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Functional identification of organic cation transporter 1 as an atenolol transporter sensitive to flavonoids

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ABSTRACT

Atenolol, a β1-adrenergic receptor blocker, is administered orally and its intestinal absorption has recently been indicated to be mediated by carrier protein and reduced markedly by ingestion of some fruit juices, such as apple and orange juices. This could be postulated to be a problem arising from the interaction of some components of fruit juices with atenolol at a transporter involved in its intestinal uptake, but the responsible transporter and its interacting components have not been identified yet. In an attempt to examine that possibility, we could successfully find that human organic cation transporter 1 (OCT1/SLC22A1), which is suggested to be expressed at the brush border membrane of enterocytes, is highly capable of transporting atenolol. In this attempt, OCT1 was stably expressed in Madin-Darby canine kidney II cells and the specific uptake of atenolol by the transporter was found to be saturable, conforming to the Michaelis-Menten kinetics with the maximum transport rate (V_{max}) of 4.00 nmol/min/mg protein and the Michaelis constant (K_m) of 3.08 mM. Furthermore, the OCT1-specific uptake was found to be inhibited by various flavonoids, including those contained in fruit juices that have been suggested to interfere with intestinal atenolol absorption. Particularly, phloretin and quercetin, which are major components of apple juice, were potent in inhibiting OCT1-mediated atenolol transport with the inhibition constants of 38.0 and 48.0 μM, respectively. It is also notable that the inhibition by these flavonoids was of the noncompetitive type. These results indicate that OCT1 is an atenolol transporter that may be involved in intestinal atenolol uptake and sensitive to fruit juices, although its physiological and clinical relevance remains to be further examined.

1. Introduction

Atenolol is a selective β1-adrenergic receptor blocker that is orally effective in the treatment of hypertension, angina pectoris, and cardiac arrhythmias [1–3]. This drug, of which the absorption is rather moderate with the bioavailability of about 50% [4], has generally been assumed to be absorbed by simple diffusion in the intestine [5]. However, based on recent findings of significant reductions in its absorption by simultaneous administration with apple juice [6] and with orange juice [7], it may be needed to assume that carrier-mediated uptake transport, which could be inhibited by some constituents of those fruit juices, is involved in its absorption. It may be possible that the disposition of this hydrophilic drug, which little undergoes metabolism and is eliminated almost exclusively (90%) by excretion into the urine in unchanged form after absorption [8], is regulated by membrane transporters.

Organic anion transporting polypeptide 1A2 (OATP1A2/SLCO1A2) and OATP2B1/SLCO2B1, which have recently been suggested to be involved in the intestinal uptake of various anionic drugs [9], can also be inhibited by such plant components, and it could lead to decreases in the absorption of their substrate drugs [10,11]. It should be of interest to note that some β1-adrenergic receptor blockers structurally analogous to atenolol have been reported to be transported by OATP1A2 and OATP2B1 [12,13], even though this class of drugs is cationic. Particularly, talinolol is known as an OATP1A2 substrate, and its absorption is also inhibited by flavonoids [12]. Thus, OATP1A2 and OATP2B1 may also contribute to the absorption of atenolol, but another possibility could not be excluded that some other transporters for cationic compounds may participate in that process. We, here, describe our successful attempt to identify human OCT1/SLC22A1 as an atenolol transporter, which may be involved in intestinal atenolol absorption. OCT1 is known to be typically expressed in the

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basolateral membrane of hepatocytes and contribute to extrusion of various cationic compounds including endogenous compounds and drugs, such as tetraethylammonium (TEA), 1-methyl-4-phenylpyrimidine (MPP^+), acetylcholine and metformin [14–16]. Diphenhydramine, atropine and desipramine are, on the other hand, known as its typical inhibitors that are not transported. Recently, its presence and potential role in drug transport has also been suggested in the intestine in the brush border and basolateral membranes.

2. Materials and methods

2.1. Materials

[^{14}C]Tetraethylammonium (TEA, 55 mCi/mmol) and [^{3}H]1-methyl-4-phenylpyridinium (MPP^+, 85 Ci/mmol) were obtained from American Radiolabeled Chemicals (St. Louis, MO, USA), [^{3}H]atenolol (3.3 Ci/mmol) was from Moravek Biochemicals (Mercury Lane, Brea, CA, USA), and [^{3}H]estrone-3-sulfate (54 Ci/mmol) was from PerkinElmer (Waltham, MA, USA). Unlabeled atenolol and Dulbecco's modified Eagle's medium (DMEM) were from Wako Pure Chemical Industries (Osaka, Japan). Fetal bovine serum (FBS) was obtained from Invitrogen (Carlsbad, CA, USA). All other regents were of analytical grade and commercially obtained.

2.2. Cell culture

Madin-Darby canine kidney II (MDCKII) cells and human embryonic kidney 293 (HEK293) cells were maintained at 37 °C and 5% CO_2 in DMEM supplemented with 10% FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin.

2.3. Plasmids

The plasmids carrying the cDNAs of OCT1/SLC22A1, OCT2/SLC22A2, OCT3/SLC22A3, OCTN1/SLC22A4 and OCTN2/SLC22A5 of human were those prepared in our previous study [17]. The plasmids carrying the cDNAs of OATP1A2 and OATP2B1 of human were prepared by using the cDNAs isolated by RT-PCR cloning from the human small intestine total RNA (Clontech, Mountain View, CA, USA) in the present study. In brief, an RT reaction was performed to obtain the cDNA mixture, using 1 μg of the total RNA, an oligo(dT) primer and ReverTra Ace (Toyobo, Osaka, Japan) as a reverse transcriptase. The cDNA of OATP1A2 was amplified by PCR, using KOD plus polymerase (Toyobo) and the following primers: forward primer, 5'-CCA GAT TTT AAG ACC AAC GC-3' and reverse primer, 5'-TTC AAA GTT CCC CAG TGT AA-3'. These primers were designed on the basis of the sequence in GenBank (accession number NM_021094.3). PCR was performed using the following conditions: predenature at 94°C for 2 min and 33 cycles of (1) denature at 98°C for 10 s, (2) annealing at 52°C for 20 s, and (3) extension at 68°C for 90 s. The second PCR was performed using the PCR product as a template and a forward primer containing XhoI restriction site (underlined), 5′-GAA CTC G AGC AAC ATG GGA GAA ACT G-3′ and a reverse primer containing XbaI restriction site (underlined), 5′-GCT CTA GAG TTG TAC AGC ATG TTC TC-3′. The amplified cDNA product was incorporated into pCI-neo vector (Promega, Madison, WI, USA) at XhoI and XbaI sites, and the sequence of the final product was determined with an automated sequencer. The cDNA of OATP2B1 (GenBank accession number NM_007256. (4) was isolated similarly by RT-PCR cloning. The primers for PCR were as follows: forward primer, 5′-TGC TTC CTC TCC CCT GCT AAG-3′ and reverse primer, 5′-GAA GGT GAT CCA GGC GAG TG-3′. The second PCR was performed using the PCR product as a template and a forward primer containing XhoI restriction site (underlined), 5′-GAA CTC GAG GTC ATG GGA CCC AGG ATA G-3′ and a reverse primer containing XbaI restriction site (underlined), 5′- GTG TCT AGA GGA GGT ACT GCT GTG GC-3′. The amplified cDNA product was incorporated into pCI-neo vector at XhoI and XbaI sites and the sequence of the final product was determined with an automated sequencer.

2.4. Preparation of MDCKII cells stably expressing OCT1

MDCKII cells were transfected with the plasmid carrying the cDNA of OCT1 by using Lipofectamine 2000 (Invitrogen) as a transfection reagent, according to the manufacturer's instructions, and cultured in DMEM supplemented with 10% FBS and 400 μg/ml G418 for 2 or 3 weeks. Antibiotic resistant clones were selected and tested for the transport of [^{3}H]MPP^+ as a probe substrate.

2.5. Uptake study in HEK293 cells transiently expressing OCTs, OATPs and OCTNs

HEK293 cells (2×10^5 cells/ml, 1 ml/well) were grown on 24-well plates coated with poly-L-lysine for 12 h, transfected with the plasmid carrying the cDNA of each transporter by using Lipofectamine 2000, and cultured for 36 h for transient expression. The transient expression of each transporter was confirmed by observing the specifically induced uptake of its typical substrate in HEK293 cells transfected with the plasmid for the transporter. The substrates were [^{3}H]TEA for OCT1,OCT2, OCT3, OCTN1 and OCTN2, and [^{3}H]estrone-3-sulfate for OATP1A2 and OATP2B1. The cells in each well were preincubated in 1 ml of substrate-free uptake buffer, that is Hanks' solution (136.7 mM NaCl, 5.36 mM KCl, 0.925 mM $CaCl_2$, 0.812 mM $MgSO_4$, 0.441 mM KH_2PO_4, 0.385 mM Na_2HPO_4, and 25 mM D-glucose) supplemented with 10 mM HEPES (pH 7.4), for 5 min and uptake assays were started by replacing the substrate-free uptake buffer for preincubation with uptake buffer containing [^{3}H]atenolol (0.25 ml). All the procedures were conducted at 37 °C. After uptake for 3 min, assays were stopped by addition of ice-cold substrate-free uptake buffer (2 ml) and the cells were washed two times with 2 ml of the same buffer. The cells were solubilized in 0.5 ml of 0.2 M NaOH solution containing 0.5% sodium dodecyl sulfate at room temperature for 1 h and the associated radioactivity was measured by liquid scintillation counting, using 3 ml of Clear-sol I (Nacalai Tesque, Kyoto, Japan) as a scintillation fluid, for the evaluation of uptake. Cellular protein content was determined by the BCA method (BCA Protein Assay Reagent Kit; Thermo Fisher Scientific, Waltham, MA, USA), using bovine serum albumin as the standard. Uptake assays were also conducted in mock cells, which were transfected with empty pCI-neo vector, to estimate nonspecific uptake.

2.6. Uptake study in MDCKII cells stably expressing OCT1

MDCKII cells stably expressing OCT1 (1.5×10^5 cells/ml, 1 ml/well) were grown on 24-well plates for 48 h to confluence. The assays of [^{3}H]atenolol uptake, which was for 3 min in regular experiments, were conducted by the same procedures as those described for assays in HEK293 cells transiently expressing OCT1. [^{3}H]MPP^+ was used as a substrate in some experiments to assess the OCT1-mediated transport characteristics of this typical substrate for comparison with those of [^{3}H]atenolol. To examine the effect of pH, Hanks' solution was supplemented with 10 mM MES, instead of HEPES, and adjusted to pH 5.5. To examine the effect of flavonoids and some other inhibitors, test compounds were added only to the uptake solution containing [^{3}H]atenolol so that they were present in the solution only during uptake period. In experiments to examine the effect of $BaCl_2$ as an agent to depolarize the plasma membrane, a chloride salt-based buffer (145 mM NaCl,