ELSEVIER

European Journal of Pharmacology 392 (2000) 11–17

ejp

www.elsevier.nl/locate/ejphar

# Inhibitory effect of novel oral hypoglycemic agent nateglinide (AY4166) on peptide transporters PEPT1 and PEPT2

Tomohiro Terada, Kyoko Sawada, Hideyuki Saito, Yukiya Hashimoto, Ken-ichi Inui *

*Department of Pharmacy, Kyoto University Hospital, Faculty of Medicine, Kyoto University, Kyoto 606-8507, Japan*

Received 21 October 1999; received in revised form 12 January 2000; accepted 4 February 2000

**Abstract**

The novel oral hypoglycemic agent nateglinide (AY4166) is a nonsulfonylurea insulin secretagogue, and its pharmacokinetic features include rapid absorption and elimination. As nateglinide is a dipeptide-like drug, we investigated the interaction of nateglinide with peptide transporters PEPT1 and PEPT2, which mediate the absorption of various peptide-like drugs. Nateglinide exhibited a potent inhibitory effect on [$^{14}$C]glycylsarcosine uptake by the human colon adenocarcinoma cell line Caco-2 and rat PEPT-transfectants. Kinetic analysis revealed that these inhibitory effects were noncompetitive. $Na^+$-coupled alanine or threonine uptake by Caco-2 cells was not inhibited by nateglinide, suggesting that the inhibitory effect of nateglinide on peptide transporters was not due to nonspecific interaction. There was little uptake of [$^{14}$C]nateglinide by peptide transporters. Various sulfonylureas, such as glibenclamide, also inhibited [$^{14}$C]glycylsarcosine uptake by rat PEPT-transfectants. In conclusion, nateglinide as well as sulfonylureas inhibit the transport activity of PEPT1 and PEPT2, although nateglinide itself is not transported by these transporters. 

*Keywords:* Drug interaction; Hypoglycemic agent; Nateglinide; Noncompetitive inhibition; Peptide transporter

## 1. Introduction

Impaired glucose-induced insulin secretion and insulin resistance are hallmarks of Type 2 diabetes (noninsulin-dependent diabetes mellitus). To compensate for defective insulin secretion, sulfonylureas have been widely used for more than 40 years in the treatment of Type 2 diabetes. However, there are several disadvantages to sulfonylurea therapy, such as excess hypoglycemia between meals due to the long duration of action of these agents.

Nateglinide (AY4166, Fig. 1) has been exploited as a new class of oral hypoglycemic agent (Shinkai et al., 1988, 1989). Although the mechanism of insulin secretion induced by nateglinide was the same as that of sulfonylureas (Fujitani and Yada, 1994; Akiyoshi et al., 1995), nateglinide exhibited a quicker and shorter lasting hypoglycemic effect than sulfonylureas due to its rapid absorption and elimination (Sato et al., 1991; Ikenoue et al., 1997). Because of these pharmacokinetic features, it was suggested that nateglinide could be beneficial in clinical use to prevent postprandial hyperglycemia without causing prolonged hypoglycemia in Type 2 diabetes patients.

Peptide transporters (PEPT1 and PEPT2) mediate the efficient absorption of a wide variety of oral peptide-like drugs in the small intestine and kidney (Inui and Terada, 1999). For example, oral β-lactam antibiotics (Saito et al., 1995; Terada et al., 1997a,b), anticancer agent bestatin (Saito et al., 1996) and angiotensine-converting enzyme inhibitors (Boll et al., 1994) are transported by these transporters. Furthermore, intestinal PEPT1 has been utilized to improve the intestinal absorption of poorly absorbed pharmacologically active agents by chemically converting to substrates for PEPT1 (Hu et al., 1989; Tsuji et al., 1990; Balimane et al., 1998; Ganapathy et al., 1998; Han et al., 1998; Sawada et al., 1999b). Because these peptide-like drugs are pharmacologically independent each other, the pharmacokinetic profiles and therapeutic efficacy may be affected in coadministration of other peptide-like drugs. Therefore, in order to evaluate the appropriate usage of peptide-like drugs, especially newly developed

* Corresponding author. Tel.: +81-75-751-3577; fax: +81-75-751-4207.

*E-mail address:* inui@kuhp.kyoto-u.ac.jp (K. Inui).

0014-2999/00/$ - see front matter 
PII: S0014-2999(00)00119-9

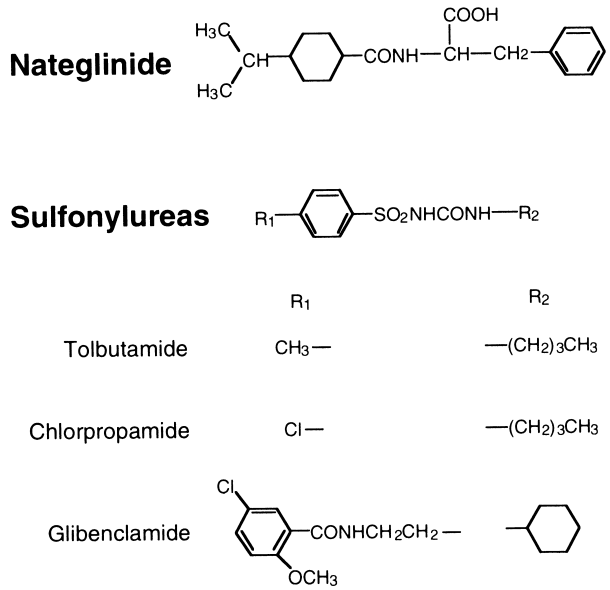


Fig. 1. Chemical structures of nateglinide and various sulfonylureas.

peptide-like drugs, it should be important to investigate their interaction with peptide transporters.

Nateglinide is a novel oral hypoglycemic agent and a dipeptide-like drug, and therefore, the present study was carried out to examine the interaction of nateglinide with peptide transporters using the human colon adenocarcinoma cell line Caco-2, and rat PEPT1- or rat PEPT2-expressing stable transfectant. Furthermore, the effects of other hypoglycemic agents, sulfonylureas, on peptide transporters were also investigated.

## 2. Materials and methods

### 2.1. Materials

Nateglinide and [$^{14}$C]nateglinide (9.00 MBq/mmol) were supplied by Ajinomoto (Yokohama, Japan). [$^{14}$C]glycylsarcosine (1.78 GBq/mmol) was obtained from Daiichi Pure Chemicals (Ibaraki, Japan), and [$^{3}$H]alanine (2.07 TBq/mmol) and [$^{3}$H]threonine (485 GBq/mmol) were from Amersham Int. (Buckinghamshire, UK). Glycylsarcosine, tolbutamide and chlorpropamide were obtained from Sigma (St. Louis, MO). Glibenclamide was purchased from Wako (Kyoto, Japan). All other chemicals used were of the highest purity available. Fig. 1 shows the chemical structures of nateglinide and various sulfonylureas.

### 2.2. Cell culture

Caco-2 cells (passage 18) were obtained from the American-Type Culture Collection (ATCC HTB37) and cultured as described previously (Inui et al., 1992). Parental LLC-PK$_1$ cells were obtained from the American-Type Culture Collection (ATCC CRL-1392). LLC-PK$_1$ cells transfected with rat PEPT1 cDNA (LLC-rPEPT1), with rat PEPT2 cDNA (LLC-rPEPT2) and with empty vector (LLC-pBK) were produced and cultured as described previously (Terada et al., 1997a, b). For uptake studies, $2 \times 10^5$ cells were inoculated into 35-mm plastic dishes in 2 ml of complete culture medium.

### 2.3. Uptake studies by cell monolayers

Uptake measurements were performed as described previously (Terada et al., 1997b). The incubation medium contained (millimolar) NaCl, 145; KCl, 3; $CaCl_2$, 1; $MgCl_2$, 0.5; D-glucose, 5, and 2-(*N*-morpholino)ethanesulfonic acid, 5 (pH 6.0) or HEPES, 5 (pH 7.4). In $Na^+$-free medium, NaCl was replaced with *N*-methyl-D-glucamine. Hypoglycemic agents were dissolved in acetonitrile, and final concentrations of acetonitrile in the incubation medium were 0.5%. The protein contents of cell monolayers solubilized in 1N NaOH were determined by the method of Bradford (1976), using a Bio-Rad Protein Assay Kit (Bio-Rad, Richmond, CA) with bovine γ-globulin as the standard.

### 2.4. Statistical analysis

Data were analyzed statistically by one-way analysis of variance followed by Scheffé's test.

## 3. Results

### 3.1. Inhibitory effects of nateglinide on glycylsarcosine uptake by peptide transporters

Caco-2 cells have been used as a model for studying the functions of peptide transporters (Inui et al., 1992; Saito and Inui, 1993), and it was reported that human PEPT1 was expressed in Caco-2 cells (Liang et al., 1995). First, we examined the effects of nateglinide on [$^{14}$C]glycylsarcosine uptake by Caco-2 cells and rat PEPT1- or PEPT2-expressing transfectant (LLC-rPEPT1 and LLC-rPEPT2 cells, respectively). As shown in Fig. 2, [$^{14}$C]glycylsarcosine uptake was not affected in the presence of 0.05 mM nateglinide, but was markedly inhibited by 0.5 mM nateglinide in Caco-2, LLC-rPEPT1 and LLC-rPEPT2 cells. The solvent used for nateglinide, acetonitrile showed no effect on [$^{14}$C]glycylsarcosine uptake in Caco-2 cells. Nateglinide did not affect the [$^{14}$C]glycylsarcosine uptake in LLC-pBK cells, the mock-transfectant. Fig. 3 shows the effects of increasing concentrations of nateglinide on [$^{14}$C]glycylsarcosine uptake. [$^{14}$C]glycylsarcosine uptake by all cells was inhibited by nateglinide in a concentration-dependent manner, and $IC_{50}$ values were calculated to be 290 μM in Caco-2 cells, 160 μM in LLC-rPEPT1 cells, and 70 μM in LLC-rPEPT2 cells.

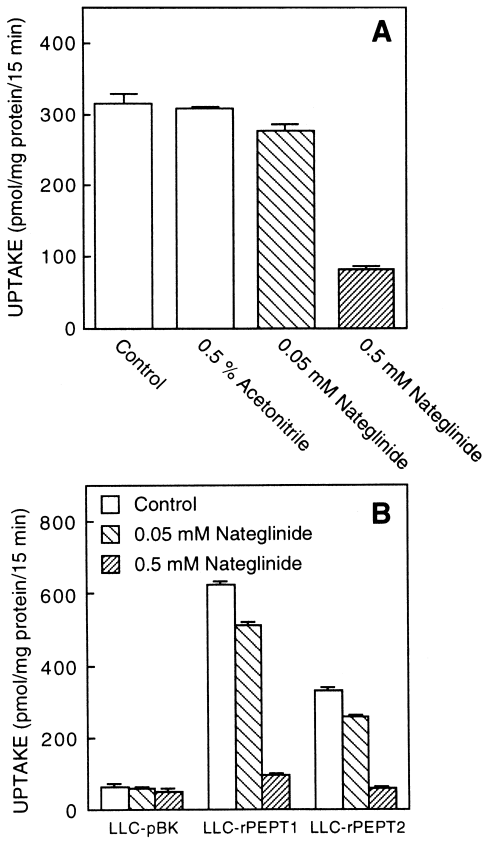


Fig. 2. Effects of nateglinide on [$^{14}$C]glycylsarcosine uptake by peptide transporters. Caco-2 cells (A) or LLC-pBK, LLC-rPEPT1 and LLC-rPEPT2 cells (B) were incubated with incubation medium containing [$^{14}$C]glycylsarcosine (20 μM, pH 6.0) in the absence or presence of nateglinide (0.05 and 0.5 mM) for 15 min at 37°C. Nateglinide was resolved in acetonitrile, and the final concentration of acetonitrile in the incubation medium was 0.5%. Each column represents the mean ± S.E.M. of three monolayers.

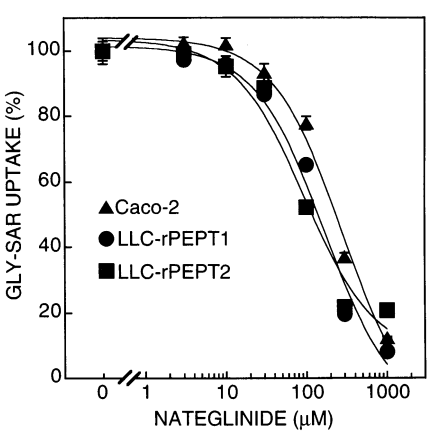


Fig. 3. Inhibition of [$^{14}$C]glycylsarcosine uptake in the presence of increasing concentrations of nateglinide by Caco-2 (▲), LLC-rPEPT1 (●) and LLC-rPEPT2 cells (■). The cell monolayers were incubated with incubation medium containing [$^{14}$C]glycylsarcosine (20 μM, pH 6.0) for 15 min at 37°C in the absence or presence of nateglinide. Each point represents the mean ± S.E.M. of three monolayers. When error bars are not shown, they are smaller than the symbols.

### *3.2. Inhibition kinetics of nateglinide on glycylsarcosine uptake*

We then investigated the kinetics of the inhibitory effect of nateglinide on [$^{14}$C]glycylsarcosine uptake by LLC-rPEPT1 and LLC-rPEPT2 cells. Fig. 4 shows the concentration dependence of [$^{14}$C]glycylsarcosine uptake in the absence or presence of nateglinide. The concentrations of nateglinide used corresponded to the respective $IC_{50}$ values for both transporters. The insets of Fig. 4 show Eadie-Hofstee plots after correction of the nonsaturable components. The presence of nateglinide markedly decreased the maximal velocities ($V_{max}$ value for control vs. that in the presence of nateglinide: 41 vs. 10 for PEPT1; 2.0 vs. 0.9 for PEPT2, nmol/mg protein/15 min). On the other hand, the apparent affinities were comparable ($K_m$ values for control vs. that in the presence of nateglinide: 0.9 vs. 1.4 for PEPT1; 0.1 vs. 0.07 for PEPT2, mM).

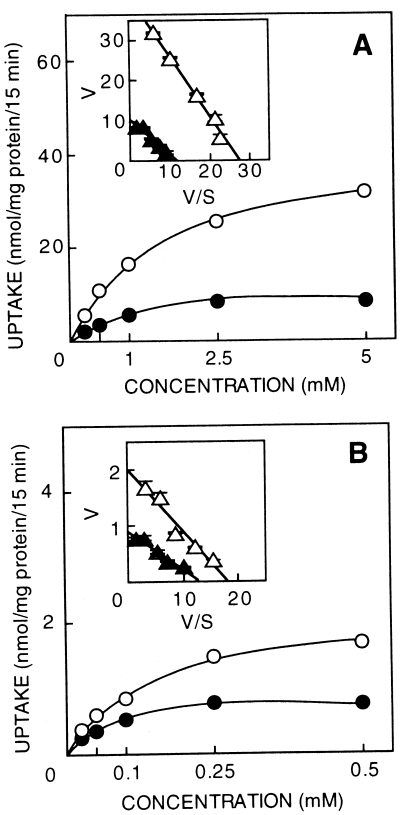


Fig. 4. Concentration dependence of [$^{14}$C]glycylsarcosine uptake in the absence or presence of nateglinide in LLC-rPEPT1 (A) and LLC-rPEPT2 cells (B). The cell monolayers were incubated with incubation medium containing various concentrations of [$^{14}$C]glycylsarcosine (pH 6.0) for 15 min at 37°C in the absence (○) or presence (●) of 160 μM (A) or 70 μM (B) nateglinide. Nonspecific uptake was evaluated by measuring [$^{14}$C]glycylsarcosine uptake in the presence of 20 mM glycyl-L-leucine, and the results are shown after correction for the nonsaturable component. Inset: Eadie-Hofstee plots of glycylsarcosine uptake after correction for the nonsaturable component. Each point represents the mean ± S.E.M. of three monolayers. When error bars are not shown, they are smaller than the symbols.

These results showed that nateglinide noncompetitively inhibited [$^{14}$C]glycylsarcosine uptake by both PEPT1 and PEPT2.

### 3.3. Effects of nateglinide on alanine and threonine uptake by Caco-2 cells

To confirm whether the inhibitory effect of nateglinide was specific to peptide transporters, we examined the effects of nateglinide on [$^{3}$H]alanine and [$^{3}$H]threonine uptake by Caco-2 cells. Alanine (Pan and Stevens, 1995) and threonine (Kekuda et al., 1997) uptake by Caco-2 cells were reported to be $Na^+$-dependent and carrier-mediated. As shown in Fig. 5, [$^{3}$H]alanine and [$^{3}$H]threonine uptake by Caco-2 cells were decreased under $Na^+$-free conditions

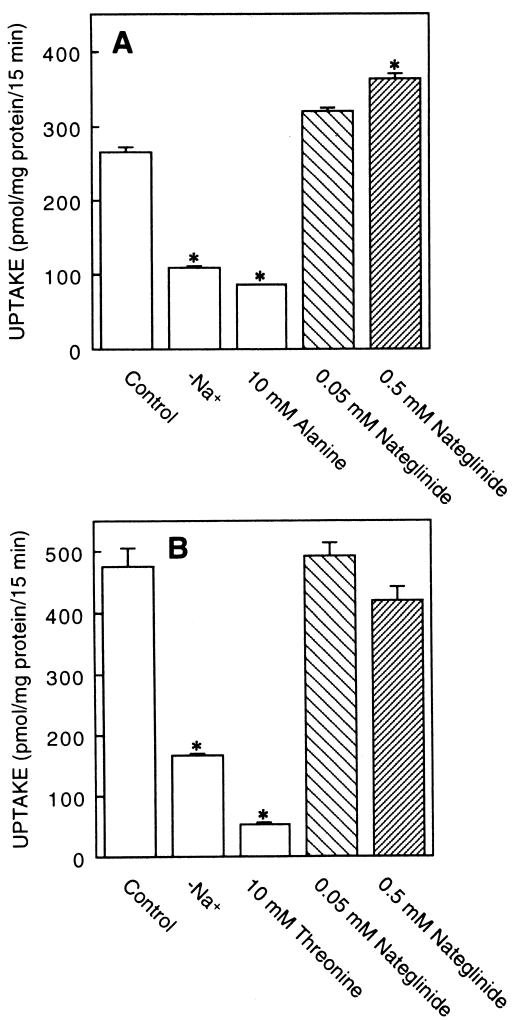


Fig. 5. Effects of nateglinide on [$^{3}$H]alanine (A) and [$^{3}$H]threonine (B) uptake by Caco-2 cells. Caco-2 cells were incubated with incubation medium containing [$^{3}$H]alanine or [$^{3}$H]threonine (20 μM, pH 6.0) in the absence or presence of each unlabeled amino acid (10 mM) or nateglinide (0.05 and 0.5 mM) for 15 min at 37°C. To examine the $Na^+$ dependence of alanine and threonine transport, the cell monolayers were incubated with $Na^+$-free incubation medium. Each column represents the mean ± S.E.M. of three monolayers. $^{*}P < 0.05$, significantly different from control.

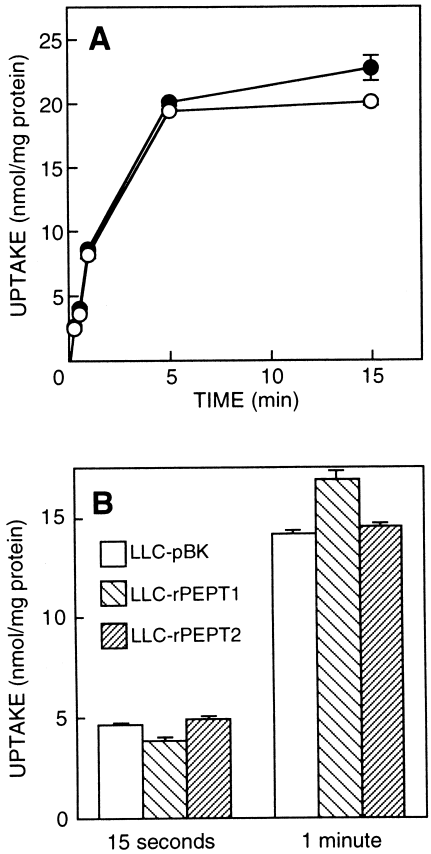


Fig. 6. Uptake of [$^{14}$C]nateglinide by peptide transporters. (A) Time course of [$^{14}$C]nateglinide uptake by Caco-2 cells. Caco-2 cells were incubated for the specified periods at 37°C with incubation medium containing [$^{14}$C]nateglinide (300 μM, pH 6.0) in the absence (○) or presence (●) of 10 mM glycylsarcosine. Each point represents the mean ± S.E.M. of three monolayers. When error bars are not shown, they are smaller than the symbols. (B) Uptake of [$^{14}$C]nateglinide by LLC-pBK, LLC-rPEPT1 and LLC-rPEPT2 cells. Cells were incubated for 15 s or 1 min at 37°C with incubation medium containing [$^{14}$C]nateglinide (300 μM, pH 6.0). Each column represents the mean ± S.E.M. of three monolayers.

and inhibited by unlabeled excess amino acids, which suggested that [$^{3}$H]alanine and [$^{3}$H]threonine uptake were indeed $Na^+$-dependent and carrier-mediated. In contrast to the inhibitory effect of nateglinide on [$^{14}$C]glycylsarcosine uptake, neither [$^{3}$H]alanine nor [$^{3}$H]threonine uptake was inhibited by nateglinide.

### 3.4. Nateglinide uptake by peptide transporters

Then, we examined whether nateglinide per se was transported by peptide transporters. Fig. 6A shows the time course of [$^{14}$C]nateglinide uptake by Caco-2 cells in the absence or presence of excess glycylsarcosine. [$^{14}$C]nateglinide uptake was increased rapidly, but was not inhibited by glycylsarcosine. In addition, [$^{14}$C]nateglinide uptake at 1 min by Caco-2 cells was not inhibited by excess oral β-lactam antibiotics or unlabeled nateglinide (data not shown). Fig. 6B shows the uptake of [$^{14}$C]nateglinide by

LLC-pBK, LLC-rPEPT1 and LLC-rPEPT2 cells. There were no differences in [$^{14}$C]nateglinide uptake among the three transfectants. Similar results were observed using water-injected and rat PEPT1 cRNA-injected oocytes (data not shown). These findings suggested that nateglinide was not transported by PEPT1 and PEPT2, but was transported by passive diffusion. However, the contribution of other transporter(s) to nateglinide uptake can not be excluded.

### 3.5. Effects of various oral hypoglycemic agents on glycylsarcosine uptake by PEPT-transfectants

Finally, we examined the effects of other hypoglycemic agents; i.e., sulfonylureas, on [$^{14}$C]glycylsarcosine uptake by LLC-rPEPT1 and LLC-rPEPT2 cells. As shown in Fig. 7, [$^{14}$C]glycylsarcosine uptake by both transfectants was inhibited significantly by tolbutamide and chlorpropamide as well as nateglinide. The solvent acetonitrile showed no effect on [$^{14}$C]glycylsarcosine uptake in both transfectants. Due to the solubility problem, we examined the effect of glibenclamide on [$^{14}$C]glycylsarcosine uptake in a separate experiment. Glibenclamide at 50 μM also inhibited the [$^{14}$C]glycylsarcosine uptake by LLC-rPEPT1 cells (control 826 ± 19 pmol/mg protein/15 min; glibenclamide 316 ± 4 pmol/mg protein/15 min, mean ± S.E.M. of three monolayers, $P < 0.05$) and by LLC-rPEPT2 cells (control 212 ± 5 pmol/mg protein/15 min; glibenclamide 74 ± 1 pmol/mg protein/15 min, mean ± S.E.M. of three monolayers, $P < 0.05$). Glibenclamide was shown to be the most potent inhibitor among the hypoglycemic agents examined.

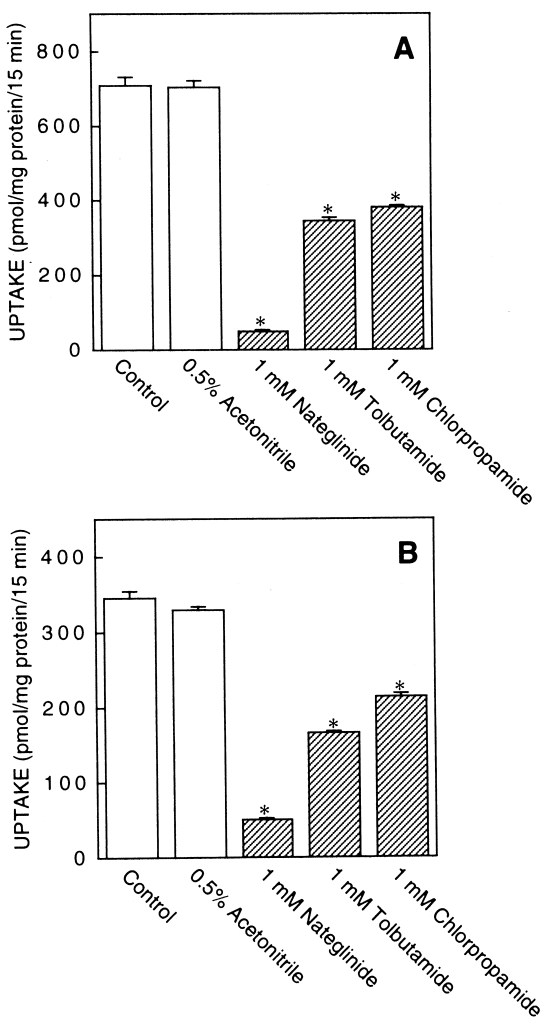


Fig. 7. Effects of various hypoglycemic agents on [$^{14}$C]glycylsarcosine uptake by LLC-rPEPT1 (A) and LLC-rPEPT2 cells (B). The cell monolayers were incubated with incubation medium containing [$^{14}$C]glycylsarcosine (20 μM, pH 6.0) for 15 min at 37°C in the absence or presence of each inhibitor at 1 mM. Each inhibitor was resolved in acetonitrile, and the final concentration of acetonitrile in the incubation medium was 0.5%. Each column represents the mean ± S.E.M. of three monolayers. * $P < 0.05$, significantly different from control.

## 4. Discussion

The present study have clearly demonstrated that nateglinide have the inhibitory effects on the glycylsarcosine uptake by PEPT1 and PEPT2. The inhibitory effects of this agent on both transporters were characterized as kinetically noncompetitive. Recently, we found that [$^{14}$C]glycylsarcosine uptake by PEPTs was inhibited by glibenclamide in a noncompetitive fashion, and it was suggested that this agent was bound to a site distinct from the glycylsarcosine binding site (Sawada et al., 1999a). An angiotensin-converting enzyme inhibitor, quinapril, was also reported to noncompetitively inhibit glycylsarcosine uptake by binding to the different site from the substrate binding site (Akarawut et al., 1998). As similar to these agents, nateglinide may bind to the transporter proteins at site other than substrate binding site. The amide bond of nateglinide seemed to be important for the interaction with peptide transporters, because alanine and threonine transport was not inhibited by nateglinide. Although it is unclear whether nateglinide, glibenclamide and quinapril share a common binding site, these agents will be useful compounds to investigate the mechanisms of transport of peptide transporters.

The present findings may have clinical implications because nateglinide and various sulfonylureas inhibited the function of peptide transporters. At therapeutic doses (90 mg every 8 h), local concentrations of nateglinide in the intestine may be reached to millimolar level, and if so, the concentrations are greater than the $IC_{50}$ values of this agent for rat (160 μM) or human (290 μM) PEPT1. Furthermore, we recently reported that the *Ki* value of glibenclamide for rat PEPT1 was 25 μM (Sawada et al., 1999a), which was smaller than the $IC_{50}$ value of nateglinide. Taken together, these findings indicated the possibility of drug interaction at intestinal absorption level between these antidiabetic agents and peptide-like drugs. For exam-

ple, when Type 2 diabetes patients treated with nateglinide or glibenclamide are coadministered oral β-lactam antibiotics, the oral bioavailability of β-lactam antibiotics may be reduced. In vivo studies are needed to verify whether this interaction occurs in vivo, and collecting clinical data with combinations of these drugs are also important.

Nateglinide exhibited a more rapid and shorter lasting hypoglycemic effect than sulfonylureas (Sato et al., 1991; Ikenoue et al., 1997). Ikenoue et al. (1997) reported that the plasma concentration of nateglinide after oral administration increased rapidly and then decreased with a short biological half-life ($t_{max}$, $0.63 \pm 0.35$ h; $t_{1/2}$, 1.8 h). On the other hand, the plasma concentration of glibenclamide was found to increase gradually followed by a slow decrease ($t_{max}$, $3.17 \pm 0.75$ h; $t_{1/2}$, 6.3 h) (Ikenoue et al., 1997). Based on these findings, the rapid and short-term hypoglycemic effect of nateglinide was suggested to be due to its pharmacokinetic profile; i.e., rapid absorption and elimination (Ikenoue et al., 1997). We were interested in whether the intestinal PEPT1 was involved in the rapid absorption of nateglinide. However, in the present study, nateglinide was demonstrated to be not transported by peptide transporters. This result suggested that intestinal PEPT1 did not mediate the rapid intestinal absorption of this agent. This may have been due to the structural features of nateglinide, a D-phenylalanine derivative, because peptide transporters were reported to be stereoselective (Daniel et al., 1992; Lister et al., 1995). Alternatively, it may be a reason that nateglinide does not have an α-amino group, of which component is one of the important factors for recognition by peptide transporters (Terada et al., 1998). Further studies are needed to elucidate the mechanism of rapid intestinal absorption of nateglinide.

In conclusion, we found that the new oral hypoglycemic agent nateglinide was a noncompetitive inhibitor of peptide transporters, although this agent per se was not transported via peptide transporters. In addition, we demonstrated that various sulfonylureas also inhibited the function of peptide transporters. These findings should provide important insight into the biochemical and clinical characteristics of peptide transporters.

## Acknowledgements

This work was supported in part by a Grant-in-Aid for Scientific Research (B) and a Grant-in-Aid for Scientific Research on Priority Areas (No. 296) from the Ministry of Education, Science, Sports and Culture of Japan, and by grants from the Uehara Memorial Foundation and from the Japan Research Foundation for Clinical Pharmacology.

## References

Akarawut, W., Lin, C.J., Smith, D.E., 1998. Noncompetitive inhibition of glycylsarcosine transport by quinapril in rabbit renal brush border membrane vesicles: effect on high-affinity peptide transporter. J. Pharmacol. Exp. Ther. 287, 684–690.

Akiyoshi, M., Kakei, M., Nakazaki, M., Tanaka, H., 1995. A new hypoglycemic agent, A-4166, inhibits ATP-sensitive potassium channels in rat pancreatic β-cells. Am. J. Physiol. 268, E185–E193.

Balimane, P.V., Tamai, I., Guo, A., Nakanishi, T., Kitada, H., Leibach, F.H., Tsuji, A., Sinko, P.J., 1998. Direct evidence for peptide transporter (PepT1)-mediated uptake of a nonpeptide prodrug, valacyclovir. Biochem. Biophys. Res. Commun. 250, 246–251.

Boll, M., Markovich, D., Weber, W.M., Korte, H., Daniel, H., Murer, H., 1994. Expression cloning of a cDNA from rabbit small intestine related to proton-coupled transport of peptides, β-lactam antibiotics and ACE-inhibitors. Pfluegers Arch. 429, 146–149.

Bradford, M.M., 1976. 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.

Daniel, H., Morse, E.L., Adibi, S.A., 1992. Determinants of substrate affinity for the oligopeptide/$H^+$ symporter in the renal brush border membrane. J. Biol. Chem. 267, 9565–9573.

Fujitani, S., Yada, T., 1994. A novel D-phenylalanine-derivative hypoglycemic agent A-4166 increases cytosolic free $Ca^{2+}$ in rat pancreatic β-cells by stimulating $Ca^{2+}$ influx. Endocrinology 134, 1395–1400.

Ganapathy, M.E., Huang, W., Wang, H., Ganapathy, V., Leibach, F.H., 1998. Valacyclovir: a substrate for the intestinal and renal peptide transporters PEPT1 and PEPT2. Biochem. Biophys. Res. Commun. 246, 470–475.

Han, H.K., de Vrueh, R.L.A., Rhie, J.K., Covitz, K.M.Y., Smith, P.L., Lee, C.P., Oh, D.M., Sadée, W., Amidon, G.L., 1998. 5′-Amino acid esters of antiviral nucleosides, acyclovir, and AZT are absorbed by the intestinal PEPT1 peptide transporter. Pharm. Res. 15, 1154–1159.

Hu, M., Subramanian, P., Mosberg, H.I., Amidon, G.L., 1989. Use of the peptide carrier system to improve the intestinal absorption of L-α-methyldopa: carrier kinetics, intestinal permeabilities, and in vitro hydrolysis of dipeptidyl derivatives of L-α-methyldopa. Pharm. Res. 6, 66–70.

Ikenoue, T., Akiyoshi, M., Fujitani, S., Okazaki, K., Kondo, N., Maki, T., 1997. Hypoglycaemic and insulinotropic effects of a novel oral antidiabetic agent, (-)-*N*-(*trans*-4-isopropylcyclohexanecabonyl)-D-phenylalanine (A-4166). Br. J. Pharmacol. 120, 137–145.

Inui, K., Terada, T., 1999. Dipeptide transporters. In: Amidon, G.L., Sadée, W. (Eds.), Membrane Transporters as Drug Targets Kluwer Academic/Plenum, New York, NY, pp. 269–288.

Inui, K., Yamamoto, M., Saito, H., 1992. Transepithelial transport of oral cephalosporins by monolayers of intestinal epithelial cell line Caco-2: specific transport systems in apical and basolateral membranes. J. Pharmacol. Exp. Ther. 261, 195–201.

Kekuda, R., Torres-Zamorano, V., Fei, Y.-J., Prasad, P.D., Li, H.-W., Mader, L.D., Leibach, F.H., Ganapathy, V., 1997. Molecular and functional characterization of intestinal $Na^+$-dependent neutral amino acid transporter $B^0$. Am. J. Physiol. 272, G1463–G1472.

Liang, R., Fei, Y.-J., Prasad, P.D., Ramamoorthy, S., Han, H., Yang-Feng, T.L., Hediger, M.A., Ganapathy, V., Leibach, F.H., 1995. Human intestinal $H^+$/peptide cotransporter. Cloning, functional expression, and chromosomal localization. J. Biol. Chem. 270, 6456–6463.

Lister, N., Sykes, A.P., Bailey, P.D., Boyd, C.A.R., Bronk, J.R., 1995. Dipeptide transport and hydrolysis in isolated loops of rat small intestine: effect of stereospecificity. J. Physiol. (London) 484, 173–182.

Pan, M., Stevens, B.R., 1995. Differentiation- and protein kinase C-dependent regulation of alanine transport via system B. J. Biol. Chem. 270, 3582–3587.

Saito, H., Inui, K., 1993. Dipeptide transporters in apical and basolateral membranes of the human intestinal cell line Caco-2. Am. J. Physiol. 265, G289–G294.

Saito, H., Okuda, M., Terada, T., Sasaki, S., Inui, K., 1995. Cloning and characterization of a rat $H^+$/peptide cotransporter mediating absorp-

tion of β-lactam antibiotics in the intestine and kidney. J. Pharmacol. Exp. Ther. 275, 1631–1637.

Saito, H., Terada, T., Okuda, M., Sasaki, S., Inui, K., 1996. Molecular cloning and tissue distribution of rat peptide transporter PEPT2. Biochim. Biophys. Acta 1280, 173–177.

Sato, Y., Nishikawa, M., Shinkai, H., Sukegawa, E., 1991. Possibility of ideal blood glucose control by a new oral hypoglycemic agent, *N*-[(*trans*-4-isopropylcyclohexyl)-carbonyl]-D-phenylalanine (A-4166), and its stimulatory effect on insulin secretion in animals. Diabetes Res. Clin. Pract. 12, 53–59.

Sawada, K., Terada, T., Saito, H., Hashimoto, Y., Inui, K., 1999a. Effects of glibenclamide on glycylsarcosine transport by the rat peptide transporters PEPT1 and PEPT2. Br. J. Pharmacol. 128, 1159–1164.

Sawada, K., Terada, T., Saito, H., Hashimoto, Y., Inui, K., 1999b. Recognition of L-amino acid ester compounds by rat peptide transporters PEPT1 and PEPT2. J. Pharmacol. Exp. Ther. 291, 705–709.

Shinkai, H., Nishikawa, M., Sato, Y., Toi, K., Kumashiro, I., Seto, Y., Fukuma, M., Dan, K., Toyoshima, S., 1989. *N*-(cyclohexylcarbonyl)-D-phenylalanines and related compounds. A new class of oral hypoglycemic agents. 2. J. Med. Chem. 32, 1436–1441.

Shinkai, H., Toi, K., Kumashiro, I., Seto, Y., Fukuma, M., Dan, K., Toyoshima, S., 1988. *N*-acylphenylalanines and related compounds. a new class of oral hypoglycemic agents. J. Med. Chem. 31, 2092–2097.

Terada, T., Saito, H., Inui, K., 1998. Interaction of β-lactam antibiotics with histidine residue of rat $H^+$/peptide cotransporters. PEPT1 and PEPT2. J. Biol. Chem. 273, 5582–5585.

Terada, T., Saito, H., Mukai, M., Inui, K., 1997a. Characterization of stably transfected kidney epithelial cell line expressing rat $H^+$/peptide cotransporter PEPT1: localization of PEPT1 and transport of β-lactam antibiotics. J. Pharmacol. Exp. Ther. 281, 1415–1421.

Terada, T., Saito, H., Mukai, M., Inui, K., 1997b. Recognition of β-lactam antibiotics by rat peptide transporters, PEPT1 and PEPT2, in LLC-PK1 cells. Am. J. Physiol. 273, F706–F711.

Tsuji, A., Tamai, I., Nakanishi, M., Amidon, G.L., 1990. Mechanism of absorption of the dipeptide α-methyldopa-phe in intestinal brush-border membrane vesicles. Pharm. Res. 7, 308–309.