British Journal of Pharmacology (1996) 119, 189–190

SPECIAL REPORT

Depression of glutamatergic transmission by nociceptin in the neonatal rat hemisected spinal cord preparation *in vitro*

[1]E.S.L. Faber, J.P. Chambers, R.H. Evans & G. Henderson

Department of Pharmacology, School of Medical Sciences, University Walk, Bristol BS8 1TD

The present study explored the action of nociceptin, the putative endogenous ligand for the orphan opioid receptor (ORL1), on the rat hemisected spinal cord preparation. Electrical stimulation of a dorsal root evokes a glutamatergic population ventral root potential (DR-VRP) in the corresponding ventral root. Low intensity stimulation evokes two A fibre-mediated components; a compound action potential of motonuerones superimposed on a population e.p.s.p. (excitatory postsynaptic potential); at higher stimulus intensities sufficient to activate C fibres a more prolonged population e.p.s.p. is evoked. All three components were depressed by nociceptin in a concentration-dependent manner with IC_{50} values (s.e.mean) of 119±2 nM ($n=4$), 241±3 nM ($n=4$) and 32±2 nM ($n=4$), respectively. The depressant actions of nociceptin (30 nM and 300 nM) were not reversed by the opioid antagonist naloxone (1 μM). Nociceptin (100 nM and 300 nM) had no effect on the afferent volleys in the dorsal root. Nociceptin therefore appears to be acting as an inhibitory peptide at the spinal level through a naloxone-insensitive opioid receptor.

Keywords: Nociceptin; spinal cord; ventral root potentials; naxolone

Introduction Nociceptin (Meunier *et al.*, 1995), or orphanin FQ (Reinscheid *et al.*, 1995), is a 17 amino acid peptide (Phe-Gly-Gly-Phe-Thr-Gly-Ala-Arg-Lys-Ser-Ala-Arg-Lys-Leu-Ala-Asn-Gln) which is the putative endogenous ligand for the orphan ORL1 receptor (Mollereau *et al.*, 1994). The ORL1 receptor structurally resembles the opioid receptors and is negatively coupled to adenylyl cyclase (Mollereau *et al.*, 1994). Nociceptin has some homology with the dynorphin family of peptides but lacks activity at μ, κ and δ opioid receptors. It has been shown to be pro-nociceptive in behavioural tests since it induces hyperalgesia when administered intracerebroventricularly to mice (Meunier *et al.*, 1995). Furthermore, molecular studies have demonstrated the presence of the ORL1 receptor transcript in the spinal cord (Wick *et al.*, 1994). For this reason the aim of the present study was to investigate the action of nociceptin on synaptic transmission in the hemisected spinal cord preparation.

Electrical stimulation of the dorsal root of the neonatal rat hemisected spinal cord preparation evokes a ventral root potential (DR-VRP) in the corresponding ipsilateral ventral root (Otsuka & Konishi, 1974). There are several distinct components of the DR-VRP which can be measured over progressively longer time sweeps (Figure 1). Low intensity stimulation activates the A fibre-mediated components of the DR-VRP comprising the initial compound action potential of motoneurones (MSR) superimposed on a population excitatory postsynaptic potential (Lte.p.s.p). High intensity stimulation evokes a prolonged population e.p.s.p. (Hte.p.s.p.) which lasts for tens of seconds (Akagi *et al.*, 1985). The Hte.p.s.p. is thought to reflect a nociceptive reflex for several reasons: the threshold of activation corresponds to that of C fibre primary afferents (Akagi *et al.*, 1985), it can be evoked by peripheral noxious stimulation and it can be depressed by analgesics such as opioids (Yanagisawa *et al.*, 1985). All of these components can be abolished by ionotropic glutamate receptor antagonists (Evans, 1989).

Methods Spinal cords were prepared from neonatal Sprague-Dawley rats (aged between 3 and 6 days, 8–14 g body weight) as previously described (Otsuka & Konishi, 1974). The hemisected spinal cords were superfused with artificial cerebral spinal fluid (ACSF) at a rate of 2 ml min^{-1} and maintained at a temperature of 25–27°C. The ACSF consisted of (mM): NaCl 118, $NaHCO_3$ 24, glucose 12, $CaCl_2$ 1.5, KCl 3, $MgCl_2$ 1.2. and was gassed with O_2/CO_2 (95%/5%) pH 7.4. Drugs were applied to the preparation in known concentrations by adding them to the superperfusate.

The A fibre-mediated MSR and Lte.p.s.p. were evoked by a single 0.5 ms pulse at three times threshold, where threshold was the intensity at which a response first appeared in the ventral root. The C fibre-mediated Ht e.p.s.p. was evoked by a single supramaximal 0.5 ms pulse. The compound action potential (CAP) of the dorsal root was evoked by a single supramaximal 0.5 ms pulse applied to the sciatic nerve and recorded from the dorsal root. The evoked potentials were measured over 1280 samples for each of the sweep times as illustrated in Figure 1. The action of drugs on each of the components was assessed, after a 20 to 35 min period required for equilibrium effect, by measuring the peak amplitude of the MSR, the areas under the curve of the Lte.p.s.p. and Hte.p.s.p., and the peak amplitude of the A and C waves of the CAP in the dorsal root. Each concentration of nociceptin was applied separately following washout of the previous concentration; these values were compared with control values measured immediately before drug application. Results are expressed as mean±s.e.mean.

Drugs and chemicals Naloxone hydrochloride was obtained from Sigma and nociceptin was synthesised in the Molecular Recognition Centre at the University of Bristol.

Results Figure 1 shows the depressant action of nociceptin on the three components of the DR-VRP. Nociceptin (300 nM) depressed the peak amplitude of the MSR to 38±4% of the control value ($n=4$, Figure 1a). The area under the curve of the Lte.p.s.p. was depressed by nociceptin (300 nM) to 56±6% of the control value ($n=5$, Figure 1b) and that of the Hte.p.s.p. was depressed to 24±4% of the control value ($n=5$, Figure 1c) Each of these effects was reversible on washout of the drug. The depressant actions of 300 nM nociceptin ($n=4$, Figure 1) and 30 nM nociceptin ($n=3$) were not, however, reversed by application of naloxone (1 μM). Nociceptin depressed each of the components of the DR-VRP in a concentration-dependent

[1] Author for correspondence.

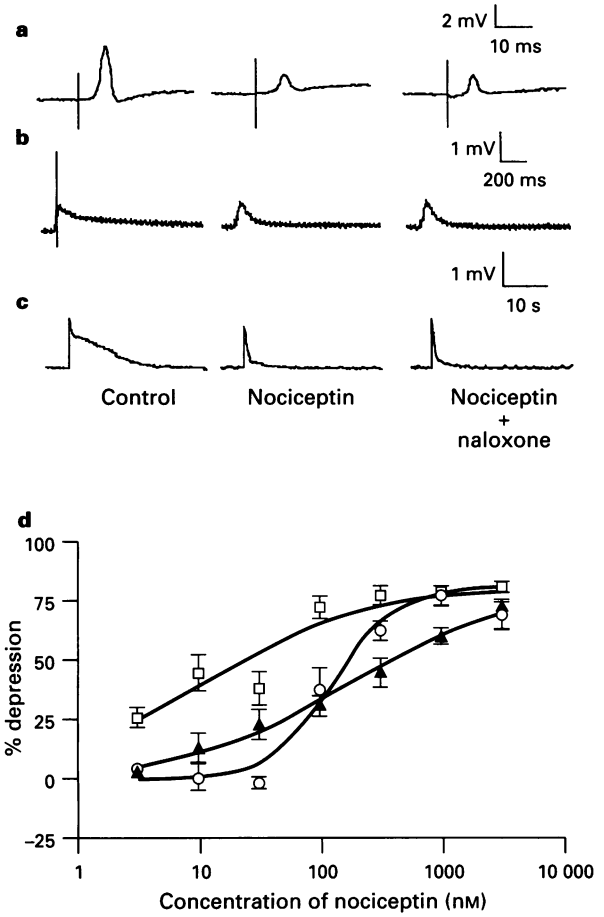

Figure 1 The effects of nociceptin on the three components of the glutamatergic population ventral root potential induced by electrical stimulation of a dorsal root (DR-VRP). Nociceptin (300 nM) significantly depressed the compound action potential of motoneurones (MSR) (a), the Lte.p.s.p. (b) and the Hte.p.s.p. (c). These effects were not reversed by 1 μM naloxone (right panels). (d) The depressant actions of nociceptin on the MSR ($n=4$, ○), Lte.p.s.p. ($n=4$, ▲) and the Hte.p.s.p. ($n=4$, □) were concentration-dependent.

manner (Figure 1d) giving significantly different ($P<0.0001$, Bonferroni Multiple comparisons test) IC_{50} values of 119 ± 2 nM for the MSR ($n=4$), 241 ± 3 nM for the Lte.p.s.p. ($n=4$) and 32 ± 2 nM for the Hte.p.s.p. ($n=4$). Approximately 20% of each component was resistant to the maximum concentration of nociceptin tested (10 μM). An action on the afferent volley was not involved in the nociceptin-induced depression because 100 nM and 300 nM nociceptin had no effect on the A or C wave of the dorsal root CAP ($n=4$, data not shown).

Discussion The results demonstrate that nociceptin depressed each of the components of the DR-VRP in a concentration-dependent manner. This depression was unlikey to have involved opioid receptors as it was not antagonised by naloxone at a concentration that abolishes opiate-induced depression. In support of this is the finding that opiates unlike nociceptin do not depress the MSR of the present preparation (Yanagisawa *et al.*, 1985). Depression of all components of the DR-VRP by nociceptin suggests a non-specific action. However, our results show that nociceptin had no effect on the afferent volley in the dorsal root. Under the present experimental conditions the DR-VRP is abolished by ionotropic glutamate antagonists (Evans, 1989). Thus different test preparations will be required in order to show whether the depressant action of nociceptin extends to other forms of excitatory transmission.

The Hte.p.s.p. has a complex concentration-effect profile compared to the A fibre-mediated responses (Figure 1d). This is possibly due to greater complexity in the polysynaptic pathways involved and may be the reason for the IC_{50} for depression of the Hte.p.s.p. being lower than those for the low threshold components. Whatever the underlying reason for this higher sensitivity it does suggest that nociceptin has an antinociceptive action.

In summary, these results provide evidence for nociceptin exerting inhibitory actions on glutamatergic transmission at the spinal level. Further studies are required to confirm that these actions are mediated through the ORL1 receptor. The greater sensitivity of the Hte.p.s.p. to nociceptin compared to the A fibre-mediated components may be due to the polysynaptic nature of the Hte.p.s.p. or it could suggest a selective action on nociceptive pathways.

References

AKAGI, H., KONISHI, S., OTSUKA, M. & YANAGISAWA, M. (1985). The role of substance P as a neurotransmitter in the reflexes of slow time courses in the neonatal rat spinal cord. *Br. J. Pharmacol.*, **84,** 663–673.

EVANS, R.H. (1989). The pharmacology of segmental transmission in the spinal cord. *Prog. Neurobiol.*, **33,** 255–279.

MEUNIER, J.-C., MOLLEREAU, C., TOLL, L., SUAUDEAU, C., MOISAND, C., ALVINERIE, P., BUTOUR, J.-L., GUILLEMOT, J-C., FERRERA, P., MONSERRAT, B., MAZARGUIL, H., VASSART, G., PARMENTIER, M. & COSTINTIN, J. (1995). Isolation and structure of the endogenous agonists of opioid receptor-like ORL1 receptor. *Nature*, **377,** 532–535.

MOLLEREAU, C., PAMENTIER, 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.

OTSUKA, M. & KONISHI, M. (1974). Electrophysiology of the mammalian spinal cord in vitro. *Nature*, **252,** 733–734.

REINSCHEID, R.K., NOTHACKER, H.-P., BOURSON, A., ARDATI, A., HENNINGSON, R.A., BUNZOW, J.R., GRANDY, D.K., LANGEN, H., MONSMA, JR., F.J. & CIVELLI, O. (1995). Orphanin FQ: a neuropeptide that activated an opioidlike G protein-coupled receptor. *Science*, **270,** 792–794.

WICK, M.J., MINNERATH, S.R., LIN, X., ELDE, R., LAW, P.-Y. & LOH, H.-H. (1994). Isolation of a novel cDNA encoding a putative membrane receptor with high homology to the cloned μ, δ and κ opioid receptors. *Mol. Brain. Res.*, **117,** 1609–1611.

YANAGISAWA, M.T., MURAKOSHI, T., TAMAI, S. & OTSUKA, M. (1985). Tail-pinch method in vitro and the effects of some antinociceptive compounds. *Eur. J. Pharmacol.*, **106,** 231–239.

(Received June 19, 1996
Accepted July 19, 1996)