Current Topics

Cellular Physiology of Channels and Transporters in Gastrointestinal Tracts

The Glycine Transporter GLYT1 in Human Intestine: Expression and Function

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Received January 4, 2011

Glycine is a well-documented cytoprotective agent and protects mammalian intestine against ischemia-reperfusion injury, irradiation and experimentally induced colitis. The specific glycine transporter GLYT1 is found throughout the human intestine where it is responsible for some 30—50% of glycine uptake into intestinal epithelial cells across the basolateral membrane and appears to function to maintain glycine supply to enterocytes and colonocytes. This paper reviews current knowledge of GLYT1 and presents recent evidence supporting its essential role in glycine mediated cytoprotection in intestinal absorptive cells. Regulatory mechanisms involved in intestinal expression of GLYT1 are discussed and the potential of glycine for use as an anti-inflammatory, protective agent in the management of inflammatory bowel disease examined.

Key words GLYT1; glycine; intestine; glutathione

1. INTRODUCTION

The gastrointestinal tract expresses a wide range of amino acid and peptide transporters. These include transporters with broad range specificity, such as the peptide transporter PepT1 (solute carrier family 15, member A1 (SLC15A1)) and members of the system A and system N sodium-coupled neutral amino acid transporters (SLC38 family), as well as those with restricted substrate specificity. While the principle function of many of these transporters is the absorption of dietary nutrients increasing evidence suggests that others have more specialised roles. An example of this, and the focus of this review, is the glycine transporter GLYT1 (SLC6A9), a member of the sodium- and chloride-dependent neurotransmitter transporter family SLC6. In common with other members of the family, which includes transporters for γ-aminobutyrate (GABA), dopamine and serotonin, GLYT1 is dependent on both sodium and chloride and structurally is predicted to contain twelve membrane spanning domains. Also consistent with other members of the family it is expressed at high levels in neuronal tissues where it restricts glycine function at glycineric synapses by facilitating glycine uptake into surrounding glial cells and modulates excitatory neurotransmission at glutamatergic synapses where it is a co-agonist at N-methyl-D-aspartate (NMDA) receptors (reviewed in). Functionally it is most similar to another glycine transporter and member of the same family, SLC6A5 or GLYT2, with which it shares approximately 50% sequence identity. Both are highly specific and exhibit high affinity for glycine, but while GLYT2 accepts only glycine, GLYT1 can transport both glycine and sarcosine. This difference was exploited in the early days of research into these transporters, allowing differentiation of the two, but more recently inhibitors specific to either one or the other have facilitated such work.

First isolated from rodent tissues at least three different GLYT1 mRNA variants, each encoding alternative protein isoforms, have been described. The most well characterised of these, GLYT1A and GLYT1B (transcript variants 3 and 1, respectively) have been identified in a number of mammals including rat, mouse, human and cattle. These transcripts differ only in the very 5’ region and are generated by the use of alternative promoters. They generate proteins which diverge in the N-terminal region; originally thought to differ only in that the proximal ten amino acids of GLYT1A were replaced by an alternate 15 amino acids in GLYT1B recent re-analysis of the sequence data revealed that the two isoforms actually differ in length by 19 base pairs with the original GLYT1B sequence extended N-terminally by a further fourteen residues. The sequence difference between GLYT1A and B has little effect on protein function and the two isoforms have identical substrate specificity and similar transport kinetics. What does differ is site of expression, driven possibly by alternate regulation within the specific promoter of each variant. GLYT1B shows a more restricted pattern of expression than GLYT1A and is highly expressed only in the brain and spinal cord; GLYT1A is expressed in neuronal tissues and many peripheral organs. In addition to these several other GLYT1 mRNA variants have been reported. In human a third isoform, GLYT1C, was isolated from the brain and is generated by alternative splicing. It is identical to GLYT1B with the exception of an additional exon included between the first and second exons of that transcript, at a point corresponding to amino acid position 30 of GLYT1B and resulting in a protein extended by 54 amino acids. Again this variation appears to have little effect on function and GLYT1C shows similar transport characteristics to isoforms A and B. Apart from brain, GLYT1C mRNA was detected only in kidney where expression was at a very low level. Three other human GLYT1 sequence variants, each lacking a different exon, were found to be non-functional and thought most likely to be the product of aberrant mRNA splicing. Two further transcript variants, GLYT1E and F, have been described in bovine tissues. Although these variants have sequences with potential to encode proteins with a novel C-terminus that significantly alters
protein function allowing interaction with the GABA<sub>C</sub> receptor ρ1 subunit and changing transport kinetics, the lack of corroborating evidence from other species suggests that they are either unique to this species or possibly rare and unstable intermediates of the splicing process.

2. GLYT1 IN INTESTINE

2.1. Characterisation   GLYT1 mRNA and protein are found throughout the human intestine. In the small intestine, the protein is located along the length of the crypt-villus axis and mRNA expression does not vary significantly from duodenum to ileum. Similarly mRNA expression within the colon is consistent through all regions and the protein is detectable throughout the crypt. In both enterocytes and colonocytes, GLYT1 is restricted to the plasma membrane and located at both apical and basolateral surfaces.

While it has not been possible to determine by functional or immunological methods the GLYT1 isoform’s present in intestine, as suitable antibodies and selective inhibitors remain elusive, studies at the molecular level have shown that the predominant transcript variant is that which encodes GLYT1A. Although several other GLYT1 transcript variants were detectable in human intestine by polymerase chain reaction (PCR), including those for GLYT1B and GLYT1C and a number which were previously undescribed, these were all found at very low concentration and detection was inconsistent, suggesting again that some if not all arose as unstable intermediates or errors of the splicing process.

Transport function of GLYT1 in human intestine has been characterised using models of intestinal absorptive cells derived from colonic carcinomas, the Caco-2 and HCT-8 cell lines. Although of similar origin these cells show significant differences in culture. Caco-2 cells spontaneously differentiate to resemble the absorptive enterocytes of the small intestine whereas HCT-8 cells are more colonic in nature. Both cell lines express GLYT1 with the protein located, as in the tissue which they model, at both apical and basal membrane surfaces. In Caco-2 cells, glycine uptake consists of three components with varying ionic dependence, namely Na<sup>+</sup>-independent, Na<sup>+</sup>-dependent and Na<sup>+</sup>- and Cl<sup>-</sup>-dependent, and is fourfold greater at the basolateral than at the apical membrane. To determine the contribution of GLYT1 to total glycine uptake, measurements were made in the presence of excess alanine, included to block glycine uptake via neutral amino acid transporters such as SNAT2. Under these conditions total glycine transport at the basolateral membrane was reduced by approximately 60% and of the remaining activity 91% was both Na<sup>+</sup>- and Cl<sup>-</sup>-dependent. Some 74% of alanine insensitive transport was also inhibited by sarcosine, a characteristic of GLYT1 mediated transport, suggesting that overall GLYT1 accounts for 30—50% of glycine uptake at the basolateral membrane. In the post-prandial state, restricted availability of glycine in the gut lumen limits absorption across the apical membrane and glycine is acquired predominantly by the action of GLYT1 operating in the influx direction at the basolateral membrane. Nutrient deprivation (4Aa) results in stimulation of activating transcription factor 4 (ATF4) and, among other effects, enhanced transcription of the GLYT1 gene. Similarly, the build-up of unfolded protein in the endoplasmic reticulum (ER) triggers the ER stress response and, in contrast, oxidative stress caused by excess reactive oxygen species (ROS) stimulates basolateral uptake of glycine by GLYT1 in a mechanism as yet undetermined but apparently independent of transcriptional activation. Oxidative stress-enhanced influx of glycine increases intracellular glutathione concentration, reduces ROS concentration and improves cell viability.

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transporter. Basolateral GLYT1 activity accounted for ca. 70% of alanine insensitive Na<sup>+</sup>- and Cl<sup>-</sup>-dependent glycine uptake and as such was the predominant mechanism for glycine uptake into these cells. Measurements made using a GLYT2 specific inhibitor, ALX-1393, confirmed molecular data indicating the absence of this transporter in human intestinal cells.

2.2. Function   That GLYT1 activity predominates at the basolateral membrane leads to questions about its function. Uptake of dietary glycine at the apical membrane may involve GLYT1 activity, however other transporters such as the proton dependent peptide transporter PepT1 which is expressed at high levels in enterocytes and Caco-2 cells may be more significant in this process. In the post-prandial state, as a result of glycine influx, cytoplasmic glycine concentration will be high in comparison with that in the serosa and under these circumstances it is likely that GLYT1 functions as a specific exit pathway allowing transport of absorbed glycine towards the blood. That GLYT1, which has a stoichiometry of 2Na<sup>+</sup>/Cl<sup>-</sup>/glycine, is able to function in this

Fig. 1. Proposed Action and Regulation of GLYT1 in Intestinal Cells

(A) In the fed state, intestinal epithelial cells acquire glycine from the gut lumen across the apical membrane via a range of amino acid and peptide transporters including GLYT1. Some glycine is retained by the cell and the remainder exits across the basolateral membrane, including possibly by GLYT1, for transport to other organs. (B) In the fasting state, restricted availability of glycine in the gut lumen limits absorption across the apical membrane and glycine is acquired predominantly by the action of GLYT1 operating in the influx direction at the basolateral membrane. Nutrient deprivation (4Aa) results in stimulation of activating transcription factor 4 (ATF4) and, among other effects, enhanced transcription of the GLYT1 gene. Similarly, the build-up of unfolded protein in the endoplasmic reticulum (ER) triggers the ER stress response and, in contrast, oxidative stress caused by excess reactive oxygen species (ROS) stimulates basolateral uptake of glycine by GLYT1 in a mechanism as yet undetermined but apparently independent of transcriptional activation. Oxidative stress-enhanced influx of glycine increases intracellular glutathione concentration, reduces ROS concentration and improves cell viability.
dual role, mediating both glycine export and uptake given appropriate electrochemical gradients has been demonstrated in several experimental models including mouse cerebellar slices, HEK293 and Chinese hamster ovary (CHO) cells, and \textit{Xenopus laevis} oocytes. However, so far studies in intestinal cells have measured only glycine uptake and so this putative role in efflux of dietary glycine from enterocytes remains to be confirmed.

To address the question of the role of basolateral GLYT1 in glycine uptake into intestinal cells we need to consider the functions of glycine. The mammalian intestine appears to require significant amounts of glycine; in pigs around 40% of dietary glycine is retained in the intestine and associated organs. As well as an obvious role in protein synthesis, glycine is a source of energy and is used in synthesis of creatinine and glutathione. The role of glycine as a protective agent in a number of tissues is also now well established. It has been shown to protect kidney, liver, lung and heart as well as intestine against a range of insults and there is now a significant body of evidence demonstrating that these protective effects are the result of multiple modes of action. In cells that express the glycine receptor, a glycine gated chloride channel, glycine inhibits activation of the channel, culminating in the prevention of calcium influx into the cell and its consequential damaging effects. Glycine is an immunomodulatory agent, inhibits macrophage activation, reduces inflammation and inhibits apoptosis. In the intestine, glycine limits the damage caused by intestinal reperfusion injury, abdominal irradiation and can prevent or reduce the severity of experimentally induced colitis. As in other tissues, the protection appears to be at least bi-modal. Tsune et al. demonstrated that dietary glycine was able to reduce intestinal inflammation in rats when given 2 d after administration of the damaging agent trinitrobenzenesulphonic acid (TNBS), whereas the work of Lee et al. showed a necessity for glycine administration before reperfusion to prevent ischemia/reperfusion injury.

That GLYT1 may play a role in glycine-mediated cytoprotection was suggested by the work of Harding et al. who identified it as a target of a co-ordinated stress response. Working in mouse fibroblasts they described how a range of challenges, including oxidative and endoplasmic reticulum stress (ER-stress), stimulated phosphorylation of elongation initiation factor 2-alpha (eIF2\textalpha) leading to activation of activating transcription factor 4 (ATF4). Targets of ATF4 were found to include genes encoding proteins involved in the synthesis and uptake of amino acids, including those for GLYT1 and X\textsubscript{C}. Like GLYT1, X\textsubscript{C} is a highly substrate specific amino acid transporter exchanging cystine for glutamate. It plays a significant role in glutathione synthesis as the cystine it imports into cells is rapidly reduced to cysteine, one of the component amino acids, with glycine and glutamate, of the antioxidant. Knock-out of xCT, a subunit of X\textsubscript{C}, results in lowered plasma glutathione concentration. GLYT1 mRNA was up-regulated by ATF4 activated in response to amino acid limitation and ER-stress.

To investigate the potential role of GLYT1 in intestinal cell protection we established models of oxidative injury in the Caco-2 and HCT-8 cell lines. Oxidative injury was chosen for investigation as it is a significant component of intestinal inflammatory diseases such as Crohn’s disease and ulcerative colitis. The oxidising agent tert-butylhydroperoxide (t-BOOH) stimulated production of reactive oxygen species in intestinal cells, reduced intracellular glutathione concentration and led to reduced cell viability. When cells were pre-treated with glycine these effects were all ameliorated, suggesting a mechanism whereby glycine stimulated or maintained glutathione concentration, thus altering overall redox status of the cell and restricting injury. When pre-treatment regimes included the GLYT1 inhibitor ALX-5407, given concurrently with glycine, the protective effects were abolished, confirming the necessity for glycine accumulation in the cells and GLYT1 functionality. However, in this experimental model, expression of GLYT1 mRNA was not up-regulated in response to oxidative stress suggesting an alternative mechanism of activation to that identified in mouse fibroblasts by Harding et al.

### 2.3. Regulation

At this time, little is known of GLYT1 regulation in intestine. GLYT1 mRNA expression is increased, compared to orally fed-controls, in the proximal intestine of rats transferred to total parenteral nutrition, reflecting its putative role in maintaining glycine supply to enterocytes. In Caco-2 cells, it is down-regulated by protein kinase C (PKC) at both functional and molecular levels and shows a biphasic response to high glycine concentration with mRNA expression being initially up-regulated but then pressed on longer exposure. PKC regulation of GLYT1B has also been demonstrated and more thoroughly investigated in the C6 glioma and HEK293 embryonic kidney cells. Here phorbol esters, activators of PKC, decreased glycine uptake by reducing maximal transport rate but appeared not to phosphorylate any of the five predicted PKC phosphorylation sites present in GLYT1B. Subsequent investigations have shown that PKC activation accelerates ubiquitination and internalisation by endocytosis of GLYT1B through a clathrin-dependent pathway and that this requires the involvement of lysine residues, particularly lysine 619, of the transporter. As this residue is common to all GLYT1 isoforms this may be the regulatory mechanism also involved in PKC-inhibition of intestinal GLYT1 where GLYT1A is the predominant isoform.

GLYT1 function is also dependent on the delivery of the correctly folded molecule to the plasma membrane. An evolutionarily conserved C-terminal motif, R(575)L(576)(X(8))D(585), is required for trafficking GLYT1 through the endoplasmic reticulum as is glycosylation of four \(N\)-glycosylation sites located on a large extracellular loop between transmembrane domains 3 and 4 of the molecule. Also implicated in membrane targeting are di-leucine motifs present in the C-terminus of the molecule which with an unknown motif in the specific N-terminal region of GLYT1B targeted this isoform to the basolateral membrane of kidney epithelial cells when heterologously expressed. GLYT1A located to both apical and basal membranes, consistent with distribution of GLYT1 in intestinal cells where GLYT1A mRNA is the predominant transcript.

### 2.4. Therapeutic Potential

The evidence of intestinal glycine cytoprotection provided here is indicative of a preventative role for GLYT1-involving mechanisms in the management of inflammatory bowel diseases (IBD). These are generally long-term conditions characterised by periods of active inflammation and remission. Promoting the antioxi-
vant status of intestinal cells during quiescent periods may increase resistance to injury and thus prolong the interval between bouts of active disease. Although the availability of cysteine is generally seen as the rate limiting factor in glutathione synthesis, low protein diets which lead to abnormalities of blood glutathione synthesis are also associated with increased excretion of 5-oxoproline indicative of a lack of glycine rather than cysteine.51—53 This with the evidence presented here suggests that enhancing glycine uptake by GLYT1 has the potential to preferentially alter cellular redox status and thus reduce recurrence of cellular injury and inflammation.

The therapeutic effectiveness of oral glycine as a treatment for schizophrenia has been examined over the past fifteen or so years. Patients with schizophrenia exhibit lower serum glycine levels,50 glycine-serine ratio55 and reduced glutamatergic neurotransmission60 than healthy controls. It was hypothesised that oral glycine would lead to increased glycine concentration in excitable glutamatergic synapses where glycine is a full agonist and thus enhance neurotransmission.50 While data shows variable responses in the treatment of schizophrenia,57 leading to the investigation and development of specific GLYT1 inhibitors as a means of potentiating synaptic glycine concentration,58,59 patients in these trials showed increased serum glycine concentration, with no reported adverse side effects, suggesting that dietary glycine supplementation may be a means of increasing glycine availability to the intestinal epithelium via basolateral GLYT1 activity as well as by apical uptake. This may be of particular significance if, as has been argued, the source of an amino acid, whether obtained at the basal or apical membrane, dictates its use by enterocytes25 and is of relevance to the distal regions of the intestine where luminal glycine concentration is low.

3. CONCLUSION

GLYT1 functions at the basolateral membrane of enterocytes and colonicocytes to maintain glycine supply required for intestinal epithelial health. Glycine acts as a cytoprotective agent in the intestine and has been shown to be non-toxic, even at high dose, indicating a possible therapeutic or preventative role in the management of intestinal disorders. Further elucidation of the effects of glycine on GLYT1 activity and intestinal glutathione content in an in vivo model and the regulatory mechanisms involved may lead to improved management of intestinal inflammatory disease.

REFERENCES