

Oleoyl l-carnitine inhibits glycine transport by glyt2

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Glycine is an inhibitory neurotransmitter in the spinal cord and brain stem, where it activates strychnine-sensitive glycine receptors. It can also act as an excitatory neurotransmitter throughout the brain and spinal cord, where it is a co-agonist with glutamate at the N-methyl-D-aspartate (NMDA) subtype of glutamate receptor. Two subtypes of glycine transporters, GLYT1 and GLYT2, are used to regulate extracellular glycine concentrations and have the potential to modulate the dynamics of both inhibitory glycinergic and excitatory glutamatergic neurotransmission (Eulenburg *et al.*, 2005). GLYT1 is expressed in glial cells surrounding both excitatory and inhibitory synapses, whereas GLYT2 is expressed in presynaptic inhibitory glycinergic neurons (Kim *et al.*, 1994). Characterization of the different physiological roles of the two GlyT subtypes has opened the possibility of pharmacologically manipulating glycine concentrations as potential means to treat schizophrenia (GLYT1 inhibitors) (Atkinson *et al.*, 2001; Aubrey & Vandenberg, 2001) and pain (GLYT2 inhibitors) (Connor *et al.*, 2010; Sur & Kinney, 2004; Vuong *et al.*, 2008).

We have previously revealed that subtle differences in lipid-based compounds produces selective GlyT inhibitors (Pearlman *et al.*, 2003; Wiles *et al.*, 2006). The free fatty acid, arachidonic acid, selectively inhibits GLYT1, while the related acyl-amino acid, N-arachidonyl glycine, is a selective GLYT2 inhibitor. In this study we investigated the influence of a series of endogenous acylcarnitines (lauroyl L-carnitine, palmitoyl L-carnitine, oleoyl L-carnitine, N-oleoyl glycine) on GLYT1 and GLYT2. Transporters were expressed in *Xenopus laevis* oocytes and activity was monitored using two-electrode voltage clamp. All lipids tested acted as inhibitors at GLYT2. Oleoyl-L-carnitine inhibits glycine transport by GLYT2 with an IC_{50} of 340 nM, which is 15-fold more potent than the previously identified lipid inhibitor N-arachidonyl-glycine. Oleoyl-L-carnitine has a slow onset of inhibition and a slow washout. The speed of washout can be enhanced by inclusion of the lipid chelator β -cyclodextrin in the wash solution, which suggests that the site of action of oleoyl-L-carnitine is closely associated with the cell membrane. Using a series of chimeric GLYT1/2 transporters and point mutant transporters we have identified an isoleucine residue in extracellular loop 4 of GLYT2 that confers differences in oleoyl-L-carnitine sensitivity between GLYT2 and GLYT1.

Oleoyl-L-carnitine is a potent non-competitive inhibitor of GLYT2. Previously identified GLYT2 inhibitors show potential as analgesics and the identification of oleoyl-L-carnitine as a novel GLYT2 inhibitor may lead to new ways of treating pain

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