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Research Report

M-channels modulate network excitatory activity induced by 4-aminopyridine in immature rat substantia gelatinosa *in vitro*

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ARTICLE INFO

Article history:
Accepted 24 March 2013
Available online 6 April 2013

Keywords:
Dorsal horn
Oscillatory activity
KCNQ
Nociception
Potassium channels
M-current

ABSTRACT

There is strong evidence that M-currents modulate peripheral sensory afferent excitability and that altered M-current efficacy may underpin aspects of pain-induced nociceptor sensitization. Less clear is the role of the M-current in regulating central excitability within spinal dorsal horn nociceptive circuitry. In this study, an *in vitro* model of central hyperexcitability that uses the potassium channel blocker 4-aminopyridine (4-AP) to induce large amplitude population spikes and 4–12 Hz oscillatory activity within rat spinal substantia gelatinosa (SG) has been used to determine the impact of pharmacological modulation of the M-current on central excitability. The M-current enhancers Retigabine (10 and 30 μM) and Flupirtine (30 μM) had a depressant effect on 4-AP-induced excitation in SG such that the frequency of large amplitude population spikes and the power of 4–12 Hz oscillatory activity were both significantly reduced. In contrast, the M-current blockers XE911 (5 μM) or Linopirdine (20 μM) significantly potentiated 4–12 Hz oscillatory activity as evidenced by significant increases in the parameters of power amplitude and power area but had no effect on large amplitude population spikes. These data indicate that pharmacological modulation of the M-current can influence excitability of nociceptive circuitry especially under conditions of central hyperexcitability, as may occur in chronic pain conditions. It is not clear whether these effects reflect a direct effect on interneurones localized to SG or indirectly via sensory afferent terminals. Nonetheless, these central actions should be taken into account alongside peripheral actions in terms of evaluating the potential therapeutic analgesic potency of novel M-current enhancers.

1. Introduction

4-Aminopyridine is a non-specific K^+ channel blocker that induces oscillatory activity in many areas of the central nervous system (CNS) including the spinal ventral horn (VH) (Taccola and Nistri, 2004) and the dorsal horn (DH) *in vivo* (Sandkuhler and Eblen-Zajjur, 1994) and *in vitro* (Chapman et al., 2009). In the immature rat substantia gelatinosa (SG) *in vitro*, 4-AP elicits two

Abbreviations: aCSF, artificial cerebrospinal fluid; 4-AP, 4-aminopyridine; DH, dorsal horn; SG, substantia gelatinosa; VH, ventral horn

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http://dx.doi.org/10.1016/j.brainres.2013.03.045

types of network-based activity that consist of large amplitude field population spiking activity and low amplitude voltage oscillations arising from inter-spike baseline noise (Chapman et al., 2009). The latter has a distinctive rhythmic characteristic oscillating within the 4–12 Hz frequency band which is similar to the dominant frequency exhibited by spontaneous activity within DH *in vivo* (Sandkuhler and Eblen-Zajjur, 1994). The functional significance of rhythmicity within SG is not yet established but it appears to be an intrinsic property of DH circuitry (Asghar et al., 2005; Sandkuhler and Eblen-Zajjur, 1994). Since this region of the DH is a critical integrator of nociceptive sensory input a better understanding of contributors to network behavior and underlying excitatory mechanisms is needed. The important contributions of ionotropic $GABA_A$-and glycine-mediated inhibition and glutamatergic excitation to the generation of 4-AP network activity in SG *in vitro* have been described (Chapman et al., 2009). Synchronization in SG was co-dependent on both ionotropic chemical and gap junction-supported electrical synapses (Chapman et al., 2009). The core role of ionic conductances in VH synchronicity linked to fictive locomotion is documented (Grillner, 2003). By comparison, there are few equivalent studies for DH rhythmicity and the functional role of voltage-gated channels localized to this region either on intrinsic neurones or on presynaptic terminals is unknown.

KCNQ (K_v7) channels are the molecular substrates of the well-characterized non-inactivating M-type K^+ current which acts to stabilize membrane excitability and dysfunction of this channel may be linked to channelopathies that involve hyperexcitability (Brown and Passmore, 2009). The pathology of neuropathic and inflammatory pain involves both peripheral and central sensitization (von Hehn et al., 2012). Peripheral nociceptive sensory neurones express functional M-channels (Passmore et al., 2003) that are inhibited by inflammatory mediators (Linley et al., 2012). Block of the M-current by 10,10-*bis*(4-pyridinylmethyl)-9(10H)-anthracenone (XE991) sensitizes nociceptor afferents, especially Aδ fibers (Passmore et al., 2012). Pharmacological inhibition of M-channels by intra-plantar injection of XE991 produces pain-like behaviors whereas enhancement by openers such as ethyl N-(2-amino-4-(4-flourobenzylamino)-phenyl) (Retigabine) and the triaminopyridine derivative Flupirtine (2-amino-3-ethoxy-carbonylamino-6-4-fluoro-benzylamino-pyridine) can at least partially compensate for diminished M-current efficacy (Passmore et al., 2012). To date, most studies of M-currents and pain have emphasized effects on peripheral excitability (Linley et al., 2012; Passmore et al., 2012) but there are indications that modified central excitability may also be a factor. In *in vitro* rat spinal cord, pharmacological M-current enhancement diminishes DH and motoneurone excitability (Rivera-Arconada and Lopez-Garcia, 2005). In a rat model of neuropathic pain, *in vivo* electrophysiology revealed inhibition of electrically and naturally evoked DH neuronal responses by Retigabine (Passmore et al., 2003).

In this study, we have used extracellular electrophysiology and the 4-AP model of hyperexcitability in the SG of immature rat DH *in vitro* to further explore the putative central role of the M-current by investigating the actions of the anticonvulsant M-current enhancers Retigabine and Flupirtine (Brown and Passmore, 2009; Dost et al., 2004). The M-current blockers 3,3-*bis*(4-pyridinylmethyl)-1-phenyllindolin-2-one (Linopirdine) and XE991 were used to test the putative existence of an endogenous M-current tone within SG circuitry activated by 4-AP.

2. Results

2.1. 4-AP-induced excitation in SG

In SG, 4-AP (25 μM) elicits two types of excitatory activity (Fig. 1) comprising large amplitude field population spikes

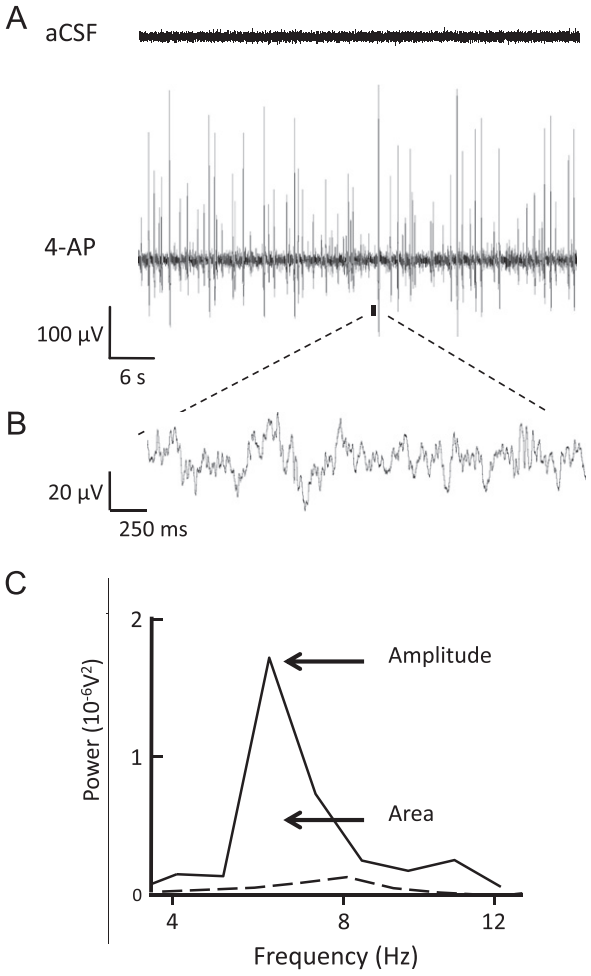

Fig. 1 – 4-AP-induced excitatory activity recorded extracellularly from substantia gelatinosa in rat spinal cord *in vitro*. A, In drug-free aCSF (upper trace) little or no spontaneous activity is recorded but in the presence of aCSF containing 25 μM 4-AP (lower trace) large amplitude population spikes of variable height and frequency are evident. B, Rhythmic oscillatory activity, which can be most easily recorded within the inter-spike interval, is also induced by more a prolonged application of 4-AP (>30 min). C, Power spectra derived from 4-AP-induced oscillatory activity reveal a dominant frequency of between 4–12 Hz (solid line) compared to drug-free aCSF (dashed line). Power amplitude and power area values were derived from spectra for quantification of drug effects on 4–12 Hz oscillatory activity.