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SPECIAL REPORT

Increase by the ORL_1 receptor (opioid receptor-like$_1$) ligand, nociceptin, of inwardly rectifying K conductance in dorsal raphe nucleus neurones

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The actions of the endogenous ORL_1-receptor (opioid receptor-like$_1$) ligand, nociceptin, on the membrane properties of rat dorsal raphe nucleus neurones were examined by use of whole-cell patch clamp recording in brain slices. Nociceptin produced an outward current in all neurones tested, with an EC_{50} of 12±2 nM. Dynorphin A (100 nM to 1 μM) produced little outward current. Outward currents reversed polarity near the predicted K^+ equilibrium potential in both 2.5 mM (measured/predicted = −105 mV/−104 mV) and 6.5 mM (measured/predicted = −80 mV/−77 mV) extracellular K^+. The conductance increase was larger between −120 and −130 mV than between −70 and −80 mV, demonstrating that the nociceptin-induced K current was due to an increased inwardly rectifying K conductance. The outward current produced by nociceptin was similar to, and occluded by, high concentrations of baclofen, demonstrating actions on the same population of K channels. Naloxone (1 μM) failed to inhibit outward currents produced by nociceptin. These results are consistent with the reported high density of ORL_1 receptor mRNA in dorsal raphe nucleus and with inhibitory actions of nociceptin in cells expressing ORL_1.

Keywords: Orphan receptor; ORL_1 receptor; opioid receptor; nociceptin; potassium channel; dorsal raphe nucleus

Introduction The ORL_1 (opioid receptor-like$_1$) orphan receptor was recently identified on the basis of close homology with the predicted amino acid sequence opioid receptors (e.g. Mollereau *et al.*, 1994; Lachowicz *et al.*, 1995). When ORL_1 receptors were expressed in heterologous systems, e.g. CHO cell lines, etorphine (Mollereau *et al.*, 1994) and dynorphin A (Zhang & Yu, 1995) weakly inhibited cyclic AMP formation, but other opioids were inactive (Mollereau *et al.*, 1994; Reinscheid *et al.*, 1995; Lachowicz *et al.*, 1995; Meunier *et al.*, 1995; Zhang & Yu, 1995). A heptadecapeptide, nociceptin (Meunier *et al.*, 1995), structurally resembling dynorphin A, has more recently been identified as a potent and efficacious endogenous agonist of the ORL_1 receptor (Meunier *et al.*, 1995; Reinscheid *et al.*, 1995).

In situ hybridization studies have demonstrated high densities of mRNA expressing ORL_1 receptors in a number of brain regions (Lachowicz *et al.*, 1995), but the actions of nociceptin on neurones in these regions is unknown. We considered the dorsal raphe (DR) to be a good candidate to explore the functional effects of nociceptin, firstly because DR neurones express high densities of ORL_1 receptor mRNA (Lachowicz *et al.*, 1995). Secondly, μ-, δ- and κ-opioid receptor actions, which might otherwise confound characterization of agonist effects on ORL_1 receptors, have not been reported to occur in DR neurones (e.g. Williams *et al.*, 1988). Finally, functional coupling to a K conductance increase has been characterized in DR neurones for other receptors, e.g. 5-HT_1 and $GABA_B$ receptors, which are known to couple via inhibitory G-proteins (e.g. Williams *et al.*, 1988).

Methods Sprague-Dawley rats, 9 to 18 days old were anaesthetized (halothane), brain slices containing DR prepared, and whole-cell patch clamp recordings were made as previously described (Osborne *et al.*, 1996). DR neurones were readily identified using Nomarski optics within the translucent region ventral to the aqueduct. Liquid junction potentials of −11 mV were corrected for. Unless otherwise indicated, the holding potential was −60 mV throughout. All data are expressed as mean±s.e.mean.

Nociceptin (Mollereau *et al.*, 1995) was synthesized (>95% pure by h.p.l.c.) by Chiron Mimotopes (Clayton, Vic., Australia). Baclofen and 5-HT creatinine sulphate were obtained from Sigma (St Louis, MO, U.S.A.) and naloxone hydrochloride was from Research Biochemicals Incorporated (Natick, MA, U.S.A.). Dynorphin A (1–17) was from the National Institutes on Drug Abuse (NIDA, U.S.A.).

Results Figure 1a demonstrates that nociceptin (300 nM) produced an outward current of similar amplitude to that produced by baclofen (30 μM) in the same DR neurone. The maximum outward currents produced by nociceptin (66±8 pA, n = 17 at ⩾0.3 μM), baclofen (65±13 pA, n = 6 at 30 μM) and 5-HT (56±11 pA, n = 5 at 30 μM) did not differ. No additional current was produced by supra-maximal concentrations of nociceptin (⩾0.3 μM) in the presence of baclofen (30 μM, n = 2, raw data not shown). The concentration-response relationship of nociceptin (Figure 1b) was fitted by a logistic function (curve in Figure 1b), yielding an EC_{50} of 12±2 nM. Dynorphin A (1 μM, n = 3) produced little outward current (Figure 1b). Naloxone (1 μM) had no effect on outward currents produced by nociceptin (0.3 μM, n = 2, raw data not shown).

The resting conductance determined from current-voltage relationships (Figure 2) showed little or no inward rectification with slope conductances of 4.1±0.6 nS and 4.6±0.7 nS measured from −60 to −90 mV and −110 to −130 mV, respectively (not significant, paired t = 1.1, d.f. 6). Nociceptin increased the conductances to 4.5±0.5 nS (not significant, paired t = 1.3, d.f. = 6) and 6.6±0.8 nS (paired t = 5.2, $P<0.005$, d.f. = 6) when measured over the same potentials. The nociceptin-induced conductance increase showed significant inward rectification (paired t = 3.5, $P<0.02$, 6 d.f.). The nociceptin-induced current reversed polarity at −105±3 mV in 2.5 mM extracellular K^+ (n = 7, Figure 2),

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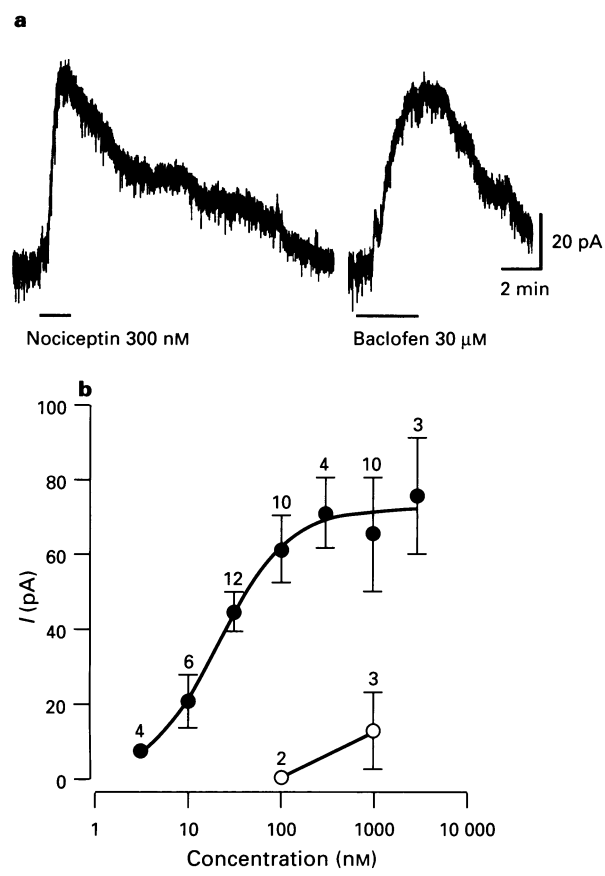

Figure 1 The effect of nociceptin on membrane currents in DR neurones. (a) Membrane currents induced by nociceptin (0.3 μM) and baclofen (30 μM) in a single DR neurone. Drugs were superfused for periods shown by bars. (b) Concentration-response relationship of outward currents induced in DR neurones by nociceptin (●) and dynorphin A (○).

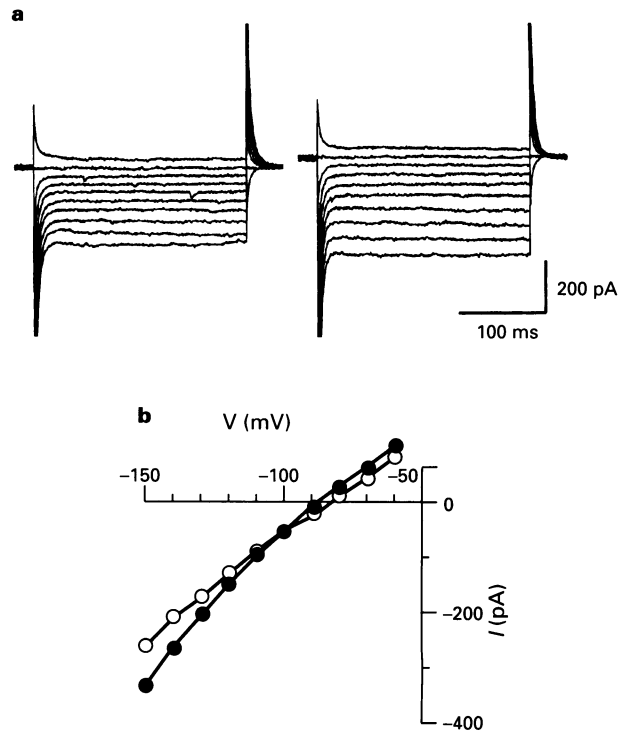

Figure 2 Nociceptin increases inwardly rectifying K conductance of DR neurones. (a) Voltage command steps 250 ms in duration were made in 10 mV increments from −50 to −140 mV from a holding potential of −60 mV. The resulting currents in the absence (left) and presence (right) of nociceptin (0.3 μM) in a single neurone are shown. (b) The current-voltage relationship (○ control; ● in nociceptin) is plotted from the amplitudes of evoked currents shown in (a).

and at -80 ± 5 mV in 6.5 mM K^+ ($n = 3$), in close agreement with the predicted Nernst equation values for a K conductance of −104 mV and −77 mV in 2.5 mM and 6.5 mM extracellular K^+, respectively.

Discussion The present results demonstrate that the ORL_1 receptor ligand, nociceptin, potently and efficaciously increases inwardly rectifying K conductance of DR neurones. These results are consistent with the reported presence of ORL_1 receptor mRNA in DR neurones (Lachowicz *et al.*, 1995), and with the reported G-protein-mediated coupling of cloned ORL_1 receptors to the activation of K channels (Zhang & Yu, 1995) and inhibition of adenylate cyclase (Meunier *et al.*, 1995; Reinscheid *et al.*, 1995). K currents induced by high concentrations of nociceptin were also occluded in the presence of high concentrations of baclofen, indicating an action on the same population of K channels.

Nociceptin almost certainly acts on ORL_1 receptors to increase K conductance of DR neurones. DR neurones have not been reported to express opioid receptors (e.g. Williams *et al.*, 1988), and in the present study naloxone (1 μM) had no effect on nociceptin-induced currents, ruling out an opioid-receptor interaction. Confirmation that nociceptin does indeed increase K conductance of DR neurones via activation of ORL_1 receptors will require identification of suitable ORL_1 receptor antagonists.

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