



SPECIAL REPORT

Nociceptin induced inhibition of K^+ evoked glutamate release from rat cerebrocortical slicesBeverley Nicol, ¹David G. Lambert, David J. Rowbotham, *Darren Smart & *Alexander T. McKnight

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Nociceptin, an endogenous ligand for the orphan receptor ORL_1 , has recently been described. In this study we have shown that nociception inhibits 46 mM K^+ -stimulated glutamate release from rat perfused cerebrocortical slices with an IC_{50} of 51 nM. At 100 nM the inhibition amounted to $68 \pm 14\%$ and was naloxone (10 μ M)-insensitive excluding an activation of μ , δ and κ opioid receptors. These data demonstrate the functional coupling of ORL_1 in glutamatergic neurones and implicates a role for nociceptin in glutamatergic neurotransmission.

Keywords: Nociceptin; orphanin FQ; orphan receptor; glutamate release; rat cerebrocortical slices

Introduction The structure, distribution and pharmacology of an 'orphan' G-protein-coupled receptor with some homology to opioid receptors has been described (Bunzow *et al.*, 1994; Mollereau *et al.*, 1994). In 1995 Meunier *et al.*, and Reinscheid *et al.*, reported the isolation of a heptadecapeptide (H-Phe-Gly-Gly-Phe-Thr-Gly-Ala-Arg-Lys-Ser-Ala-Arg-Lys-Leu-Ala-Asn-Gln-OH) named nociceptin or orphanin FQ which displayed nanomolar binding affinity for ORL_1 in radioligand binding studies and was hyperalgesic when injected intracerebroventricularly (icv) in the mouse. In addition to inhibiting cyclic AMP formation (Meunier *et al.*, 1995; Reinscheid *et al.*, 1995) nociceptin has recently been shown to increase inwardly rectifying K^+ currents in dorsal raphe nucleus neurones (Vaughan & Christie, 1996), inhibit N-type voltage-sensitive Ca^{2+} channels and enhance the carbachol-stimulated increase in $[Ca^{2+}]_i$ in SH-SY5Y cells (Connor *et al.*, 1996) and inhibit tachykinin release from peripheral sensory neurones (Giuliani & Maggi, 1996). High levels of expression of ORL_1 have been reported in rat cortex (Bunzow *et al.*, 1994) and the post receptor coupling is similar to that seen with opioid receptors (Resisine, 1995). As we have shown that opioid receptor occupation inhibits glutamate release from K^+ depolarized rat cerebrocortical slices (Nicol *et al.*, 1996) we have examined the effects of nociceptin on glutamate release. We report that nociceptin produced a dose-dependent naloxone-insensitive release of glutamate.

Methods Female Wistar rats (200–250 g) were killed by cervical dislocation and decapitation. The cerebral cortex was rapidly dissected and placed into ice-cold oxygenated (95% O_2 , 5% CO_2) Krebs buffer, pH 7.4 of the following composition (mM): NaCl 115, KCl 4.7, $CaCl_2$ 2.0, $MgCl_2$ 1.2, $NaHCO_3$ 25 and glucose 8.8 Slices ($350 \times 350 \mu$ m) were cut and washed three times in fresh Krebs buffer prior to agitation in a shaking water bath set at $37^\circ C$ for 40 min; 1 ml of gravity packed slices were pipetted into a perfusion chamber constructed from the barrel of a 2 ml syringe with a custom-made flow diffuser as a modified plunger. Slices were perfused at $37^\circ C$ for 60 min at 1 ml min^{-1} prior to collection of 2 min fractions for the estimation of glutamate concentration. A 2 min pulse of 46 mM K^+ (Na^+ adjusted) was applied following 6 min of perfusion (S_1). Slices were perfused for a further 30 min, prior to the second application of a 2 min pulse of 46 mM K^+ (S_2). Fractions were collected for 8 min after S_2 . Nociceptin (10–

1000 nM in the presence of 30 μ M bestatin, amastatin, captopril, phosphoramidon and 0.1% BSA to prevent breakdown) was applied immediately after S_1 until the end of the experiment (also present during S_2). In some experiments, naloxone (10 μ M) was also included. Perfusate glutamate concentrations were measured fluorimetrically based on the conversion of NADP to NADPH by glutamate dehydrogenase; 480 μ l of perfusate was incubated with NADP (1.0 mM) and glutamate dehydrogenase (30 u). The resulting production of NADPH was detected fluorimetrically at excitation and emission wavelengths of 366 nm and 430 nm respectively in a Perkin-Elmer LS50B spectrofluorimeter, and compared with a known set of glutamate standards. Glutamate release was expressed relative to the mean of the first three basal samples collected and S_2/S_1 ratios calculated. All data are presented as mean \pm s.e.mean. IC_{50} value for nociceptin was obtained by computer-assisted curve fitting of the composite curve using GRAPH-PAD-PRIZM. Statistical comparisons of paired samples were made using Wilcoxon Rank sum test and considered significant when $P < 0.05$.

Results Depolarization of rat cerebrocortical slices with 46 mM K^+ produced a monophasic release of glutamate for both S_1 and S_2 (Figure 1a) with a mean S_2/S_1 ratio from 26 control experiments of 0.97 ± 0.06 . Nociceptin produced a dose-dependent reduction in the S_2/S_1 ratio with an estimated IC_{50} of 51 nM (Figure 1a,b). At 100 nM, the inhibition amounted to $64 \pm 8\%$ (Figure 1a). Naloxone (10 μ M) did not reverse nociceptin (100 nM) inhibition of glutamate release (Figure 1c).

Discussion We demonstrate that nociceptin inhibits K^+ -evoked glutamate release from rat cerebrocortical slices, in a concentration-dependent manner, with an IC_{50} of 51 nM. IC/EC_{50} values of 42 nM and 60 nM were reported by Connor *et al.* (1996) for N-type Ca^{2+} channel current inhibition and the enhancement of carbachol-stimulated increase in $[Ca^{2+}]_i$ in SH-SY5Y human neuroblastoma. In addition, nociceptin enhances an outward K^+ conductance in dorsal raphe neurones with an EC_{50} of 12 nM (Vaughan & Christie, 1996). A combination of Ca^{2+} channel inhibition and hyperpolarization, resulting from enhanced outward K^+ fluxes, is likely to be the underlying mechanism of glutamate inhibition observed in the present study. In these reports and the present study, naloxone was ineffective, confirming an action at non- μ , δ , or κ opioid receptors. The physiological significance of inhibition of glutamate release in cerebral cortex is unclear but if inhibition of glutamatergic transmission is mirrored in the hippocampus

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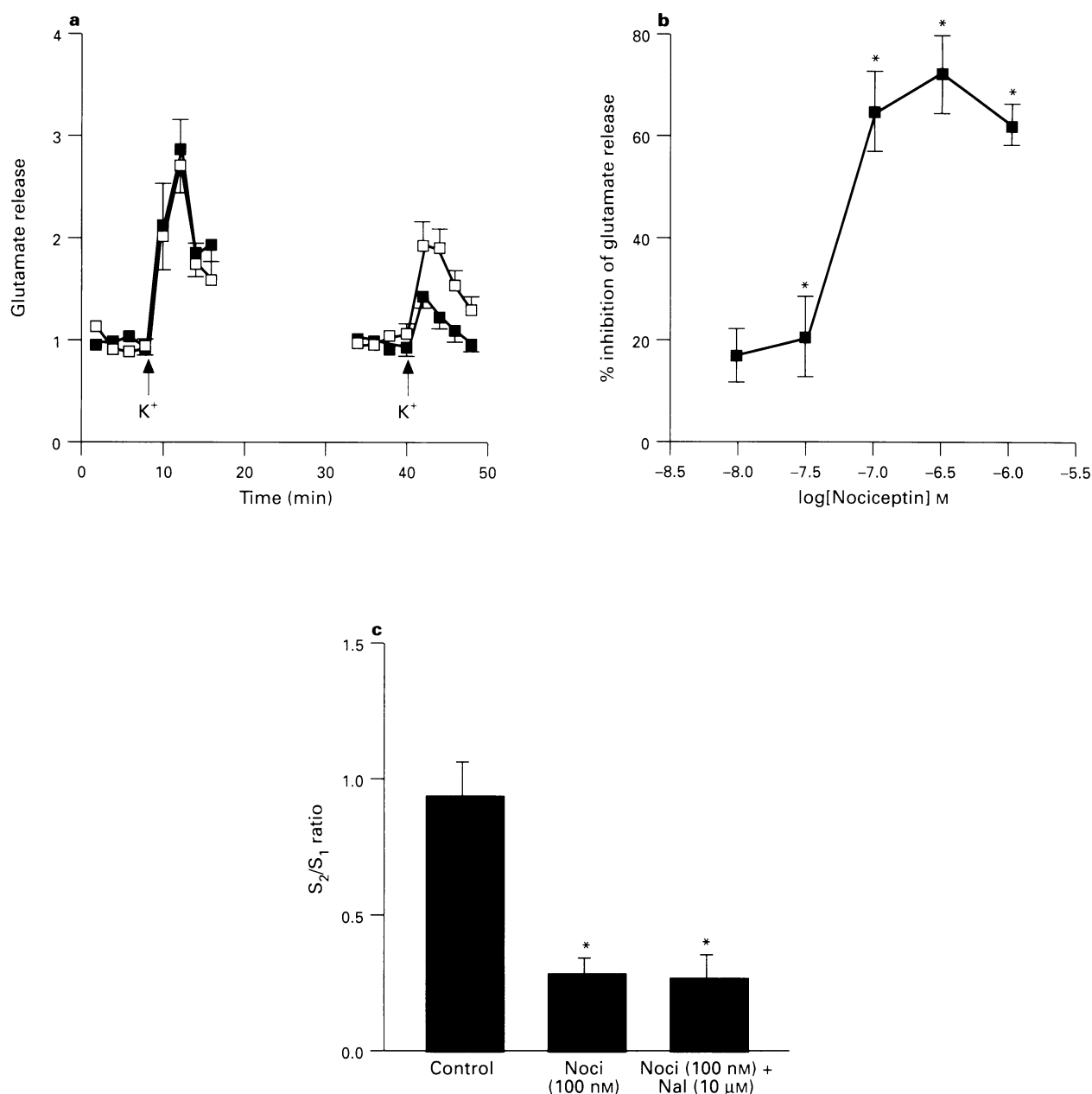


Figure 1 Nociceptin inhibits glutamate release from rat cerebrocortical slices. In panel (a) the effect of 100 nM nociceptin (■) applied between two 2 min challenges (arrows) of 46 mM K⁺, S₁ and S₂ is shown; (□) control. Data are presented relative to the mean of the first three fractions collected. In panel (b) a dose-dependent inhibition of the S₂/S₁ ratio is presented and in panel (c) the effects of naloxone (Nal, 10 μM) on the nociceptin (Noci, 100 nM) inhibition of S₂/S₁ ratio is shown. All data are mean ± s.e.mean from at least 5 independent determinations. **P* < 0.05 significantly reduced compared with paired control.

where high levels of ORL₁ expression are found (Bunzow *et al.*, 1994; Mollereau *et al.*, 1994) then an involvement in learning and memory must be suspected. ORL₁ is also located in the cerebellum, albeit at low levels (Mollereau *et al.*, 1994) and

i.c.v. nociceptin produced ataxia and loss of righting reflex in mice (Reinscheid *et al.*, 1995). Clearly further studies of ORL₁ receptor-mediated inhibition of glutamate release are required in these areas.

References

- BUNZOW, J.R., SEAZ, C., MORTURD, M., BOUVIER, C., WILLIAMS, J.T., LOW, M. & GRANDY, D.K. (1994). Molecular cloning and tissue distribution of a putative member of the rat opioid receptor gene family that is not a μ , δ or κ opioid receptor type. *FEBS Lett.*, **347**, 284–288.
- CONNOR, M., YEO, A. & HENDERSON, G. (1995). The effect of nociceptin on Ca²⁺ channel current and intracellular Ca²⁺ in the SH-SY5Y human neuroblastoma cell line. *Br. J. Pharmacol.*, **118**, 205–207.
- GIULIANI, S. & MAGGI, C.A. (1996). Inhibition of tachykinin release from peripheral endings of sensory nerves by nociceptin, a novel opioid peptide. *Br. J. Pharmacol.*, **118**, 1567–1569.
- MEUNIER, J.C., MOLLEREAU, C., TOLL, L., SUAUDEAU, C., MOISAND, C., ALVINERIE, P., BUTOUR, J.L., GUILLEMOT, J.C., FERRARA, P., MONSARRAT, B., MAZARGUIL, H., VASSART, G., PARMENTIER, M. & COSTENTIN, J. (1995). Isolation and structure of the endogenous agonist of opioid receptor-like ORL₁ receptor. *Nature*, **377**, 532–535.

- MOLLEREAU, C., PARMENTIER, M., MAILLEUX, P., BUTOUR, J.L., MOISAND, C., CHALON, P., CAPUT, D., VASSART, G. & MEUNIER, J.C. (1994). ORL1, a novel member of the opioid receptor family. *FEBS Lett.*, **341**, 33–38.
- NICOL, B., ROWBOTHAM, D.J. & LAMBERT, D.G. (1996). Is glutamate release from rat cerebrocortical slices opioid receptor subtype selective? *Br. J. Anaesthesia.*, **77**, 282P.
- REISCHEID, R.K., NOTHACKER, H.P., BOURSON, A., ARDATI, A., HENNINGSSEN, R.A., BUNZOW, J.R., GRANDY, D.K., LANGEN, H., MONSMA, Jr, F.J. & CIVELLI, O. (1995). Orphanin FQ: A neuropeptide that activates an opioid like G protein-coupled receptor. *Science*, **270**, 792–794.
- REISINE, T. (1995). Opiate receptors. *Neuropharmacology*, **34**, 463–472.
- VAUGHAN, C.W. & CHRISTIE, M.J. (1996). Increase by the ORL1 receptor (opioid receptor-like₁) ligand, nociceptin, of inwardly rectifying K conductance in dorsal raphe nucleus neurones. *Br. J. Pharmacol.*, **117**, 1609–1611.

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