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Function of mGlu1 receptors in the modulation of nociceptive processing in the thalamus

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ABSTRACT

As postsynaptic metabotropic subtype 1 (mGlu1) receptors are present in the thalamus, we have investigated the effect of potentiating and antagonising mGlu1 receptors on responses of thalamic neurones to noxious sensory stimulation. Extracellular recordings were made *in vivo* with multi-barrel iontophoretic electrodes from single neurones in the thalamus of urethane-anaesthetised rats. Responses to iontophoretic applications of the Group I mGlu agonist 3,5-dihydroxy-phenylglycine (DHPG) were selectively potentiated by co-application of the mGlu1 positive allosteric modulator Ro67-4853, whereas they were selectively reduced upon co-application of the mGlu1 receptor orthosteric antagonist LY367385. This indicates that thalamic DHPG responses are mediated primarily via mGlu1 receptors, consistent with the high postsynaptic levels of this receptor in the thalamus. Furthermore, potentiation of DHPG responses by Ro67-4853 were greater when the initial DHPG response was of a low magnitude. Ro67-4853 also potentiated responses of thalamic neurones to noxious thermal stimulation, whilst having little effect on the baseline activity of nociceptive neurones. By contrast, nociceptive responses were reduced by LY367385. In a further series of experiments we found that inactivation of somatosensory cortex by cooling resulted in a reduction of thalamic nociceptive responses. These results underline the importance of mGlu1 receptors in the processing of sensory information in the thalamus, particularly with respect to nociceptive responses. Furthermore, the involvement of mGlu1 receptors may reflect the activity of descending cortico-thalamic afferents.

1. Introduction

The neurotransmitter pharmacology of nociceptive processing is of great importance in our understanding of pain mechanisms and therapy. Understanding of the neurotransmitters and receptors involved in nociception is extensive at the level of the peripheral nervous system and the spinal cord (Lesage, 2004; Millan, 1999). However, at higher levels of the neuraxis, our knowledge is less comprehensive. The ventrobasal thalamus (VB) is a pivotal processing point for the integration of somatosensory information ascending from the spinal cord with a prominent descending cortico-thalamic input from Layer 6 of the corresponding somatosensory cortex (Sherman, 2012). Both of these pathways use glutamate as their excitatory transmitter. Previous work from this laboratory and others has shown that responses of thalamic neurones to noxious peripheral stimuli are largely mediated by glutamate receptors, in particular the ionotropic NMDA receptor (Bordi and Quartaroli, 2000; Eaton and Salt, 1990; Kolhekar et al., 1997) and the metabotropic glutamate (mGlu) receptors, mGlu1 and mGlu5 (Eaton et al., 1993; Salt and Binns, 2000; Salt and Turner, 1998).

There are eight mGlu receptor subtypes (mGlu1-mGlu8) that can be placed into three groups (Group I, II, III) based on sequence homology, intracellular transduction cascade, and agonist/antagonist pharmacology (Niswender and Conn, 2010). Group I receptors (mGlu1, mGlu5) are often (but not exclusively) localised postsynaptically where they may couple to inositol phosphate metabolism and enhance post-synaptic excitability via changes in K^+ conductances and/or modulation of ionotropic glutamate receptors (Niswender and Conn, 2010). In addition to selective agonists and antagonists, a novel class of pharmacological agents acting at mGlu receptors has more recently become available, the positive allosteric modulators (PAMs) (Nicoletti et al., 2011). PAMs act at sites on

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the receptor distinct from the orthosteric ligand (glutamate) binding site and enhance the activity of receptors in response to orthosteric agonists (Nicoletti et al., 2011). This has advantages in that PAMs can be highly selective for single mGlu receptor subtypes and can function to potentiate the physiological activation of receptors by endogenous glutamate. Both of these properties are useful for the investigation of physiological processes. We have shown that one such PAM, Ro67-4853 (Knoflach et al., 2001), can be used in *in vivo* experiments to potentiate responses mediated via mGlu1 receptors in an activity-dependent manner (Salt et al., 2012). Thus, Ro67-4853 is an appropriate tool to investigate possible mGlu1 involvement in thalamic nociceptive processing.

The present study aimed to investigate the involvement and function of mGlu1 receptors in nociceptive processing in the thalamus. We have achieved this by potentiating mGlu1 receptor mediated responses with the selective PAM Ro67-4853 and by reducing mGlu1 receptor activation using the selective antagonist LY367385 (Clark et al., 1997) whilst recording nociceptive responses of thalamic neurones. Furthermore, given the association of mGlu1 receptors with cortico-thalamic pathways (Godwin et al., 1996; Martin et al., 1992; Vidnyanszky et al., 1996), we investigated the effect of somatosensory cortex inactivation on nociceptive responses of thalamic neurones. We show that it is possible to substantially change nociceptive responses of thalamic neurones by increasing or decreasing the degree of mGlu1 receptor activation and that nociceptive responses of thalamic neurones are dependent upon a functional cortico-thalamic projection. This is important in understanding sensory processing and the design of novel analgesic therapies, and underlines the critical role of mGlu1 receptors in sensory processing in the thalamus.

2. Methods

Experiments were carried out in male adult Wistar rats (270–400 g) anaesthetised with urethane (1.2 g/kg, I.P.), as detailed previously (Salt, 1987; Salt and Binns, 2000; Salt et al., 2012). Animals were purchased from Harlan (UK) and were housed on a 12 h light/dark cycle with unlimited access to food and water. All procedures were subject to local ethical committee review, were approved by the Home Office (UK) and were in accordance with the Animals (Scientific Procedures) Act 1986. Electroencephalogram and electrocardiogram were monitored throughout and anaesthesia was maintained by additional I.P. administration of urethane as required. An approximately 5 mm-square unilateral craniotomy centred over the thalamus (3 mm lateral to the midline, 5 mm rostral to the inter-aural line (Paxinos and Watson, 1988)) was made and the dura resected to expose the surface of the cortex. In addition to overlying the thalamus, this area of cortex contains the hindlimb and trunk/tail representation of the S1 somatosensory cortex (Chapin and Lin, 1984). In some experiments the craniotomy was surrounded by a small open chamber cemented to the skull that could be filled with either mineral oil or physiological saline; in the remaining experiments the surface of the brain was protected by agar (2% in physiological saline).

Recording electrodes were stereotaxically lowered into the thalamus using a stepping microdrive. Extracellular recordings were made from single neurones in the VB and immediately dorsal thalamus using either tungsten-in-glass electrodes or, for pharmacological experiments, the central barrel of seven-barrel glass iontophoretic electrodes. Single neurone action potential spikes were gated using a hardware spike-discriminator whose output pulses were timed and recorded by a CED1401 interface and computer system with Spike2 software. The amplitude and shape of the gated action potentials were monitored throughout the recording session. Neurones were identified on the basis of their stereotaxic location (AP +5.0 mm from *lambda*, Lateral 2.9 mm from midline, Depth 4.6–5.2 mm from surface) and their responses to somatosensory (nociceptive and non-nociceptive) stimuli, as described previously (Guilbaud et al., 1980; Peschanski et al., 1980, 1983; Salt and Binns, 2000). Nociceptive responses were evoked by immersion of part of either the contralateral hindpaw or the tail in water of 52C for 20 s. Responses to such stimuli were typically increases in action potential firing during the course of the stimulus and outlasting the stimulus by up to 2 min, as described previously (Peschanski et al., 1980). Similar response profiles were observed irrespective of the recording electrode type or the type of craniotomy preparation. Noxious stimuli were repeated at regular 5-min intervals in experiments where modulation of nociceptive responses was investigated (see below).

For pharmacological experiments, substances under investigation were applied in the recording location from the six outer barrels of the electrode using the iontophoretic technique (Stone, 1985) with a Neurophore BH2 system. Each of the outer barrels contained a selection from one of the following substances: NMDA (N-methyl-D-aspartate, 50 mM, pH 8.0 in 150 mM NaCl); AMPA (S-*alpha*-Amino-3-hydroxy-5-methyl-4-isoxazolepropionate, 50 mM, pH 8.0 in 150 mM NaCl); LY367385 (100 mM in water, pH 8.0); DHPG (S-3,5-dihydroxy-phenylglycine, 50 mM in 150 mM NaCl, pH 3.5); Ro67-4853 (2 mM in 10% DMSO in 150 mM NaCl, pH 7.5), vehicle control (10% DMSO in 150 mM NaCl, pH 7.5), Pontamine Sky Blue dye (2% in 1 M NaCl), 1 M NaCl. All pharmacological agents were obtained from Tocris Bioscience (UK), apart from Ro67-4853, which was a gift from Roche Pharmaceuticals (Switzerland). DHPG was ejected as a cation, all other substances were ejected as anions. Agents were prevented from diffusing from the iontophoresis barrel by applying a retaining current (5–15 nA) of opposite polarity to the ejection current. Automatic current balancing was routinely performed through the 1 M NaCl-containing barrel. In experiments where the effects of either the PAM or antagonist on agonist responses were evaluated, regular repeated cycles of agonist ejections were set up and initiated by the computer system which also produced online peristimulus-histograms (PSTHs) of single-neurone activity. Agonist ejection parameters were adjusted so as to produce sub-maximal responses. The effects of the PAM or antagonist on agonist effects were assessed by continuous concurrent iontophoretic application of these agents during several cycles of agonist ejection. Thus, although it is not possible to give absolute tissue concentrations of agents, we are confident that the doses used are within the range of producing physiologically relevant and pharmacologically selective effects. In experiments where the effects of either the PAM or antagonist on nociceptive responses were investigated, these agents were ejected with similar iontophoretic parameters to those found to be effective on responses to agonists.

In experiments designed to investigate the influence of cortical activity on thalamic responses, we inactivated the S1 somatosensory cortex by cooling (Clemo and Stein, 1986; Diamond et al., 1992). After responses to noxious stimuli of a neurone were established, the warm physiological saline in the well overlying the cortex was gently aspirated and replaced with chilled (4C) saline every 2–3 min for up to 10 min and the effect on the nociceptive responses noted. Finally, the saline was replaced with warm saline and nociceptive responses were further recorded. In separate experiments, a miniature thermocouple was inserted into the cortex to a depth of 1 mm and it was found that the cooling procedure reduced the temperature at this point to 15C. When the thermocouple was moved down into the body of the thalamus, no change in temperature could be detected when the cortical cooling procedure was performed.

Responses to agonists or noxious stimuli were quantified as the number of action potentials evoked by agonist ejection or stimulus, from which PSTHs were plotted. The effects of the PAM, the antagonist, or cortical inactivation on these responses were assessed by calculating the agonist or stimulus response during these experimental manipulations as a percentage of the response under control conditions. In the case of nociceptive responses, distinct 'initial' and 'maintained' response components were computed: the initial component was the number of action potential spikes evoked during the nociceptive stimulus whereas the maintained response was the number of action potential spikes occurring during the minute immediately after the stimulus. Data from individual neurones were used to compute mean values of effects (±s.e.m.). Statistical comparisons of these values under control conditions and during experimental manipulations were made using the Wilcoxon Signed Rank test. Results were deemed to be significant when $P < 0.05$.

3. Results

Recordings were made from 25 neurones that were characterised as nociceptive thalamic neurones on the basis of their stereotaxic location and responses to noxious stimuli directed at the contralateral limbs and tail as described previously (Guilbaud et al., 1980; Peschanski et al., 1980). Typically these neurones were located above or lateral to the vibrissal representation in the VB complex, had low spontaneous firing rates (0.05–2.4 spikes per second) and responded to noxious stimuli with a graded increase in firing rate that outlasted the stimulus, as described previously (Eaton and Salt, 1990; Guilbaud et al., 1980; Peschanski et al., 1980; Salt and Binns, 2000).

Iontophoretic application of the Group I agonist DHPG (20–80 nA, 10–20 s) caused increases in action potential firing rate of the thalamic neurones, as previously described (Salt and Binns, 2000; Salt et al., 2012). Previous work from this laboratory has indicated that this excitatory response to DHPG is mediated predominantly via mGlu1 receptors (Salt and Binns, 2000). In order to evaluate the ability of Ro67-4853 to potentiate mGlu1 receptor mediated responses of nociceptive thalamic neurones, we co-applied this PAM (50–150 nA) during regular ejections of DHPG. We found that this potentiated the DHPG responses to 282 ± 87%