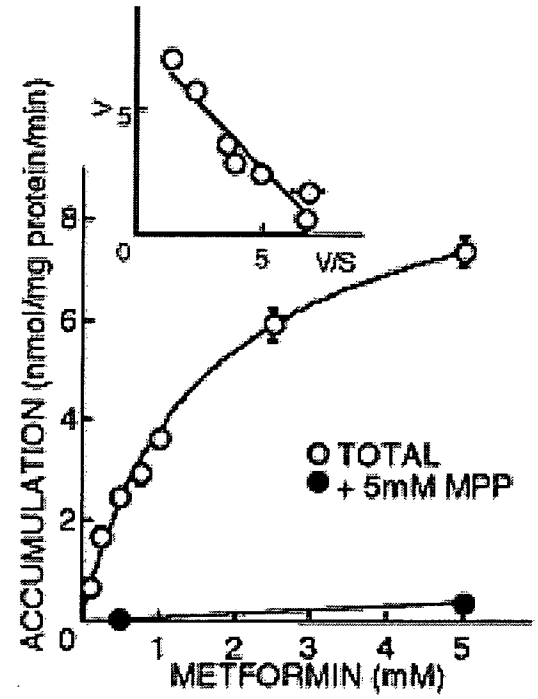

Fig. 2. Concentration dependence of [^{14}C]metformin transport by hOCT2. hOCT2 transfectants were incubated at 37°C for 2 min with various concentrations of [^{14}C]metformin (100, 250, 500, 750, 1000, 2500, and 5000 μM) in the absence (○) or presence (●) of 5 mM 1-methyl-4-phenylpyridinium (pH 7.4). Each point represents the mean ± SE of three monolayers from a typical experiment. Inset: Eadie-Hofstee plots of metformin uptake after correction for non-saturable components. V, uptake rate (nmol mg protein^{-1} min^{-1}); S, metformin concentration (mM).

Effect of Membrane Potential on the Transport of [^{14}C]Metformin and [^{14}C]Tetraethylammonium by hOCT2

Next, we examined the effect of membrane potential on the accumulation of [^{14}C]metformin and [^{14}C]tetraethylammonium in HEK293 cells expressing hOCT2 (Fig. 3). The accumulation of [^{14}C]metformin in hOCT2-expressing cells decreased in the presence of high K^+ (145 mM) medium (Fig. 3A) similarly as observed for [^{14}C]tetraethylammonium (Fig. 3B). Furthermore, the accumulation of [^{14}C]metformin and [^{14}C]tetraethylammonium *via* hOCT2 decreased in the presence of Ba^{2+} (9.2 mM), a nonselective blocker of K^+ channels. The mean percent of control values ± SE of [^{14}C]metformin accumulation obtained from three separate experiments using three monolayers were 24.8 ± 2.8% and 59.0 ± 9.9% for high K^+ and Ba^{2+} media, respectively ($p < 0.01$ vs. control). The values ± SE of [^{14}C]tetraethylammonium accumulation were 17.8 ± 2.7% and 39.7 ± 9.6% for high K^+ and Ba^{2+} media, respectively ($p < 0.01$ vs. control).

Inhibition of hOCT2-Mediated Transport of [^{14}C]Metformin by Cationic Drugs

To assess the potencies of cationic drugs to cause drug-interactions with hOCT2-mediated metformin transport, we examined the inhibitory effects of several cationic compounds on the uptake of [^{14}C]metformin by the hOCT2-expressing cells (Fig. 4). Then, we calculated the IC_{50} values of cationic compounds from the inhibition plots as described in "Materials and Methods." Tetraethylammonium (a typical substrate for the renal organic cation transporter), 1-methyl-4-phenylpyridinium (a cationic neurotoxin), procainamide and quinidine (antiarrhythmic drugs), trimethoprim (an antibiotic), and cimetidine and ranitidine (H_2 receptor antagonists) inhibited the uptake of [^{14}C]metformin by hOCT2-expressing cells in a dose-dependent manner. As summarized in Table I, 1-methyl-4-phenylpyridinium showed the most potent inhibitory effect, whereas procainamide and cimetidine had moderate inhibitory effects on the transport of metformin by hOCT2. Furthermore, biguanides inhibited the transport of [^{14}C]metformin by hOCT2 in the following order: phenformin > buformin > metformin.

DISCUSSION

In the current study, we characterized [^{14}C]metformin transport using hOCT2-expressing HEK293 cells, and assessed drug interactions between metformin and cationic drugs using [^{14}C]metformin as a tracer. [^{14}C]metformin uptake was markedly enhanced in HEK293 cells stably transfected with hOCT2 (Fig. 1). The uptake of [^{14}C]metformin was dependent on membrane potential (Fig. 3), being consistent with the functional characteristics of OCT2 (13,19). To our knowledge, this is the first demonstration showing direct evidence of metformin transport by hOCT2. Considering that hOCT2 is the dominant organic cation transporter expressed in the basolateral membranes of the human renal cortex (10), hOCT2 should play a relevant role in the transport of metformin across basolateral membranes in the human kidney.

The accumulation of [^{14}C]metformin in hOCT2-expressing cells was saturated at high concentrations (Fig. 2). Although the driving force of both compounds by hOCT2 seems to be common, that is, the membrane potential (Fig. 3), the apparent affinity of metformin to hOCT2 (K_m = 1.38 mM) was much lower than that of tetraethylammonium

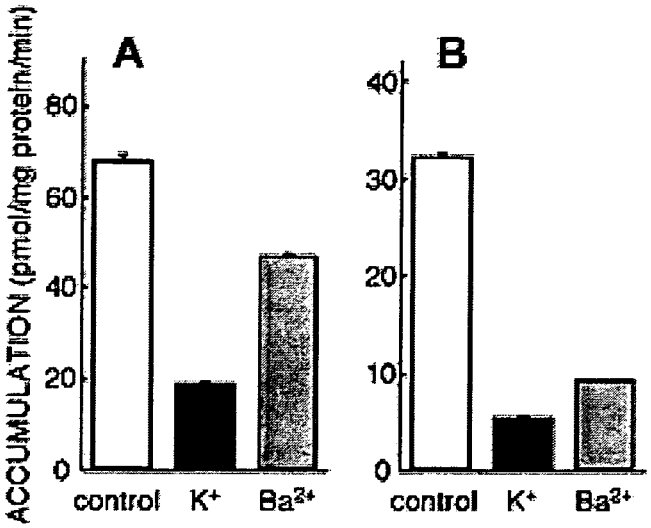

Fig. 3. Effect of membrane potential on [^{14}C]metformin (A) and [^{14}C]tetraethylammonium (B) transport by hOCT2. The cells transfected with hOCT2 were incubated with respective buffers at 37°C for 2 min with 10 μM [^{14}C]metformin (A) or 5 μM [^{14}C]tetraethylammonium (B) (pH 7.4). Each column represents the mean ± SE of three monolayers from a typical experiment.

(K_m = 431 μM) (13). Because the maximum plasma concentration of metformin was reported to be 9–12 μM after a single oral administration of metformin HCl (850 mg) in patients with type 2 diabetes mellitus (20–22) and up to 15 μM and 25 μM in healthy elderly patients and patients with moderate chronic renal impairment, respectively (23), the transport of metformin by hOCT2 should not saturate at therapeutic concentrations. Moreover, these results seem to be

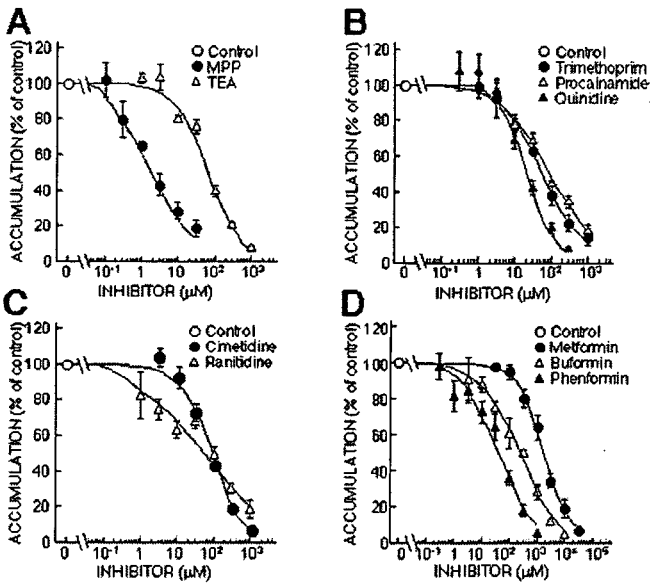

Fig. 4. Effects of cationic compounds on [^{14}C]metformin transport by hOCT2. HEK 293 cells transfected with hOCT2 were incubated at 37°C for 2 min with 10 μM [^{14}C]metformin (pH 7.4) in the presence of (A) 1-methyl-4-phenylpyridinium (MPP, ●) or tetraethylammonium (TEA, △); (B) trimethoprim (●), procainamide (△), or quinidine (▲); (C) cimetidine (●) or ranitidine (△); (D) metformin (●), buformin (△), or phenformin (▲). Each point represents the mean ± SE of three to six separate experiments using three monolayers.

Table I. The Apparent IC_{50} Values of Various Cationic Compounds for [^{14}C]Metformin Uptake by hOCT2

Compounds	Apparent IC_{50} values for [^{14}C]metformin uptake (μM)
MPP	2.99 ± 1.15
Quinidine	17.4 ± 5.7
Phenformin	55.3 ± 12.5
Trimethoprim	60.0 ± 19.0
TEA	66.6 ± 2.3
Cimetidine	72.6 ± 11.4
Ranitidine	74.6 ± 32.2
Procainamide	79.8 ± 7.5
Buformin	203 ± 63
Metformin	1840 ± 370

See experimental conditions in the legend of Fig. 4. The apparent IC_{50} values were calculated from inhibition plots (Fig. 4) by nonlinear regression analysis as described in "Materials and Methods." The data represent the mean ± SE of three to six separate experiments using three monolayers. MPP, 1-methyl-4-phenylpyridinium; TEA, tetraethylammonium.

comparable with the reports by Sambol *et al.* (20) that renal clearance of metformin was not changed by single dosings between 850 mg and 2550 mg, respectively.

In the current study, we demonstrated the inhibition of hOCT2-mediated [^{14}C]metformin transport by various cationic drugs, and then calculated respective IC_{50} values (Fig. 4 and Table I). Of the organic cations tested, quinidine or procainamide inhibited [^{14}C]metformin transport with IC_{50} values of 17.4 μM and 79.8 μM, respectively, which were comparable to the plasma concentrations of these drugs. It is reported that the plasma concentration of *N*-acetylprocainamide, a major metabolite of procainamide, also inhibit renal organic cation transporters (24). Moreover, the plasma procainamide concentration was elevated, when the drug was administered concomitantly with cimetidine (25). Therefore, we consider that concomitant administration of quinidine or procainamide with metformin should decrease the tubular secretion of metformin by interrupting hOCT2.

Somogyi *et al.* (7) reported the presence of drug interactions between metformin and cimetidine, where renal clearance of metformin was reduced by cimetidine, while cimetidine disposition was not altered by concomitantly administered metformin. According to the fact that apparent K_m value of metformin transport by hOCT2 was about 10-fold larger than that of cimetidine (K_m = 145 μM, unpublished observation), it seems to be reasonable that renal disposition of metformin, but not of cimetidine, was decreased by the drug interaction between these drugs. Dresser *et al.* (16) reported that IC_{50} value of metformin on hOCT2-mediated [^{3}H]cimetidine transport was 1700 μM. Because the value was approximately 100-fold higher than the therapeutic concentration of metformin, we consider that their results may indicate the unchanged disposition of cimetidine by concomitant administration of metformin, which was observed by Somogyi *et al.* (7).

The transport of [^{14}C]metformin was inhibited dose-dependently by cimetidine. This phenomenon apparently seems to be comparable to the observation by Somogyi *et al.* (7) that cimetidine inhibited the tubular secretion of metformin, and thereby increased its plasma concentration. The IC_{50} value of cimetidine for metformin transport (72.6 ± 11.4 μM)

was moderately higher than the plasma concentration of cimetidine, i.e., a single oral dose of 200 mg of cimetidine to the patients with normal renal function gave C_{max} values of between 2.3 μM and 6.8 μM (26). These data suggest that contribution of hOCT2 in the drug interaction between metformin and cimetidine would be minimum. However, it is known that systemic clearance of cimetidine decreases in the patients with renal dysfunction (27) and in the elderly (28), and thereby increases plasma concentrations of cimetidine. Therefore, the role of hOCT2 in the drug interactions between metformin and cimetidine could be relatively large in the patients with decreased renal function.

In the current study, the order of the potencies of biguanides to inhibit [^{14}C]metformin transport was phenformin > buformin > metformin. Dresser *et al.* (16) also demonstrated that the potency of phenformin to inhibit [^{3}H]cimetidine transport by hOCT2 was higher than that of metformin, suggesting consistency with the data in the present study. Interestingly, Wang *et al.* (14,15) also reported the order of affinity of biguanides to the rOCT1 was phenformin > buformin > metformin, suggesting similar substrate spectrum between OCT1 and OCT2. This phenomenon seems to be reasonable, because substrate spectrums were similar between OCT1 and OCT2 as demonstrated by us (11,12). Because the information regarding the relationship between plasma concentration of biguanides and blood lactose levels is limited (21), the role of hOCT2 in the biguanides-induced lactic acidosis should be clarified in subsequent studies.

In conclusion, hOCT2 is involved in the basolateral membrane transport of metformin in the human kidney. hOCT2 could also play a relevant role in the drug interaction between metformin and some cationic drugs.

ACKNOWLEDGMENTS

This work was supported in part by a grant-in-aid for Comprehensive Research on Aging and Health from the Ministry of Health, Labor and Welfare of Japan, by a grant-in-aid for Scientific Research from the Ministry of Education, Science, Culture and Sports of Japan, and by the 21st Century COE program "Knowledge Information Infrastructure for Genome Science."

REFERENCES

1. P. Marchetti, R. Giannarelli, A. di Carlo, and R. Navalesi. Pharmacokinetic optimisation of oral hypoglycaemic therapy. *Clin. Pharmacokinet.* **21**:308–317 (1991).
2. T. B. Klepser and M. W. Kelly. Metformin hydrochloride: An antihyperglycemic agent. *Am. J. Health Syst. Pharm.* **54**:893–903 (1997).
3. I. W. Campbell. Metformin and the sulphonylureas: The comparative risk. *Horm. Metab. Res. Suppl.* **15**:105–111 (1985).
4. C. R. Sirtori, G. Franceschini, M. Galli-Kienle, G. Cighetti, G. Galli, A. Bondioli, and F. Conti. Disposition of metformin (N,N-dimethylbiguanide) in man. *Clin. Pharmacol. Ther.* **24**:683–693 (1978).
5. P. J. Pentikäinen, P. J. Neuvonen, and A. Penttilä. Pharmacokinetics of metformin after intravenous and oral administration to man. *Eur. J. Clin. Pharmacol.* **16**:195–202 (1979).
6. A. Somogyi and M. Muirhead. Pharmacokinetic interactions of cimetidine 1987. *Clin. Pharmacokinet.* **12**:321–366 (1987).
7. A. Somogyi, C. Stockley, J. Keal, P. Rolan, and F. Bochner. Reduction of metformin renal tubular secretion by cimetidine in man. *Br. J. Clin. Pharmacol.* **23**:545–551 (1987).
8. V. Gorboulev, J. C. Ulzheimer, A. Akhoundova, I. Ulzheimer-Teuber, U. Karbach, S. Quester, C. Baumann, F. Lang, A. E. Busch, and H. Koepsell. Cloning and characterization of two human polyspecific organic cation transporters. *DNA Cell Biol.* **16**: 871–881 (1997).
9. L. Zhang, M. J. Dresser, A. T. Gray, S. C. Yost, S. Terashita, and K. M. Giacomini. Cloning and functional expression of a human liver organic cation transporter. *Mol. Pharmacol.* **51**:913–921 (1997).
10. H. Motohashi, Y. Sakurai, H. Saito, S. Masuda, Y. Urakami, M. Goto, A. Fukatsu, O. Ogawa, and K. Inui. Gene expression levels and immunolocalization of organic ion transporters in the human kidney. *J. Am. Soc. Nephrol.* **13**:866–874 (2002).
11. Y. Urakami, M. Okuda, S. Masuda, H. Saito, and K. Inui. Functional characteristics and membrane localization of rat multispecific organic cation transporters, OCT1 and OCT2, mediating tubular secretion of cationic drugs. *J. Pharmacol. Exp. Ther.* **287**: 800–805 (1998).
12. Y. Urakami, M. Okuda, S. Masuda, M. Akazawa, H. Saito, and K. Inui. Distinct characteristics of organic cation transporters, OCT1 and OCT2, in the basolateral membrane of renal tubules. *Pharm. Res.* **18**:1528–1534 (2001).
13. Y. Urakami, M. Akazawa, H. Saito, M. Okuda, and K. Inui. cDNA cloning, functional characterization, and tissue distribution of an alternatively spliced variant of organic cation transporter hOCT2 predominantly expressed in the human kidney. *J. Am. Soc. Nephrol.* **13**:1703–1710 (2002).
14. D. S. Wang, J. W. Jonker, Y. Kato, H. Kusuhara, A. H. Schinkel, and Y. Sugiyama. Involvement of organic cation transporter 1 in hepatic and intestinal distribution of metformin. *J. Pharmacol. Exp. Ther.* **302**:510–515 (2002).
15. D. S. Wang, H. Kusuhara, Y. Kato, J. W. Jonker, A. H. Schinkel, and Y. Sugiyama. Involvement of organic cation transporter 1 in the lactic acidosis caused by metformin. *Mol. Pharmacol.* **63**:844–848 (2003).
16. M. J. Dresser, G. Xiao, M. K. Leabman, A. T. Gray, and K. M. Giacomini. Interactions of n-tetraalkylammonium compounds and biguanides with a human renal organic cation transporter (hOCT2). *Pharm. Res.* **19**:1244–1247 (2002).
17. Y. Urakami, N. Kimura, M. Okuda, and K. Inui. Creatinine transport by basolateral organic cation transporter hOCT2 in the human kidney. *Pharm. Res.* **21**:976–981 (2004).
18. M. M. Bradford. A Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**:248–254 (1976).
19. M. Okuda, H. Saito, Y. Urakami, M. Takano, and K. Inui. cDNA cloning and functional expression of a novel rat kidney organic cation transporter, OCT2. *Biochem. Biophys. Res. Commun.* **224**: 500–507 (1996).
20. N. C. Sambol, J. Chiang, M. O'Conner, C. Y. Liu, E. T. Lin, A. M. Goodman, L. Z. Benet, and J. H. Karam. Pharmacokinetics and pharmacodynamics of metformin in healthy subjects and patients with noninsulin-dependent diabetes mellitus. *J. Clin. Pharmacol.* **36**:1012–1021 (1996).
21. A. J. Scheen. Clinical pharmacokinetics of metformin. *Clin. Pharmacokinet.* **30**:359–371 (1996).
22. M. B. Davidson and A. L. Peters. An overview of metformin in the treatment of type 2 diabetes mellitus. *Am. J. Med.* **102**:99–110 (1997).
23. N. C. Sambol, J. Chiang, E. T. Lin, A. M. Goodman, C. Y. Liu, L. Z. Benet, and M. G. Cogan. Kidney function and age are both predictors of pharmacokinetics of metformin. *J. Clin. Pharmacol.* **35**:1094–1102 (1995).
24. T. Katsura, M. Takano, Y. Tomita, M. Yasuhara, K. Inui, and R. Hori. Characteristics of organic cation transporter in rat renal basolateral membrane. *Biochim. Biophys. Acta* **1146**:197–202 (1993).
25. A. Somogyi, A. McLean, and B. Heinzow. Cimetidine-procainamide pharmacokinetic interaction in man: Evidence of competition for tubular secretion of basic drugs. *Eur. J. Clin. Pharmacol.* **25**:339–345 (1983).
26. R. Larsson, G. Bodemar, and B. Norlander. Oral absorption of cimetidine and its clearance in patients with renal failure. *Eur. J. Clin. Pharmacol.* **15**:153–157 (1979).
27. P. A. Bjaeldager, J. B. Jensen, N. E. Larsen, and E. F. Hvidberg. Elimination of oral cimetidine in chronic renal failure and during haemodialysis. *Br. J. Clin. Pharmacol.* **9**:585–592 (1980).
28. A. Somogyi, H. G. Rohner, and R. Gugler. Pharmacokinetics and bioavailability of cimetidine in gastric and duodenal ulcer patients. *Clin. Pharmacokinet.* **5**:84–94 (1980).

Available online at www.sciencedirect.com
SCIENCE DIRECT
Biochemical Pharmacology 69 (2005) 993–999
Biochemical Pharmacology
www.elsevier.com/locate/biochempharm

Restored expression and activity of organic ion transporters rOAT1, rOAT3 and rOCT2 after hyperuricemia in the rat kidney

Yasushi Habu [a], Ikuko Yano [a], Masahiro Okuda [a], Atsushi Fukatsu [b], Ken-ichi Inui [a,*]

[a] *Department of Pharmacy, Kyoto University Hospital, Faculty of Medicine, Kyoto University, Sakyo-ku, Kyoto 606-8507, Japan*
[b] *Division of Artificial Kidneys, Kyoto University Hospital, Faculty of Medicine, Kyoto University, Sakyo-ku, Kyoto 606-8507, Japan*

Received 22 October 2004; accepted 13 December 2004

Abstract

We previously reported that in hyperuricemic rats, renal impairment occurred and organic ion transport activity decreased, accompanied with a specific decrease in the expression of rat organic anion transporters, rOAT1 and rOAT3, and organic cation transporter, rOCT2. In the present study, we investigated the reversibility of the organic ion transport activity and expression of organic ion transporters (slc22a) during recovery from hyperuricemia. Hyperuricemia was induced by the administration of a chow containing uric acid and oxonic acid, an inhibitor of uric acid metabolism. Four days after discontinuance of the chow, the plasma uric acid concentration returned to the normal level, and renal functions such as creatinine clearance and BUN levels were restored, although the recovery of tubulointerstitial injury was varied in sites of the kidney. Basolateral uptake of *p*-aminohippurate (PAH) and tetraethylammonium (TEA), and both protein and mRNA levels of rOAT1, rOAT3 and rOCT2 in the kidney gradually improved during 14 days of recovery from hyperuricemia. Basolateral PAH transport showed a higher correlation with the protein level of rOAT1 ($r^2 = 0.80$) than rOAT3 ($r^2 = 0.34$), whereas basolateral TEA transport showed a strong correlation with rOCT2 protein ($r^2 = 0.91$). The plasma testosterone concentration, which is a dominant factor in the regulation of rOCT2, was gradually restored during the recovery from hyperuricemia, but the correlation between the plasma testosterone level and rOCT2 protein expression in the kidney was not significant. These results suggest that the regulation of organic ion transporters, rOAT1, rOAT3 and rOCT2, by hyperuricemia is reversible, and the organic ion transport activity restores according to the expression levels of these transporters.

Keywords: Renal transport; Organic anion transporter; Organic cation transporter; slc22a; Rats; Hyperuricemia

1. Introduction

Urinary excretion of various compounds including endogenous metabolites, drugs and xenobiotics is an important physiological function of the renal proximal tubules. In renal tubules, membrane transport systems mediate the tubular secretion, and several isoforms of organic anion and cation transporters have been characterized [1,2]. Two organic anion transporters, OAT1 and OAT3, at the renal basolateral membrane mediated the renal tubular secretion of several anionic drugs including *p*-aminohippurate (PAH), nonsteroidal anti-inflammatory drugs, methotrexate and cephalosporins [3–8]. On the other hand, organic cation transporters, OCT1 and OCT2, were localized to basolateral membranes of renal tubular cells, and contributed to the transport of many cationic compounds including tetraethylammonium (TEA), cimetidine, monoamines and procainamide [8–12].

We previously reported that renal organic anion and cation transport activity across the basolateral membrane was decreased in hyperuricemic rats, accompanied with decreased expression of some organic ion transporters, rOAT1, rOAT3 and rOCT2 [13]. In contrast, the renal expression levels of rOCT1, OAT-K1 and OAT-K2, kidney-specific organic anion transporters, and organic anion transporting polypeptide 1 (oatp1) were unchanged in hyperuricemic rat kidney [13]. Renal clearances of methotrexate and cimetidine were also decreased in hyperuricemic rats, suggesting that the down-regulation of rOAT1, rOAT3 and rOCT2 partly accounts for the decreased renal disposition of these drugs [13].

Altered expression of renal organic ion transporters has been reported using several animal models with renal impairment induced by cisplatin and by subtotal nephrect-

Abbreviations: PAH, *p*-aminohippurate; TEA, tetraethylammonium

* Corresponding author. Tel.: +81 75 751 3577; fax: +81 75 751 4207.
E-mail address: inui@kuhp.kyoto-u.ac.jp (K.-i. Inui).

0006-2952/$ – see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.bcp.2004.12.004

omy [14–17]. However, little information is available on the alterations in the expression of renal organic ion transporters during the recovery from renal impairment. On one hand, renal functions were decreased in hyperuricemic rats induced by the administration of a chow containing uric acid and oxonic acid, an inhibitor of uric acid metabolism [18]. After administration of the chow was discontinued, renal functions such as the fractional reabsorption of sodium and phosphate were improved, although the urine concentrating ability, calcium reabsorption and the capacity to excrete ammonium remained impaired [18]. Therefore, renal functions including renal organic ion transport might change at different rates during recovery from hyperuricemia in rats.

In the present study, we examined the alteration of organic ion transport activity and the expression of organic ion transporters including rOAT1, rOAT3 and rOCT2 in the kidney during recovery from hyperuricemia in rats.

2. Materials and methods

2.1. Materials

D-[1-^{3}H(*N*)]-mannitol (973 GBq/mmol) and *p*-[glycyl-1-^{14}C]-aminohippuric acid (1.9 GBq/mmol) were obtained from Perkin-Elmer™ Life Sciences. [1-^{14}C] Tetraethylammonium bromide (2.04 GBq/mmol) was obtained from American Radiolabeled Chemicals. Oxonic acid and uric acid were purchased from Aldrich and Wako Pure Chemical Industries, respectively. All other chemicals used were of the highest purity available.

2.2. Animals

The animal experiments were performed in accordance with the Guidelines for Animal Experiments of Kyoto University. The experimental protocol was approved by the Animal Research Committee, Graduate School of Medicine, Kyoto University. Male Wistar rats weighing 190–235 g were fed ground rat chow and water freely for 10–24 days. Hyperuricemia was induced with ground standard rat chow containing 2.5% uric acid and 5% oxonic acid for 10 days as described in our previous report (0-day recovery group) [13]. During recovery from hyperuricemia, the rats were fed standard rat chow for 4, 7 or 14 days (4, 7 or 14-day recovery group).

2.3. Biochemical tests

The concentration of blood urea nitrogen (BUN) and creatinine in plasma and urine were measured by the urease–indophenol method and Jaffé method using kits obtained from Wako Pure Chemical Industries, respectively. Plasma uric acid concentration was determined by high performance liquid chromatography as described in the previous report [13]. Plasma concentration of testosterone was measured with an enzyme immunoassay kit (Cayman Chemical Company).

2.4. Histological analyses

Kidneys of rats during recovery from hyperuricemia were removed and immediately fixed for 1 day at room temperature in carnoy fixative (ethanol:chloroform:acetic acid = 6:3:1) and preserved in 70% ethanol. Conventional histological sections were stained with periodic acid-Schiff reagent [8].

2.5. Uptake of PAH and TEA into renal slices

Renal slices were prepared with a Stadie–Riggs microtome and the uptake of [^{14}C]PAH (5 μM, 0.93 kBq/mL) or [^{14}C]TEA (5 μM, 1.03 kBq/mL) were measured as previously described [13]. [^{3}H]Mannitol (5 μM, 22.8 kBq/mL) was used to calculate the extracellular trapping and nonspecific uptake of [^{14}C]PAH and [^{14}C]TEA as well as to evaluate the viability of slices.

2.6. Western blot analyses

Preparation of crude membrane fractions and Western blot analyses were performed as previously reported [13].

2.7. Northern blot analyses

Total RNA was extracted from the kidney using TRIZOL™ reagent (Invitrogen Co.). Then, Northern blot analyses were performed as previously described [13].

2.8. Statistical analyses

The statistical significance of differences between mean values was calculated using the non-paired *t*-test, or by the one-way analysis of variance with the Scheffé test for post hoc analysis. *P*-values of <0.05 were considered significant.

3. Results

Several physiological and biochemical parameters were measured during the recovery period of hyperuricemia in rats (Fig. 1). The body weight gradually increased during recovery for 14 days. Plasma uric acid returned to the normal level during the initial 4 days of recovery. Improvement of BUN, plasma creatinine and creatinine clearance during the initial 4 days suggested that renal functions have been recovered quickly. In contrast, urine volume returned to normal more slowly than the above parameters.

Histological analyses of the kidney were performed and shown in Fig. 2. The tubular lumen was dilated in a diffuse

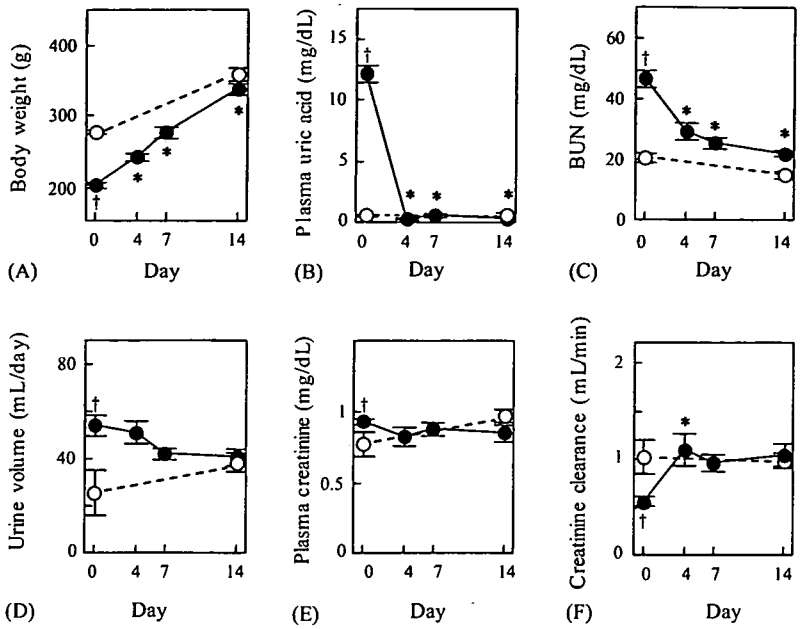

Fig. 1. Physiological and biochemical parameters during recovery from hyperuricemia. At 4, 7 and 14 days recovery from hyperuricemia, body weight (A), plasma uric acid (B), BUN (C), urine volume (D), plasma creatinine (E) and creatinine clearance (F) were measured (●). Open circles represent the values of normal rats. Each point represents the mean ± S.E. of 5–13 rats. $^{\dagger}P < 0.05$, vs. normal rats. $^{*}P < 0.05$, vs. hyperuricemic rats on day 0.

area filled with detached tubular epithelial cells and was infiltrated by mono- and poly-nuclear cells in hyperuricemic rat kidney (0-day recovery group) as previously reported [13]. Moreover, inflammatory cells also infiltrated into the interstitium and in tubules [13]. This tubulointerstitial pathology was observed in the diffuse area on day 4, and visible in the focal area on day 7, which was almost disappeared on day 14 (Fig. 2).

We then evaluated basolateral organic anion and cation transport activity by the uptake of PAH and TEA into renal slices (Fig. 3). The PAH and TEA uptake into renal slices in the 0-day recovery group were much lower than those in normal rats. In the 4-days recovery group, PAH and TEA uptake were significantly increased compared with those in 0-day recovery group. The PAH and TEA uptake in the 14-days recovery groups achieved similar uptake activity to those in control group at 30 or 60 min.

The expression of rOAT1, rOAT3 and rOCT2 protein during the recovery period of hyperuricemic rats was examined by Western blot analyses (Fig. 4). The expression of these transporters of 0-day recovery group was significantly decreased in hyperuricemic rats compared with normal rats, and the expression levels of rOAT1 and rOAT3 in 7- and 14-days recovery group significantly increased compared with those in 0-day recovery group. The expression of rOCT2 protein gradually increased until 14-days of recovery.

The mRNA expression of rOAT1 and rOCT2 during recovery from hyperuricemia was analyzed by Northern blot analyses (Fig. 5). The mRNA expression of both transporters in the 0-day recovery group was much lower than that in normal rats, and the rOAT1 mRNA expression significantly increased in the 7- and 14-days recovery group compared with 0-day recovery group. Recovery of rOCT2 mRNA expression was significant on day 14. Moreover, the expression of rOAT1 and rOCT2 protein was significantly correlated with that of mRNA, respectively (rOAT1, $r^2 = 0.66$, $P < 0.05$; rOCT2, $r^2 = 0.45$, $P < 0.05$).

We investigated the correlation between basolateral PAH and TEA transport and the protein level of rOAT1, rOAT3 and rOCT2 (Fig. 6). Initial PAH transport at 15 min across basolateral membrane showed a high correlation with the protein level of rOAT1 ($r^2 = 0.80$, $P < 0.05$), but not with that of rOAT3 ($r^2 = 0.34$, $P = 0.35$). On one hand, basolateral TEA transport showed a high correlation with the protein level of rOCT2 ($r^2 = 0.91$, $P < 0.05$).

Finally, we measured plasma concentration of testosterone, which was the dominant factor mediating sex-related difference in the expression of rOCT2 [19]. Plasma testosterone concentration was decreased in the 0-day recovery group (control, 3.23 ± 0.59 ng/mL; 0 day, 1.08 ± 0.10 ng/mL; mean ± S.E., $n = 6$) and was gradually increased during the recovery period of hyperuricemia (4 days, 1.68 ± 0.45 ng/mL; 7 days, 3.61 ± 0.24 ng/mL; 14 days, 4.51 ± 1.26 ng/mL; mean ± S.E., $n = 6$). However, plasma testosterone levels and the expression of rOCT2 protein did not show a significant linear correlation (Fig. 7, $r^2 = 0.14$, $P = 0.14$).

4. Discussion

Hyperuricemia is often the first clinical manifestation of gout and accompanies renal failure. Recently, serum uric

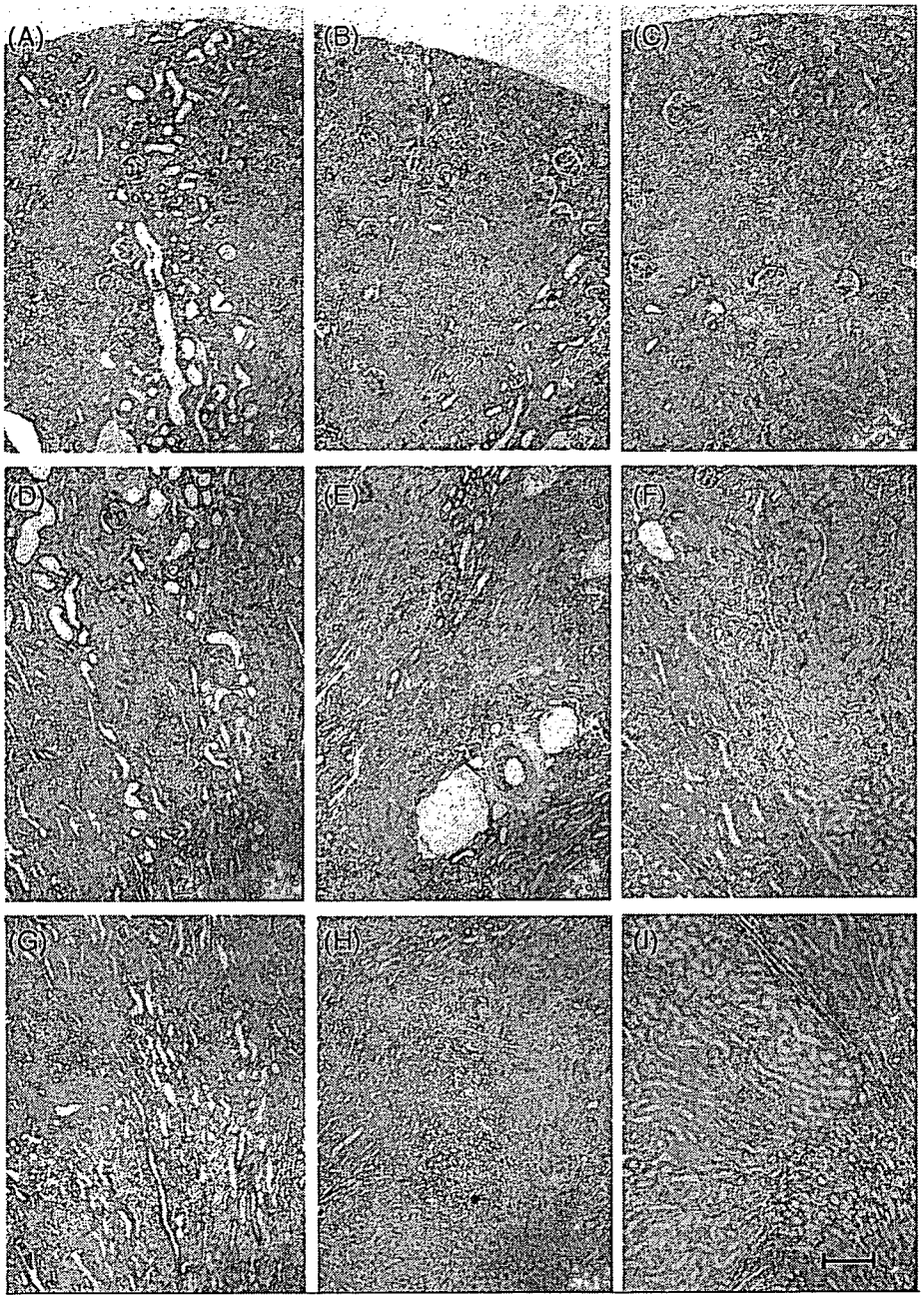

Fig. 2. Periodic acid-Schiff staining of cortex (A–C), outer medulla (D–F) and inner medulla (G–I) in the serial picture from each typical rat kidney at 4—(A, D and G), 7—(B, E and H) and 14—(C, F and I) days recovery from hyperuricemia; bar = 100 μm.

acid was found to be an independent risk factor for development of renal insufficiency in a study of 6403 subjects [20]. Mazaali et al. reported that crystal-independent mechanism, mediated in part by activation of the renin-angiotensin system and downregulation of NO synthase expression, contributed to renal injury in hyperuricemia [21]. Actually, we did not observe urate crystals in any part of kidneys in our experiments (Fig. 2). We previously reported that plasma creatinine and BUN levels increased, and that basolateral organic ion transport activity decreased, accompanied with specifically decreased expression of rOAT1, rOAT3 and rOCT2 in the kidney of hyperuricemic rats [13]. In this study, we investigated renal organic ion transport activity and the expression of rOAT1, rOAT3 and rOCT2 in kidney during recovery from hyperuricemia.

During the initial 4 days, BUN, plasma creatinine and creatinine clearance were restored significantly (Fig. 1), suggesting that renal functions recovered quickly. On the other hand, the recovery of tubulointerstitial injury was varied in sites of the kidney, probably because of the different severity of damage as shown in Fig. 2. Organic anion and cation transport activity across basolateral membranes and the expression of organic ion transporters, rOAT1, rOAT3 and rOCT2 in the kidney also gradually improved (Figs. 3 and 4). Interestingly, basolateral PAH

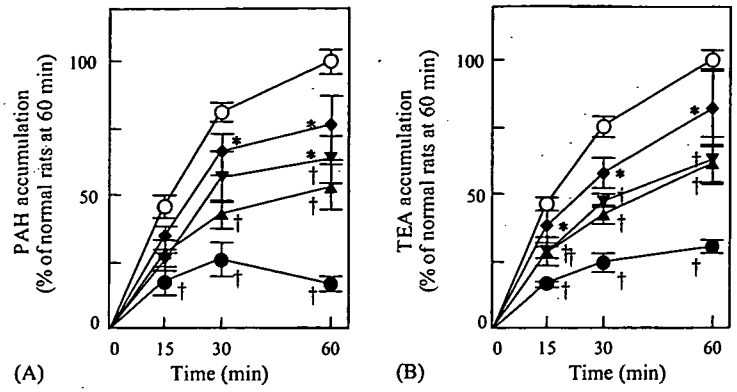

Fig. 3. Accumulation of PAH (A) and TEA (B) into renal slices during the recovery from hyperuricemia. Renal slices from normal (○) and hyperuricemic rats at 0: ●, 4: ▲, 7: ▼ and 14: ◆ days of recovery were incubated at 25 °C in incubation buffer containing 5 μM [^{14}C]PAH or 5 μM [^{14}C]TEA for the periods indicated. D-[^{3}H]Mannitol was used to estimate the extracellular trapping and non-specific uptake of [^{14}C]PAH and [^{14}C]TEA. Each point represents the mean ± S.E. for 5–8 slices from different rats. $^{\dagger}P < 0.05$, vs. normal rats. $^{*}P < 0.05$, vs. hyperuricemic rats on day 0.

transport showed a higher correlation with the protein level of rOAT1 than that of rOAT3 (Fig. 6), demonstrating that rOAT1 was a dominant transporter mediating the basolateral uptake of PAH. Basolateral TEA transport showed a high correlation with the protein level of rOCT2 (Fig. 6). As far as we know, this is the first report demonstrating the restoration of renal organic ion transporters (slc22a) during recovery from renal impairment, accompanying the change of basolateral uptake of PAH and TEA in the kidney.

The expression of rOCT2 is regulated by testosterone [19]. In contrast to rOCT2, renal expression of rOAT1 and rOCT1 was not changed between male and female, suggesting little or no contribution of testosterone in the regulation of renal rOAT1 and rOCT1 [22]. In addition, both plasma testosterone levels and the expression levels of rOCT2 decreased in 5/6 nephrectomized rats, where the expression levels of rOCT2 were restored by the administration of physiological concentrations of testosterone [17]. In clinical cases, a lower serum testosterone level was reported to be associated with chronic renal failure [23,24]. In the present study, both rOCT2 expression and plasma testosterone concentration decreased in hyperuricemia, and were restored to normal during recovery from hyperuricemia. However, the correlation between rOCT2 expression and plasma testosterone concentration was not significant. These results suggested that the decreased plasma testosterone level was one of the determinants of rOCT2 expression in hyperuricemic rats.

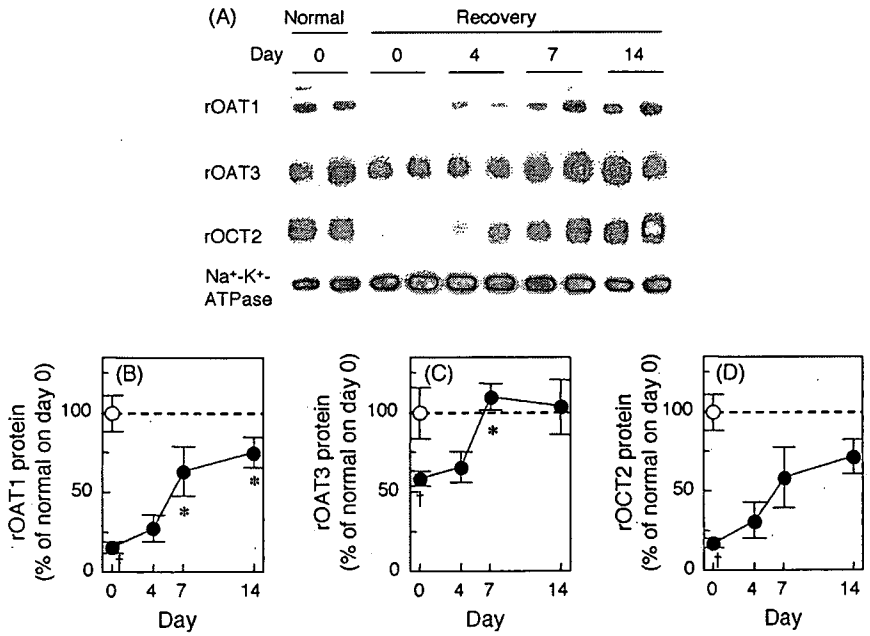

Fig. 4. Western blot analyses of rOAT1, rOAT3 and rOCT2 in crude membrane fractions from the kidneys during recovery from hyperuricemia. Crude membrane fractions from the kidneys of normal and hyperuricemic rats at 0, 4, 7 and 14 days of recovery were separated by SDS-PAGE. rOAT1, rOAT3, rOCT2 and Na^{+}-K^{+}-ATPase α-1 subunit were identified with each antibody. The results in a typical experiment are shown in panel (A). The ratio of rOAT1 (B), rOAT3 (C) and rOCT2 (D) density to Na^{+}-K^{+}-ATPase α-1 subunit density. Each point represents the mean ± S.E. for three normal (○) and four hyperuricemic (●) rats from two experiments. $^{\dagger}P < 0.05$, vs. normal rats. $^{*}P < 0.05$, vs. hyperuricemic rats on day 0.

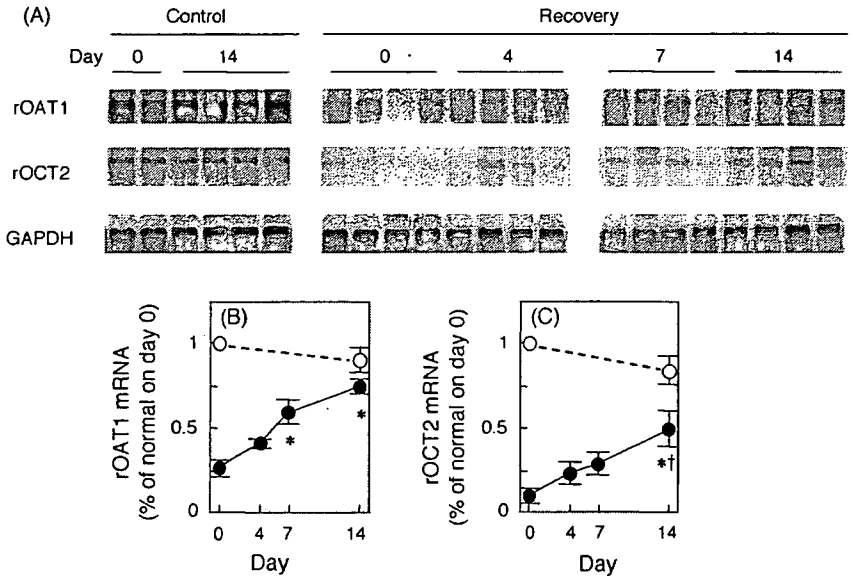

Fig. 5. Northern blot analyses of rOAT1 and rOCT2 during recovery from hyperuricemia. Total RNA (5 μg) from the kidneys of normal and hyperuricemic rats at 0, 4, 7 and 14 days of recovery was hybridized with rOAT1, rOCT2 and GAPDH cDNA probes under high stringency. The results in a typical experiment are shown in panel (A). Densitometry of rOAT1 (B) and rOCT2 (C) mRNAs was corrected for loading with GAPDH mRNA. Each point represents the mean ± S.E. for two to four normal (○) and four hyperuricemic (●) rats. $^{\dagger}P < 0.05$, vs. normal rats. $^{*}P < 0.05$, vs. hyperuricemic rats on day 0.

The dosage regimen of various drugs for patients with renal insufficiency is generally determined according to the value of creatinine clearance. However, renal excretion of ionic drugs into urine is mediated not only by glomerular filtration but also by tubular secretion via organic anion and cation transporters. Actually, the dosage schedule based on creatinine clearance was demonstrated to be inadequate for ampicillin and cephalexin dosing in some patients with renal insufficiency [25]. Recently, we reported that the elimination rate of cefazolin, which is a substrate of hOAT3, was significantly correlated with the levels of hOAT3 mRNA in humans [26]. In the present study, basolateral uptake of PAH and TEA in the kidney was significantly correlated with the expression of rOAT1 and

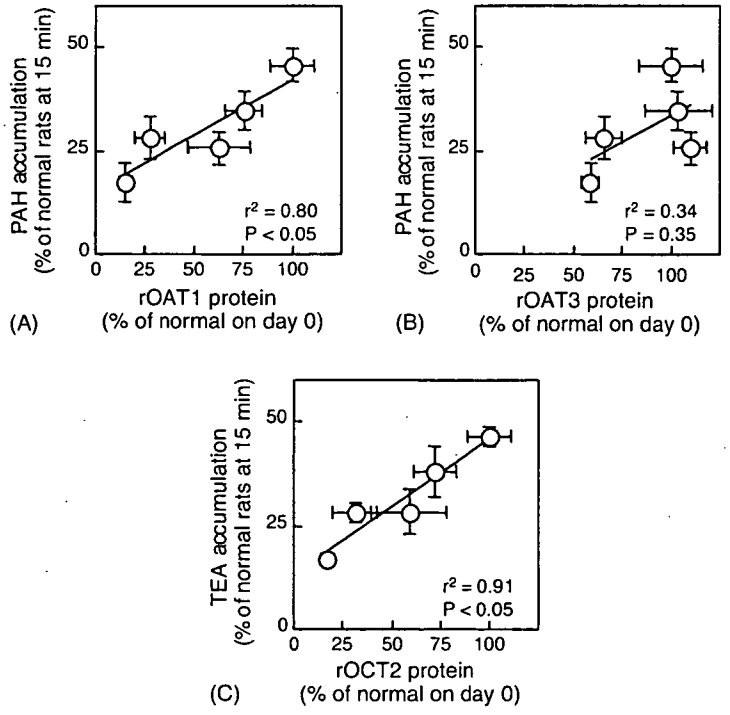

Fig. 6. Correlation between protein levels of rOAT1 (A), rOAT3 (B) and rOCT2 (C) and initial uptake activity of PAH (A and B) and TEA (C) by the renal slices during recovery from hyperuricemia. The linear regression was obtained with the mean values of rOAT1, rOAT3 and rOCT2 at protein levels and initial uptake activity of PAH and TEA. Each point represents the mean ± S.E. obtained in Figs. 3 and 4.

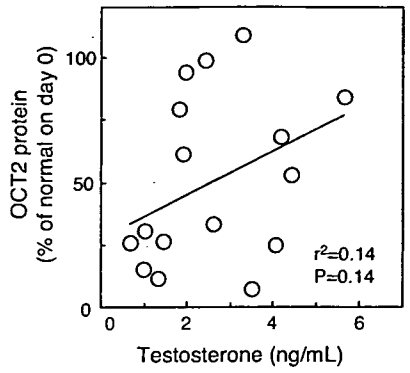

Fig. 7. Correlation between plasma testosterone and rOCT2 protein levels in the kidney during recovery from hyperuricemia. The linear regression was obtained with the plasma concentration of testosterone and rOCT2 protein levels of 17 rats during recovery from hyperuricemia.

rOCT2, respectively. Moreover, the recovery rate of rOAT1, rOAT3 and rOCT2 expression was slower than that of creatinine clearance. Based on these findings, dosage regiments according to the activity of organic anion and cation transport in addition to creatinine clearance should be helpful for precise dosage regimen in the patients with renal dysfunctions.

In conclusion, renal expression of organic ion transporters, rOAT1, rOAT3 and rOCT2, was reversibly regulated by hyperuricemia, accompanying the change of organic ion transport. Although further clinical investigations on the expression levels of drug transporters in several disease states are needed, the expression profiles of drug transporters may be useful information for understanding the alteration of renal drug secretion.

Acknowledgments

This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

References

[1] Pritchard JB, Miller DS. Mechanisms mediating renal secretion of organic anions and cations. Physiol Rev 1993;73:765–96.
[2] Inui K, Masuda S, Saito H. Cellular and molecular aspects of drug transport in the kidney. Kidney Int 2000;58:944–58.
[3] Sekine T, Watanabe N, Hosoyamada M, Kanai Y, Endou H. Expression cloning and characterization of a novel multispecific organic anion transporter. J Biol Chem 1997;272:18526–9.
[4] Sweet DH, Wolff NA, Pritchard JB. Expression cloning and characterization of ROAT1. The basolateral organic anion transporter in rat kidney. J Biol Chem 1997;272:30088–95.
[5] Cha SH, Sekine T, Fukushima J, Kanai Y, Kobayashi Y, Goya T, et al. Identification and characterization of human organic anion transporter 3 expressing predominantly in the kidney. Mol Pharmacol 2001; 59:1277–86.
[6] Sekine T, Cha SH, Endou H. The multispecific organic anion transporter (OAT) family. Pflügers Arch 2000;440:337–50.
[7] Uwai Y, Okuda M, Takami K, Hashimoto Y, Inui K. Functional characterization of the rat multispecific organic anion transporter OAT1 mediating basolateral uptake of anionic drugs in the kidney. FEBS Lett 1998;438:321–4.
[8] Motohashi H, Sakurai Y, Saito H, Masuda S, Urakami Y, Goto M, et al. Gene expression levels and immunolocalization of organic ion transporters in the human kidney. J Am Soc Nephrol 2003;13:866–74.
[9] Gründemann D, Gorboulev V, Gambaryan S, Veyhl M, Koepsell H. Drug excretion mediated by a new prototype of polyspecific transporter. Nature 1994;372:549–52.
[10] Okuda M, Saito H, Urakami Y, Takano M, Inui K. cDNA cloning and functional expression of a novel rat kidney organic cation transporter, OCT2. Biochem Biophys Res Commun 1996;224:500–7.
[11] Koepsell H, Gorboulev V, Arndt P. Molecular pharmacology of organic cation transporters in kidney. J Membr Biol 1999;167:103–17.
[12] Urakami Y, Okuda M, Masuda S, Akazawa M, Saito H, Inui K. Distinct characteristics of organic cation transporters, OCT1 and OCT2, in the basolateral membrane of renal tubules. Pharm Res 2001;18:1528–34.
[13] Habu Y, Yano I, Takeuchi A, Saito H, Okuda M, Fukatsu A, et al. Decreased activity of basolateral organic ion transports in hyperuricemic rat kidney: roles of organic ion transporters, rOAT1, rOAT3 and rOCT2. Biochem Pharmacol 2003;66:1107–14.
[14] Huang Q, Dunn II RT, Jayadev S, DiSorbo O, Pack FD, Farr SB, et al. Assessment of cisplatin-induced nephrotoxicity by microarray technology. Toxicol Sci 2001;63:196–207.
[15] Demeule M, Brossard M, Béliveau R. Cisplatin induces renal expression of P-glycoprotein and canalicular multispecific organic anion transporter. Am J Physiol Renal Physiol 1999;277:F832–40.
[16] Laouari D, Yang R, Veau C, Blanke I, Friedlander G. Two apical multidrug transporters, P-gp and MRP2, are differently altered in chronic renal failure. Am J Physiol Renal Physiol 2001;280:F636–45.
[17] Ji L, Masuda S, Saito H, Inui K. Down-regulation of rat organic cation transporter rOCT2 by 5/6 nephrectomy. Kidney Int 2002;62:514–24.
[18] Brown EA, Kliger AS, Hayslett JP, Finkelstein FO. Renal function in rats with acute medullary injury. Nephron 1980;26:64–8.
[19] Urakami Y, Okuda M, Saito H, Inui K. Hormonal regulation of organic cation transporter OCT2 expression in rat kidney. FEBS Lett 2000;473:173–6.
[20] Iseki K, Oshiro S, Tozawa M, Iseki C, Ikemiya Y, Takishita S. Significance of hyperuricemia on the early detection of renal failure in a cohort of screened subjects. Hypertens Res 2001;24:691–7.
[21] Mazaali M, Hughes J, Kim Y-G, Jefferson JA, Kang D-H, Godon KL, et al. Elevated uric acid increases blood pressure in the rat by a novel crystal-independent mechanism. Hypertension 2001;38: 1101–1106.
[22] Urakami Y, Nakamura N, Takahashi K, Okuda M, Saito H, Hashimoto Y, et al. Gender differences in expression of organic cation transporter OCT2 in rat kidney. FEBS Lett 1999;461:339–42.
[23] Joven J, Villabona C, Rubies-Prat J, Espinel E, Galard R. Hormonal profile and serum zinc levels in uraemic men with gonadal dysfunction undergoing haemodialysis. Clin Chim Acta 1985;148:239–45.
[24] Mitchell R, Bauerfeld C, Schaefer F, Scharer K, Rovertson WR. Less acidic forms of luteinizing hormone are associated with lower testosterone secretion in men on haemodialysis treatment. Clin Endocrinol 1994;41:65–73.
[25] Hori R, Okumura K, Kamiya A, Nihira H, Nakano H. Ampicillin and cephalexin in renal insufficiency. Clin Pharmacol Ther 1983;34: 792–8.
[26] Sakurai Y, Motohashi H, Ueo H, Masuda S, Saito H, Okuda M, et al. Expression levels of renal organic anion transporters (OATs) and their correlation with anionic drug excretion in patients with renal diseases. Pharm Res 2004;21:61–7.

Am J Physiol Gastrointest Liver Physiol 288: G664–G670, 2005.
First published November 4, 2004; doi:10.1152/ajpgi.00270.2004.

Increased protein level of PEPT1 intestinal H^+-peptide cotransporter upregulates absorption of glycylsarcosine and ceftibuten in 5/6 nephrectomized rats

Yuriko Shimizu, Satohiro Masuda, Kumiko Nishihara, Lin Ji, Masahiro Okuda, and Ken-ichi Inui

Department of Pharmacy, Kyoto University Hospital, Faculty of Medicine, Kyoto University, Sakyo-ku, Kyoto, Japan

Submitted 22 June 2004; accepted in final form 3 November 2004

Shimizu, Yuriko, Satohiro Masuda, Kumiko Nishihara, Lin Ji, Masahiro Okuda, and Ken-ichi Inui. Increased protein level of PEPT1 intestinal H^+-peptide cotransporter upregulates absorption of glycylsarcosine and ceftibuten in 5/6 nephrectomized rats. *Am J Physiol Gastrointest Liver Physiol* 288: G664–G670, 2005. First published November 4, 2004; doi:10.1152/ajpgi.00270.2004.—In chronic renal failure (CRF), dietary protein is one of the factors that deteriorates residual renal functions. Numerous studies have indicated that the products of protein digestion are mainly absorbed as small peptides. However, how small peptides are absorbed in CRF remains poorly understood. H^+-coupled peptide transporter (PEPT1/*SLC15A1*) plays an important role in the absorption of small peptides and peptide-like drugs in the small intestine. Because dietary protein intake is one of the risk factors for renal failure, the alteration of intestinal PEPT1 might have implications in the progression of renal disease as well as the pharmacokinetics of peptide-like drugs. In this study, we examined the alteration of intestinal PEPT1 in 5/6 nephrectomized (5/6 NR) rats, extensively used as a model of chronic renal failure. Absorption of [^{14}C]glycylsarcosine and ceftibuten was significantly increased in 5/6 NR rats compared with sham-operated rats, without a change in intestinal protease activity. Western blot analysis indicated that the amount of intestinal PEPT1 protein in 5/6 NR rats was increased mainly at the upper region. On the other hand, the amount of intestinal PEPT1 mRNA was not significantly different from that of sham-operated rats. These findings indicate that the increase in absorption of small peptides and peptide-like drugs, caused by the upregulation of intestinal PEPT1 protein, might contribute to the progression of renal failure as well as the alteration of drug pharmacokinetics.

renal failure; H^+-coupled peptide transporter; intestine

SMALL PEPTIDES, including di- and tripeptides, are the main products of protein digestion in the gut lumen (2, 13). The absorption of small peptides is mediated by H^+-coupled peptide transporter (PEPT1), which is localized at the brush-border membranes of intestinal epithelial cells. Furthermore, PEPT1 mediates the absorption of a broad range of peptide-like drugs, such as β-lactam antibiotics, the anti-cancer agent bestatin, and angiotensin-converting enzyme (ACE) inhibitors (10, 12, 21). Recently, Gangopadhyay et al. (8) reported that uncontrollable diabetes has a profound effect on the expression of intestinal PEPT1.

In chronic renal failure (CRF), morphological and enzymatic abnormalities have been found in the small intestinal mucosa of patients (7) and model rats at 12 wk after nephrectomy (9). For example, the activities of sucrase and maltase were reduced (9), and the expression of intestinal cytochrome *P*-450 was downregulated in rats at 6 wk after nephrectomy (11). However, the activity of some dipeptidases was significantly but weakly increased or unchanged in the isolated brush-border membranes from the nephrectomized rat intestinal mucosa at 8 wk after surgery (31). It seems plausible to presume that the absorptive function of the mucosa in CRF is disturbed. Recently, the impairment of intestinal P-glycoprotein function was reported in CRF rats (30). In CRF, dietary protein is considered to impair residual renal function, and therefore, patients with CRF are recommended to take a low-protein diet to prevent uremia (25). However, the regulation of intestinal PEPT1 in CRF remains unclear. On the basis of this background, we have hypothesized that the alteration of intestinal PEPT1 has implications not only in the pharmacokinetics of peptide-like drugs but also in the progression of renal failure in patients.

In the present study, we examined the functional and expressional changes of intestinal PEPT1 in 5/6 nephrectomized (5/6 NR) rats and demonstrated the effect of CRF on the intestinal absorptive rates of small peptides and peptide-like drugs. We report in this article that the activity of the intestinal peptide transporter was increased in CRF, which was caused by an upregulation of PEPT1 expression at the protein level.

MATERIALS AND METHODS

Materials. [^{14}C]glycylsarcosine (Gly-Sar; 1.78 GBq/mmol) was obtained from Daiichi Pure Chemicals (Ibaraki, Japan). Ceftibuten was from Shionogi (Osaka, Japan). All other chemicals were of the highest purity available.

Animals and their treatment. Male Wistar albino rats (200–220 g) were nephrectomized as described previously (16, 26). Briefly, the right kidney was removed, and the posterior and anterior apical segmental branches of the left renal artery were individually ligated. Sham-operated (sham) animals were used as controls. After surgery, animals were allowed to recover from anesthesia and surgery in cages, with free access to water and standard rat chow containing 23.6% (wt/wt) of protein.

To examine renal function, we maintained rats in metabolic cages for 24 h before the experiments. The blood urea nitrogen (BUN) was determined using the urease-indophenol method. The levels of creatinine in plasma and urine were determined using the Jaffé reaction. For these measurements, we used assay kits from Wako Pure Chemical Industries (Osaka, Japan). The concentration of urinary albumin was measured using an ELISA kit (NEPHRAT II; Exocell, Philadelphia, PA). The intestinal protease activity in the mucosal homogenate of duodenum, jejunum, or ileum was determined using a protease assay kit (Calbiochem, La Jolla, CA) according to the manufacturer's instructions. Data are expressed as percent activity by using trypsin (10 μg) as a positive control. The animal experiments were performed in accordance with the "Guidelines for Animal Experiments of Kyoto University." All protocols were previously approved by the Animal Research Committee, Graduate School of Medicine, Kyoto University.

Address for reprint requests and other correspondence: K. Inui, Dept. of Pharmacy, Kyoto Univ. Hospital, Sakyo-ku, Kyoto 606-8507, Japan (E-mail: inui@kuhp.kyoto-u.ac.jp).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.