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Postsynaptic mGluR mediated excitation of neurons in midbrain periaqueductal grey

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ABSTRACT

Metabotropic glutamate (mGlu) receptors modulate pain from within the midbrain periaqueductal grey (PAG). In the present study, the postsynaptic mGlu receptor mediated effects on rat PAG neurons were examined using whole-cell patch-clamp recordings in brain slices. The selective group I agonist DHPG (10 μM) produced an inward current in all PAG neurons tested which was associated with a near parallel shift in the current–voltage relationship. By contrast, the group II and III mGlu receptor agonists DCG-IV (1 μM) and L-AP4 (3 μM) produced an outward current in only 10–20% of PAG neurons tested. The DHPG induced current was concentration dependent (EC_{50} = 1.4 μM), was reduced by the mGlu1 antagonist CPCCOEt (100 μM), and was further reduced by CPCCOEt in combination with the mGlu5 antagonist MPEP (10 μM). The glutamate transport blocker TBOA (30 μM) also produced an inward current, however, this was largely abolished by CNQX (10 μM) plus AP5 (25 μM). Slow EPSCs were evoked following train, but not single shock stimulation, which were enhanced by TBOA (30 μM). The TBOA enhancement of slow EPSCs was abolished by MPEP plus CPCCOEt. These findings indicate that endogenously released glutamate, under conditions in which neurotransmitter spill-over is enhanced, activates group I mGlu receptors to produce excitatory currents within PAG. Thus, postsynaptic group I mGlu receptors have the potential to directly modulate the analgesic, behavioural and autonomic functions of the PAG.

This article is part of a Special Issue entitled 'Metabotropic Glutamate Receptors'.

1. Introduction

Metabotropic glutamate G-protein-coupled (mGlu) receptors exert a wide range of cellular actions within the central nervous system (Anwyl, 1999). Based on their sequence homology and biochemical and pharmacological profiles, mGlu receptors have been classified into three main subtypes, namely group I (mGlu1 and 5), group II (mGlu2 and 3) and group III (mGlu4, 6, 7 and 8) receptors (Pin and Duvoisin, 1995). There is strong behavioural and electrophysiological evidence that mGlu receptors are involved in nociceptive processing, although most of these studies have focussed on actions at the level of the peripheral nociceptor and the spinal cord (Neugebauer, 2002). mGlu receptors are also present in a number brain regions involved in the modulation of nociceptive information, including the amygdala and the midbrain periaqueductal grey (PAG) (Ohishi et al., 1995; Shigemoto et al., 1992; Tamaru et al., 2001).

The PAG plays a pivotal role in integrating an animal's somatomotor, autonomic and behavioural responses to threat, stress and pain, and is a major site of the analgesic actions of opioids and cannabinoids (Fields et al., 2006; Keay and Bandler, 2001). Microinjection of mGlu receptor agonists into brain regions such as the PAG can be either antinociceptive, or pronociceptive, depending upon the mGlu receptor subtype and pain model used (Kim et al., 2002; Maione et al., 1998, 2000; Marabese et al., 2007b). This analgesia could be mediated via distinct pre- and postsynaptic mechanisms. We have previously shown that, like opioids and cannabinoids, activation of presynaptic $G_{i/o}$-coupled group II and III mGlu receptors directly inhibits GABAergic synaptic transmission within the PAG (Drew and Vaughan, 2004). In addition, activation of postsynaptic G_q-coupled group I mGlu receptors inhibits GABAergic synaptic transmission indirectly through a process of retrograde endocannabinoid signalling and presynaptic cannabinoid CB1 receptors (Drew et al., 2008; Drew and Vaughan, 2004). The postsynaptic effects of group I, II and III mGlu receptor activation on rat PAG neurons *in vitro* are unknown and were the subject of the present study.

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2. Materials and methods

2.1. Animals and slice preparation

Experiments were carried out on male and female Sprague-Dawley rats (15–24 days old), following the guidelines of the National Health and Medical Research Council 'Australian code of practice for the care and use of animals for scientific purposes' and with the approval of the Royal North Shore Hospital Animal Care and Ethics Committee. Animals were deeply anaesthetized with isoflurane, decapitated and coronal midbrain slices (300 μm) containing PAG were cut using a vibratome (VT1000S, Leica Microsystems, Nussloch, Germany) in ice-cold artificial cerebrospinal fluid (ACSF), of the following composition: (in mM): NaCl 126, KCl 2.5, NaH_2PO_4 1.4, $MgCl_2$ 1.2, $CaCl_2$ 2.4, glucose 11, $NaHCO_3$ 25, as described previously (Drew et al., 2005). The slices were maintained at 34 °C in a submerged chamber containing ACSF equilibrated with 95% O_2 and 5% CO_2. Individual slices were then transferred to a chamber and superfused continuously (1.8 ml min^{-1}) with ACSF at 34 °C.

2.2. Drug solutions

DL-2-Amino-5-phosphonovaleric acid (AP5), (±)-baclofen, 7-(Hydroxyimino)-cyclopropa[b]chromen-1a-carboxylate ethyl ester (CPCCOEt), (2S,3S,4R)-3-(Carboxymethyl)-4-isopropylpyrrolidine-2-carboxylic acid (dihydrokainic acid, DHK), (RS)-3,5-dihydroxyphenylglycine (DHPG), picrotoxin and strychnine hydrochloride were obtained from Sigma (Sydney, Australia); L-(+)-2-amino-4-phosphonobutyric acid (L-AP4), (2S)-3-[[(1S)-1-(3,4-Dichlorophenyl)ethyl]amino-2-hydroxypropyl](phenylmethyl)phosphinic acid hydrochloride (CGP55845), 3-[2-[4-(4-Fluorobenzoyl)-1-piperidinyl]ethyl]-2,4[1H,3H]-quinazolinedione tartrate (ketanserin), (2S,2′R,3′R)-2-(2′,3′-dicarboxycyclopropyl)glycine (DCG-IV), and DL-threo-β-benzyloxyaspartic acid (TBOA) from Tocris Cookson (Bristol, UK); 6-Cyano-7-nitroquinoxaline-2,3-dione disodium (CNQX), 2-Methyl-6-(phenylethynyl)pyridine hydrochloride (MPEP), SR95531, (S)-N-tert-Butyl-3-(4-(2-methoxyphenyl)-piperazin-1-yl)-2-phenylpropanamide dihydrochloride (WAY100135) and tetrodotoxin (TTX) from Abcam Biochemicals (Cambridge, U.K.). Stock solutions of all drugs were made in distilled water, except MPEP and TBOA which were made in dimethylsulfoxide. All agents were diluted to working concentrations in ACSF (solvent ≤ 0.03% v/v) immediately before use and applied by superfusion.

2.3. Electrophysiology and analysis

PAG neurons were visualized using infrared Dodt contrast gradient optics on an upright microscope (BX51; Olympus, Tokyo, Japan). Whole-cell voltage-clamp recordings at −60 mV (liquid junction potential corrected) were made using an Axopatch 200B (Molecular Devices, Sunnyvale, USA) with an internal solution comprising (mM): K-gluconate 95, KCl 30, NaCl 15, $MgCl_2$ 2, HEPES 10, EGTA 11, MgATP 2, NaGTP 0.3 and QX-314 3; with pH of 7.3 and osmolality of 280–285 mosmol l^{-1}. Series resistance (<25 MΩ) was compensated by 80% and continuously monitored during experiments. In some experiments EPSCs (excitatory postsynaptic currents) were electrically evoked via a unipolar glass electrode (tip diameter 5–20 μm) placed 50–200 μm from the recording electrode (10–55 V, 100–200 μs). Single and train stimuli (2–20 per train, rate = 100 s^{-1}) were delivered every 12 s.

Recordings of postsynaptic currents and evoked EPSCs were filtered (0.5, 2 kHz low-pass filter) and sampled (1, 10 kHz) for analysis (Axograph X, Axograph Scientific Software, Sydney, Australia). Neurons were considered to respond to an agonist if it produced a current of greater than 5 pA (approximately 1 standard deviation of the noise level) and reversed upon washout. Agonist induced currents were measured as the difference between the peak current during agonist application compared to that immediately prior to application. The total charge transfer of evoked EPSCs was measured as the area of the EPSC relative to the baseline over a 10 ms period prior to stimulation. The time constant of the evoked EPSC decay phase was fit to an exponential using the least square method based. The EPSCs had mixed decay phase kinetics, with some being best fit by one and others by two exponentials; this was decided by the fit with the lowest sums of squared errors. The tau values (decay time constant) presented are the weighted average. All numerical data are expressed as mean ± SEM. Statistical comparisons of mean drug effects were made using paired Student's *t*-test, and comparisons between multiple treatment groups with a one-way ANOVA (using Dunnett's, or the Newman–Keuls corrections for post-hoc comparisons), or two-way ANOVA (using a Bonferroni correction for post-hoc comparisons). Comparisons of proportions were made using Chi-squared, or Fisher's exact tests. Differences were considered significant if $p < 0.05$.

3. Results

3.1. Group I, but not group II and III mGlu receptor activation produces an inward current in most PAG neurons

We first examined the postsynaptic effects of the group I, II and III mGlu receptor agonists DHPG, DCG-IV and L-AP4 on PAG neurons, using concentrations which we have previously shown to produce maximal presynaptic inhibition within PAG (Drew and Vaughan, 2004). DHPG (10 μM) produced an inward current in all neurons tested throughout the ventrolateral, lateral and dorsolateral PAG (Fig. 1A, B, mean current = −27 ± 3 pA, $p < 0.0001$, $n = 25$). By contrast, DCG-IV (1 μM) and L-AP4 (3 μM) had no effect in most neurons, producing an outward current in 10% and 18% of PAG neurons, respectively (Fig. 1A, B, n = 2/20 and 4/22). When averaged across all neurons, DCG-IV and L-AP4 did not produce a significant change in membrane current (Fig. 1B, mean currents = 3 ± 2 pA and 1 ± 1 pA, p = 0.2 and 0.4). Subsequent application of the $GABA_B$ agonist baclofen (10 μM) produced an outward current in all of these neurons which was reversed by the $GABA_B$ antagonist CGP55845 (1 μM) (Fig. 1A, 33 ± 4 pA, $n = 25$).

The inward current produced by DHPG (10 μM) was associated with a near parallel inward shift in the current–voltage relationship (Fig. 1C). The mean slope conductance in these neurons was 1.4 ± 0.3 nS and 2.5 ± 0.7 nS in the absence and 1.6 ± 0.4 nS and 2.3 ± 0.6 nS in the presence of DHPG, when measured between −60/−90 mV and −110/−130 mV (n = 6). By contrast, subsequent application of baclofen (10 μM) produced an outward current which reversed polarity at −110 ± 4 mV (Fig. 1C, $n = 6$). In these neurons, baclofen (10 μM) increased the slope conductance to 1.9 ± 0.4 nS and 3.3 ± 0.7 nS, when measured between −60/−90 mV and −110/−130 mV ($n = 6$).

3.2. DHPG acts via postsynaptic mGlu1 and mGlu5 receptors

DHPG produced an inward current over concentrations ranging from 0.3 to 30 μM (n = 4–14). The inward current produced by

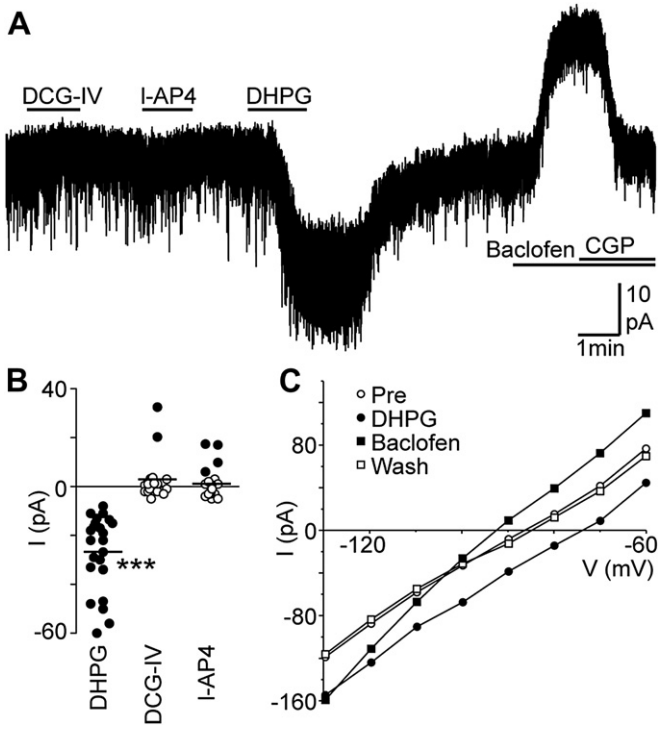

Fig. 1. The group I mGlu receptor agonist DHGP produces an inward current in all PAG neurons. (A) Current trace of a PAG neuron during superfusion of DCG-IV (1 μM), L-AP4 (3 μM), DHPG (10 μM), baclofen (10 μM) and CGP55845 (CGP, 1 μM). (B) Scatter plot of the inward currents produced by DHPG, DCG-IV and L-AP4, with the bars indicating the mean current. (C) Current–voltage relationship for a PAG neuron before (Pre), during DHPG, then during baclofen and following washout of baclofen (Wash). Membrane currents were evoked by voltage steps in 10 mV increments from −60 mV to −130 mV (250 ms duration). In (B) responders are shown as filled circles and non-responders as open circles; *** denotes $p < 0.0001$. (A) and (C) are from different neurons.