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Lipid inhibitors of high affinity glycine transporters: Identification of a novel class of analgesics

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ABSTRACT

Glycine plays a key role in regulating inhibitory neurotransmission in the spinal cord and concentrations of glycine in the CNS are regulated by two subtypes of high affinity glycine transporters, GlyT1 and GlyT2. In this mini review we will discuss a series of lipid inhibitors of GlyT2 that show promise as analgesics in the treatment of neuropathic and inflammatory pain. N-arachidonyl-glycine inhibits the rate of transport by GlyT2, but has very little or no activity on GlyT1. We will discuss structure–activity studies of the actions of related lipids on GlyT2 and also the characterization of a more potent lipid inhibitor of GlyT2, oleoyl-L-carnitine. Both N-arachidonyl-glycine and oleoyl-L-carnitine show specificity for GlyT2 over GlyT1, which has allowed the use of chimeric GlyT1/GlyT2 transporters to begin characterizing the molecular basis for specificity and mechanism of action of these lipid inhibitors. Although our understanding of the molecular basis for lipid inhibition is still in its infancy, it appears that extracellular loop 4 of GlyT2 plays an important role in the inhibitory mechanism.

1. Introduction

Neurotransmitter transporters are the targets for a variety of therapeutic drugs, including antidepressants, anticonvulsants and also drugs of abuse, such as cocaine and methamphetamine. These membrane bound proteins are particularly good drug targets because they have the capacity to selectively manipulate neurotransmitter concentrations and thereby enhance or diminish signalling through particular brain pathways. High affinity glycine transporters (GlyTs) regulate extracellular concentrations of glycine and provide a novel therapeutic target for neurological disorders and pain management. However, there are currently no drugs on the market that selectively modulate GlyTs.

Glycine can act as both an inhibitory and an excitatory neurotransmitter. It activates strychnine-sensitive glycine receptors and is a co-agonist with glutamate on the N-methyl-D-aspartate (NMDA) subtype of glutamate receptors. Thus, GlyTs have the potential to influence both inhibitory glycinergic and excitatory glutamatergic neurotransmission (Bergeron et al., 1998; Eulenburg et al., 2005). The GlyTs are members of the Na^+/Cl^- dependent family of neurotransmitter transporters, which also include transporters for γ-aminobutyric acid, noradrenaline, dopamine and serotonin (Kim et al., 1994; Liu et al., 1992, 1993). Mammalian members of this family share 20–25% amino acid sequence identity with the prokaryotic transporter $LeuT_{Aa}$, the crystal structure of which serves as a useful template for unravelling the functional implications of transporter structures (Yamashita et al., 2005). Two human subtypes of GlyTs have been cloned, 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 associated with presynaptic inhibitory glycinergic neurons (Kim et al., 1994; Liu et al., 1992, 1993; Zafra et al., 1995a,b).

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. For example, GlyT1 inhibitors are thought to provide potential treatments for schizophrenia, while inhibition of GlyT2 is associated with alleviation of pain (Sur and Kinney, 2004; Kinney et al., 2003; Eulenburg et al., 2005). In this review we will focus on the ability of a series of endogenous lipids that modulate GlyT function (Fig. 1). The knowledge gained from these studies providing novel avenues to not only expand our knowledge of transporter physiology, but also providing potential novel therapeutics.

2. N-arachidonyl Acid – an endogenous analgesic?

Fatty acids are abundant throughout native mammalian tissues, where they are known to play important roles as energy sources and signalling molecules. More recently, interest has been developing into understanding the potential of these molecules to interact with, and modulate the function of, transmembrane proteins. At

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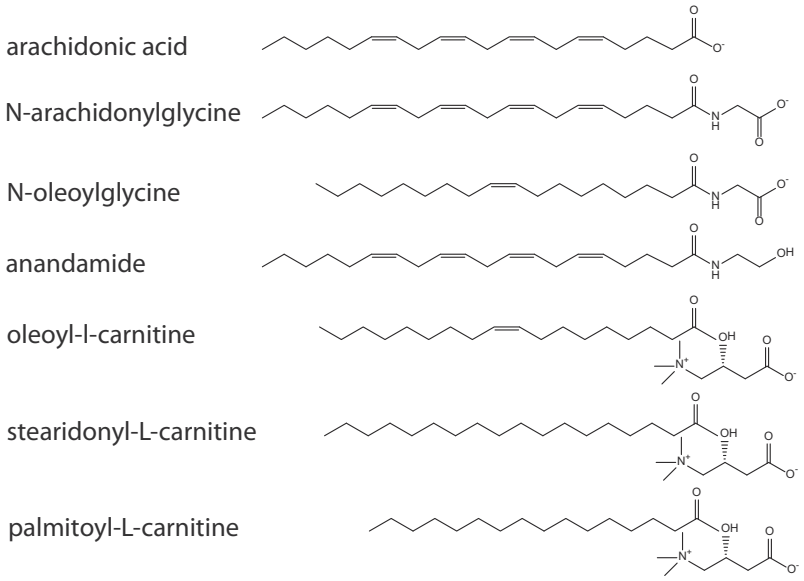

Fig. 1. Chemical structures of lipids that modulate GlyT function: arachidonic acid, N-arachidonyl-glycine, N-oleoyl-glycine, anandamide, oleoyl-L-carnitine, palmitoyl-L-carnitine and stearidonyl-L-carnitine.

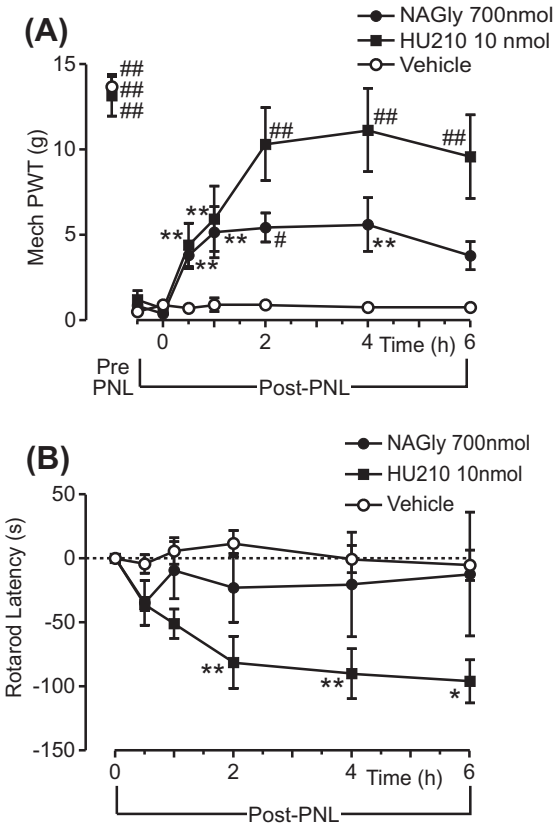

Fig. 2. Analgesic effects of NAGly. (A) NAGly reduces mechanical allodynia. Animals received an injection of 700 nmol of NAGly or 30 nmol of HU210 14 days after partial nerve ligation and then responses to stimulation by von frey hairs recorded over a 6 h period. HU210 is more effective as an analgesic than NAGly. (B) In contrast to HU210, NAGly does not affect motor function. After the same drug treatment as in A, animals were tested for their ability to remain on the rotorod. Figure adapted from Figs. 1 and 3 of Vuong et al. (2008).

the simplest level, fatty acids consist of a hydrocarbon tail attached to a hydrophilic head group, with the carboxylic acid being the simplest head group. The hydrophobic nature of the tail group lends itself to interactions with cell membranes, while the head group has the potential to provide for polar interactions with, for example, transmembrane proteins.

The endogenous cannabinoids, arachidonyl ethanolamide (anandamide) and 2-arachidonyl glycerol, have been studied for their ability to relieve pain. These compounds act as agonists at the cannabinoid receptors, CB1 and CB2, alleviating inflammatory pain by reducing allodynia and hyperalgesia (Hohmann and Suplita, 2006; Rice et al., 2002). However, non-selective cannabinoid agonists also produce unwanted motor and psychotropic effects *via* their activity at CB1 receptors (Herzberg et al., 1997; Fox et al., 2001; Scott et al., 2004) (Fig. 2). Structure–activity studies of the CB1 receptor provoked interested in the arachidonyl amino acids, including N-arachidonyl glycine (NAGly) (Connor et al., 2010).

NAGly was first synthesized as a structural analogue of anandamide (Sheskin et al., 1997). It was subsequently identified in mammals (Huang et al., 2001), with the highest levels found in the spinal cord and small intestine (Huang et al., 2001). In animal models of formalin-induced pain co-injection of NAGly with the formalin suppressed the tonic pain phase (Huang et al., 2001). These findings were complemented by studies demonstrating that intrathecal injection of NAGly reduced both mechanical allodynia and thermal hyperalgesia in a model of inflammatory pain (Succar et al., 2007) and reduced mechanical allodynia in a nerve-injury induced animal model of neuropathic pain (Vuong et al., 2008) (Fig. 2). The CB1 receptor agonist, HU-210, also alleviates pain, but in contrast to NAGly, the actions of HU-210 are blocked by CB1 and CB2 receptor antagonists (Succar et al., 2007; Vuong et al., 2008). Thus, the analgesic actions of NAGly are not mediated by cannabinoid receptors. The dose dependence of NAGly activity and the lack of effect by the degradation products, glycine and arachidonic acid, imply that the analgesic activity of NAGly is mediated by modulation of a specific protein.

A site of action of many analgesic agents within the spinal cord is the superficial dorsal horn. In an attempt to elucidate the mechanism of action of NAGly at a cellular level, the effect of NAGly on synaptic neurotransmission in the superficial dorsal horn was assessed (Jeong et al., 2010). Brief exogenous application of glycine to dorsal horn slices generates strychnine-sensitive inward currents that decay due to glycine uptake by glycine transporters. NAGly prolongs the time course of the glycine-evoked currents,

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