DMD Fast Forward. Published on September 29, 2008 as doi:10.1124/dmd.108.022418
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High-affinity Interaction of Sartans with H^+/Peptide Transporters

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DMD Fast Forward. Published on September 29, 2008 as DOI: 10.1124/dmd.108.022418
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Running title page: Interaction of AT_1 antagonists with H^+/peptide transporters

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Number of text pages: 14

Number of tables: 1

Number of figures: 4

Number of references: 20

Number of words in abstract: 238

Number of words in introduction: 284

Number of words in discussion: 1162

Abbreviations: Gly-Sar, glycylsarcosine; PEPT1, H^+/peptide transporter 1; PEPT2, H^+/peptide transporter 2

DMD Fast Forward. Published on September 29, 2008 as DOI: 10.1124/dmd.108.022418
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ABSTRACT

Sartans are very effective drugs for treatment of hypertension, heart failure and other cardiovascular disorders. They antagonize the effects of angiotensin II at the AT_1 receptor and display oral bioavailability rates of 13 – 80%. Because some sartans sterically resemble dipeptide derivatives, we investigated whether they are transported by peptide transporters. We first assessed the effects of sartans on [^{14}C]Gly-Sar uptake into Caco-2 cells expressing PEPT1 and into SKPT cells expressing PEPT2. Losartan, irbesartan, valsartan and eprosartan inhibited [^{14}C]Gly-Sar uptake into Caco-2 cells in a competitive manner with K_i values of 24, 230, 390 and > 1000 µM. Losartan and valsartan also strongly inhibited the total transepithelial flux of [^{14}C]Gly-Sar across Caco-2 cell monolayers. In SKPT cells, [^{14}C]Gly-Sar uptake was inhibited with K_i values of 2.2 µM (losartan), 65 µM (irbesartan), 260 µM (valsartan) and 490 µM (eprosartan). We determined by the two-electrode voltage clamp technique whether the compounds elicited transport currents by PEPT1 or PEPT2 when expressed in *Xenopus laevis* oocytes. No currents were observed for any of the sartans but the compounds strongly and reversibly inhibited peptide-induced currents. Uptake of valsartan, losartan and cefadroxil was quantified in HeLa cells after heterologous expression of hPEPT1. In contrast to cefadroxil, no PEPT1-specific uptake of valsartan and losartan was found. We conclude that the sartans tested in this study display high-affinity interaction with H^+/peptide transporters but are not transported themselves. However, they strongly inhibit hPEPT1-mediated uptake of dipeptides and cefadroxil.

Introduction

Sartans such as losartan, valsartan, irbesartan and eprosartan are blockers of the angiotensin II type 1 receptor. They have proven to be effective in the treatment of hypertension, renal diseases, heart failure, ventricular hypertrophy, dilation, arrhythmias and dysfunction with overall reduced cardiovascular morbidity and mortality and less negative side effects than the classical angiotensin converting enzyme inhibitors (for review see Perico and Remuzzi, 1998; Jack, 2000; Jugdutt, 2006; Ramasubbu et al., 2007). Valsartan has gained considerable interest last year when Wang and coworkers (2007) discovered that it lowers brain ß-amyloid protein levels in a mouse model and significantly reduces Alzheimer disease-type neuropathology and cognitive deterioration, even when delivered at a dose lower than that used for hypertension treatment in humans.

After oral administration as the primary route, sartans display bioavailability levels in the range of 13% (eprosartan) to 80% (irbesartan) (Dominiak, 1999). Because some sartans sterically resemble di- and tripeptide derivatives, the question arises whether these drugs interact with peptide transporters. Di- and tripeptides are taken up into intestinal cells by the low-affinity H^+/peptide cotransporter PEPT1. In the kidney tubule, di- and tripeptides are reabsorbed mainly by the high-affinity H^+/peptide cotransporter PEPT2 (for review see Daniel and Kottra, 2004; Brandsch et al., 2008). Ekins and coworkers (2005), when searching the Comprehensive Medicinal Chemistry database in a pharmacophore-based approach for new hPEPT1 ligands, retrieved - among many other drugs - losartan as a possible substrate or inhibitor of hPEPT1. Transport of sartans by peptide transporters could explain their oral bioavailabilities and their renal reabsorption. On the other hand, high-affinity inhibition of peptide transporters may have consequences for the absorption of peptides and drugs that are peptide transporter substrates such as oral ß-lactam antibiotics or valacyclovir.

Materials and Methods

Materials. The human cell lines Caco-2 and HeLa were obtained from the German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany). The rat renal cell line SKPT was provided by U. Hopfer (Case Western Reserve University, Cleveland, USA). pBluescript II SK(-), pBluescript-hPEPT1 and pBluescript-hPEPT2 were kind donations of V. Ganapathy (Medical College of Georgia, Augusta, USA). [Glycine-1-^{14}C]Gly-Sar (specific radioactivity 53 mCi/mmol) was custom synthesized by GE Healthcare (Buckinghamshire, UK). Gly-Sar, Gly-Gln, cefadroxil and cephalexin were from Sigma-Aldrich (Deisenhofen, Germany). Eprosartan was from Solvay (Hannover, Germany), irbesartan from Sanofi-Aventis (Chilly-Mazarin, France), losartan from MSD Sharp & Dohme (Haar, Germany) and valsartan from Novartis (Basel, Switzerland).

Culture of Caco-2 and SKPT Cells and Uptake Studies. Caco-2 and SKPT cells were cultured as described previously (Theis et al., 2002; Knütter et al., 2004; Luckner and Brandsch, 2005). Both lines were subcultured in 35-mm disposable petri dishes (Sarstedt, Nümbrecht, Germany) at a seeding density of 0.8 x 10^6 cells per dish. Uptake of [^{14}C]Gly-Sar was measured 4 days (SKPT) or 7 days (Caco-2) after seeding. Uptake buffer was 25 mM Mes/Tris (pH 6.0) containing 140 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, 0.8 mM $MgSO_4$, 5 mM glucose, [^{14}C]Gly-Sar (10 µM) and unlabeled sartans (0 – 3.16 mM, pH readjusted if necessary). After incubation for 10 min the monolayers were quickly washed four times with ice-cold uptake buffer, solubilized and prepared for liquid scintillation spectrometry. Transepithelial flux of [^{14}C]Gly-Sar across Caco-2 cell monolayers cultured on permeable filters was measured as described (Neumann et al., 2004). After washing the inserts at pH 7.5 for 10 min, uptake at 37°C was started by adding 1.5 ml uptake buffer (pH 6.0) containing [^{14}C]Gly-Sar with or without 1 mM sartan to the donor side. Samples were taken from the receiver compartment (2.6 ml buffer, pH 7.5) and replaced with fresh buffer. The radioactivity in the

samples was measured by liquid scintillation spectrometry. After 2 h, the filters supporting the monolayers were quickly washed four times with ice-cold uptake buffer, cut out of the plastic insert and transferred to scintillation vials.

***Xenopus laevis* Oocytes Expressing PEPT1 and PEPT2 and Electrophysiology.** Surgically removed oocytes from female *Xenopus laevis* (African Xenopus Facility, Knysna, South Africa) were separated by collagenase treatment and were injected with 30 nl of RNA solution containing 30 ng of rabbit PEPT1 or rabbit PEPT2 cRNA (Knütter et al., 2004; Theis et al., 2002). After 3 - 6 days the two-electrode voltage clamp technique was applied to characterize responses in current (I) to substrate addition (1 mM). Current-voltage (I-V_m) relationships were measured in the potential range from –160 to +80 mV. I-V_m measurements were made immediately before and 30 s after substrate application when current flow reached steady state.

Heterologous Expression of hPEPT1 and hPEPT2 in HeLa Cells and Uptake Studies. HeLa cells were routinely cultured with Dulbeccos` Minimum Essential Medium with Glutamax, supplemented with 10% fetal bovine serum and gentamicin (45 µg/ml). The cDNA of human PEPT1 and PEPT2 was cloned into pcDNA3 using the pBluescript constructs as a template for the PCR and Xho I and BamH I as restriction sites. The resulting pcDNA3-hPEPT1/2 constructs were confirmed by sequencing. Human PEPT1 and human PEPT2 were heterologously expressed in HeLa cells using pcDNA3 hPEPT1 or hPEPT2 cDNA constructs (1 µg/well) and Turbofect (1.5 µl/well; Fermentas, St. Leon-Rot, Germany) according to manufacturers' protocols. The transfection was done 1 h post seeding in 24 well plates, and 20-24 h post transfection uptake of [^{14}C]Gly-Sar (20 µM, pH 6.0) in the absence or presence of increasing concentrations of Gly-Sar, valsartan or losartan was measured at room temperature. After incubation for 10 min the monolayers were quickly washed four times with ice-cold uptake buffer, solubilized and prepared for liquid scintillation spectrometry.

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Transfected HeLa cells were also used for uptake studies with the unlabeled drugs. Cells were incubated with unlabeled cefadroxil, losartan or valsartan (1 mM) at pH 6.0 for 30 min at room temperature. Subsequently, the monolayers were quickly washed four times with ice-cold uptake buffer and 500 µl bidest. water was added. After freezing and thawing three times, the resulting suspension was transferred to 1.5 ml tubes, homogenized with a 25 gauge needle and centrifuged in a table top centrifuge at 13 000 rpm for 45 min. The supernatant was centrifuged again at 13 000 rpm for 45 min and prepared for HPLC measurements.

HPLC Measurements. Samples were analyzed according to the laboratory standard HPLC (La-Chrom®, Merck-Hitachi, Darmstadt, Germany) with a diode array detector and a Purospher® STAR RP-18 endcapped column (125-4, 5 µm; Merck, Darmstadt, Germany). The eluent was 45% acetonitril/55% H_2O with 0.1% trifluoroacetic acid (pH 1.9) for losartan and valsartan and 15% acetonitril/85% H_2O with 0.1% trifluoroacetic acid (pH 1.9) for cefadroxil. UV-detection was done at 232 nm, the injection volume was 20 µl, the flow rate 0.75 ml/min and the column temperature 34°C. Cephalexin was used as internal standard for cefadroxil whereas losartan was used as internal standard for valsartan and vice versa. The retention time were between 2.5 and 8 min.

Calculations and Statistics. All data are given as the mean ± S.E. of three to five independent experiments. The kinetic parameters were calculated by non-linear regression methods and confirmed by linear regression of the respective Eadie-Hofstee plots. IC_{50} values (i.e. concentration of the unlabeled sartans or dipeptides necessary to inhibit 50% of carrier-mediated [^{14}C]Gly-Sar uptake) were determined by non-linear regression using the logistical equation for an asymmetric sigmoid (allosteric Hill kinetics): $y = Min + (Max-Min)/(1 + (X/IC_{50})^{-P})$ where Max is the initial Y value, Min the final Y value and the power P represents Hills' coefficient. Inhibition constants (K_i) were calculated from IC_{50} values according to the method developed by Cheng and Prusoff (1973).

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Results

Inhibition of [^{14}C]Gly-Sar Transport at Caco-2 and SKPT Cells by Sartans. We studied the interaction of the four sartans losartan, irbesartan, valsartan and eprosartan with PEPT1 and PEPT2 in competition assays using [^{14}C]Gly-Sar as a reference substrate. The intestinal cell line Caco-2 constitutively expressing PEPT1 (Knütter et al., 2004; Neumann et al., 2004) and the renal cell line SKPT constitutively expressing PEPT2 (Theis et al., 2002; Luckner and Brandsch, 2005) were used. Uptake of [^{14}C]Gly-Sar into Caco-2 cells in the presence of an inwardly directed H^+ gradient showed concentration-dependent inhibition by losartan, irbesartan, valsartan and eprosartan (Fig. 1A). From the competition curves we calculated K_i values between 24 µM and 2.2 mM (Table 1). The competitor with the highest affinity was losartan followed by irbesartan, valsartan and eprosartan. At SKPT cells the four sartans also inhibited [^{14}C]Gly-Sar uptake with K_i values ranging between 2.2 µM and 0.49 mM (Fig. 1A, Table 1). To rule out unspecific effects on cell membrane integrity or on the proton gradient as the driving force for Gly-Sar uptake, we also studied the effect of sartans on proton-coupled [^{3}H]proline transport (Metzner et al., 2008). Eprosartan, losartan and valsartan (1 mM) did not affect [^{3}H]proline uptake at Caco-2 cells (97.9 ± 4.3%, 83.0 ± 3.8 % and 94.9 ± 1.2 % of control, respectively).

Caco-2 cells were also cultured for 21 days on permeable filter membranes in Transwell chambers. With this technique, we measured the total transepithelial [^{14}C]Gly-Sar flux across cell monolayers at pH 6.0 in the absence or presence of unlabeled sartans (Fig. 1B). The mean transepithelial electrical resistance of the Caco-2 cell monolayers at the day of the experiment was 576 ± 11 $\Omega \cdot cm^2$. The transepithelial [^{14}C]Gly-Sar flux (20 µM, control) was 1.48 ± 0.1 $\% \cdot h^{-1} \cdot cm^{-2}$, which is in agreement with previously published values (Neumann et al., 2004). In the presence of losartan, valsartan and eprosartan (1 mM), the [^{14}C]Gly-Sar flux was decreased to 0.23 ± 0.01, 0.93 ± 0.05 and 1.31 ± 0.1 $\% \cdot h^{-1} \cdot cm^{-2}$, respectively (Fig. 1B). The

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same rank order of inhibition was observed when the [^{14}C]Gly-Sar contents in cells after completion of the flux measurements (2 h) were analyzed (Fig. 1B, inset): The [^{14}C]Gly-Sar accumulation within the cells was inhibited by the sartans by 16 to 61%. The degree of inhibition of both the total transepithelial [^{14}C]Gly-Sar flux and cellular [^{14}C]Gly-Sar accumulation corresponds well with the drug affinity constants obtained for PEPT1 in the influx competition experiments.

The calculation of IC_{50} values by non-linear regression revealed Hill coefficients of around 1 for all inhibition data as it has to be expected for competitive inhibition. In order to determine the effects of sartans on the kinetic parameters of Gly-Sar uptake, we studied the relationship between the Gly-Sar uptake rates and extracellular Gly-Sar concentration in Caco-2 (Fig. 2A, C) and SKPT (Fig. 2B, D) cells. In the absence of valsartan, the Michaelis constant, K_t, of Gly-Sar uptake at Caco-2 cells was 1.1 ± 0.1 mM and the maximal velocity of uptake, V_{max}, was 39.4 ± 1.0 nmol · mg of protein^{-1} per 10 min (Fig. 2A). The corresponding kinetic constants obtained in the presence of 0.5 mM valsartan were (K_t) 3.2 ± 0.4 mM and (V_{max}) 46.3 ± 2.3 nmol · mg of protein^{-1} per 10 min. Hence, valsartan at a concentration close to its K_i value increased the K_t value of Gly-Sar uptake by about 3-fold whereas the V_{max} value was not altered significantly. For SKPT cells, the K_t value of Gly-Sar uptake was 0.14 ± 0.02 mM and the V_{max} value was 6.9 ± 0.3 nmol · mg of protein^{-1} per 10 min (Fig. 2B). The corresponding kinetic constants obtained in the presence of 0.4 mM valsartan were (K_t) 0.28 ± 0.03 mM and (V_{max}) 6.13 ± 0.09 nmol · mg of protein^{-1} per 10 min. Valsartan thereby increased the K_t of Gly-Sar uptake by PEPT2 also 2-fold without affecting V_{max}. Similarly, losartan at a concentration of 50µM and 5 µM, respectively, increased the K_t values of Gly-Sar uptake 2.5-fold at Caco-2 cells and 2-fold at SKPT cells without affecting the V_{max} value (Fig. 2C and D, insets).

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Two-electrode Voltage Clamp Technique in *Xenopus laevis* Oocytes Expressing PEPT1 or PEPT2. Inhibition of [^{14}C]Gly-Sar uptake does not necessarily mean that the sartans themselves are transported by peptide transporters. To assess their possible transport by PEPT1 and PEPT2, the two-electrode voltage clamp technique with *X. laevis* oocytes expressing the H^+/peptide transporters was applied (Knütter et al., 2004; Theis et al., 2002). In contrast to transporter substrates, no currents were observed for any of the sartans (Fig. 3A, B). The currents elicited by the four sartans (1 mM) are expressed as percent of the current induced by the dipeptide Gly-Gln (1 mM) measured in the same oocyte (Fig. 3B). In Figure 3C and 3D representative currents elicited by Gly-Gln and losartan for PEPT1 and PEPT2 as a function of membrane potential are shown. Losartan - despite its very high affinity for PEPT1 and PEPT2 - failed to evoke any currents whereas Gly-Gln (1 mM) caused high inward currents. Importantly, the currents evoked by Gly-Gln were strongly inhibited by losartan. Moreover, this inhibition was fully reversible, i.e. washing the oocyte and adding Gly-Gln again completely restored the inward directed currents.

Uptake of Losartan and Valsartan in HeLa-hPEPT1 and HeLa-hPEPT2 Cells and their Effect on Cefadroxil Uptake . The inhibition of dipeptide uptake by losartan and valsartan was also studied in competition assays with human PEPT1 and human PEPT2 heterologously expressed in HeLa cells (Fig. 4A). Again, [^{14}C]Gly-Sar was used as a reference substrate. The uptake of [^{14}C]Gly-Sar was almost completely inhibited by excess amounts of unlabeled Gly-Sar with K_i values of 0.71 ± 0.03 mM at HeLa-hPEPT1 and 0.25 ± 0.02 mM at HeLa-hPEPT2. Losartan inhibited [^{14}C]Gly-Sar uptake into HeLa-hPEPT1 and HeLa-hPEPT2 cells with very high affinity with K_i values of 52 ± 5 µM and 2.4 ± 0.3 µM, respectively (Fig. 4A). For valsartan K_i values of 0.56 ± 0.07 mM and 0.11 ± 0.01 mM were obtained confirming the results obtained at Caco-2 cells expressing human PEPT1 and SKPT cells expressing rat PEPT2.

Electrophysiological measurements at PEPT1- and PEPT2-expressing oocytes only detect electrogenic transport of substrates. Electroneutral transport of the four sartans by PEPT1 and PEPT2 could not be ruled out. Therefore, in the next series of experiments, we directly measured the uptake of losartan and valsartan into HeLa-pcDNA3 and HeLa-hPEPT1 cells (Fig. 4B). The PEPT1 substrate cefadroxil served as positive control. The cells were incubated with valsartan, losartan or cefadroxil at a concentration of 1 mM for 30 min, washed and solubilized. The intracellular drug concentrations were determined by quantitative HPLC. Compared to the uptake of cefadroxil, uptake of valsartan and in particular uptake of losartan was high but, more importantly, there was no difference between cells transfected with empty vector and cells transfected with hPEPT1. In contrast, cefadroxil is accumulated at least 30-fold in hPEPT1-HeLa cells compared to mock cells (Fig. 4B). However, losartan and valsartan at a concentration of 1 mM were able to inhibit the hPEPT1-mediated cefadroxil uptake (1 mM) strongly by 87% and 69 %, respectively (Fig. 4C).

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Discussion

In this study, we investigated systematically whether the sartans losartan, irbesartan, valsartan and eprosartan interact with the H^+/peptide transporters PEPT1 and PEPT2. First, we studied the effect of the drugs on the transport of a reference dipeptide, Gly-Sar, into human intestinal cells (Caco-2) and rat renal cells (SKPT) expressing PEPT1 or PEPT2, respectively (Theis et al., 2002; Knütter et al., 2004; Neumann et al., 2004; Luckner and Brandsch, 2005). Losartan, irbesartan, valsartan and eprosartan inhibited [^{14}C]Gly-Sar uptake into Caco-2 cells in a competitive manner with K_i values of 24, 230, 390 and > 1000 µM. The affinity constants of prototype substrates of PEPT1 (dipeptides and tripeptides) are typically in the range of 100 µM to 1 mM. The $K_{i\ PEPT1}$ of Ala-Pro, for example, measured under identical conditions is 140 ± 10 µM (Knütter et al., 2008). The sartans tested in this study therefore interact with PEPT1 with affinities similar to those of natural dipeptide substrates. According to our classification of PEPT1 substrates and inhibitors (Brandsch et al., 2008), losartan, irbesartan and valsartan are considered as high-affinity ligands.

In SKPT cells, [^{14}C]Gly-Sar uptake was inhibited with K_i values of 2.2 µM (losartan), 65 µM (irbesartan), 260 µM (valsartan) and 490 µM (eprosartan). For comparison, the $K_{i\ PEPT2}$ of Ala-Pro is 14 ± 1 µM (Knütter et al., 2008). Again, losartan displayed the highest affinity. According to our classification of PEPT2 substrates and inhibitors (Brandsch et al., 2008), losartan and irbesartan are high-affinity ligands, valsartan and eprosartan interact with medium affinity. Comparing the rank order of compounds for Gly-Sar uptake inhibition we conclude that there are no major differences in the sartan recognition pattern between hPEPT1 and rPEPT2. The higher affinity of most compounds to PEPT2 compared to PEPT1 is a well known phenomenon.

The results obtained at HeLa cells expressing human PEPT1 and human PEPT2 confirmed the inhibition constants measured at intestinal or renal cells and rule out that the differences in

affinity constants between human PEPT1 and rat PEPT2 are due to species differences. The result that sartans do not inhibit [^{3}H]proline uptake at Caco-2 cells rules out the possibility that their effects on Gly-Sar transport are due to effects on cell membrane integrity or on the proton gradient as the driving force for PEPT1 and PEPT2. At the same time, the results show that sartans are no substrates for the proton-coupled amino acid cotransporter hPAT1 (Metzner et al., 2008).

Our results suggest a competitive type of inhibition of Gly-Sar uptake by the sartans. First, the calculation of IC_{50} values by non-linear regression revealed Hill coefficients of around 1 for all inhibition data as it has to be expected for competitive inhibition. Second, valsartan and losartan only affected the K_t value of Gly-Sar transport but not the maximal velocity of uptake (V_{max}). The sartans are therefore acting either as competing transporter substrates or as non-transported competitive inhibitors. To decide this question, we applied the two-electrode voltage clamp technique with *X. laevis* oocytes expressing either PEPT1 or PEPT2. Because of the cotransport with H^+, transport of substrates usually elicits inwardly directed currents (Theis et al., 2002; Daniel and Kottra, 2004; Knütter et al., 2008). Interestingly, all four sartans failed to elicit any significant currents. Hence, they are not transported by PEPT1 or PEPT2 in an electrogenic manner. The voltage-dependent inward currents induced by Gly-Gln uptake were, however, strongly inhibited by the sartans. This inhibition was fully reversible suggesting again a competitive mode of action.

Theoretically, the possibility exists that sartans are transported by PEPT1 and PEPT2 in an electroneutral manner. In other words, so far we could not rule out that sartans are taken up by the H^+/peptide cotransporters without a net charge (H^+) crossing the oocyte membrane. Therefore, in a third approach, we measured directly, by HPLC analysis, the PEPT1-specific uptake of losartan and valsartan into HeLa cells expressing human PEPT1. There was no

difference between cells transfected with empty vector and cells transfected with hPEPT1. Losartan and valsartan are not transported by PEPT1, in contrast to cefadroxil.

Compared to the uptake of cefadroxil, where uptake in mock cells was below HPLC quantification limit, the uptake rates of valsartan and in particular uptake of losartan were quite high. This might be due to the much higher lipophilicity of the sartans compared to cefadroxil. Simple transcellular diffusion could perhaps even explain the oral bioavailability of sartans. Alternatively, the question arises whether sartans, if not transported by PEPT1, are substrates for other membrane transport systems. Results published by Yamashiro and coworkers (2006) and Yamada and coworkers (2007) suggest that organic anion-transporting polypeptide OATP1B1 and OATP1B3, organic anion transporters OAT1 and OAT3, multidrug resistance-associated protein (MRP) 2 and other systems contribute to hepatic and renal transport of olmesartan and valsartan. According to Edwards et al. (1999) the four sartans studied here are secreted in the kidney by a carrier similar, if not identical, to the classic *para*-aminohippurate transporter (OATs). The intestinal absorption of sartans via such transport systems should be investigated in the future. With regard to efflux back into the intestinal lumen, it is relevant that losartan has been shown to be a P-glycoprotein substrate (Young et al., 2006).

Our results obtained in this study regarding the interaction of sartans with peptide transporters suggest that the sartans interact with PEPT1 and PEPT2 with high affinity but are not transported themselves. Their oral bioavailability cannot be explained by the activity of PEPT1. The high-affinity interaction with PEPT1 and PEPT2, however, might have consequences for the absorption of other drugs that are substrates of PEPT1. We have shown here that losartan and valsartan, when given simultaneously at equimolar concentrations, strongly inhibit the hPEPT1-specific uptake of cefadroxil. Such drug drug interactions depend on the affinity constants of the drugs in relation to their effective concentrations in the

intestinal lumen. The affinity constants of many drugs for hPEPT1 are known (for review see tables in Brandsch et al., 2008). The constants of four sartans have been measured in this study. According to Dominiak (1999) the daily doses of sartans given during therapy are as follows: losartan 50-100 mg, valsartan 80-160 mg, eprosartan 600-800 mg and irbesartan 75-300 mg. Estimating the local drug concentration in the luminal intestinal fluid that comes in contact with the epithelial cell layer, concentrations of around 50 to 500 µM are conceivable. This concentration range corresponds to the constants of the drugs for Gly-Sar uptake inhibition (20 to 2000 µM). Because PEPT1 is the predominant membrane protein that mediates uptake of orally available ß-lactam antibiotics, valacyclovir and other peptidomimetic drugs it could be speculated that sartans, when given simultaneously for a longer treatment period may alter the bioavailability and pharmacokinetics of PEPT1 substrates. On the other hand, depending on their affinity and their respective local concentration, PEPT1 substrates can reduce the interaction potential of sartans with the carrier protein. Whether such effects occur at the human intestinal epithelium and whether there might be consequences for drug pharmacokinetics has to be investigated *in vivo*.

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Acknowledgements

We thank Rainer Reichlmeir for excellent technical assistance, Martin G. Hahn for determination of the IC_{50} values of valsartan at Caco-2 and SKPT cells and Stefanie Geißler for determination of the IC_{50} values of Gly-Sar at HeLa cells.

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Footnote:

This study was supported by the State Saxony-Anhalt Life Sciences Excellence Initiative (M.B.) and by the Deutsche Forschungsgemeinschaft, grant KO 1605/2-4 (G.K.).

Fig. 1. Effect of sartans on [^{14}C]Gly-Sar uptake in Caco-2 and SKPT cells. A: Uptake of [^{14}C]Gly-Sar was measured in Caco-2 cells and in SKPT cells (10 µM [^{14}C]Gly-Sar, pH 6.0, 10 min, n = 3 - 4) in the presence of increasing concentrations of sartans (0 – 3.16 mM). Uptake rates measured in the absence of inhibitor were taken as 100%. B: Effect of sartans on total transepithelial flux and intracellular accumulation of [^{14}C]Gly-Sar at Caco-2 cell monolayers. [^{14}C]Gly-Sar was added to the apical (donor) compartment of the Transwell® chambers in uptake buffer (pH 6.0) with or without 1 mM sartan. After the time intervals indicated, samples were taken from the receiver compartment (pH 7.5) and replaced with buffer. Inset: [^{14}C]Gly-Sar content in cells on filters cut out of the plastic inserts after the flux measurements. Data are shown as means ± S.E., n = 3.

Fig. 2. Effects of valsartan and losartan on the saturation kinetics of Gly-Sar uptake into Caco-2 cells (A, C) and SKPT cells (B, D). Uptake of Gly-Sar was measured at pH 6.0 for 10 min. The results represent saturable uptake values after correction for the non-saturable component. Inset: Eadie-Hofstee transformations of the data (v = uptake rate in nmol · 10 min^{-1} · mg protein^{-1}; S = Gly-Sar in mM). n = 4.

Fig. 3. Sartan-induced inward currents in *Xenopus laevis* oocytes expressing PEPT1 or PEPT2. A: Typical recordings of inward currents in oocytes expressing PEPT1 (-60 mV) in the presence of 1 mM Gly-Gln, valsartan and irbesartan. B: Currents induced by losartan, irbesartan, valsartan and eprosartan as the percentage of the current induced by 1 mM Gly-Gln (PEPT1, -60 mV; PEPT2, -160 mV; n = 4 - 13). C, D: Steady-state I-V relationships were measured by the two-electrode voltage clamp technique in oocytes expressing PEPT1 (C) or PEPT2 (D) superfused with modified Barth-solution at pH 6.5 and 1 mM losartan and/or 1mM Gly-Gln. The membrane potential was stepped symmetrically to the test potentials shown and substrate-dependent currents were recorded as the difference measured in the absence and the presence of substrates. (NI Oocyte: non injected oocyte).

Fig. 4. Uptake at HeLa cells transfected with pcDNA3, pcDNA3-hPEPT1 or pcDNA3-hPEPT2. A: Effect of Gly-Sar, valsartan and losartan on [^{14}C]Gly-Sar uptake. Uptake of [^{14}C]Gly-Sar was measured in transfected HeLa cells (20 µM [^{14}C]Gly-Sar, pH 6.0, 10 min, n = 4) in the presence of increasing concentrations of sartans (0 – 3.16 mM). Uptake rates measured in the absence of inhibitor were taken as 100%. B: Uptake of losartan, valsartan and cefadroxil in HeLa-pcDNA3 and HeLa-hPEPT1 cells. Uptake was measured in transfected HeLa cells (pH 6.0, 30 min, n = 3-5) in the presence of 1 mM losartan, valsartan or cefadroxil. * under limit of quantification (0.155 nmol/30 min per 0.7 x 10^6 cells). C: Effect of losartan and valsartan on cefadroxil uptake. Uptake of cefadroxil was measured in transfected HeLa cells (1 mM, pH 6.0, 30 min, n = 3) in the presence of 1 mM sartans.

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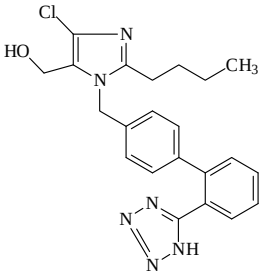
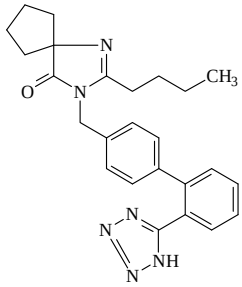
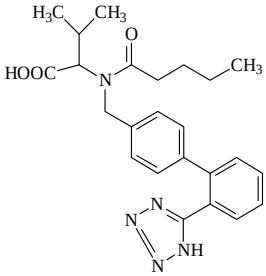
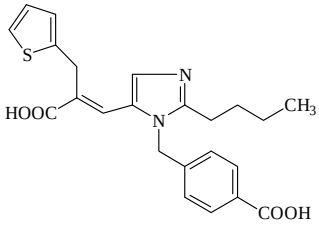
TABLE 1

Inhibition constants (K_i) of four sartans for the inhibition of [^{14}C]Gly-Sar uptake in Caco-2 cells expressing PEPT1 and SKPT cells expressing PEPT2

Compound	Structure	Caco-2 cells $K_{i\ PEPT1}$ (μM)	SKPT cells $K_{i\ PEPT2}$ (μM)
Losartan		24 ± 1	2.2 ± 0.2
Irbesartan		230 ± 30	65 ± 10
Valsartan		390 ± 40	260 ± 20
Eprosartan		> 1000 (≈ 2 200)[#]	490 ± 40

Structures were obtained from the Scifinder database. Constants (± S.E. n = 4) were derived from the competition curves shown in figure 1A. [#]K_i value extrapolated beyond measurement range because of limited solubility of compound. See Figure 1A for maximal substrate concentrations used.

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Fig. 1

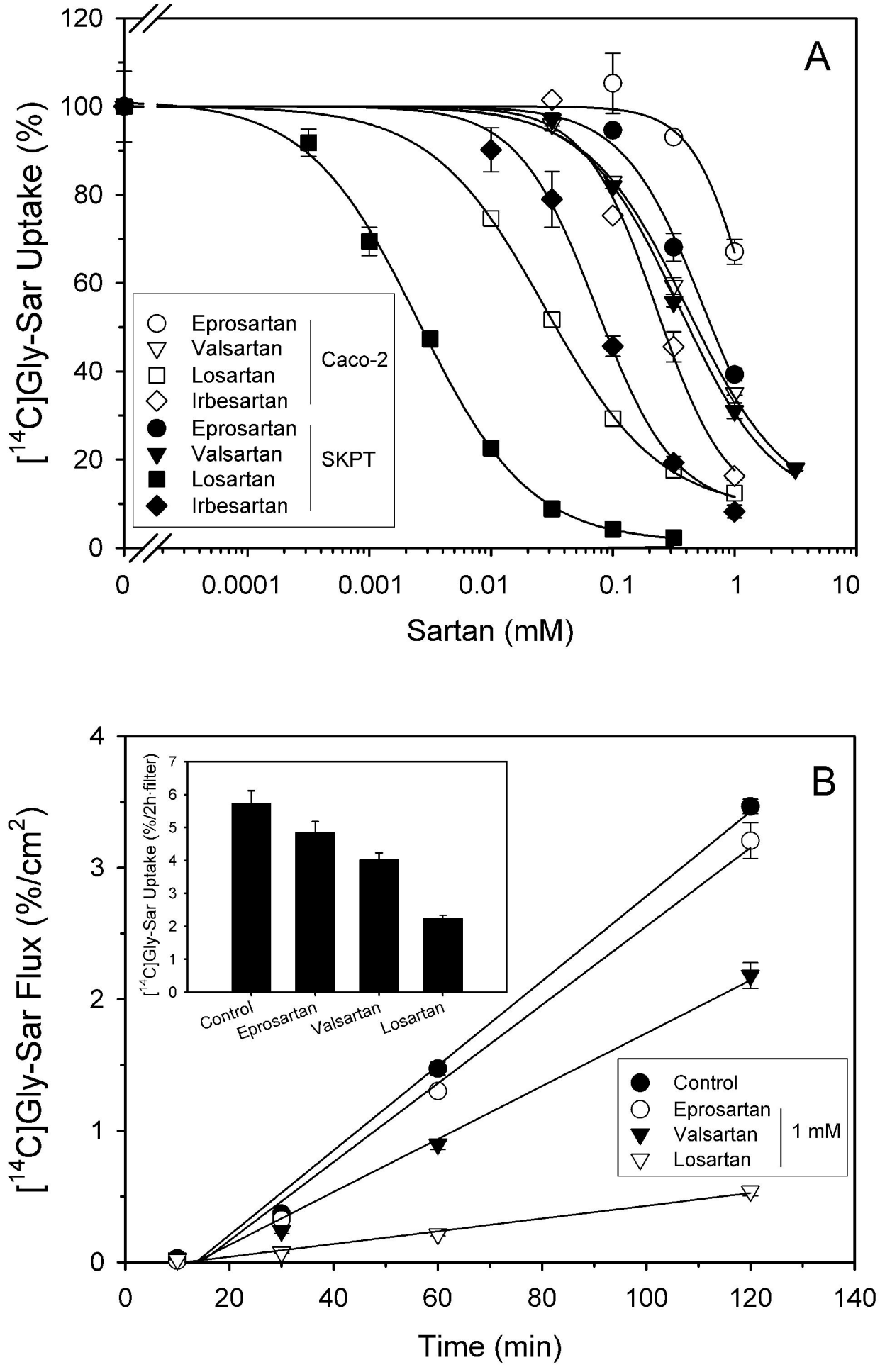
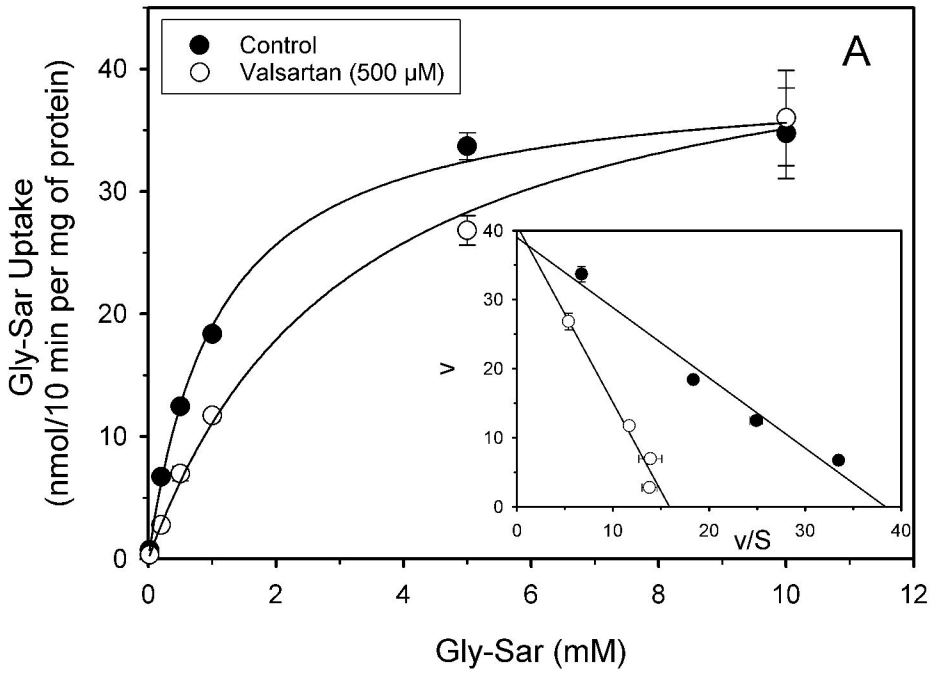
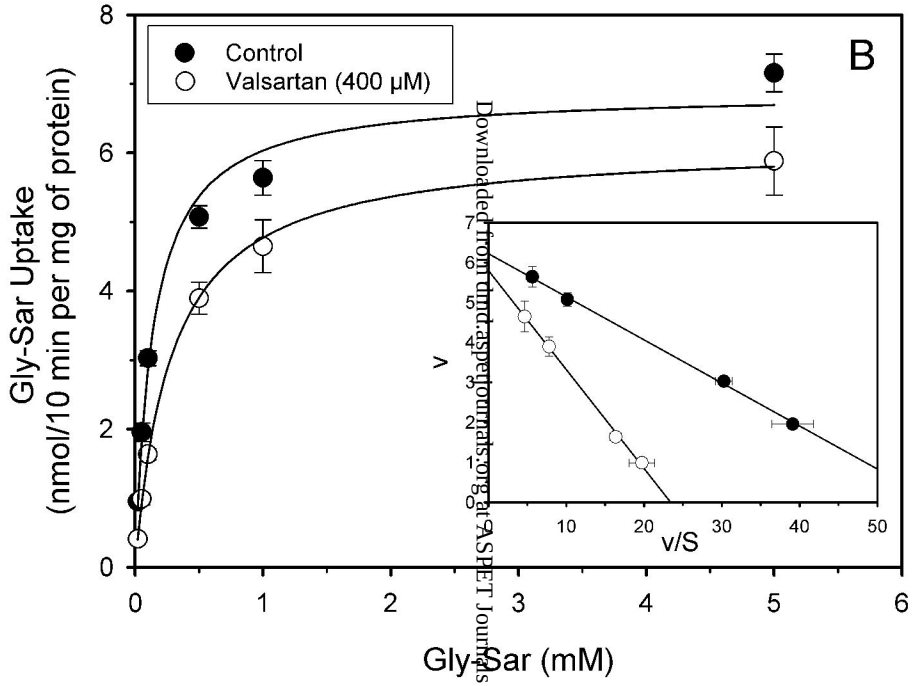
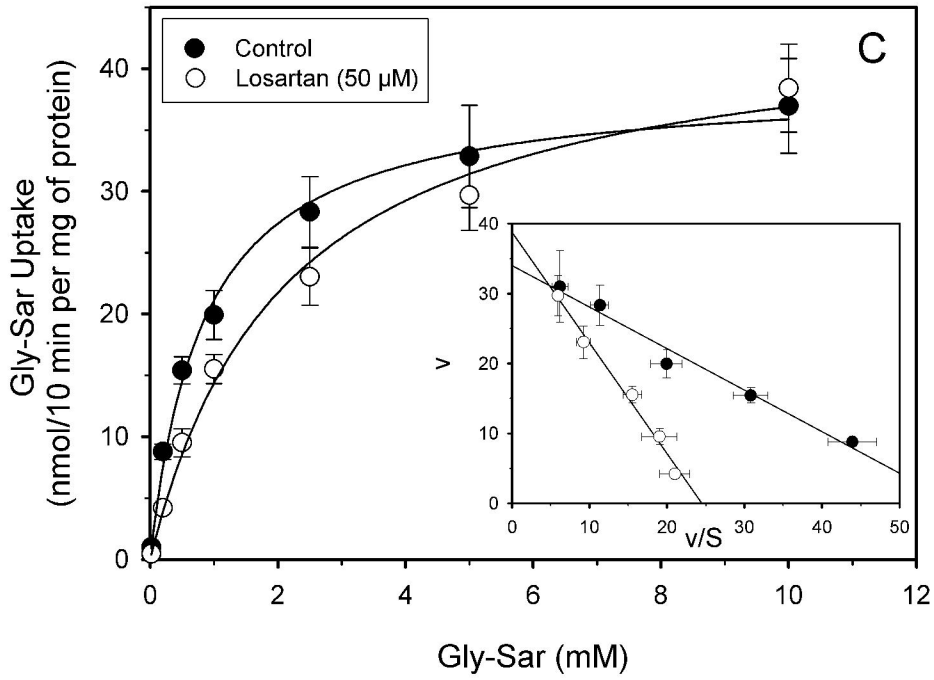


Fig. 2







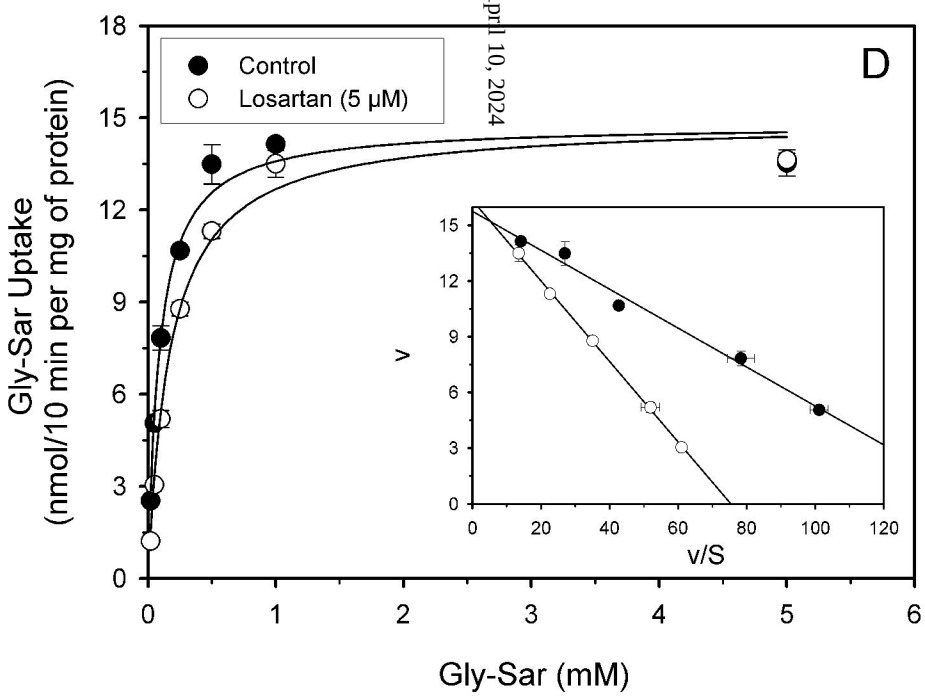
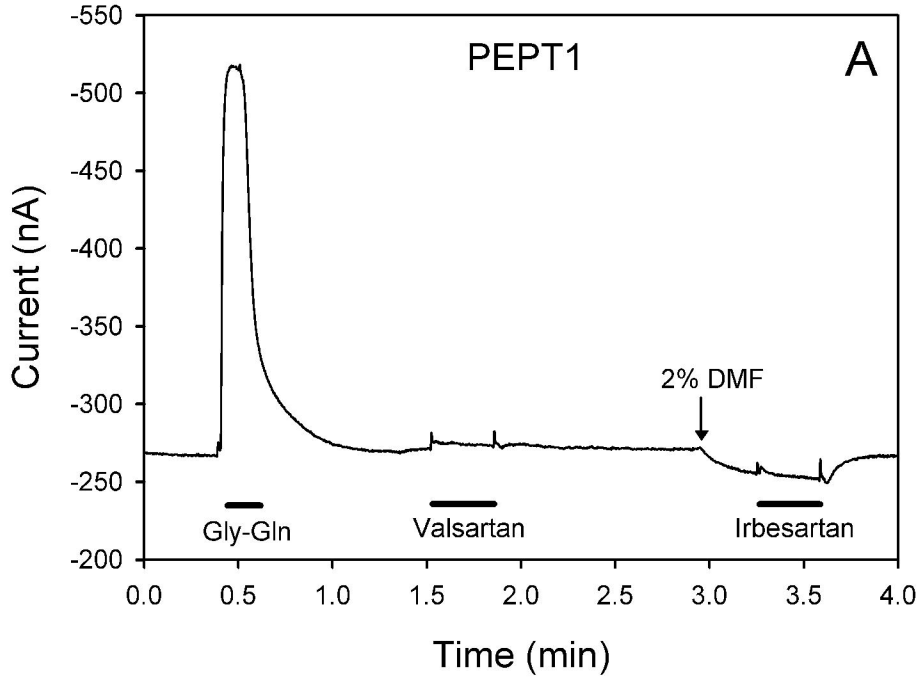
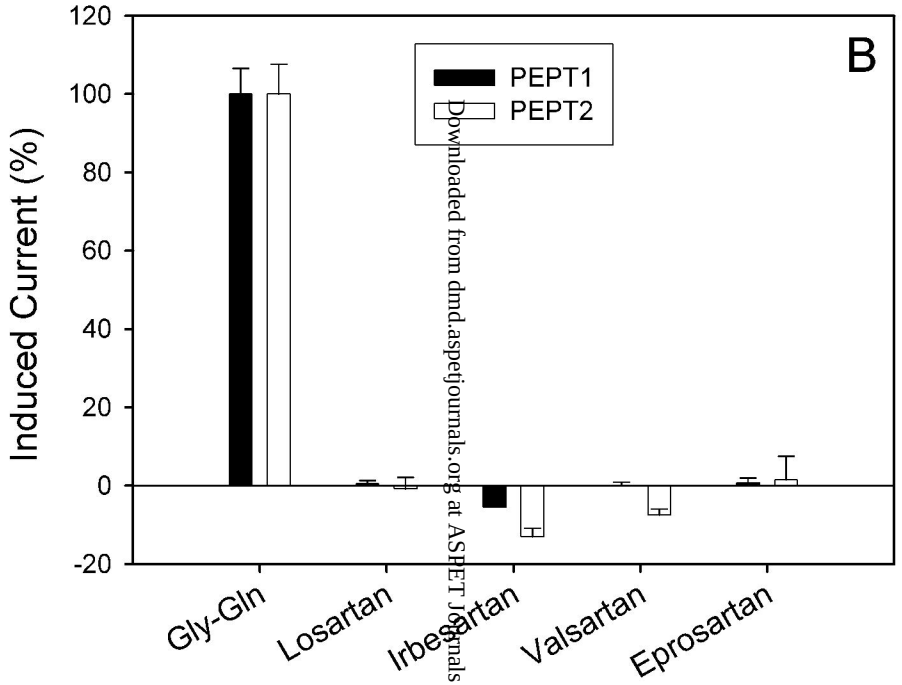
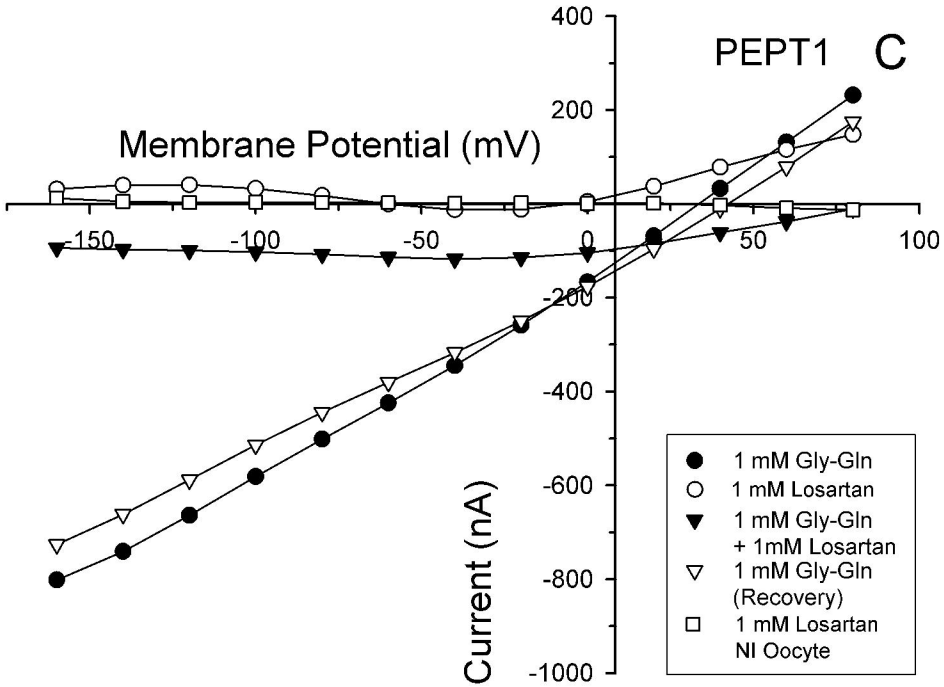


Fig. 3







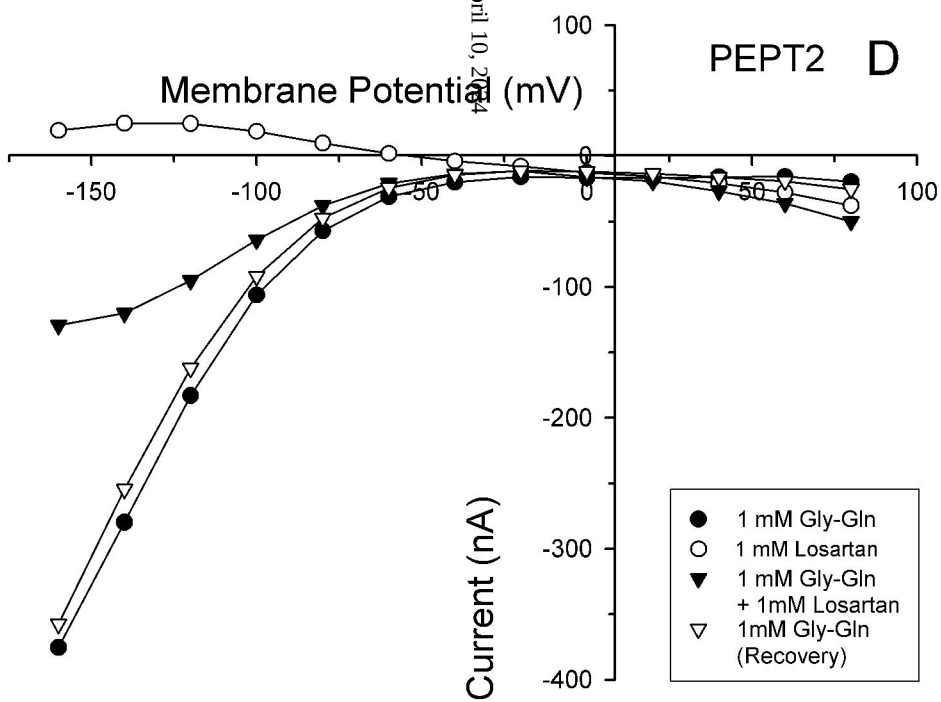


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Fig. 4