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Glycine transporter2 inhibitors: Getting the balance right

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ABSTRACT

Neurotransmitter transporters are targets for a wide range of therapeutically useful drugs. This is because they have the capacity to selectively manipulate the dynamics of neurotransmitter concentrations and thereby enhance or diminish signalling through particular brain pathways. High affinity glycine transporters (GlyTs) regulate extracellular concentrations of glycine and provide novel therapeutic targets for neurological disorders.

1. Introduction

Neurotransmitter transporters are targets for a wide range of therapeutically useful drugs. This is because they have the capacity to selectively manipulate the dynamics of neurotransmitter concentrations and thereby enhance or diminish signalling through particular brain pathways. High affinity glycine transporters (GlyTs) regulate extracellular concentrations of glycine and provide novel therapeutic targets for neurological disorders (Eulenburg et al., 2005; Vandenberg et al., 2014).

Glycine is both an inhibitory and an excitatory neurotransmitter. It activates strychnine-sensitive inhibitory glycine receptors and is a co-agonist with glutamate on the excitatory N-methyl-D-aspartate (NMDA) subtype of glutamate receptors. Thus, GlyTs have the potential to influence both inhibitory glycinergic and excitatory glutamatergic neurotransmission (Berger et al., 1998; Eulenburg et al., 2005). Two human subtypes of GlyTs have been identified, GlyT1 and GlyT2. GlyT1 is predominantly expressed in glial cells surrounding both excitatory and inhibitory synapses, whereas GlyT2 is predominantly expressed in the brainstem and spinal cord where it is associated with presynaptic inhibitory glycinergic neurons (Liu et al., 1992, 1993; Kim et al., 1994; Zafra et al., 1995a, 1995b; Kinney et al., 2003).

The GlyTs are members of the Na^+/Cl^- dependent family of neurotransmitter transporters, that also includes transporters for γ-aminobutyric acid, noradrenaline, dopamine and serotonin (Liu et al., 1992, 1993; Amara and Kuhar, 1993; Kim et al., 1994; Broer and Gether, 2012). Characterisation of the different physiological roles of the two GlyT subtypes has opened the possibility of pharmacologically manipulating glycine concentrations as a potential means to treat specific disorders. GlyT1 inhibitors are thought to provide potential treatments for schizophrenia, alcohol addiction and pain, while inhibition of GlyT2 has the potential to alleviate pain (Sur and Kinney, 2004; Eulenburg et al., 2005; Harvey and Yee, 2013; Vandenberg et al., 2014).

Inhibitory glycinergic neurons are highly abundant in the dorsal horn of the spinal cord, particularly in lamina III (Pfeiffer et al., 1984; Mitchell et al., 1993; Zeilhofer et al., 2012), and contribute to inhibition of nociceptive signalling. These neurons have important roles in segregating nociceptive and non-noxious information pathways (von Hehn et al., 2012; Zeilhofer et al., 2012) and dysfunction of glycinergic systems in the spinal cord, together with GABAergic systems (Yaksh, 1989; Sivilotti and Woolf, 1994; Sorkin and Puig, 1996; Lynch and Callister, 2006; Torsney and MacDermott, 2006; Lu et al., 2013), contribute to allodynia associated with neuropathic and inflammatory pain. In this review we will discuss the pharmacological properties of inhibitors of the GlyT2 subtype of glycine transporters and their potential use for the treatment of neuropathic and inflammatory pain. As part of this discussion we will address the potential for unwanted effects of GlyT2 inhibitors. Transient inhibition of neuronal GlyT2 can initially elevate extracellular glycine concentrations and stimulate glycinergic neurotransmission, but paradoxically, long term inhibition

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may lead to a reduced capacity to load glycine into synaptic vesicles and reduce glycinergic neurotransmission (Fig. 1). These considerations will need to be addressed if the therapeutic potential of GlyT2 inhibitors is to be realised.

2. The roles of GlyT2 in controlling inhibitory glycinerigic neurotransmission

Vesicular glycine release from presynaptic terminals leads to a rapid increase in synaptic glycine concentrations to approximately 3 mM (Beato, 2008) and activation of postsynaptic inhibitory glycine receptors. The termination of glycine neurotransmission is achieved by a combination of diffusion of glycine from the synapse and active uptake of glycine by GlyT1 into glial cells and GlyT2 into the presynaptic neurons (Eulenburg et al., 2005; Beato, 2008). Glycine transport by GlyT1 is coupled to the co-transport of 2 Na + ions and a Cl-ion, which ensures that GlyT1 reduces the extracellular glycine concentration to approximately 100 nM whilst maintaining the intracellular glial glycine concentration at approximately 2 mM. The GlyT2 transporter is a more powerful transporter being coupled to the co-transport of 3 Na + ions and a Cl-ion, which allows GlyT2 to reduce synaptic glycine concentrations further to approximately 10 nM, whilst maintaining a neuronal glycine concentration of 20 mM. It appears that presynaptic expression of GlyT2 is the critical factor for ensuring that intracellular glycine concentrations are significantly greater than GABA concentrations and sufficient for loading synaptic vesicles with glycine via the vesicular inhibitory amino acid transporter, VIAAT (Roux and Supplisson, 2000; Supplisson and Roux, 2002; Aubrey et al., 2007; Rousseau et al., 2008).

GlyT2 knockout mice have been generated and they show very marked alterations in glycine neurotransmission. The mice are normal at birth but develop spasticity, tremor and an inability to right and die towards the end of the second postnatal week (Gomeza et al., 2003). The lack of presynaptic GlyT2 prevents the accumulation of sufficient intracellular glycine for loading of synaptic vesicles. Thus, glycine neurotransmission is absent. Initially, these observations deterred the development of therapeutically useful GlyT2 inhibitors because of the potential for loss of glycine transmission with their prolonged use. However, the alternative approach of partial knock down of GlyT2 using siRNAs provides promising data that suggests that GlyT2 inhibitors have the potential to be therapeutic in the treatment of neuropathic pain. Knockdown of GlyT2 to approximately 30% of wild type levels (which may mimic partial inhibition of transport) provides analgesia in rat models of allodynia associated with neuropathic pain (Morita et al., 2008). The equilibrium glycine concentration gradient achieved by GlyT2 is determined by the ion gradients across the cell membrane, whereas the rate at which equilibrium is achieved will be determined by the number of glycine transporters (Roux and Supplisson, 2000; Supplisson and Roux, 2002). Thus, partial inhibition will not alter the equilibrium glycine concentration gradient, but will prolong the time required to reach equilibrium, allowing greater glycine receptor activity. It appears that partial inhibition of glycine transport in the siRNA knock-down experiments allows sufficient glycine uptake for loading of presynaptic glycinergic vesicles to maintain, or even prolong, glycinergic neurotransmission.

Intrathecal administration of glycine has been shown to prevent mechanical allodynia in animal models of neuropathic pain and also to reduce thermal hyperalgesia (Simpson et al., 1997; Huang and Simpson, 2000), whilst the glycine receptor antagonist strychnine has been show to induce mechanical hyperalgesia and allodynia (Yaksh, 1989). These studies demonstrate that manipulation of glycine concentrations has the potential to alleviate pain. In the following sections we will review the pharmacological properties of a range of GlyT2 inhibitors and highlight some of the key features of the various compounds that may impact of their suitability for further development as analgesics for the treatment of pain.

3. ALX1393

ALX1393 is a potent GlyT2 inhibitor with an IC_{50} of 10–25 nM and an approximate 200-fold selectivity for GlyT2 over GlyT1 (Caulfield et al., 2001; Mingorance-Le Meur et al., 2013) (Fig. 2). ALX1393 has been studied in animal models of pain using either intravenous or intrathecal injections (Xu et al., 2005; Morita et al., 2008; Haranishi et al., 2010; Nishikawa et al., 2010; Mingorance-Le Meur et al., 2013). A single intravenous injection of 0.01 mg/kg ALX1393 reduces mechanical allodynia over a 4 h period. These effects remain for up to 24 h and then return to pre-drug injection levels over the course of 4 days. Intrathecal injections of 10 ng of ALX1393 show similar reductions in mechanical allodynia associated with three separate models of chronic pain: nerve ligation injury; the streptozotocin-induced diabetic pain model; and the

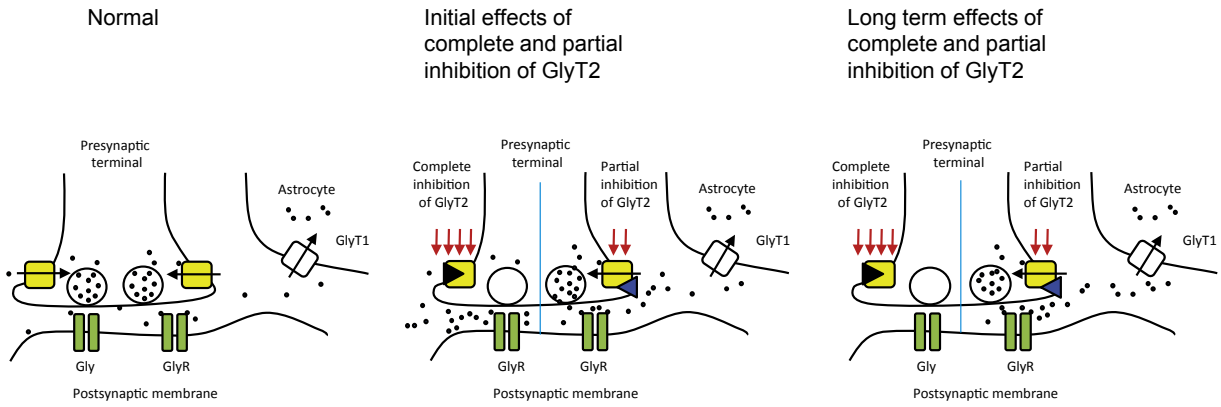

Fig. 1. Schematic diagrams of an inhibitory glycinergic synapse showing the functional consequences of complete and partial inhibition of GlyT2. Under normal conditions, Glycine is released from synaptic vesicles to activate postsynaptic glycine receptors (GlyR) and neurotransmission is terminated by presynaptic glycine reuptake by GlyT2 (yellow rectangle) and also uptake via GlyT1 expressed on surrounding astrocytes. In the initial phase, complete (left hand side; black triangle) or partial (right hand side; blue triangle) inhibition of GlyT2 will elevate the synaptic glycine concentration and enhance GlyR activity. After prolonged complete inhibition of GlyT2 (black triangle), the cytosolic presynaptic glycine concentration will drop such that synaptic vesicle loading will cease preventing subsequent glycine neurotransmission. In contrast, partial prolonged inhibition (blue triangle) will slow the clearance of glycine in the synapse, but will not change the equilibrium presynaptic cytosolic glycine concentration and thus allow subsequent glycine neurotransmission.