Characterization of monocarboxylate transporter 6: expression in human intestine and transport of the antidiabetic drug nateglinide

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Running title: Nateglinide transport via MCT6

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Abbreviations:
CHC, alpha-cyano-4-hydroxycinnamic acid; MCT, monocarboxylate transporter; OAT, organic anion transporter; PAH, p-aminohippuric acid; PEPT, peptide transporter; SLC, solute carrier; TAT1, T-type amino acid transporter.
ABSTRACT

Monocarboxylate transporter 6, encoded by SLC16A5, is a member of the monocarboxylate transporter (MCT) family. Nateglinide, an oral hypoglycemic agent, quickly reaches the maximal serum concentration after its pre-meal administration. Although the functional existence of uptake systems for nateglinide in intestine has been demonstrated, these transport systems have not yet been identified at the molecular level. The aim of this study was to demonstrate the localization of MCT6 in the human small intestine, and characterize the transport properties of nateglinide via MCT6. Immunohistochemical analysis of human small intestine revealed that anti-MCT6 antiserum stained the luminal side of the epithelial cells. When expressed in Xenopus laevis oocytes, MCT6-mediated uptake of [14C]nateglinide was sensitive to extracellular pH- and membrane potential. Furthermore, the Kᵢ value of nateglinide (45.9 μM) for MCT6 was lower than those previously reported in Caco-2 cell and rat intestinal brush-border membrane vesicles. In addition, probenecid, fluorescein, valproic acid, and salicylic acid, which are inhibitors of nateglinide uptake in Caco-2 cells and rat intestine, did not inhibit the uptake of nateglinide via MCT6. These results suggest that MCT6 may play a role in the intestinal absorption of nateglinide, although other transporters are also likely involved.
INTRODUCTION

Monocarboxylate transporter 6 (MCT6), previously known as MCT5 and encoded by SLC16A5, is a member of the monocarboxylate transporter (MCT) family (Price et al., 1998; Halestrap and Meredith, 2004). We have previously shown that MCT6 transports bumetanide, nateglinide, probenecid, and prostaglandin F2α, but not L-lactic acid and L-tryptophan, which are typical substrates for MCT1–MCT4, and T-type amino acid transporter 1 (TAT1), respectively (Murakami et al., 2005). MCT6-mediated bumetanide uptake was pH- and membrane potential-dependent. However, the properties of MCT6-mediated transport of other substrates remain to be investigated in detail.

Nateglinide is an oral antidiabetic agent that acts directly on pancreatic beta-cells to stimulate insulin secretion. It quickly reaches maximal serum concentration after pre-meal administration and is eliminated quite rapidly after oral administration (McLeod, 2004). The pharmacokinetic features of nateglinide may be attributable to its rapid intestinal absorption. Because nateglinide is an anionic compound with pKa 3.1, it exists predominantly in an ionized form at physiological pH in the intestine. Moreover, the chloroform/water partition coefficient of nateglinide is reported to be 0.2 at pH 6.8 (Daiichisankyo, 2010), indicating that nateglinide is hydrophilic. These physicochemical
features are incompatible with rapid absorption by passive diffusion, suggesting that nateglinide is absorbed via a specific transport system(s) in the intestine.

Previous studies have shown that the uptake of nateglinide by the human colon adenocarcinoma cell line, Caco-2, occurs via an $H^+$-driven transport system (Okamura et al., 2002) and fluorescein/$H^+$ cotransport system (Itagaki et al., 2005a). In rat intestinal brush-border vesicles, it has been shown that the ceftibuten/$H^+$ cotransport system, which is identical to the fluorescein/$H^+$ cotransport system, is involved in the uptake of nateglinide (Itagaki et al., 2005b; Saito et al., 2006). Furthermore, nateglinide inhibits rat peptide transporters PEPT1 and PEPT2, rat organic anion transporter 1 (Oat1), and human MCT1, but is not transported by these transporters (Terada et al., 2000; Uwai et al., 2000; Okamura et al., 2002). Thus, the nateglinide transport mechanism responsible for intestinal absorption, although characterized to some extent, has not yet been elucidated at molecular level.

In the present study, we aimed to investigate the potential role of MCT6 in the absorption mechanisms of nateglinide. Our results suggest that MCT6 may play a role in the absorption of nateglinide.
MATERIALS AND METHODS

Chemicals

$[^{14}\text{C}]$Nateglinide and nateglinide were kindly supplied by Ajinomoto Co., Inc. (Tokyo, Japan). $[^3\text{H}]$Bumetanide was purchased from American Radiolabeled Chemicals (St. Louis, MO, USA). All other chemicals were of the highest grade commercially available.

Immunohistochemical analysis

Polyclonal MCT6-specific antiserum was raised by immunizing rabbits with synthetic peptide corresponding to the COOH terminus of MCT6 (residues 456–470, CQSSRQPRPAGVNKH). The immunogen peptide shows no significant sequence homology with other MCTs. A paraffin section of human small intestine was obtained from BioChain Institute, Inc. (Hayward, CA, USA); no further detail was available about which part of the small intestine had been sampled. Sections were dewaxed, rehydrated, and incubated with 3% $\text{H}_2\text{O}_2$ to eliminate endogenous peroxidase activity. After rinsing in 0.05 M Tris-buffered saline containing 0.1% Tween 20 and blocking in 10% goat serum for 40 min, sections were treated with primary rabbit polyclonal antiserum (1:100; 4°C, overnight) or nonimmune rabbit serum as a negative control (Universal negative controls,
Dako Co., Carpinteria, CA, USA). Thereafter, the sections were incubated with a secondary goat polyclonal antibody against rabbit immunoglobulin, conjugated to peroxidase labeled-dextran polymer (EnVision+™, Dako Co., CA, USA). This step was followed by incubation with diaminobenzidine and hydrogen peroxide (DAB+, Dako Co., CA, USA). The sections were counterstained with hematoxylin and examined under light microscopy.

cRNA synthesis and injection into *Xenopus* oocytes

pSP64T-MCT6 (Murakami et al., 2005) was linearized by digestion with *Bam* HI (Takara Bio Inc, Shiga, Japan) and transcribed into cRNA in vitro using a SP6-mMESSAGE mMACHINE Kit (Ambion, Inc., TX, USA), according to the manufacturer’s instruction. Concentration and quality of cRNA were determined by UV spectrophotometry and denaturing formaldehyde-agarose gel electrophoresis.

Oocytes were obtained from female *Xenopus laevis*, as previously described (Kobayashi et al., 2004). Oocytes (stage V–VI) were treated with collagenase A (2.0 mg/ml; Roche Diagnostics, Mannheim, Germany) in oocyte Ringer’s 2 solution (83 mM NaCl, 2 mM KCl, 1 mM MgCl2, and 5 mM HEPES, pH 7.5) for at least 1 h at room temperature.
temperature. Defolliculated oocytes were injected with cRNA or distilled water (Gibco, Invitrogen Co., CA, USA), and incubated in Barth’s buffer (88 mM NaCl, 1 mM KCl, 0.33 mM Ca(NO₃)₂, 0.41 mM CaCl₂, 0.82 mM MgSO₄, 2.4 mM NaHCO₃, 10 mM HEPES, 50 ng/mL gentamicin sulfate, pH 7.6) at 18°C for 2 to 3 days.

Uptake studies

For uptake experiments, 8–20 oocytes were transferred into 24-well or 48-well multi-dishes and preincubated in uptake buffer (82.5 mM NaCl, 2.5 mM KCl, 1 mM NaHPO₄, 1 mM MgCl₂, 15 mM HEPES, pH 7.4 adjusted with Tris). The uptake buffer containing the radiolabeled drug was added to initiate uptake after removal of preincubation buffer, and the mixture was incubated at room temperature at pH 7.4 or 6.0, except for the pH-dependence experiment. pH was adjusted with 2-(N-morpholino)ethanesulfonic acid/Tris (pH 5.5 and 6.0) or HEPES/Tris (pH 7.4 and 8.0). The Na⁺-free uptake buffer contained 100 mM ([KCl]+[choline-Cl]), 1.8 mM CaCl₂, 1 mM MgCl₂, and 5 mM HEPES. The concentration of potassium in the uptake buffer was in the range of 2–100 mM in the Na⁺-free condition, in which KCl plus choline-Cl are equal to 100 mM. Uptake was terminated by the addition of ice-cold uptake buffer, and oocytes were washed.
at least 3 times. The oocytes were divided between 1–8 scintillation vials and dissolved in 200 μl of 10% sodium dodecyl sulfate. The solution was slowly shaken for 1 h. Scintillation cocktail was added, and the radioactivity was counted using a liquid scintillation counter.

Since uptake of nateglinide in this study by uninjected oocytes was essentially the same as that of oocytes injected with water (Murakami et al., 2005), uninjected oocytes were used as the control instead of water-injected oocytes throughout this study. The uptake by oocytes (uptake; μl/oocyte) is presented as the ratio of radioactivity in the sample (dpm/oocyte) to the initial concentration in the uptake buffer (dpm/μl).

MCT6-specific uptake was calculated by subtraction of the uptake by control oocyte from that by MCT6 cRNA-injected oocytes. The MCT6-specific uptake rates of nateglinide were obtained from the uptake value at 60 min. To estimate the kinetic parameters of the saturable uptake via MCT6, the Michaelis-Menten equation

\[ \frac{J}{C} = \frac{J_{\text{max}}}{K_t + C} \]  

was fitted to the experimental data by means of nonlinear least-squares regression analysis (Yamaoka et al., 1981). J and C represent the uptake rate (pmol/oocyte/time) and the concentration of substrate (μM), respectively. J_{\text{max}} and K_t represent the maximum uptake
rate for the saturable component and the half-maximal concentration, respectively.

Efflux study

Efflux of $[^{14}\text{C}]$nateglinide from oocytes was measured in uptake buffer (pH 7.4) at room temperature after preincubation for 5 min in ice-cold uptake buffer, followed by injection of 50 nl $[^{14}\text{C}]$nateglinide in uptake buffer. At the designated time, 100 μl of solution was sampled, and an equivalent volume of uptake buffer was added as replacement. After all samples were collected, the oocytes were washed in ice-cold uptake buffer and lysed to measure the amount remaining in oocytes. The efflux rates were calculated using 3 time points, viz., 10, 20, and 30 min.

Statistical analysis

Statistical analysis was performed using the unpaired Student’s $t$-test or analysis of variance (ANOVA), followed by Dunnett’s test. A difference between means was considered statistically significant when the P-value was less than 0.05.
RESULTS

Immunohistochemical analysis

At first, we performed an immunohistochemical analysis to elucidate the localization of MCT6 in the human small intestine. As shown in Figures 1A and B, the antiserum against MCT6 peptide stained at the luminal side of the epithelial cells lining the villi. In intestinal crypts, both sides of the epithelial cells were stained (Figure 1C). Incubation with nonimmune rabbit serum diminished immunoreactivity (Figure 1D).

Extracellular pH- and membrane potential-dependence of [14C]nateglinide uptake via MCT6

We next investigated the properties of nateglinide uptake by MCT6. The amount of [14C]nateglinide taken up by oocytes injected with MCT6 cRNA was significantly higher than that by uninjected oocytes (Figure 2A). MCT6-specific uptake increased linearly up to 60 min, with the regression line passing through the origin. Subsequently, the uptake experiments were performed at 60 min. The uptake rate of [14C]nateglinide by oocytes injected with MCT6 cRNA was also higher than that by uninjected oocytes at pH 7.4 and 8.0 (Figure 2B). At pH 6.0 and 5.5, the differences in MCT6-specific uptake and the uptake by control oocytes were smaller than those at pH 7.4 and 8.0. The uptake rate of
[14C]nateglinide via MCT6 was increased with increasing extracellular pH, indicating that MCT6 activity was sensitive to pH (Figure 2B).

Membrane potential was then changed by varying extracellular K+ concentration. The uptake rate of [14C]nateglinide by uninjected oocytes was not substantially affected by extracellular K+ concentration (Figure 2C). On the other hand, the MCT6-specific uptake rate increased with increasing extracellular K+ concentration (Figure 2C), indicating that MCT6 is sensitive to membrane potential.

Concentration dependence of nateglinide uptake via MCT6

We examined the concentration-dependent uptake of nateglinide mediated by MCT6. As shown in Figure 3A, the MCT6-specific uptake rate of nateglinide was saturable by increasing substrate concentration. The kinetic parameters obtained for nateglinide uptake at pH 7.4 were $K_t \ 45.9 \ \mu M$ (19.4, 108.4; mean – S.D., mean + S.D., respectively) and $J_{max} \ 32.5 \pm 8.4 \ pmol \ per \ oocyte \ per \ 60 \ min$ (mean ± S.D. of 4 separate experiments).
Analysis of the inhibitory mechanism of nateglinide on bumetanide uptake via MCT6

As we have previous reported (Murakami et al., 2005), bumetanide is a useful tool for investigating the functional properties of MCT6. To elucidate substrate recognition by MCT6, we investigated the mechanism of MCT6 inhibition by nateglinide. Figure 3B shows a Lineweaver-Burk plot of the effects of nateglinide on the uptake of bumetanide. In the presence of nateglinide, the slope was increased and the X-intercept remained unchanged, indicating that nateglinide is a noncompetitive inhibitor of bumetanide uptake via MCT6. Kinetic analysis using the Michaelis-Menten equation for noncompetitive inhibition yielded an inhibitory constant (K_i) value of 5.4 ± 0.2 μM (estimate ± S.D.) for nateglinide, a K_i value of 118 ± 16 μM for bumetanide, and a J_max value of 44.5 ± 6.0 pmol per oocyte per 30 min for bumetanide.

Efflux of nateglinide via MCT6

To clarify whether MCT6 mediates translocation of nateglinide across the plasma membrane, we examined the ability of oocytes to expel nateglinide via MCT6; efflux studies were carried out at pH 7.4. As shown in Figure 4A, the efflux of [14C]nateglinide from MCT6-expressing oocytes was increased in a time-dependent manner and was higher
than the efflux from water-injected oocytes and uninjected oocytes. Nateglinide efflux via MCT6 was depressed in the presence of a high concentration of nateglinide or bumetanide (Figure 4B).

Effect of various compounds on $[^{14}\text{C}]$nateglinide uptake via MCT6

To compare the properties of MCT6-mediated nateglinide uptake with those of the carrier responsible for the uptake of nateglinide by Caco-2 cells, we next investigated the effects of various compounds on the MCT6-specific uptake of $[^{14}\text{C}]$nateglinide. In the presence of 10 mM alpha-cyano-4-hydroxycinnamic acid (CHC), which is known to be an inhibitor of nateglinide uptake by Caco-2 cells, uptake was reduced by approximately 70%. However, salicylic acid (10 mM), valproic acid (10 mM), probenecid (1 mM), and fluorescein (1 mM), which have been shown to inhibit uptake of nateglinide by Caco-2 cells (Okamura et al., 2002; Itagaki et al., 2005a), did not affect the MCT6-specific uptake of $[^{14}\text{C}]$nateglinide (Figure 5). Succinic acid (10 mM), methotrexate (5 mM), $p$-aminohippuric acid (PAH) (10 mM), and L-lactic acid (10 mM), which do not significantly decrease the uptake of nateglinide by Caco-2 cells, also did not affect the MCT6-specific uptake of $[^{14}\text{C}]$nateglinide.
DISCUSSION

In this study, we investigated the expression of MCT6 in the human small intestine and compared the transport properties of nateglinide via MCT6 with those previously reported for Caco-2 cells (Okamura et al., 2002; Itagaki et al., 2005). Our results indicated that MCT6 is located on the apical side of human intestinal villous epithelial cells and mediates the transmembrane transport of nateglinide, suggesting that MCT6 may be involved in the absorption of nateglinide. However, discrepancies between the MCT6-mediated transport properties of nateglinide in oocytes and those in Caco-2 cells suggested that MCT6 is not the main transporter for nateglinide in Caco-2 cells.

It has been demonstrated by northern blot analysis that SLC16A5 mRNA is highly expressed in the placenta and kidney (Price et al., 1998), and RT-PCR has revealed that this gene is expressed in Caco-2 cells (Hadjiagapiou, et al., 2000). However, the expression and localization of MCT6 in the human small intestine has been unknown to date. In this study, our immunohistochemical analysis showed that the luminal surface of intestinal villous epithelial cells reacted with antiserum to the COOH-terminal peptide of MCT6. Moreover, the luminal membranes of intestinal epithelial cells are involved in the first step of drug absorption, and exhibit carrier-mediated transport activity for several drugs. Based
on this finding and our previous finding that MCT6 can transport some drugs, including
bumetanide, probenecid, and nateglinide (Murakami et al., 2005), we speculated that
MCT6 would be involved in the absorptive transport of some drugs. On the other hand,
crypt cells were also stained, on both sides. Because the intestinal crypt contains stem cells
supplying villous epithelial cells, MCT6 may shift its localization from being present on
both sides, to being present on the luminal surface, in a differentiation-dependent manner.
Further analysis will be required to elucidate the change of MCT6 subcellular localization
during epithelial differentiation.

The uptake of bumetanide via MCT6 has been shown to be pH- and membrane
potential-sensitive, but not proton gradient-dependent (Murakami et al., 2005). When the
membrane was depolarized, the uptake of bumetanide via MCT6 increased. When
intracellular and extracellular K⁺ concentration was equal, the uptake of [³H]bumetanide by
MCT6 was significantly higher than that in standard uptake buffer. The present study
demonstrated that the MCT6-specific uptake rate of nateglinide increased with rising
extracellular K⁺ concentration. Membrane potential sensitivity is a common property of
MCT6-mediated transport of various substrates. On the other hand, the pH-sensitivity of
MCT6 activity varies between bumetanide and nateglinide. These observations are
consistent with the idea that the MCT6-mediated transport mechanism may facilitate the diffusion of organic anions along the electrochemical potential gradient, and that the pH gradient across the cell membrane is not the driving force in MCT6-mediated transport (Murakami et al., 2005).

The inhibitory effect of nateglinide on bumetanide uptake via MCT6 was characterized as kinetically noncompetitive. This result suggests that nateglinide binds to MCT6 at sites other than the bumetanide-binding site. Different binding sites may be another explanation for the difference in pH-sensitivity between bumetanide and nateglinide. The $K_i$ of nateglinide for inhibition of the MCT6-mediated transport of bumetanide is lower than its $K_t$ value for transport. This may suggest the presence of more than 1 binding site for substrates on the MCT6 molecule. Further studies will be needed to test this hypothesis.

We did not focus on the effect of a sodium gradient on the transport of nateglinide via MCT6, because a previous study did not indicate sodium-dependence of the uptake of bumetanide via MCT6. The MCT6-specific uptake of nateglinide under Na$^+$-free conditions (Figure 2C) was roughly the same as under normal conditions (Figure 2A). These data indicated that the transport of nateglinide via MCT6 is not influenced by
extracellular sodium ion concentrations.

MCT6 is unlikely to be the main molecule responsible for nateglinide transport in Caco-2 cells, because the Kᵢ value of nateglinide (45.9 μM) for MCT6-expressing oocytes is lower than that in Caco-2 cells (448 μM and 240 μM, respectively) (Okamura et al., 2002; Itagaki et al., 2005) and rat intestinal brush-border membrane vesicles (120 μM) (Itagaki et al., 2005). In addition, probenecid, fluorescein, valproic acid, and salicylic acid, which are inhibitors of nateglinide uptake in Caco-2 cells (Okamura et al., 2002; Itagaki et al., 2005) and rat intestinal brush-border membrane vesicles (Itagaki et al., 2005), were unable to inhibit the uptake of nateglinide via MCT6. These results suggest that uptake systems in Caco-2 cells and rat intestinal brush-border membranes involve mechanisms other than MCT6. The intestinal concentration of nateglinide (molecular weight 317.42) estimated from a single dose (90 mg) and 250 mL water is 1.13 mM and 25-fold higher than the Kᵢ value of nateglinide for MCT6. This means that MCT6 may be saturated at the clinical dose.

From our previous Caco-2 study, we have speculated that a lower affinity system, which is suited to the intestinal concentration of nateglinide, may contribute most to the absorption of nateglinide. However, nothing is currently known about the inhibitory effect
of the inhibitors probenecid, fluorescein, valproic acid, and salicylic acid on nateglinide uptake in human intestine. In the duodenum, the intraluminal pH is about pH 6. The pH gradually increases in the small intestine from pH 6 to about pH 7.4 in the terminal ileum (Fallingborg, 1999). This study demonstrated that the transport of nateglinide via MCT6 was increased in neutral pH, which suggests that MCT6 may contribute to absorption in lower intestine. There remains the possibility that nateglinide may inhibit absorption of other drugs via MCT6, although this is not experimentally supported at present.

CHC has been used as an inhibitor for MCT1–MCT4 (Bröer et al., 1998; Bröer et al., 1999; Yoon et al., 1997; Dimmer et al., 2000). This study firstly demonstrated that CHC inhibits a transporter other than MCTs. Further studies are required to elucidate whether CHC inhibits the transport of another substrate via MCT6 and, if so, to clarify the mechanism of MCT6 inhibition by CHC.

In conclusion, MCT6 mediates transmembrane transport of nateglinide and is expressed on the apical side of the intestinal epithelium. These results suggest that MCT6 may contribute to the absorption of nateglinide. In addition, the affinity and inhibition profiles of MCT6 expressed in *Xenopus laevis* oocytes derived here are not in agreement with the previous reports for nateglinide uptake in Caco-2 cells and rat intestine, suggesting
that other unidentified transporter(s) may be involved in nateglinide uptake. Further studies are needed to quantitatively assess the contribution of MCT6 and other transporter(s) to the intestinal absorption of nateglinide.
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Authorship Contributions

Participated in Research Design: Kohyama, Shiokawa

Conducted Experiments: Kohyama, Shiokawa

Performed Data Analysis: Kohyama, Shiokawa

Wrote or contributed to the writing of the manuscript: Kohyama, Shiokawa, Ohbayashi, Kobayashi, Yamamoto
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Footnotes

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FIGURE LEGENDS

Figure 1 Localization of MCT6 protein in human small intestine.

Immunohistochemical detection of MCT6 protein in the human small intestine using anti-MCT6 antiserum, which was raised by immunizing rabbits with synthetic peptide corresponding to the COOH terminus of MCT6 (residues 456–470, CQSSRQPRPAGVNKH). A, C, D: Low magnification view (× 200) in human small intestine. B: High magnification view (× 400) showing the villi of the small intestine. D: Immunoreactivity is absent in small intestine incubated with nonimmune serum. Immunoreactivity of MCT6 was located in the apical membrane of villus epithelial cells (arrows in A and B) and the luminal surface (thin arrows in C) as well as the abluminal side (arrows in C) of crypts.

Figure 2 Transport properties of MCT6-mediated nateglinide uptake.

A: Time-dependent transport of nateglinide; B: pH-dependence, and C: extracellular K+ dependence of the uptake of [14C]nateglinide via MCT6. Uptake of [14C]nateglinide into MCT6-expressing (closed circles) or uninjected control oocytes (open circles) was measured at room temperature. MCT6-mediated uptake (closed squares) was calculated by
subtracting the uptake amount of control oocytes from that of MCT6-expressing oocytes.

Each point represents the mean ± S.E.M. (n=3; *, p < 0.05 versus uninjected). A: Uptake of 6 μM [14C]nateglinide was measured at pH 7.4. B: Uptake of 8 μM [14C]nateglinide was measured for 60 min. C: Uptake of 8 μM [14C]nateglinide was measured at pH 7.4 for 60 min. The concentration of potassium in the uptake buffer ranged from 2 to 100 mM in the Na+-free condition, in which [KCl] plus [choline-Cl] are equal to 100 mM.

**Figure 3 Transport characteristics of nateglinide by MCT6.**

A: Concentration dependence of the uptake of [14C]nateglinide via MCT6. Uptake of 10–80 nM [14C]nateglinide and 60 nM [14C]nateglinide, plus 100–500 μM nateglinide, by MCT6-expressing or non-injected control oocytes was measured at pH 7.4 for 60 min. MCT6-mediated uptake (closed circles) was calculated by subtracting the amount taken up by control oocytes from that of MCT6-expressing oocytes. The nateglinide uptake was saturable and fit a least-square approximation with nonlinear parameters; the result is shown by the solid line. Each point represents the mean ± S.E.M. (n = 5). B: Lineweaver-Burk plots for the kinetic study of bumetanide uptake via MCT6, and its inhibition by nateglinide. MCT6-expressing and control oocytes were incubated with the
uptake buffer containing 0.3–1000 μM bumetanide in the absence (open circles) and presence of 1 μM (closed triangles) or 10 μM nateglinide (closed squares), at room temperature and pH 7.4 for 30 min. MCT6-mediated uptake was calculated by subtracting the uptake of nateglinide by control oocytes from that of MCT6-expressing oocytes. Each point represents the mean ± S.E.M. (n = 3). The dotted lines were generated from Equation 1, using the estimated parameters.

**Figure 4 Efflux of nateglinide via MCT6.**

A: The efflux amount of radioactivity from oocytes expressing MCT6 (closed circles) was measured at pH 7.4 in the standard incubation buffer, compared with uninjected oocytes (open circles) and water-injected oocytes (open triangles). Each point represents the mean ± S.E.M. (n = 4; *P < 0.05 versus uninjected oocyte). B: Effects of 1 mM nateglinide and 1 mM bumetanide on the efflux of nateglinide via MCT6. Each value represents the mean ± S.E.M. (n = 3; *P < 0.05 versus control of MCT6-specific uptake).
**Figure 5** Cis-inhibitory effect of various compounds on the uptake of nateglinide via MCT6.

Uptake of 10 μM [\(^{14}\)C]nateglinide in MCT6-expressing oocytes or non-injected oocytes was measured at room temperature and pH 7.4 for 60 min in the presence or absence of inhibitors. MCT6-specific uptake was calculated by subtracting the amount taken up by control oocytes from that of MCT6-expressing oocytes. Each value represents the mean S.E.M. of 4 separate experiments. * P < 0.05 versus control.
Figure 2

A

Nateglinide uptake (µL/oocyte)

0.0 0.2 0.4 0.6 0.8 1.0 1.2 1.4

0 20 40 60

Time (min)

B

Nateglinide uptake rate (µL/oocyte/60 min)

0.0 0.5 1.0 1.5 2.0 2.5

5.0 6.0 7.0 8.0

pH

C

Nateglinide uptake rate (µL/oocyte/60 min)

0.0 0.5 1.0 1.5 2.0 2.5

0 50 100

K+ (mM)
Figure 3

A

Nateglinide uptake rate (µL/oocyte/60 min)

Nateglinide concentration (µM)

B

1/Bumetanide uptake rate (1/(pmol/oocyte/30 min))

1/Bumetanide concentration (1/µM)

Control
+1 µM nateglinide
+10 µM nateglinide
Fitting line
Figure 4

A

Nateglinide efflux (pmol/oocyte)

Time (min)

Uninjected oocytes
MCT6-expressing oocytes
Water-injected oocytes

B

Nateglinide efflux rate (pmol/oocyte/min)

Control
+1 mM nateglinide
+1 mM bumetanide

Uninjected oocytes
MCT6-expressing oocytes
MCT6 specific uptake

*