Pravastatin, an HMG-CoA Reductase Inhibitor, Is Transported by Rat Organic Anion Transporting Polypeptide, oatp2

Taro Tokui,[1] Daisuke Nakai,[1] Rie Nakagomi,[1] Hiromu Yawo,[2] Takaaki Abe,[2,4] and Yuichi Sugiyama[3]

Received January 7, 1999; accepted February 23, 1999

Purpose. We previously demonstrated the HMG-CoA reductase inhibitor, pravastatin, is actively taken up into isolated rat hepatocytes through multispecific organic anion transporters. The present study examined whether a newly cloned organic anion transporting polypeptide (oatp2) transports pravastatin.

Methods. We investigated functional expression of oatp2 in *Xenopus laevis* oocytes, to examine [^{14}C] pravastatin uptake.

Results. [^{14}C] Pravastatin (30 μM) uptake into oatp2 cRNA-injected oocytes was 40 times higher than that of water-injected control oocytes. The oatp2-mediated pravastatin uptake was Na^+-independent and saturable. The Michaelis-Menten constant was 37.5 ± 9.9 μM, a level comparable to that obtained in isolated rat hepatocytes in our previous study. As is the case with rat hepatocytes, the uptake of pravastatin (30 μM) was inhibited by 300 μM concentrations of taurocholate, cholate, bromosulfophthalein, estradiol-17β-glucuronide, and simvastatin acid, but not by para-aminohippurate. On the other hand, [^{14}C] simvastatin acid (30 μM) uptake of oatp2 cRNA-injected oocytes was not significantly different from that of water-injected oocytes.

Conclusions. The cloned oatp2 was identified as the transporter responsible for the active hepatocellular pravastatin uptake.

KEY WORDS: Pravastatin; HMG-CoA reductase inhibitor; organic anion transporter; oatp2; hepatic transport.

INTRODUCTION

HMG-CoA reductase inhibitors decrease serum cholesterol by inhibiting cholesterol biosynthesis in the liver (1,2). Because inhibition of cholesterol synthesis in other types of tissues may lead to side effects (3), it is of great interest to restrict the inhibitory effect to liver tissue. Pravastatin, a hydrophilic HMG-CoA reductase inhibitor (Fig. 1), has been shown to inhibit cholesterol synthesis specifically in the liver (4,5). One reason for this selectivity of pravastatin may be its highly hydrophilic nature, which restricts its distribution to a limited number of organs. Thus, we hypothesized hepatic uptake of pravastatin on the liver surface involved a specific type of mechanism associated with a so-called "multispecific anion transporter (6)."

[1] Analytical and Metabolic Research Laboratories, Sankyo Co., Ltd., Tokyo, 140-8710, Japan.

[2] Department of Neurophysiology, Tohoku University School of Medicine, Sendai, 980-8575, Japan.

[3] Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo, 113-8654, Japan.

[4] To whom correspondence should be addressed. (e-mail: takaabe @mail.cc.tohoku.ac.jp)

Previously, we demonstrated that pravastatin is taken up actively into rat hepatocytes through a multispecific anion transporter (7,8). The initial uptake of pravastatin by isolated rat hepatocytes is competitively inhibited by bile acids (taurocholate and cholate); dibromosulfophthalein, a typical ligand for the multispecific anion transporter in the liver; and simvastatin acid, an active form of highly lipophilic HMG-CoA reductase inhibitor (Fig. 1). In contrast, Na^+-independent taurocholate uptake is competitively inhibited by pravastatin. Furthermore, the hepatic permeability surface area products for indices of unbound pravastatin, evaluated in four different experimental systems (*in vivo* (9), *in vitro* liver perfusion (10), isolated cells (8), and primary cultured cells (10)), were in good agreement, which indicates the active transporter mechanism on the liver surface for this drug is entirely responsible for its initial distribution into the liver (11).

At a molecular level, however, the transport system responsible for the hepatocellular pravastatin uptake has yet to be clarified. Jacquemin *et al.* have cloned Na^+-independent organic anion transporting polypeptides (oatp1) from rat liver, as a multispecific organic anion transporter (12). oatp1 represents an 80-kDa basolateral transporter (13) that mediates the hepatocellular uptake of a wide range of amphipathic substrates, including bromosulfophthalein, bile acids, estrogen conjugates, and peptide mimetic drugs (12,14,15). However, Kouzuki *et al.* found pravastatin uptake was not stimulated in COS-7 cells expressing oatp1 (16).

Recently, we succeeded in isolating oatp2 and oatp3 from rat retina, as homologues of oatp1, and demonstrated that these transporters took up thyroid hormone T3 and T4, as well as taurocholate (17). oatp2 mRNA was exclusively expressed in the liver, brain, and retina, and oatp3 was expressed in the kidney and retina (17). Noe *et al.* also cloned oatp2 from rat brain, and showed oatp2 transported bile acids, estrogen conjugates, ouabain, and digoxin (18). Recently, we demonstrated oatp2 immunoreactivity was observed at the basolateral membrane of the hepatocytes (19).

In the present study, we examined whether oatp2 transports pravastatin, to identify the transporter responsible for hepatocellular pravastatin uptake.

MATERIALS AND METHODS

Materials

[^{14}C]Pravastatin (specific activity: 14.3 mCi/mmol) was synthesized at Amersham Japan (Tokyo, Japan). The radiochemical purity, checked by HPLC methods, was 99%. [^{14}C]Simvastatin (lactone form, specific activity: 7.22 mCi/mmol) was synthesized at Amersham Japan (Tokyo, Japan), and [^{14}C]simvastatin acid (open acid form) was prepared from [^{14}C]simvastatin as follows: 10 μmol of [^{14}C]simvastatin was added to a mixture of 100 μl ethanol and 25 μl 0.5N NaOH. After a 30 min incubation at room temperature, the mixture was diluted with 100 μl 100 mM phosphate buffer and 675 μl ethanol (final concentration 10 mM). The radiochemical purity of [^{14}C]simvastatin acid, checked by HPLC methods, was 95%.

All other chemicals were of reagent grade.

0724-8741/99/0600-0904$16.00/0

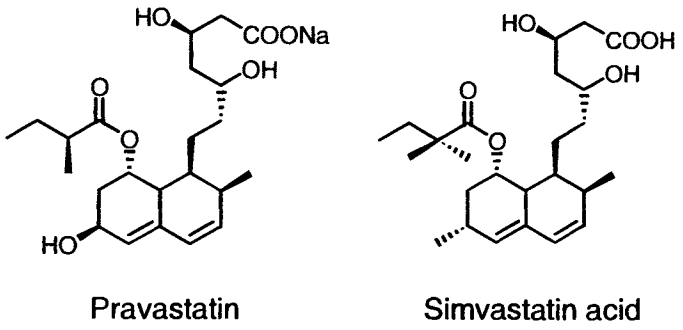

Fig. 1. Chemical structures of the HMG-CoA reductase inhibitors: pravastatin and simvastatin acid.

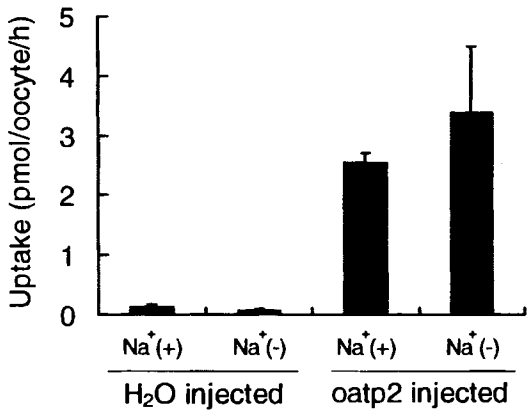

Fig. 2. Uptake of [^{14}C]pravastatin was measured in sodium chloride ($Na^+(+)$)- or choline chloride ($Na^+(-)$)-containing medium (see Material and Methods) in oatp2-cRNA- and H_2O-injected oocytes. Data are expressed as the mean ± SE of 3 to 5 uptake measurements.

Animals

Mature *Xenopus laevis* females were purchased from Hamamatsu Kyozai (Hamamatsu, Japan) and kept under standard conditions (20).

Expression of oatp2 in *Xenopus laevis* Oocytes

The coding region of oatp2 was ligated into *Xenopus* expression vector pGEM-HE, containing the *Xenopus* beta-globin 5′ and 3′ untranslated regions with some modifications (21). The complementary RNA (cRNA) was synthesized *in vitro* using T7 RNA polymerase in the presence of cap analogue m7GpppG from linearized construct. Frogs were anesthetized in an ice-cold bath containing 0.1% of ethyl m-amino benzoate (MS-222) for 30 minutes. Oocytes were removed from the ovary by laparotomy and transferred in Ca^{2+}-free OR-2 solution (in mM: 82.5 NaCl, 5 HEPES, pH 7.5, 2 KCl, 1 $MgCl_2$) containing 2 mg/ml collagenase A (Boehringer Mannheim). After 2 hours of incubation with gentle shaking at 18°C, the oocytes were washed in modified Barth's solution (in mM: 88 NaCl, 10 HEPES, pH 7.4, 1 KCl, 0.33 $Ca(NO_3)_2$, 0.41 $CaCl_2$, 0.82 $MgSO_4$, 2.4 $NaHCO_3$) containing 50 μg/ml gentamicin, 10 unit/ml penicilin, 10 μg/ml streptomicin, and 2.5 mM pyruvate. Stage V and VI oocytes were selected. After an overnight incubation at 18°C in the solution, vital oocytes were injected with 5 ng of oatp2 cRNA and cultured for 3 days at 18°C, with a daily change of the modified Barth's solution.

Uptake into Oocytes

Uptake experiments were started by incubating 6 oocytes at room temperature in 100 μl of either sodium-free or sodium-containing uptake buffer (in mM: 100 CholineCl (or NaCl), 10 HEPES/5 Tris, pH 7.5, 1 KCl, 1 $CaCl_2$, 2 $MgCl_2$) containing [^{14}C]pravastatin or [^{14}C]simvastatin acid. After the indicated intervals, uptake was terminated by the addition of 3 ml of ice-cold incubation buffer, and the oocytes were washed three times with the same ice-cold buffer. Single oocytes were dissolved in 0.5 ml of 10% (w/w) sodium dodecyl sulfate, and 4 ml of scintillation fluid (Hionic Fluor, Packard) was added. The radioactivity was determined in a Packard TriCarb 2200 CA liquid scintillation analyzer.

Determination of Kinetic Parameters

The oatp2-mediated uptake velocity (V_0) of pravastatin was calculated by subtracting the uptake in water-injected oocytes from that in cRNA-injected oocytes. The kinetic parameters for pravastatin were estimated according to the following equation: $V_0 = Vmax \cdot S/(Km + S)$, where Vmax is the maximum uptake velocity (pmol/min/oocyte), Km is the Michaelis constant (μM), and S is the pravastatin concentration in medium (μM). The above equation was fitted to the uptake data by an iterative nonlinear least-squares method using WinNonlin (Ver1.1, Scientific Consulting, Inc.).

RESULTS

oatp2-Mediated [^{14}C] Pravastatin Uptake in *Xenopus laevis* Oocytes

[^{14}C]Pravastatin (30 μM) uptake into oatp2-cRNA injected oocytes was 40 times higher than that of water-injected control oocytes (Fig. 2). The replacement of sodium in the medium by choline did not show any effect on the pravastatin uptake, thereby indicating its Na^+-independence. Figure 3 shows the time course of [^{14}C]pravastatin uptake by oocytes. As the uptake linearly increased up to 60 min, all subsequent initial uptake measurements were performed at 60 min. oatp2-mediated

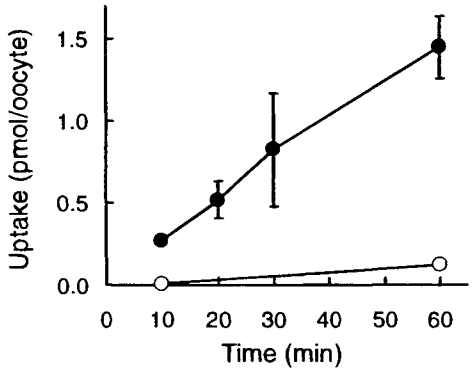

Fig. 3. Time course of [^{14}C]pravastatin (30 μM) uptake by oatp2-cRNA(●)- and H_2O (○)-injected oocytes. The uptake was measured in a $Na^+(-)$ medium. Data are expressed as the mean ± SE of 3 to 5 uptake measurements.

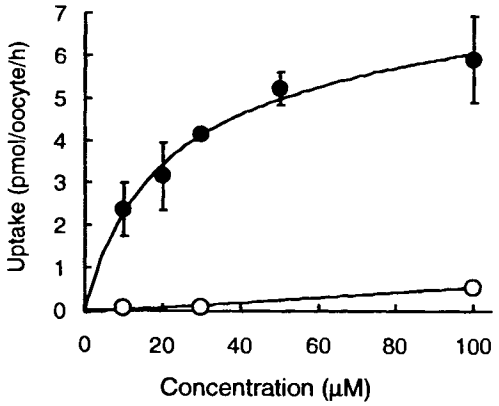

Fig. 4. Concentration dependency of oatp2-mediated [^{14}C]pravastatin uptake in oatp2-cRNA(●)- and H_2O (○)-injected oocytes. The uptake was measured in a $Na^+(-)$ medium. Data are expressed as the mean ± SE of 3 to 5 uptake measurements. The solid line is the least-squares fit of data to Eq. 1.

[^{14}C]pravastatin uptake was saturable with the Michaelis-Menten constant (Km) 37.5 ± 9.9 μM (n = 3) (Fig. 4).

Cis-Inhibition of oatp2-Mediated [^{14}C] Pravastatin by Organic Anions

The cis-inhibition study was performed to further characterize oatp2-mediated pravastatin uptake (Fig. 5). The addition of 300 μM of taurocholate or cholate inhibited the uptake of [^{14}C]pravastatin at 30 μM. The [^{14}C]pravastatin uptake was also strongly inhibited by 300 μM concentrations of bromosulfophthalein (the typical organic anion transporter substrate), estradiol-17β-glucuronide (a compound reported to be an excellent substrate for oatp2, with the Km 3 ± 1 μM (18)), and simvastatin acid. On the other hand, para-aminohippurate (a substrate for a renal organic anion exchanger OAT1(22)) did not exert any significant cis-inhibitory effect on the pravastatin uptake.

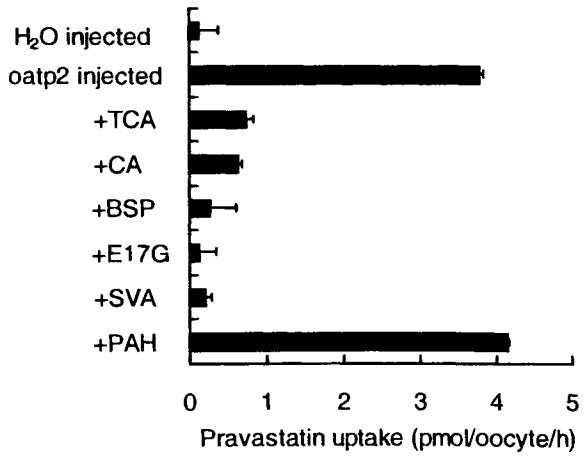

Fig. 5. Effects of bile acids and organic anions (300 μM) on [^{14}C]pravastatin (30 μM) uptake in oatp2-cRNA-injected oocytes. The uptake was measured in a Na^+ (−) medium. TCA: taurocholate; CA: cholate; BSP: bromosulfophthalein; E17G: estradiol-17β-glucuronide; SVA: simvastatin acid; PAH: para-aminohippurate. Data are expressed as the mean ± SE of 4 to 7 uptake measurements.

oatp2-Mediated [^{14}C] Simvastatin Acid Uptake in *Xenopus laevis* Oocytes

As [^{14}C]simvastatin acid uptake linearly increased up to 15 min (data not shown), the initial uptake measurements were performed at 15 min. [^{14}C]simvastatin acid uptake of oatp2-cRNA-injected oocytes up to 100 μM was not significantly different from that of water-injected oocytes (Fig. 6).

DISCUSSION

After oral administration, pravastatin was well absorbed from the gastrointestinal tract (70% in rats, and more than 50% in dogs) (23). Regarding transport across the intestinal brush-border membrane, a proton-gradient-dependent carrier-mediated mechanism has been suggested (24). Pravastatin is taken up efficiently from the circulation by the liver through a multispecific anion transporter (8). Subsequently, pravastatin is excreted to the bile via a primary active transporter system termed the "canalicular multispecific organic anion transporter" (cMOAT) (25), and enterohepatic circulation has been suggested to ensue (23). These carrier-mediated transport mechanisms contribute to efficient exposure of the target enzyme (HMG-CoA reductase) in the liver to this orally administered drug.

Though some investigators have identified a number of characteristics of pravastatin uptake into the liver (8,9,26), the molecular entity responsible for hepatocellular pravastatin uptake has not yet been clarified. In the present study, we demonstrated oatp2 mediates the uptake of pravastatin in *Xenopus laevis* oocytes. [^{14}C]pravastatin (30μM) uptake in oatp2-cRNA-injected oocytes was 40 times higher than that in water-injected control oocytes. This uptake was Na^+-independent (Fig. 2) and saturable (Fig. 4). The Michaelis-Menten constant we observed, 37.5 ± 9.9 μM, was comparable to that obtained in isolated rat hepatocytes (29.1 ± 5.8 μM). The uptake of [^{14}C]pravastatin was inhibited by bile acids, bromosulfophthalein, estradiol-17β-glucuronide, and simvastatin, but not by para-aminohippurate. All the data obtained in oatp2-expressing oocytes were in accord with those obtained in isolated rat hepatocytes (8). These data strongly suggested oatp2 was the

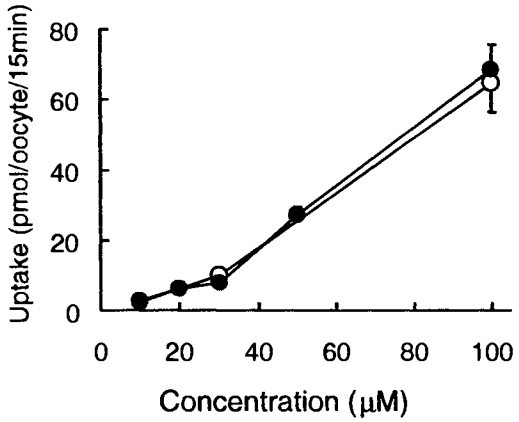

Fig. 6. Concentration dependency of [^{14}C]simvastatin acid uptake by oatp2-cRNA (●)- and H_2O (○)-injected oocytes. The uptake was measured in a $Na^+(-)$ medium. Data are expressed as the mean ± SE of 3 to 6 uptake measurements.

transporter responsible for the active hepatocellular pravastatin uptake.

On the other hand, [^{14}C]simvastatin acid uptake of oatp2-cRNA-injected oocytes was in no way different from that of water-injected oocytes (Fig. 6). The non-specific uptake clearance of [^{14}C]simvastatin acid, calculated from the uptake into H_2O-injected oocytes, was 0.04 μl/min/oocyte, i.e., about 500 times higher than that of [^{14}C]pravastatin (8×10^{-5} μl/min/oocyte). This high level of passive diffusion of [^{14}C]simvastatin acid may hide the contribution of oatp2 to the total uptake of [^{14}C]simvastatin acid. If the transporter is highly expressed in hepatocytes, the carrier-mediated transport system could contribute to the [^{14}C]simvastatin acid uptake. However, when we consider the carrier-mediated uptake clearance (18.8 μl/min/mg protein) and the non-specific uptake clearance (1.6 μl/min/mg protein) of [^{14}C]pravastatin in isolated rat hepatocytes (8), we may have to conclude that the transporters contribute only slightly to the [^{14}C]simvastatin acid uptake in the hepatocytes.

Estradiol-17β-glucuronide, a good substrate for oatp2, strongly inhibited the [^{14}C]pravastatin uptake. On the other hand, simvastatin acid strongly inhibited the [14C]pravastatin uptake, even though it was not a good substrate for oatp2 (Fig. 5). When discussing substrate specificity of a transporter, we should note a good inhibitor is not always a good substrate for the transporter.

oatp2 is highly expressed in the brain as well as in the liver, especially in the hippocampus, cerebellum, and choroid plexus (17). Nevertheless, since it has been shown that pravastatin did not distribute to the rat brain after intravenous administration (23) or in rat brain perfusion (27), we can surmise oatp2 may not contribute to the brain uptake of pravastatin. More definite studies on the exact topical and cellular localization of oatp2 in the rat brain will be required. Very recently, Li *et al.* reported glutathione was a driving force of oatp1 (28). The driving force of oatp2-mediated transmembrane substrate transport should be examined, to determine the direction of the transport.

oatp1 is highly homologous to oatp2, with an amino acid sequence identity of 77%. The reported substrate specificity was similar, and the only exception was digoxin, a cardiac glycoside (18). Digoxin was transported by oatp2- but not by oatp1-expressing oocytes. The structural components of importance for the identification of oatp and its transport are not known. Further investigations on the substrate specificities of oatp1 and oatp2 should help elucidate the multiplicity of the hepatic uptake of organic anions.

Kouzuki *et al.* recently found that pravastatin uptake by COS-7 cells transfected with oatp1 was not stimulated (16). However, it should also be noted, from this result alone we cannot conclude the contribution of oatp1 to Na^+-independent pravastatin uptake by rat hepatocytes is negligible. First, this is because the assay system Kouzuki *et al.* used may not have been adequate to detect the small transporting activities, and second, the configuration of oatp1 expressed in the COS-7 transfectant system may not have been suitable for accepting pravastatin as a substrate. Further studies should be performed to estimate the exact contribution of oatp1 to pravastatin uptake by rat hepatocytes.

It was suggested pravastatin was also taken up by human hepatocytes via an active transport system (29). The transport kinetics in human hepatocytes have not yet been reported. As a hepatic organic anion transporter in humans, OATP has been cloned by Kullak-ublick *et al.* (30). However, since human OATP was shown to be mainly expressed in the brain and was not found to accept digoxin as a substrate (18), we can assume it is not necessarily a counterpart of rat oatp1 or rat oatp2. There may be several additional clones coding for what may be closely related organic anion transporter proteins.

In summary, the cloned oatp2 was identified as the transporter responsible for active hepatocellular pravastatin uptake. To further elucidate the contribution of oatp2 to the hepatic uptake of pravastatin, the inhibition of uptake in rat hepatocytes using function-perturbing antibodies should be examined. Moreover, studies seeking to identify a human transporter responsible for pravastatin uptake will be required.

ACKNOWLEDGMENTS

This work was supported in part by research grants from the Ministry of Education, Science and Culture of Japan, and the Inamori Foundation.

REFERENCES

1. S. M. Grundy. HMG-CoA reductase inhibitors for treatment of hyper-cholesterolemia. *N. Engl. J. Med.* **319**:324–332 (1988).
2. D. R. Illingworth. HMG-CoA reductase inhibitors. *Lipidology* **2**:244–34 (1991).
3. W. A. Scott, E. M. Mahoney, and S. T. Mosley. Mechanism of action and differential pharmacology of pravastatin, a hydrophilic and selective HMG-CoA reductase inhibitor. In J. C. LaRosa (eds.), *New Advances in the Control of Lipid Metabolism*: Focus on Pravastatin, Royal Society of Medicine, 1989, pp. 1–8.
4. Y. Tsujita, M. Kuroda, Y. Shimada, K. Tanzawa, M. Arai, I. Kaneko, M. Tanaka, H. Masuda, C. Tamura, Y. Watanabe, and S. Fujii. CS-514, a competitive inhibitor of 3-hydroxyl-3-methylglutaryl coenzyme A reductase: tissue-selective inhibition of sterol synthesis and hypolipidemic effect on various animal species. *Biochim. Biophys. Acta* **1045**:115–120 (1990).
5. T. Koga, Y. Shimada, M. Kuroda, Y. Tsujita, K. Hasegawa, and M. Yamazaki. Tissue selective inhibition of cholesterol synthesis in vivo by pravastatin sodium, a 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor. *Biochim. Biophys. Acta* **877**: 50–60 (1986).
6. P. J. Meier. Transport polarity of hepatocytes. *Semin. Liver Dis.* **8**:649–661 (1988).
7. T. Komai, E. Shigehara, T. Tokui, T. Koga, M. Ishigami, C. Kuroiwa, and S. Horiuchi. Carrier-mediated uptake of pravastatin by rat hepatocytes in primary culture. *Biochem. Pharmacol.* **43**:667–670 (1992).
8. M. Yamazaki, H. Suzuki, M. Hanano, T. Tokui, T. Komai, and Y. Sugiyama. Na+-independent multispecific anion transporter mediates active transport of pravastatin into rat liver. *Am. J. Physiol.* **264**:G36–G44 (1993).
9. M. Yamazaki, T. Tokui, M. Ishigami, and Y. Sugiyama. Tissue-selective uptake of pravastatin in rats: contribution of a specific carrier-mediated uptake system. *Biopharm. Drug Disposit.* **17**:990.1–15 (1996).
10. M. Ishigami, T. Tokui, T. Komai, K. Tsukahara, M. Yamazaki, and Y. Sugiyama. Evaluation of the uptake of pravastatin by perfused rat liver and primary cultured rat hepatocytes. *Pharm. Res.* **12**:1741–1745 (1995).
11. M. Yamazaki, H. Suzuki, and Y. Sugiyama. Recent advances in carrier-mediated hepatic uptake and biliary excretion of xenobiotics. *Pharm. Res.* **13**:497–513 (1996).
12. E. Jacquemin, B. Hagenbuch, B. Stieger, A. W. Wolkoff, and P. J. Meier. Expression cloning of a rat liver Na+-independent organic anion transporter. *Proc. Natl. Acad. Sci. USA* **91**:133–137 (1994).
13. A. J. Bergwerk, X. Shi, A. C. Ford, N. Kanai, E. Jacquemin, R. D. Burk, S. Bai, P. M. Novikoff, B. Stieger, P. J. Meier, V. L. Schuster, and A. W. Wolkoff. Immunologic distribution of an

organic anion transport protein in rat liver and kidney. *Am. J. Physiol.* **271**:G231–G238 (1996).
14. X. Bossuyt, M. Muller, B. Hagenbuch, and P. J. Meier. Polyspecific drug and steroid clearance by an organic anion transporter of mammalian liver. *J. Pharmacol. Exp. Ther.* **276**:891–896 (1996).
15. U. Eckhardt, J. A. Horz, E. Petzinger, W. Stuber, M. Reers, G. Dickneite, H. Daniel, M. Wagener, B. Hagenbuch, B. Stieger, and P. J. Meier. The peptide-based thrombin inhibitor CRC 220 is a new substrate of basolateral rat liver organic anion transporting polypeptide. *Hepatology* **24**:380–384 (1996).
16. H. Kouzuki, H. Suzuki, K. Ito, R. Ohashi, and Y. Sugiyama. Contribution of organic anion transporting polypeptide to the uptake of its possible substrates into rat hepatocytes. *J. Pharmacol. Exp. Ther.* in press.
17. T. Abe, M. Kakyo, H. Sakagami, T. Tokui, T. Nishio, M. Tanemoto, H. Nomura, S. C. Hebert, S. Matsuno, H. Kondo, and H. Yawo. Molecular characterization and tissue distribution of a new organic anion transporter subtype (oatp3) that transports thyroid hormones and taurocholate, and comparison with oatp2. *J. Biol. Chem.* **273**:22395–22401 (1998).
18. B. Noe, B. Hagenbuch, B. Stieger, and P. J. Meier. Isolation of a multispecific organic anion and cardiac glycoside transporter from rat brain. *Proc. Natl. Acad. Sci.* **94**:10346–10350 (1997).
19. M. Kakyo, H. Sakagami, T. Nishio, D. Nakai, R. Nakagomi, T. Tokui, T. Naitoh, S. Matsuno, T. Abe, and H. Yawo. Immunohistochemical distribution and functional characterization of an organic anion transporting polypeptide 2 (oatp2). *FEBS Letter* (in press).
20. A. Goldin. Maintenance of *Xenopus laevis* and oocyte injection. *Methods in Enzymology* **207**:266–279 (1992).
21. E. R. Liman, J. Tytgat, and P. Hess. Subunit stoichiometry of a mammalian K+ channel determined by construction of multimeric cDNAs. *Neuron* **9**:861–871 (1992).
22. T. Sekine, N. Watanabe, M. Hosoyamada, Y. Kanai, and H. Endou. Expression cloning and characterization of a novel multispecific organic anion transporter. *J. Biol. Chem.* **272**:18526–18529 (1997).
23. T. Komai, K. Kawai, T. Tokui, Y. Tokui, C. Kuroiwa, E. Shigehara, and M. Tanaka. Disposition and metabolism of pravastatin sodium in rats, dogs and monkeys. *Eur. J. Met. Pharmacokin.* **17**:103–113 (1992).
24. I. Tamai, H. Takanaga, H. Maeda, T. Ogihara, M. Yoneda, and A. Tsuji. Proton-cotransport of pravastatin across intestinal brush-border membrane. *Pharm. Res.* **12**:1727–1732 (1995).
25. M. Yamazaki, S. Akiyama, K. Ni'inuma, R. Nishigaki, and Y. Sugiyama. Biliary excretion of pravastatin in rats: contribution of the excretion pathway mediated by canalicular multispecific organic anion transporter (cMOAT). *Drug Metab. Disposit.* **25**:1123–1129 (1997).
26. K. Zieglar and W. Stunkel. Tissue-selective action of pravastatin due to hepatocellular uptake via a sodium-independent bile acid transporter. *Biochim. Biophys. Acta* **1139**:203–209 (1992).
27. A. Saheki, T. Terasaki, I. Tamai, and A. Tsuji. In vivo and in vitro blood-brain barrier transport of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors. *Pharm. Res.* **11**:305–311 (1994).
28. L. Li, T. K. Lee, P. J. Meier, and N. Ballatori. Identification of glutathione as a driving force and leukotriene C_4 as a substrate for oatp1, the hepatic sinusoidal organic solute transporter. *J. Biol. Chem.* **273**:16184–16191 (1998).
29. A. K. van Vliet, G. C. F. van Thiel, R. H. Huisman, H. Moshage, S. H. Yap, and L. H. Cohen. Different effects of 3-hydroxyl-3-methylglutaryl-coenzyme A reductase inhibitors on sterol synthesis in various human cell types. *Biochim. Biophys. Acta* **1254**:105–111 (1995).
30. G. A. Kullak-Ublick, B. Hagenbuch, B. Stieger, A. W. Wolkoff, and P. J. Meier. Functional characterization of the basolateral rat liver organic anion transporting polypeptide. *Hepatol.* **20**:411–416 (1994).