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Effect of VGLUT inhibitors on glutamatergic synaptic transmission in the rodent hippocampus and prefrontal cortex

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ABSTRACT

Vesicular glutamate transporters (VGLUTs) are known to be important in the uptake of glutamate into vesicles in the presynaptic terminal; thereby playing a role in synaptic function. VGLUT dysfunction has also been suggested in neurological and psychiatric disorders such as epilepsy and schizophrenia. A number of compounds have been identified as VGLUT inhibitors; however, little is known as to how these compounds affect synaptic transmission. We therefore investigated the effects of structurally unrelated VGLUT inhibitors on synaptic transmission in the rodent hippocampus and prefrontal cortex.

In the CA1 and dentate gyrus regions of the *in vitro* slice preparation of mouse hippocampus, AMPA receptor-mediated field excitatory postsynaptic potentials (fEPSPs) were evoked in response to Schaffer collateral/commissural pathway stimulation. Application of the VGLUT inhibitors Rose Bengal (RB), Congo Red (CR) or Chicago Sky Blue 6B (CB) resulted in a concentration-related reduction of fEPSP amplitudes. RB (30 μM) or CB (300 μM) also depressed NMDA receptor-mediated responses in the CA1 region. The naturally occurring kynurenine Xanthurenic Acid (XA) is reported to be a VGLUT inhibitor. We found XA attenuated both AMPA and NMDA receptor-mediated synaptic transmission. The potency order of the VGLUT inhibitors was consistent with literature K_i values for VGLUT inhibition.

Impaired glutamatergic neurotransmission is believed to contribute to schizophrenia, and VGLUTs have also been implicated in this disease. We therefore investigated the effect of VGLUT inhibition in the prefrontal cortex. Application of the VGLUT inhibitors RB or CB resulted in a concentration-dependent reduction in the amplitude of glutamate receptor-mediated fEPSPs recorded in layer V/VI in response to stimulation in the forceps minor.

We conclude that VGLUT inhibitors can modulate glutamatergic synaptic transmission in the PFC and hippocampus. This could be important in the pathophysiology of nervous system disorders and might represent a target for developing novel pharmacological therapies.

1. Introduction

Glutamate is the principal excitatory neurotransmitter in the central nervous system (CNS) and the mechanisms underlying glutamatergic neurotransmission are of considerable interest for our understanding of normal synaptic function and pathophysiology. Glutamate is stored in vesicles located in the presynaptic terminal and is released into the synaptic cleft following fusion of a vesicle with the cell membrane (Sudhof, 1995; Takamori, 2006). In the presynaptic terminal, the principal role of the vesicular glutamate transporters (VGLUTs) is to load glutamate into these vesicles via a proton-dependent electrochemical gradient (Takamori, 2006; Thompson et al., 2005). In addition, VGLUTs may also affect synaptic vesicle clustering and mobility in the presynaptic terminal (Fremeau et al., 2004; Shigeri et al., 2004; Siksou et al., 2013; Takamori, 2006).

Three isoforms of VGLUT have been identified and these belong to the type I phosphate transporter (SLC17) family. VGLUT1 was first cloned in 1994 (Ni et al., 1994) and was initially identified as brain-specific Na^+-dependent inorganic phosphate transporter 1 (BNP1), but was renamed due to expression on the vesicle membrane and recognition of its ability to transport glutamate (Bellocchio et al., 2000; Takamori et al., 2000; Takamori, 2006). The two other isoforms, VGLUT2 and VGLUT3, were subsequently identified in the early 2000s (see (Takamori, 2006 and references therein). Consistent with a role in glutamatergic synaptic transmission, VGLUT 1 and 2 are expressed in glutamatergic terminals, with little overlap in expression (Fremeau et al., 2001, 2004; Fujiyama et al., 2001), although in some instances they may be co-expressed (Herzog et al., 2006). VGLUT3 may also have a role in glutamatergic

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transmission (Higley et al., 2011), but expression in neurones expressing other neurotransmitters, extrasynaptic expression and expression in peripheral tissues, such as the liver, suggests additional roles (Shigeri et al., 2004; Takamori, 2006).

VGLUTs play a role in maintaining normal synaptic function as demonstrated in studies in mice where transporters are either artificially expressed or are knocked out. Introduction of VGLUT1 into GABA-expressing neurones results in the cells producing glutamatergic autapses (Takamori et al., 2000), and in mice lacking VGLUT there are impairments of synaptic transmission, sensory processing, coordination and learning and memory (Balschun et al., 2010; Fremeau et al., 2004; Moechars et al., 2006; Seal et al., 2008, 2009; Smear et al., 2007; Wojcik et al., 2004). In addition to a role in normal synaptic activity, there is also evidence that modulation of VGLUT function or expression may play a role in neurological and psychiatric diseases including: epilepsy (Juge et al., 2010; Schallier et al., 2009), pain (Moechars et al., 2006; Seal et al., 2009) and schizophrenia (Eastwood and Harrison, 2005; Varea et al., 2012; Uezato et al., 2009; Oni-Orisan et al., 2008).

A number of compounds have been identified as VGLUT inhibitors (Pietrancosta et al., 2010; Shigeri et al., 2004; Thompson et al., 2005), and there is some evidence that these may modulate neuronal function *in vivo* (He et al., 2013). It is, however, perhaps surprising that there is little data regarding the action of these compounds on synaptic transmission. Recently, we demonstrated an effect of VGLUT inhibition on synaptic transmission in the mouse DG (Neale et al., 2013). In the current study we expand on those studies and test the action of structurally unrelated VGLUT inhibitors, Rose Bengal, Congo Red or Chicago Sky Blue 6B (Fig. 1) and the naturally occurring kynurenine and VGLUT inhibitor XA (Bartlett et al., 1998; Carrigan et al., 2002; Schwarcz et al., 2012), on glutamate receptor-mediated synaptic transmission in the prefrontal cortex and hippocampal CA1 and DG regions of the mouse.

2. Methods

2.1. Slice preparation

Adult (>4 weeks) female C57Bl6/J mice (Harlan, UK) were killed by decapitation and the brain was removed and placed into ice-cold oxygenated sucrose Krebs' medium containing (mM): sucrose 202, KCl 2, KH_2PO_4 1.25, $MgSO_4$ 10, $CaCl_2$ 0.5, $NaHCO_3$ 26, glucose 10. To record from the prefrontal cortex, 400 μm coronal slices were prepared, and for hippocampal recording 300 μm parasagittal slices were prepared with an oscillating microtome (Integraslice; Campden Instruments Ltd., Loughborough, UK). Slices were then transferred to a recovery chamber at room temperature containing oxygenated Krebs' solution (mM): NaCl 124, KCl 2, KH_2PO_4 1.25, $MgSO_4$ 1, $CaCl_2$ 2, $NaHCO_3$ 26, glucose 10.

2.2. Data acquisition and analysis

Following at least 1 h of recovery, individual slices were transferred to an interface recording chamber where they were perfused (flow rate of 0.6 ml min^{-1}) with Krebs' solution (36 °C). Extracellular field potential recordings were made with an Axoprobe 1A amplifier (Axon Instruments Ltd. USA) via a Krebs'-filled glass micropipette (resistance 3–7 MΩ), digitized (5 kHz) via a CED1401 interface and stored on a computer with Spike2 software (Cambridge Electronic Design Ltd., Cambridge, UK). To record

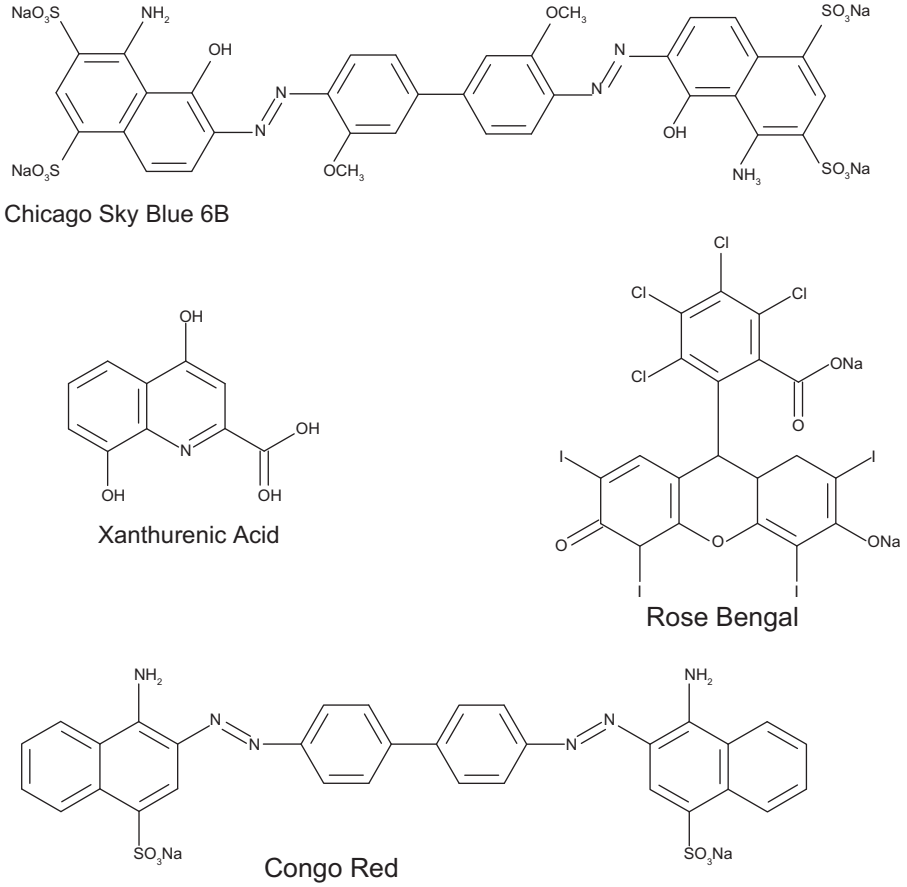

Fig. 1. Structure of the VGLUT inhibitors Rose Bengal, Chicago Sky Blue 6B, Congo Red and Xanthurenic Acid.

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