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Ethanol Inhibits Functional Activity of the Human Intestinal Dipeptide Transporter hPepT1 Expressed in *Xenopus* Oocytes

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Abstract

Background—The pathological effects of high alcohol (ethanol) consumption on gastrointestinal and hepatic systems are well recognized. However, the effects of ethanol intake on gastric and intestinal absorption and transport systems remain unclear. The present study investigates the effects of ethanol on the human peptide transporter 1 (hPepT1) which mediates the transport of di-and tripeptides as well as several orally administered peptidomimetic drugs such as *β*-lactam antibiotics (e.g., penicillin), angiotensin-converting enzyme inhibitors, the anti-neoplastic agent bestatin, and prodrugs of acyclovir.

Methods—*Xenopus* oocytes were injected with hPepT1 cRNA and incubated for 3 to 10 days. Currents induced by glycyl-sarcosine (Gly-Sar), Ala-Ala (dipeptides), penicillin and enalapril measured in the presence or absence of ethanol were determined using an 8-channel 2-electrode voltage clamp system, with a membrane potential of –70 mV and 11 voltage steps of 100 milliseconds (from +50 mV to –150 mV in –20 mV increments).

Results—Ethanol (200 mM) inhibited Gly-Sar and Ala-Ala currents by 42 and 30%, respectively, with IC_{50}s of 184 and 371 mM, respectively. Ethanol reduced maximal transport capacity (I_{max}) of hPepT1 for Gly-Sar without affecting Gly-Sar binding affinity ($K_{0.5}$ and Hill coefficient). Penicillin- and enalapril-induced currents were significantly less than those induced by dipeptides and were not inhibited by ethanol.

Conclusion—Ethanol significantly reduced transport of dipeptides via a reduction in transport capacity, rather than competing for binding sites in hPepT1. Ethanol inhibition or alteration of transport function may be a primary causative factor contributing to both the nutritional deficits as well as the immunological deficiencies that many alcoholics experience including alcohol liver disease and brain damage.

Keywords

hPepT1 Transporter; Ethanol Inhibition; Dipeptides; Penicillin; Enalapril

ALCOHOL (ETHANOL) ABUSE is a growing problem worldwide, with a per capita consumption of approximately 10 l pure ethanol per year in the United States and even higher levels in some Eastern European countries (Rehm et al., 2003). Ethanol abuse causes extensive pathological changes in the gastrointestinal (GI) tract and hepatic pancreatic system, and has been linked to more than 60 diseases (Gutjahr et al., 2001; Rehm et al., 2003). Ethanol directly

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damages the mucosal layer of the GI tract, causing erosive lesions and hemorrhages, and increases bacterial growth and toxin release in the GI tract. Increased permeability due to GI tract damage allows more of these bacterial toxins to enter the portal blood from the GI lumen, leading to endotoxemia and damage of other organs, including the liver, pancreas, and immune system (Bode and Bode, 2003; Halsted, 2004; Preedy et al., 1993).

The small intestine is the major site of nutrient absorption in the body, but to date, the effect of ethanol on this process has not been addressed. Ingested and digested nutrients such as peptides, vitamins and metal ions require endogenous solute carrier proteins (transporters) to facilitate their transport from the intestinal lumen to the portal blood for general utilization within the body (Dean et al., 2001; Fei et al., 1994; Terada et al., 2005). There are a variety of transporters expressed at the apical surface of epithelial cells lining the GI tract for transport of these species. One of these transporter systems is the human dipeptide transporter, hPepT1 (SLC15A1) (Hediger et al., 2004). The hPepT1 is a 708-amino acid protein with 12 α-helical transmembrane spanning domains (Liang et al., 1995). hPepT1 is expressed primarily in the intestine, and is also expressed to a lesser degree in the proximal tubules of the kidney and in cells of the liver and pancreas (Bockman et al., 1997; Liang et al., 1995; Thamotharan et al., 1997). The substrate specificity of hPepT1 is broad, permitting transport of many kinds of di- and tripeptides and several orally administered drugs, including β-lactam antibiotics (penicillin and amoxicillin) (Tamai et al., 1997), angiotensin-converting enzyme (ACE) inhibitors (enalapril and captopril) (Zhu et al., 2000), an anti-neoplastic agent (bestatin) and L-dopa (Tamai et al., 1998), as well as prodrugs such as valacyclovir and ganciclovir (Balimane et al., 1998; Ganapathy et al., 1998).

The present study represents the first study that specifically investigates potential ethanol-induced alterations of transport function in the human intestinal dipeptide transporter (hPepT1). We hypothesize that inhibition of the activity of this transporter could significantly reduce the absorption of nutrients, leading to the dietary and/or immunological deficiencies experienced by many alcoholics.

In the mammalian intestine, the transport of dipeptide is coupled with positively charged protons through hPepT1 inducing inward electrical current (Ganapathy and Leibach, 1991; Meredith and Boyd, 1995). Therefore, using an electrophysiological approach, we investigated the effects of ethanol on the absorption of dipeptides glycyl-sarcosine (Gly-Sar) and Ala-Ala by hPepT1, as well as an antibiotic (penicillin) and an ACE inhibitor (enalapril). The results demonstrate that ethanol inhibits hPepT1 absorption of dipeptides in a concentration-dependent manner, but does not inhibit the absorption of penicillin and enalapril.

MATERIALS AND METHODS

cDNA Construction and cRNA Synthesis

hPepT1 cDNA was subcloned into the eukaryotic expression vector pcDNA3 (Invitrogen, Carlsbad, CA) as previously described (Kulkarni et al., 2003a), and used as a template for cRNA synthesis. The hPepT1 plasmid was linearized with the restriction enzyme Bam-HI (New England BioLabs, Ipswich, MA). Transcription of the hPepT1 insert was performed using the mMESSAGE mMACHINE High Yield Capped RNA T7 Transcription Kit (Ambion Inc., Austin, TX).

Preparation of Oocytes and Expression of hPepT1

Oocytes were prepared as reported previously (Davies et al., 2005). Briefly, oocytes were excised from *Xenopus laevis* (Nasco, Ft, Atkinson, WI), enzymatically digested with 0.2% collagenase type 1A. Stage V and VI oocytes were selected and stored in incubation medium

(in mM): NaCl 96, KCl 2, $CaCl_2$ 1, $MgCl_2$ 1, HEPES 5, theophilline 0.6, pyruvic acid 2.5 with 1% horse serum, and 0.05 mg/ml gentamycin, pH 7.5. The following day, oocytes were injected with approximately 50 ng cRNA encoding the hPepT1 transporter and stored in incubation medium at 18°C. Electrophysiological recordings were conducted between 3 and 10 days after cRNA injection. Noninjected oocytes were used as a control.

Electrophysiological Recordings

An 8-channel high throughput 2-electrode voltage clamp (TEVC) system (OpusXpress 6000; Molecular Devices, Union City, CA), which automates the impalement of oocytes, fluid delivery and current recording from 8 oocytes in parallel, was used to carry out all electrophysiological recordings. Oocytes were placed in 8 recording chambers (volume ~200 μl), super-perfused with modified Barth's solution (in mM) (MBS, NaCl 88, KCl 1, $CaCl_2$ 0.91, $Ca(NO_3)_2$ 0.33, $NaHCO_3$ 2.4, $MgSO_4$ 0.8, HEPES 10, pH 7.5) at a speed of 2 ml/min and impaled with 2 glass electrodes filled with 3 M KCl (1 to 2 mega ohms). To increase the inward current carried by protons the pH of MBS solution in the bath was reduced from 7.5 to 5.5 (Mackenzie et al., 1996) during the voltage application. The membrane potential was held at −70 mV, and currents were sampled at 10 KHz and filtered at 1 KHz while 11 voltage steps of 100 milliseconds (from +50 mV to −150 mV in −20 mV increments) were applied. The following protocol was utilized: first, currents at pH 7.5 (I_{MBS}) were acquired; second, test solution 1 (pH 5.5 MBS) was delivered and currents were acquired ($I_{pH5.5}$); third, test solution 2 (pH5.5 MBS plus substrate) was delivered and currents were acquired ($I_{pH5.5+sub}$) and forth a 15-minute application of pH7.5 perfusion was applied to ensure that the substrate was completely washed out before further testing. Oocytes were exposed to test solutions for 90 seconds. For ethanol studies, ethanol was co-applied with pH 5.5 MBS in the absence of substrate to ensure that the reported concentration of ethanol in the oocyte bath was achieved. Next, the same concentration of ethanol was added to test solution 2 and applied to oocytes as described above. The maximum ethanol concentration utilized in the current study was 200 mM. We found that higher concentrations of ethanol in the presence of a pH of 5.5 resulted in the deterioration of the oocytes. The currents acquired in the presence or absence of substrates and with or without ethanol were stored on the computer for off-line analysis.

Data Analysis

The OpusXpress records currents from 8 oocytes in parallel. The currents between 70 and 90 millisecond were averaged and individually measured. The difference of the steady-state currents in the presence of substrates, with or without ethanol or at different pHs was used for analysis. The difference of the $I_{pH5.5\ +\ sub}$ and $I_{pH5.5}$ represents the substrate-induced current. To calculate the $K_{0.5}$, I_{max} and IC_{50} we incorporated a custom written computer program that linked pClamp 9.0 software, MS Excel (Microsoft, Richmond, WA), and Prism (GraphPAD Software, San Diego, CA). Steady-state data were fitted to the following equation: $I = I_{max}S^n/((K0.5)^n + S^n)$, where I is the substrates evoked current, I_{max} is the derived current maximum, S is the concentration of substrates, n is the Hill coefficient, and $K_{0.5}$ is the concentration of substrates which induced half maximal response. Data were compared using Student's *t*-test, and a *p*-value of less than 0.05 was considered statistically significant.

Determination of Ethanol Concentrations in Rat Small Intestine

This protocol was obtained from the Research Center for Liver and Pancreatic Diseases at the University of Southern California. Ethanol (35% w/v, 3 g/kg body weight) was directly injected into rat stomach through a gavage needle. Under anesthesia with 5:1 mixture of ketamine (100 mg/ml, Warner-Lambert, Morris Plains, NJ) and xylazine (20 mg/ml, 1 ml/100 g body weight, Spectrum Chemical, Gardena, CA), the abdomen was completely opened and whole small intestine exposed. A #20 gauge plastic tubing was inserted into the jejunum and ileum

separately at 60 minutes after ethanol injection to obtain liquids. The collected liquids were centrifuged at 1100 × *g* for 10 minutes, and supernatants were collected. The ethanol concentrations in the supernatants were measured using an alcohol analyzer (Model: Analyser GM, Analox Instruments, London, England).

Chemicals and Reagents

All chemicals and ethanol were USP grade or purer and were purchased from Sigma (St Louis, MO), unless otherwise indicated.

RESULTS

Effect of Ethanol on Gly-Sar-Induced Currents

In a previous study using TEVC for evaluating the functional activity of hPepT1, we showed that the current induced by the model hPepT1 substrate, Gly-Sar, is voltage- and pH-dependent (Kulkarni et al., 2003a). Here, we used a similar approach to examine the effects of ethanol on hPepT1 activity. Figure 1 illustrates a representative 8-channel recording. Steady state currents were achieved within 30 milliseconds. In control experiments, 3 mM Gly-Sar induced a minimal, nonsignificant current (2.5 pA, $n = 3$) in uninjected oocytes.

As illustrated in Fig. 2, Gly-Sar (3 mM) induced an inward current in a voltage-dependnt manner, and ethanol (200 mM) significantly inhibited the hPepT1 transport activity. After a 15-minute washout period to remove ethanol, Gly-Sar induced current completely recovered to the level of control (Fig. 2A). To investigate if ethanol affects the apparent affinity of Gly-Sar for hPepT1, 7 concentrations of Gly-Sar (0.03, 0.1, 0.3, 1, 3, 10, and 30 mM) were used to generate a concentration–response relationship in the absence and presence of ethanol (200 mM, which is IC_{50}). The induced current increased with increasing concentrations of Gly-Sar. Ethanol (200 mM) inhibited the current with a more prominent inhibitory effect at higher Gly-Sar concentrations (Fig. 2*B*). The inhibitory effect was ethanol concentration dependent, with the current induced by Gly-Sar (3 mM) in the presence of 3 concentrations of ethanol (50, 100, and 200 mM) indicating an ethanol concentration for half-maximal inhibition (IC_{50}) of 184 ± 5 mM at −110 mV (Fig. 2*C*). From these experiments, $K_{0.5}$, I_{max}, and Hill coefficient values at −110 mV were calculated (Table 1). Ethanol significantly reduced the maximal transport capacity (I_{max}), but did not significantly affect the apparent affinity ($K_{0\cdot5}$ and Hill coefficient). I_{max} for Gly-Sar in the presence or absence of ethanol is voltage-dependent with a maximal value at more hyperpolarizing potentials (−150 mV) and a decreased value at lower membrane potentials (Fig. 3*A*). $K_{0.5}$ for Gly-Sar reached a maximum at −150 mV and minimum at −50 mV, and ethanol had no significant effect on $K_{0.5}$ at any membrane potential (Fig. 3*B*). Ethanol (200 mM) inhibition of Gly-Sar transport was not affected by concentrations of Gly-Sar from 0.2 to 20 mM or by changes in membrane potentials from −110 mV to −30 mV (Table 2).

Effect of Ethanol on Ala-Ala-Induced Currents

Gly-Sar is a nonnatural dipeptide that is widely used as a model substrate for hPepT1 as it is nonhydrolyzable. To determine if ethanol exerted similar inhibitory effects on transport of a natural dipeptide, we examined hPepT1 uptake of Ala-Ala. As there are no reported electrophysiological data for Ala-Ala transport by hPepT1, we first investigated the concentration–response relationship of Ala-Ala-induced currents. As shown in Fig. 4, Ala-Ala-induced currents are also voltage- and concentration-dependent. The apparent affinity of Ala-Ala was smaller ($K_{0.5}$ = 101 ± 35 μM) and the I_{max} was larger (1.0 ± 0.1 μA) compared with those of Gly-Sar, and the Hill coefficient was 2.1 ± 0.4 ($n = 4$). Ethanol (200 mM) caused a reversible inhibitory effect on Ala-Ala-induced currents (Fig. 5*A*), similar to that for Gly-Sar. The inhibitory effect of ethanol was concentration-dependent, with an ethanol IC_{50} of 371 mM for uptake of 100 μM Ala-Ala (Fig. 5*B*).

Effect of Ethanol on Drug-Induced Currents

The experiments were repeated using penicillin and enalapril. Each drug induced a significantly smaller current than those induced by Gly-Sar and Ala-Ala at the same concentration (3 mM) (Fig. 6*A*). In contrast to the dipeptides, ethanol (200 mM) did not inhibit either penicillin- or enalaprilinduced currents (Fig. 6*B*).

Determination of the Ethanol Concentration in the Small Intestine of Rat

To determine if the ethanol IC_{50} shown in our oocyte experiments were physiologically relevant, we measured the concentrations of ethanol in liquid collected from the intestinal lumen and blood in vivo from 2 rats after ethanol (35% w/v, 3 g/kg body weight) was directly injected into the stomach through a gavage needle. The concentrations of ethanol were 384 mM in the jejunum and 36 mM in the ileum in one rat, and 36 mM in the jejunum and 34 mM in the ileum in the other rat at 60-minute postinjection. The ethanol concentrations in blood were 30 mM in both rats. The difference in ethanol concentration between the 2 rats in the jejunum is most likely due to the result of the sampling method. The fluid obtained from the small intestine for the ethanol concentration test was a mixture of ethanol, gastric, and intestinal fluids. Nonetheless, the high ethanol concentration found in one of the rats illustrates the potential for high ethanol concentration within the intestinal region that expresses the highest level of hPepT1.

DISCUSSION

The main findings of the present study are that ethanol reversibly inhibits hPepT1 transport of dipeptides, but does not affect the transport of 2 nondipeptide drugs. Transport mediated by PepT1 obeys the Michaelis–Menten equation, which is characterized by 2 parameters I_{max} and $K_{0.5}$. Ethanol inhibition of Gly-Sar transport was characterized by a decrease in I_{max} by 36% with no change in the apparent affinity $K_{0.5}$, and the extent of ethanol inhibition did not change for Gly-Sar concentrations from 0.2 to 20 mM. These results indicate that ethanol does not compete with the substrate for binding sites on the transporter. The reduced I_{max} could be due to a change in the number of the functional transporters available, or ethanol may have an allosteric effect on hPepT1 that decreases the transport efficiency.

The level of the hPepT1 transporter is influenced by hormonal regulation (Adibi, 2003; Sun et al., 2003; Thamotharan et al., 1999), diet (Erickson et al., 1995; Shiraga et al., 1999), and disease (Sundaram et al., 2005; Ziegler et al., 2002). Three mechanisms have been proposed for regulation of hPepT1 expression. First, induction of secondary messengers can alter hPepT1 expression as demonstrated by incubation with PMA (a PKC activator) and cAMP. In this work, over a timescale of minutes, PMA reduced V_{max} but did not change K_m(Brandsch et al., 1994; Muller et al., 1996). Second, alteration of translocation can alter hPepT1 expression. This work demonstrated that incubation with insulin for 60 minute increases V_{max} without affecting K_m, probably due to an insulin-mediated increase in trafficking of the transporter from intra-cellular pools to the apical membrane (Thamotharan et al., 1999). Finally, changes in expression of DNA and mRNA can alter hPepT1 expression. In this work, it was shown that incubation with thyroid hormone triiodothyronine (T3) or epidermal growth factor for 4 to 28 days decreases V_{max} without any significant change in K_m (Ashida et al., 2002; Nielsen et al., 2001).

In our current experiments, oocytes were exposed to ethanol for only 3 minutes; therefore, it is very unlikely that reduced DNA or mRNA expression could account for the ethanol-induced change in V_{max}. Reduction of the number of functional transporters available in the apical membrane through reduced trafficking of hPepT1 from intracellular pools and/or by increasing the internalization of the transporter is also possible, but the timescale of the experiments

suggests that this is also an unlikely explanation (Lee, 2000; Thamotharan et al., 1999). Moreover, the ethanol reduction in dipeptide transport versus the lack of the effect on penicillin and enalapril transport also argues against changes in the number of functional transporters on the cell membrane as a plausible reason for ethanol-induced changes in V_{max}.

In a recent investigation of the ethanol effects on ligandgated ion channels expressed in oocytes, we found that a 1-minute exposure to ethanol potentiated the ATP-induced current in the purinergic P2X3 receptor and inhibited the ATP-induced current in the purinergic P2X4 receptor (Davies et al., 2005). The underlying mechanisms for both the positive and negative effects appear to be allosteric modulation by ethanol, and there may be a similar basis for the effect of ethanol on hPepT1.

The X-ray structure of the proton-coupled 12-membrane *Escherichia coli*-lac permease transporter (Abramson et al., 2007), which may be structurally related to hPepT1, has indicated that a large conformational shift may occur upon substrate transport. Partial computer models from several laboratories (Irie et al., 2005; Kulkarni et al., 2007; Mackenzie et al., 1996) have proposed that PepT1 undergoes a series of conformational changes upon binding of H^+ and substrate. These changes result in a "flip-flop" of the protein, such that the substrate is exposed to and released into the cytosol (with concurrent translocation of the proton). The transporter then undergoes a reverse conformational change back to a pretransport case (Kulkarni et al., 2003b, 2007; Sala-Rabanal et al., 2006). The ethanol reduction of V_{max} could be due to an effect of ethanol on any of these steps.

Ethanol is linked to several diseases, and alcoholism is a leading cause of malnutrition in developed countries. The severity of the negative effects of ethanol depends on the ethanol concentration and length of exposure. Ethanol concentrations in the intestine exceeding 2% will interfere with the active transport of nutrients, and concentrations exceeding 12.5% (which is the concentration in wine) will induce more extensive damage such as hemorrhage, structural changes and increased mucosal permeability (Bode and Bode, 1992; Preedy et al., 1993; Siegmund et al., 2005). Here, we have shown that the IC_{50} for ethanol inhibition of hPePT1 is 184 mM (~0.9%) for Gly-Sar and 371 mM (~1.7%) for Ala-Ala. Furthermore, these concentrations of ethanol are achievable in the intestine in rat, since 60 minute after the application of a single dose of ethanol (35% w/v, 3 g/kg body weight), which is about the concentration of spirits, the concentration reached 384 mM in the jejunum and 36 mM in the ileum. Note, in humans, the upper small intestine is a major organ of absorption for ethanol (see reviews, Baselt and Danhof, 1987; Rajendram and Preedy, 2005). It is conceivable that the concentration of ethanol in the jejunum can reach a value of 500 to 630 mM (~2.5 to 3%, w/v) after drinking 40 g of ethanol. This would be equivalent to consuming approximately 3 or 4 alcoholic beverages (Israel et al., 1969). Moreover, concentrations of ethanol could reach 1000 mM or higher (6.5 to 9.4%) after drinking 0.8 g/kg body weight of ethanol (see review, Rajendram and Preedy, 2005). Therefore, concentrations of ethanol in excess of the IC_{50} for inhibition of hPePT1 can reasonably occur physiologically, and such concentrations could affect the activity of the hPepT1 transporter and significantly reduce uptake of dipeptides, thereby causing nutrient deficiency.

We found that the nondipeptide substrates, penicillin and enalapril, induced much smaller currents than those induced by Gly-Sar and Ala-Ala did, and that ethanol did not affect the induced current for the nondipeptide drugs. The penicillin- and enalapril-induced currents were only about 20% of the dipeptide-induced currents at the same concentration (3 mM), consistent with their higher $K_{0.5}$ (about 10 mM) of the nondipeptides (Poschet et al., 1999; Zhu et al., 2000); we determined that the natural dipeptide Ala-Ala has a $K_{0.5}$ 0.1 mM, and the $K_{0.5}$ of Gly-Sar is 1 mM. At this time, we do not know if the differences in ethanol inhibition of Gly-Sar versus the penicillin or enalapril differ. The lack of an effect of ethanol on the transport of

penicillin and enalapril could be because the nondipeptides have a different transport mechanism, compared to dipeptides, with this mechanism unaffected by ethanol. However, the growing body of knowledge regarding hPepT1-mediated transport argues for a single (albeit flexible) substrate binding site in the transporter, and so it is likely that all transported substrates have the same or similar basic mechanism. Hence, the lack of effect of ethanol on nondipeptide substrates most likely reflects a relative lack of sensitivity of the transporter for such substrates (as indicated by the reduced transport of such substrates, compared to dipeptides). That is, allosteric modulation of hPepT1 by ethanol is too subtle an effect to influence the transport of nondipeptide substrates, as the transport of these substrates already occurs through suboptimal conformational changes. Nonetheless, the observation that ethanol can influence dipeptide uptake is of importance from a pharmacological perspective, as the goal of transport-mediated oral delivery of drugs and prodrugs is to optimize the interaction and transport of designed substrates targeting hPepT1, and with increased transport efficiency, the allosteric effects of ethanol may become important.

The present study represents one of only a few studies that have begun to address the issue of ethanol/transport interactions, at the molecular level, for any of the nutritional uptake transporters. We found that ethanol reduces transport of dipeptides by reducing transport capacity, rather than competing for binding sites in human peptide transport, whereas, ethanol does not affect nondipeptide transport. The findings suggest difference in the manner of transport for nondipeptide substrates. Ethanol inhibition or alteration of transport function may be a primary causative factor contributing to both the nutritional deficits as well as the immunological deficiencies that many alcoholics experience including alcohol liver disease and brain damage. An increased understanding of the mechanism by which ethanol alters transport function can provide additional therapeutic strategies/targets for offsetting the effects of ethanol on nutrient transport.

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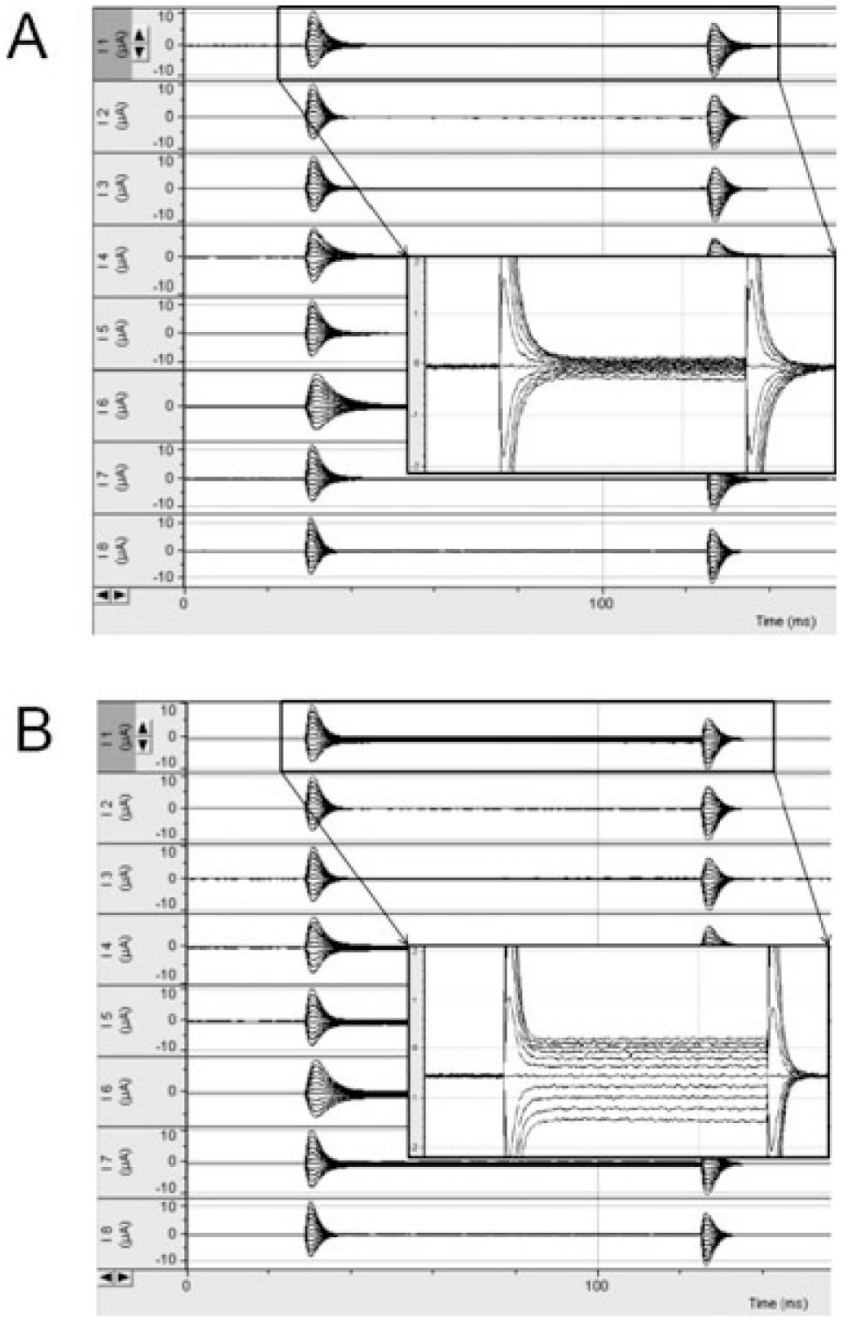

Fig. 1.

Representative current traces obtained from 8 channel TEVC OpusXpress system. Inset shows a larger image of current traces. (**A**) current traces without Gly-Sar; (**B**) current traces with 3 mM Gly-Sar. Note that I_h at −70 mV decreases after the addition of Gly-Sar.

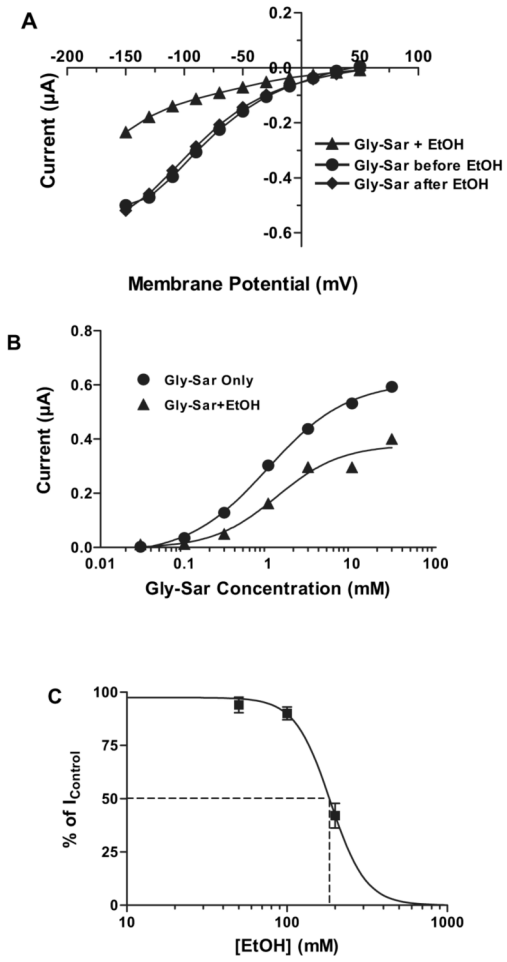

Fig. 2.

Ethanol inhibits Gly-Sar-induced currents. (**A**) Representative traces showing the current-voltage relationship for inhibition of 3 mM Gly-Sar-induced currents by 200 mM ethanol at pH 5.5. (**B**) Gly-Sar concentration-response curves in the absence and presence of 200 mM ethanol. (**C**) Ethanol inhibition curve of 3 mM Gly-Sar-induced currents (n = 6 to 10).

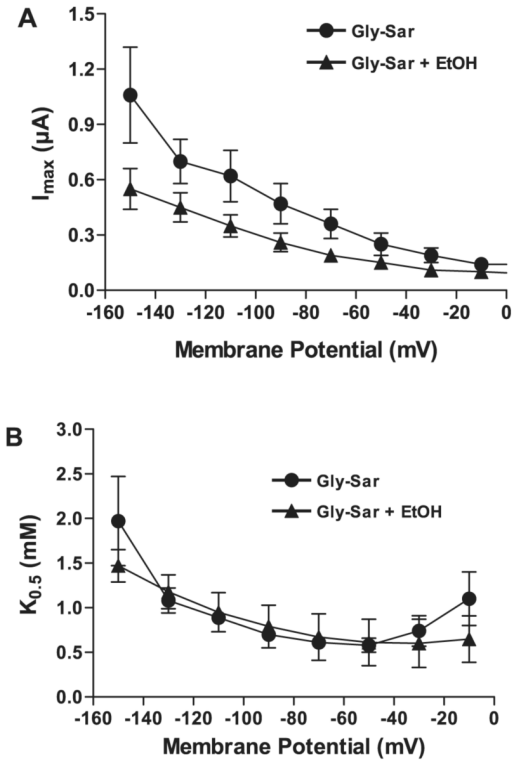

Fig. 3.

Ethanol reduces maximum current (I_{max}) but not $K_{0.5}$.(**A**)Maximum current-voltage and (**B**) $K_{0.5}$/voltage relationships (n = 4 to 9).

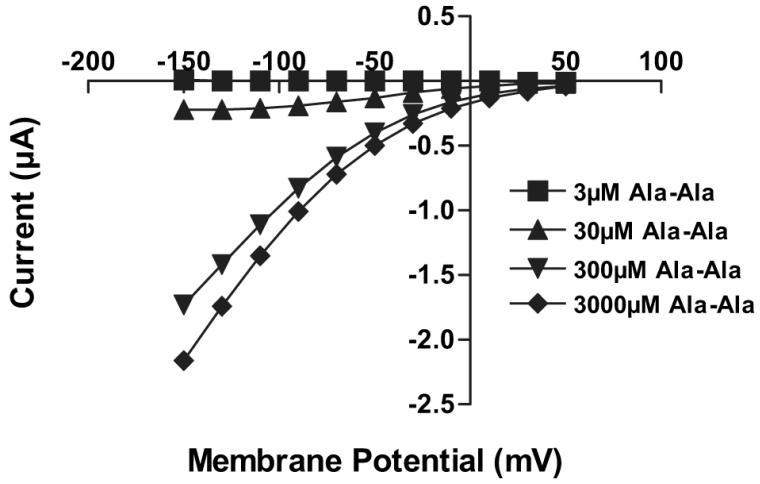

Fig. 4.
Current-voltage relationships of Ala-Ala-induced currents.

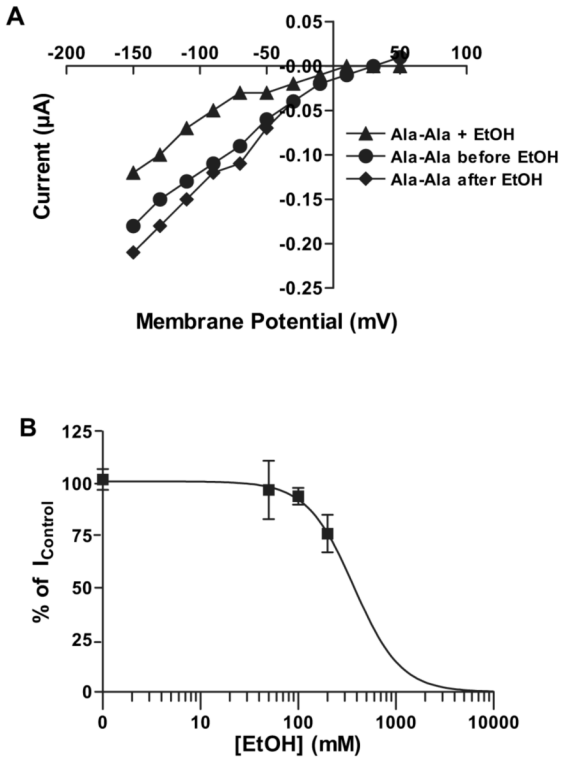

Fig. 5.

Ethanol inhibits Ala-Ala-induced currents. (**A**) Current-voltage relationship for inhibition of 100 μM Ala-Ala-induced currents by 200 mM ethanol. (**B**) Ethanol inhibition curve of 100 μM Ala-Ala-induced currents (n = 3 to 7).

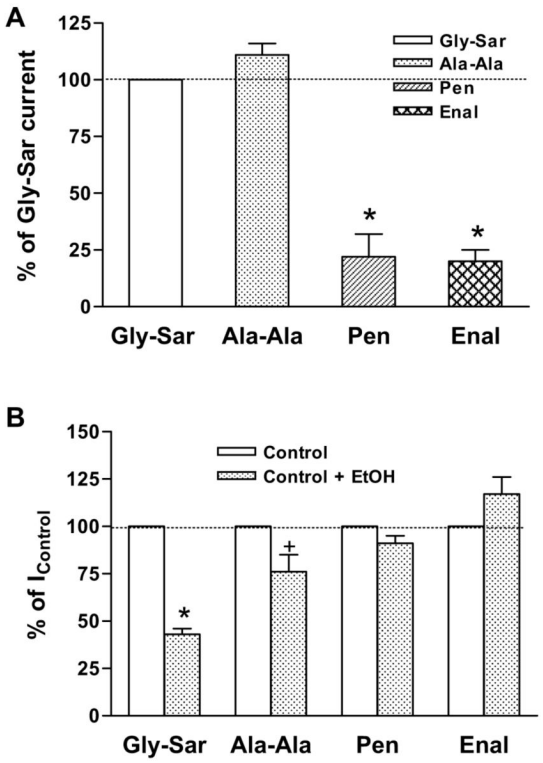

Fig. 6.

Comparison of currents induced by dipeptides and drugs. (**A**) Gly-Sar-, Ala-Ala-, penicillin (Pen)- and enalapril (Enal)-induced currents. Concentration of all substrates was 3 mM and Gly-Sar-induced current was normalized as 100%. Data represent mean ± SEM, *$p < 0.05$ compared with Gly-Sar ($n = 5$ to 7). (**B**) Gly-Sar-, Ala-Ala-, penicillin- and enalapril-induced currents in the absence and presence of 200 mM ethanol. Currents obtained in the absence of ethanol were normalized as 100%. Data represent mean ± SEM, *$p < 0.05$, +$p = 0.08$ compared with Gly-Sar-induced current ($n = 3$ to 7).

Table 1

Effect of Ethanol on the I_{max}, $K_{0.5}$ and Hill Coefficient for Gly-Sar Transport by hPepT1

	I_{max} (μA)*	$K_{0.5}$ (mM)	Hill coefficient
No ethanol	0.69 ± 0.18	1.0 ± 0.3	1.3 ± 0.1
200 mM ethanol	0.40 ± 0.12	0.9 ± 0.1	1.3 ± 0.1

I_{max}, $K_{0.5}$, and Hill coefficient were calculated in the presence of 3 mM Gly-Sar and with and without 200 mM ethanol. Membrane potential was −110 mV and pH 5.5; n = 4 to 9.

*$p < 0.01$ for no ethanol versus 200 mM ethanol.

Table 2

Effect of Gly-Sar Concentrations and Membrane Potential on % Ethanol (200mM) Inhibition of Gly-Sar Uptake by hPepT1 (*n* = 3 to 7)

Membrane potential	Gly-Sar concentration			
	0.2 mM	**1 mM**	**5 mM**	**20 mM**
−30 mV	68 ± 13	68 ± 5	66 ± 4	70 ± 4
−50 mV	66 ± 12	63 ± 4	58 ± 4	63 ± 4
−110 mV	74 ± 13	63 ± 4	60 ± 3	64 ± 2

Gly-Sar-induced currents were obtained at different concentrations of Gly-Sar and membrane potentials in the presence of 200 mM ethanol (*n* = 3 to 7).