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Postsynaptic actions of substance P on rat periaqueductal grey neurons in vitro

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Abstract

The postsynaptic actions of substance P on rat midbrain periaqueductal grey (PAG) neurons were examined using whole-cell patch-clamp recordings in brain slices. Substance P produced an inward current in a subpopulation (60%) of PAG neurons. The substance P induced current was concentration dependent (EC_{50}=27 nM) and was reduced by the NK1, NK2 and NK3 antagonists L-732,138 (20 μM), GR 159897 (3 μM) and SB 218795 (3 μM). The selective NK1, NK2 and NK3 agonists [Sar^9,$Met(O_2)^{11}$]-Substance P (100 nM), GR 64349 (300–500 nM) and senktide (300 nM) also produced inward currents in subpopulations of neurons. A greater proportion of substance P-sensitive neurons (70%) than substance P-insensitive neurons (31%) responded to the μ/δ opioid agonist met-enkephalin (10 μM). Substance P reduced the outward current produced by met-enkephalin. The reversal potential of the substance P induced current varied from −5 mV to below −140 mV in the absence of met-enkephalin, and was −105 mV in the presence of met-enkephalin. These results indicate that substance P acts via NK1, NK2 and NK3 receptors to excite subpopulations of opioid-sensitive and insensitive PAG neurons by increasing a non-selective cation conductance and by reducing a K^+ current. In addition, substance P has anti-opioid actions that are largely mediated by a reduction in the opioid induced K^+ current.

Keywords: Substance P; Opioid; Periaqueductal grey; Analgesia; Potassium current; Cation current

1. Introduction

The undecapeptide substance P belongs to the tachykinin family, is widely distributed throughout the nervous system and produces its physiological actions predominantly via the neurokinin-1 (NK1) receptor (Yokota et al., 1989; Maeno et al., 1993; Maggi, 1995). Substance P is thought to promote pain transmission because it is released by nociceptive primary afferent fibres in the spinal cord dorsal horn, where it has excitatory actions (Fields and Basbaum, 1999). In contrast, supraspinally administered substance P produces analgesia (Stewart et al., 1976; Frederickson et al., 1978; Malick and Goldstein, 1978; Mohrland and Gebhart, 1979). Furthermore, stress-induced analgesia, a supraspinally mediated phenomenon, is impaired in mice with an NK1 receptor deletion (De Felipe et al., 1998; Bester et al., 2001). These observations are consistent with the suggestion that substance P produces analgesia by activating descending pathways.

The midbrain periaqueductal grey (PAG) is a major site of the analgesic actions of opioids and forms part of a descending analgesic pathway which projects via the

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rostral ventromedial medulla (RVM) to modulate nociceptive transmission within the spinal cord (Fields and Basbaum, 1999). Ascending nociceptive spinal cord neurons, many of which contain substance P, project to brain regions such as the PAG (Hylden et al., 1986; Noguchi and Ruda, 1992). The NK1 receptor is present in the PAG and brain regions which are involved in the modulation of nociceptive information (Barbaresi, 1998; Commons and Valentino, 2002). Furthermore, noxious stimuli and stress induce substance P release within the PAG and microinjection of substance P into the PAG produces analgesia (Malick and Goldstein, 1978; Rosen et al., 1992, 2004; Xin et al., 1997).

Previous studies have shown that substance P increases a non-selective cation conductance (I_{Cat}) and suppresses a G-protein-coupled inwardly rectifying K^+ (GIRK) conductance in other brain regions (Shen and North, 1992b; Koyano et al., 1993; Aosaki and Kawaguchi, 1996). Furthermore, substance P reduces the increase in GIRK conductance produced by activation of μ-opioid and somatostatin receptors in locus coeruleus neurons (Velimirovic et al., 1995). Substance P increases the action potential firing rate of PAG neurons (Ogawa et al., 1992), however, the cellular actions of substance P within the PAG are unknown. In the present study we have examined the postsynaptic actions of substance P and its interaction with μ-opioids on PAG neurons in vitro.

2. Methods

The experimental protocol was approved by the institutional Animal Care and Ethics Committee. Male and female Sprague–Dawley rats (13–30 days old) were anaesthetised with halothane, decapitated and coronal midbrain slices containing PAG were cut (250–300 μm) in ice-cold artificial cerebrospinal fluid (ACSF), as described previously (Drew and Vaughan, 2004). The slices were maintained at 34 °C in a submerged chamber containing ACSF equilibrated with 95% O_2 and 5% CO_2. The slices were then individually transferred to a chamber and superfused continuously (2 ml min^{-1}) with ACSF (34 °C) of composition: (mM): NaCl 126, KCl 2.5, NaH_2PO_4 1.4, $MgCl_2$ 1.2, $CaCl_2$ 2.4, glucose 11, $NaHCO_3$ 25.

Whole-cell voltage-clamp recordings (holding potential −60 mV) were made from lateral and ventrolateral PAG neurons, located within the caudal two-thirds of the PAG, using an Axopatch 200B (Axon Instruments, Foster City, USA). Neurons were visualised using infrared Nomarski optics on an upright microscope (Olympus BX51). Recording electrodes contained an internal solution comprising (mM): K-gluconate 95, KCl 30, NaCl 15, $MgCl_2$ 2, HEPES 10, EGTA 11, MgATP 2, NaGTP 0.25 (pH 7.3, 280–285 mosmol l^{-1}). Series resistance (<20 MΩ) was compensated by 80% and continuously monitored during experiments. Liquid junction potentials of −10 mV were corrected for. Recordings were filtered (200 Hz low-pass filter) and sampled (500 Hz) for analysis (Axograph 4, Axon Instruments).

Stock solutions of all drugs were made in distilled water, then diluted to working concentrations using ACSF immediately before use and applied by superfusion, except for picrotoxin, which was directly added to ACSF. Baclofen and picrotoxin were obtained from Sigma (Sydney, Australia); substance P and met-enkephalin were from Auspep (Parkville, Australia); L-732,138, L-733,060, GR 159897, SB 218795, [Sar^9, $Met(O_2)^{11}$]-Substance P, GR 64349, senktide and 6-cyano-7-nitroquinoxaline-2,3-dione disodium (CNQX) were obtained from Tocris Cookson (Bristol, UK); Tetrodotoxin (TTX) was from Alomone Laboratories (Jerusalem, Israel). All numerical data are expressed as mean ± SEM, and statistical comparisons were made using χ^2 tests for differences among proportions and paired, or unpaired *t*-tests. Differences were considered significant if $P<0.05$.

3. Results

3.1. Substance P acts upon a subpopulation of opioid-sensitive PAG neurons

Maximal concentrations of substance P (100–300 nM) produced an inward current (mean current = −33 ± 3 pA) in a subpopulation of PAG neurons (60%, $n=64/106$) voltage clamped at −60 mV (Fig. 1). The proportion of substance P (100–300 nM) responding neurons was similar in the lateral (67%, $n=37/55$) and ventrolateral PAG (53%, $n=25/47$; $\chi^2=2.1$, $P>0.05$). In addition, the current produced by substance P was similar in the absence (−34 ± 3 pA, $n=41$) and in the presence (−31 ± 5 pA, $n=23$) of the non-NMDA antagonist CNQX (5 μM), picrotoxin (100 μM) and TTX (300 nM) ($P>0.05$, unpaired *t*-test). The current produced by substance P was concentration dependent (Fig. 2; $EC_{50}=27\pm8$ nM, slope factor = 1.1 ± 0.3), with substantial desensitisation being observed at concentrations above 100 nM.

Substance P (100–300 nM) was more likely to produce an inward current in neurons that responded with an outward current to the μ/δ-opioid agonist met-enkephalin (10 μM) than in neurons that did not respond to met-enkephalin (Fig. 1). Substance P produced an inward current in 70% ($n=53/76$) and 31% ($n=8/26$) of met-enkephalin responders and non-responders, respectively (Fig. 1C; $\chi^2=12.2$, $P<0.001$). The substance P induced current was −34 ± 3 pA and −25 ± 4 pA in met-enkephalin responders and