Pergamon

www.neuroscience-ibro.com

PII: S0306-4522(01)00354-2

Neuroscience Vol. 107, No. 2, pp. 275–281, 2001

0306-4522/01 $20.00+0.00

BIDIRECTIONAL ACTIONS OF NOCICEPTIN/ORPHANIN FQ ON Aδ-FIBRE-EVOKED RESPONSES IN RAT SUPERFICIAL SPINAL DORSAL HORN *IN VITRO*

R. RUSCHEWEYH and J. SANDKÜHLER*

Institute of Physiology and Pathophysiology, University of Heidelberg, Im Neuenheimer Feld 326, D-69120 Heidelberg, Germany

Abstract—The present study investigated the modulatory actions of nociceptin/orphanin FQ on excitatory glutamatergic transmission in spinal dorsal horn. In transverse spinal cord slices with an attached dorsal root, mono- and polysynaptic Aδ-fibre-evoked extracellular field potentials were recorded from superficial dorsal horn. Nociceptin/orphanin FQ showed bidirectional effects on monosynaptic transmission with a potentiation at lower concentrations (100–300 nM) and a dose-dependent depression at higher concentrations (1–3 μM). The polysynaptic field potential was dose-dependently depressed by nociceptin/orphanin FQ (100 nM–3 μM). None of the actions of nociceptin/orphanin FQ was reversed by the non-specific opioid receptor antagonist naloxone, the *N*-methyl-D-aspartate receptor antagonist D-2-amino-5-phosphonovaleric acid or the peptide nocistatin.

The bidirectional actions of nociceptin/orphanin FQ on the monosynaptic field potential may provide an *in vitro* model for the bidirectional actions of nociceptin/orphanin FQ in behavioural studies showing hyperalgesia at low doses of intrathecal nociceptin/orphanin FQ and analgesia at higher doses.

Key words: nociception, analgesia, nocistatin, ORL-1 receptor, primary afferent, synaptic transmission.

Fine primary afferent nerve fibres including nociceptive Aδ-fibres terminate in superficial spinal dorsal horn. Modification of synaptic strength between these afferents and second order neurones in spinal cord is considered a cellular mechanism of some forms of hyperalgesia and analgesia (Moore et al., 2000; Sandkühler, 2000). Nociceptin/orphanin FQ is a recently discovered endogenous peptide (Reinscheid et al., 1995; Meunier et al., 1995) that together with its putative receptor, the orphan opioid or ORL-1 receptor (Wick et al., 1994), seems to be capable of modulating the nociceptive system in a way independent of the classical opioid system (see Ito et al., 2000 for a recent review). Nociceptin/orphanin FQ has been shown to be active at multiple sites of nociceptive transmission, ranging from peripheral nociceptors (Inoue et al., 1998) to nociceptive centres in the brain (Mogil et al., 1996; Morgan et al., 1997). *In vivo* studies showed bidirectional actions of spinally administered nociceptin/orphanin FQ. At low doses, nociceptin/orphanin FQ facilitated nociceptive responses in behavioural studies. In contrast, nociceptive responses were depressed at higher doses (Ito et al., 2000; Nakano et al., 2000; Inoue et al., 1999). Previous *in vitro* studies failed to identify bidirectional effects of nociceptin/orphanin FQ, only a dose-dependent inhibition of synaptic transmission between unidentified primary afferent nerve fibres and second order neurones in the superficial spinal dorsal horn has been described (Liebel et al., 1997; Lai et al., 1997). Thus, the nature of the bidirectional effects of nociceptin on spinal nociception remains unknown.

Spinal cord slices with long dorsal roots provide the means to differentiate synaptic activity evoked in spinal dorsal horn by primary afferent Aβ-, Aδ- and C-fibres. Here, we used simultaneous field potential recordings of mono- and polysynaptic Aδ-fibre-evoked activity in superficial dorsal horn of spinal cord slices with attached long dorsal roots to further identify the site of action of intrathecal nociceptin/orphanin FQ. Some of these results have been published in abstract form (Ruscheweyh and Sandkühler, 1999).

*Corresponding author. Tel.: +49-6221-544052; fax: +49-6221-544047.

E-mail address: sandkuhler@urz.uni-heidelberg.de (J. Sandkühler).

Abbreviations: AMPA, α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid; CNQX, 6-cyano-7-nitro-quinoxaline-2,3-dione; D-AP-5, D-2-amino-5-phosphonovaleric acid; EPSC, excitatory postsynaptic current; HEPES, *N*-(2-hydroxyethyl)piperazine-*N*′-(2-ethanesulphonic acid); NMDA, *N*-methyl-D-aspartate; ORL-1 receptor, opioid-receptor-like-1 receptor.

EXPERIMENTAL PROCEDURES

Preparation of spinal cord slices

Transverse, 500-μm-thick slices each with one 7–14-mm-long dorsal root attached were obtained from lumbar spinal cord of Sprague–Dawley rats (18–28 days old; Zentralinstitut für Versuchstierzucht, Hannover). Experiments were in accordance with institutional and federal regulations for the use of experimental animals. Slices were incubated in a solution that con-

sisted of (in mM): NaCl 124, KCl 5, KH_2PO_4 1.3, $CaCl_2$ 2.4, $MgSO_4$ 1.3, $NaHCO_3$ 26, glucose 15, and that was equilibrated with 95% O_2, 5% CO_2, pH 7.4. A single slice was then transferred to the recording chamber where it was superfused with oxygenated recording solution at 3 ml/min. The recording solution was identical to the incubation solution except for (in mM): NaCl 127, KCl 1.9 and $CaCl_2$ 4.3. Experiments were conducted at room temperature (20–24°C). All efforts were made to minimise the number of animals used and their suffering.

Recording and stimulation techniques

A suction electrode was used for electrical stimulation of the dorsal root with a constant current stimulator (World Precision Instruments, Sarasota, FL, USA). Field potential recordings were made with glass microelectrodes from the superficial spinal dorsal horn. When filled with (in mM) NaCl 135, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 1, HEPES 5 (pH adjusted to 7.2 with NaOH), electrodes had DC tip resistances between 2 and 7 MΩ. Low-pass filter was set to 1000 Hz, amplification 500× (Axopatch 200B, Axon Instruments). The field potentials were digitised by an A/D converter card (Data Translation DT 2821) and stored in a computer for off-line analysis using the software package Experimenter's Workbench, version 4.0 (Data Wave Technologies).

Experimental protocol

Recording sites were optimised for two clearly distinguishable signals in response to dorsal root stimulation. The elevated Ca^{2+} concentration in the recording solution (4.3 mM) was used to amplify the second signal, as described recently (Ruscheweyh and Sandkühler, 2000). Stimulation thresholds for Aδ-fibre-evoked potentials were around 0.1 mA (0.1-ms pulse width), latencies were approximately 2.5 ms for the first signal and 10–11 ms for the second signal, and amplitudes were at least 400 μV. Test pulses were given at 15-s intervals and at a supramaximal intensity for Aδ-fibres (0.7 mA, 0.1 ms). After obtaining stable amplitudes for both signals for at least 15 min, a drug was added to the superfusion solution. One experiment was performed per slice.

Data analysis

Two consecutive field potentials were averaged and the strength of monosynaptic transmission from primary afferent Aδ-fibres was quantified by measuring the baseline-to-peak amplitude of the first signal. Strength of polysynaptic transmission was evaluated by measuring the onset-to-peak amplitude of the second signal. The latencies from the beginning of the artefact to the peak of each signal were also measured. The mean amplitudes and the mean latencies of 10 consecutive averaged test responses prior to drug application served as controls. The mean amplitudes and latencies of 10 consecutive average responses at the end of the drug application were used to assess the effect of the drug. For calculating the conduction velocity, the latency from the beginning of the artefact to the onset of the first signal was measured. All values are expressed as mean ± S.E.M. Statistical comparisons were made using the non-parametric Wilcoxon rank sum test. $P < 0.05$ was considered significant. The software package Mathematica, version 3.0 (Wolfram Research, Champaign, IL, USA) was used.

Application of drugs

All drugs were added to the superfusion solution at known concentrations. Drugs and their sources were as follows: nociceptin/orphanin FQ (100–3000 nM, Tocris, Bristol, UK), nocistatin (3 μM, Tocris), naloxone hydrochloride (naloxone, 10 μM, Sigma, Deisenhofen, Germany) and D-2-amino-5-phosphonovaleric acid (D-AP-5, 50 μM, Tocris). Stock solutions were prepared of each drug by dissolving the drug in distilled water. Stock solutions were stored in aliquots at −20°C.

RESULTS

Two clearly distinguishable extracellular field potentials were recorded in superficial spinal dorsal horn in response to electrical stimulation of the dorsal root at Aδ-fibre intensity (0.7 mA, 0.1 ms, Fig. 1A). As described previously (Ruscheweyh and Sandkühler, 2000), the early potential represents an Aδ-fibre-evoked monosynaptic field potential while the late deflection corresponds to a polysynaptic field potential evoked by Aδ-fibres. Both field potentials were abolished by bath application of 6-cyano-7-nitro-quinoxaline-2,3-dione (CNQX; 5 μM) (Ruscheweyh and Sandkühler, 2000), indicating that they were mediated by postsynaptic α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA)/kainate receptors.

Effects of nociceptin/orphanin FQ on the mono- and polysynaptic spinal field potentials

Bath application of nociceptin/orphanin FQ differentially affected the mono- and the polysynaptic Aδ-fibre-evoked spinal field potentials. The effect on the mono-

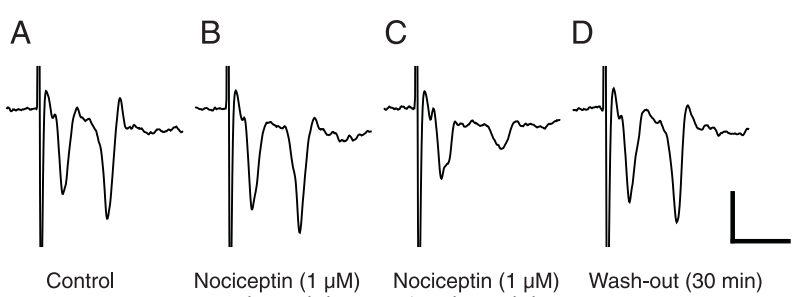

Fig. 1. The effects of nociceptin on mono- and polysynaptic Aδ-fibre-evoked field potentials in one typical experiment. (A) Original trace of Aδ-fibre-evoked field potentials recorded in superficial dorsal horn of a spinal cord slice in response to electrical stimulation (0.7 mA, 0.1 ms) of the attached long dorsal root. An early and a late potential following the artefact can be clearly distinguished. The early potential corresponds to a monosynaptic Aδ-fibre-evoked potential while the late potential corresponds to a polysynaptic Aδ-fibre-evoked potential. (B) Five minutes after beginning of wash-in of nociceptin (1 μM), the monosynaptic potential is potentiated to about 120% of control. (C) After a longer wash-in period (15 min) of nociceptin (1 μM), both potentials are clearly depressed. (D) Thirty minutes after beginning wash-out of nociceptin, both potentials have returned to control values (calibration bars: 1 mV, 10 ms).

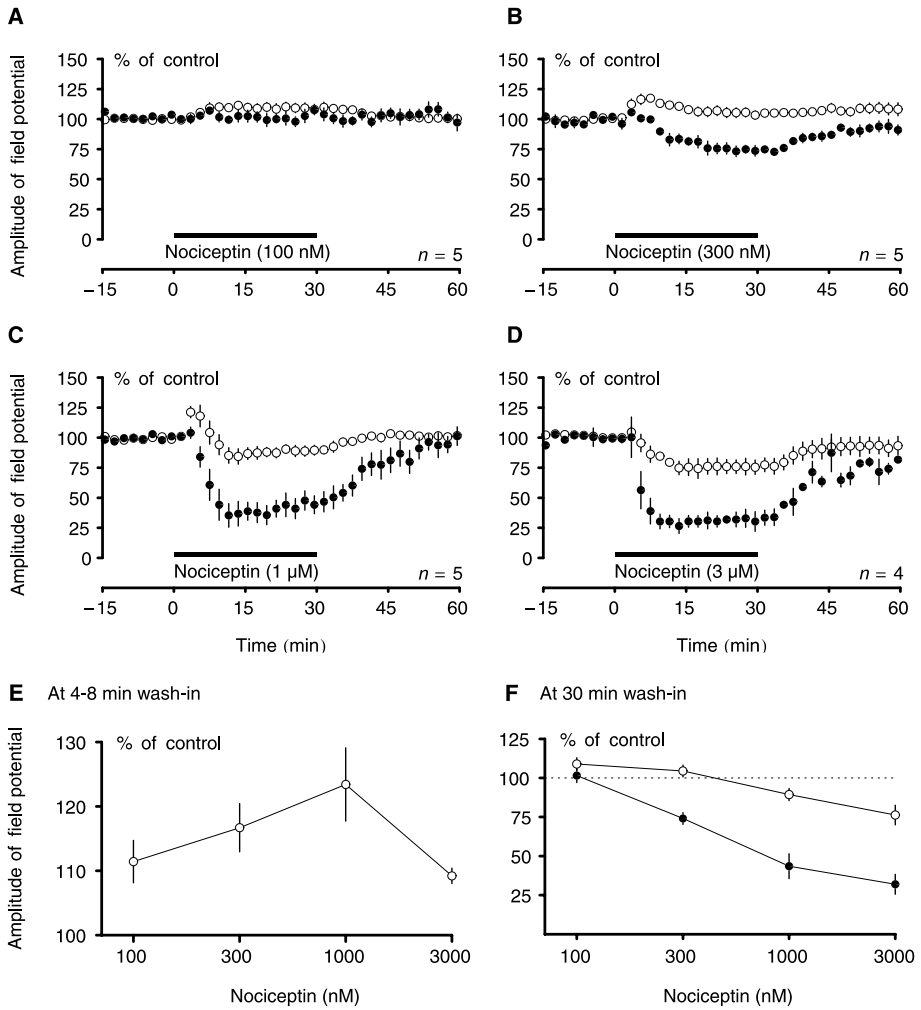

Fig. 2. Nociceptin dose-dependently depressed the polysynaptic potential (closed circles) and had a bidirectional effect on the monosynaptic potential (open circles). (A–D) Mean time courses of the actions of nociceptin (100 nM–3 μM) are shown. (E) Dose–response curve for the maximal potentiation that nociceptin induced in the monosynaptic potential during the first few minutes of wash-in. (F) Dose–response curve for the depression induced by nociceptin at the end of the 30-min wash-in period (open circles: monosynaptic potential, closed circles: polysynaptic potential).

synaptic field potential was bidirectional, showing potentiation of synaptic strength at low concentrations (100–300 nM) and depression at higher concentrations (1–3 μM). Depressions were preceded by a potentiation during the first 4–8 min of wash-in of nociceptin/orphanin FQ at high concentrations probably reflecting the slowly rising nociceptin/orphanin FQ concentration in the recording chamber during wash-in (Figs. 1 and 2 and Table 1).

The effect on the polysynaptic potential, in contrast, showed no bidirectional behaviour but a dose-dependent depression by nociceptin/orphanin FQ (100–3000 nM, Fig. 2). This inhibition was accompanied by an increase in latency of the polysynaptic potential (coefficient of

Table 1. Effects of nociceptin on Aδ-fibre-evoked mono- and polysynaptic field potentials

	Concentration	*n*	Early potential		Late potential	
Nociceptin	100 nM	5	109 ± 4	**	102 ± 5	
	300 nM	5	104 ± 4		74 ± 4	**
	1 μM	5	89 ± 4	**	44 ± 8	**
	3 μM	4	76 ± 6	**	32 ± 6	**
Nocistatin	3 μM	4	99 ± 4	n.s.	89 ± 9	**
Nocistatin/nociceptin	3 μM/1 μM	4	93 ± 5	n.s.†	58 ± 11	n.s.†
Naloxone/nociceptin	10 μM/1 μM	3	89 ± 3	n.s.†	29 ± 14	n.s.†
D-AP-5/nociceptin	50 μM/1 μM	4	87 ± 4	n.s.†	64 ± 13	n.s.†

Values are amplitudes of the mono- and polysynaptic potentials in per cent of control after 30 min of wash-in of nociceptin or nocistatin. *n*, number of experiments; n.s., not significantly different from control ($P > 0.05$); **significantly different from control ($P < 0.01$); n.s.† not significantly different from the action of nociceptin alone ($P > 0.05$).

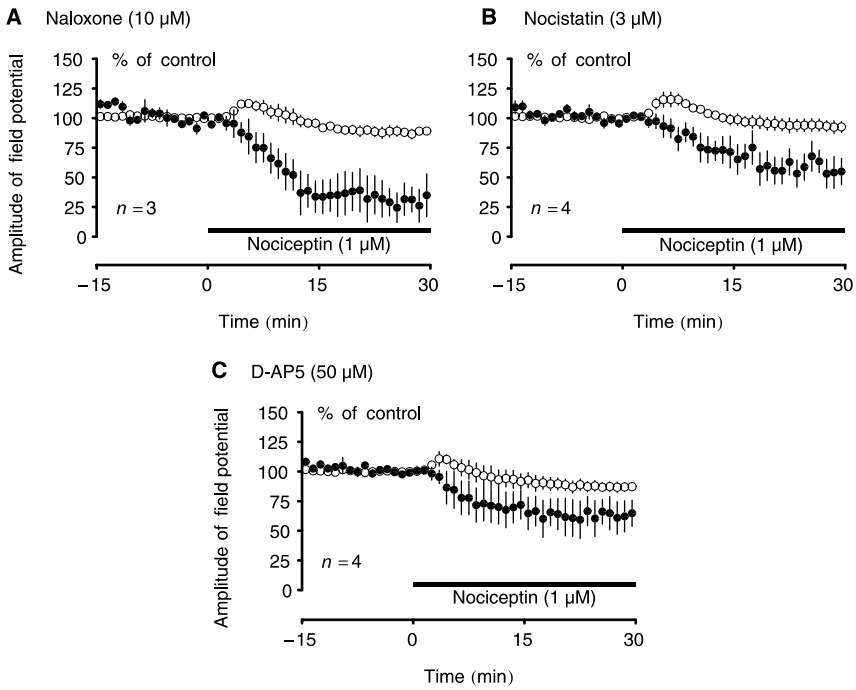

Fig. 3. Neither naloxone nor nocistatin nor D-AP-5 prevented any of the effects of nociceptin. (A–C) Time courses of the action of nociceptin (1 µM) in the presence of the different blockers.

correlation: 0.72, Fig. 4). All effects were reversible at wash-out of nociceptin/orphanin FQ. Table 1 summarises the effects and Fig. 2A–D shows the mean time courses. Fig. 1 shows a typical example.

Lack of effect of naloxone, D-AP-5 and nocistatin on nociceptin/orphanin FQ-induced effects

The non-specific opioid receptor antagonist naloxone was used to examine the possibility that the actions of nociceptin/orphanin FQ were mediated via classical opioid receptors. At a concentration of 10 µM, naloxone failed to significantly modulate the effects of nociceptin/orphanin FQ (1 µM) on mono- or polysynaptic field potentials (Fig. 3A, Table 1). At this concentration naloxone has been shown to abolish the effects of the µ-opioid receptor agonist morphine under identical experimental conditions (Ruscheweyh and Sandkühler, 2000).

Furthermore, the *N*-methyl-D-aspartate (NMDA) receptor antagonist D-AP-5 (50 µM) and the endogenous peptide nocistatin (3 µM), which originates from the same prepropeptide as nociceptin/orphanin FQ, have both been shown to antagonise some actions of nociceptin/orphanin FQ under certain circumstances (Hara et al., 1997; Okuda-Ashitaka et al., 1998; Nicol et al., 1998), but failed to significantly modify any of the effects of nociceptin/orphanin FQ (1 µM, Fig. 3B,C, Table 1). Nocistatin (3 µM) alone had no effect on the monosynaptic field potential and induced a small but significant depression of the polysynaptic field potential (Table 1).

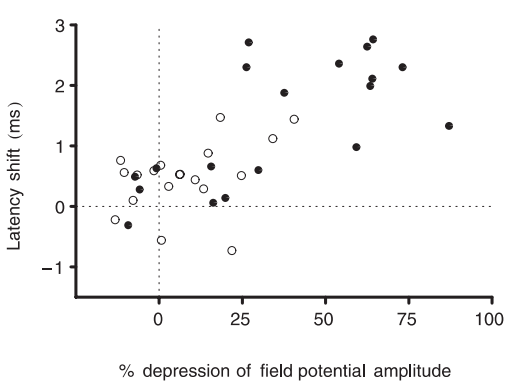

Fig. 4. The nociceptin-induced amplitude depression of the polysynaptic potential was positively correlated with a shift to longer peak latencies of the polysynaptic potential. Each closed circle represents the depression of the amplitude of the late potential and the corresponding latency shift after application of nociceptin (100 nM–3 µM) in a single experiment. The open circles represent the values after wash-out of nociceptin. The coefficient of correlation between amplitude depression and latency shift was 0.71.

DISCUSSION

Nociceptin/orphanin FQ is a recently discovered 17-amino acid peptide (Reinscheid et al., 1995; Meunier et al., 1995) that is the putative endogenous ligand of the orphan opioid receptor ORL-1 (Wick et al., 1994). The ORL-1 receptor is a G-protein-coupled receptor with homology to the classical opioid receptors that is abundant in spinal dorsal horn, particularly in lamina II (Anton et al., 1996; Monteillet-Agius et al., 1998). Nociceptin/orphanin FQ, which structurally resembles endogenous opioids (Ito et al., 2000), is also present in spinal dorsal horn (Riedl et al., 1996; Schulz et al., 1996; Lai et

al., 1997). Rhizotomy studies show that nociceptin/orphanin FQ originates mainly from intrinsic spinal neurones (Riedl et al., 1996). In spite of its structural homology to classical opioids, nociceptin/orphanin FQ does not bind to classical opioid receptors and the ORL-1 receptor does not bind classical opioids (Henderson and McKnight, 1997) suggesting that the nociceptin/ORL-1 system may modulate nociception in spinal cord independently of the classical opioid system.

From behavioural studies, intrathecal nociceptin/orphanin FQ is known to have bidirectional effects on nociception. At low doses, it induces spontaneous nociceptive behaviour (Inoue et al., 1999; Sakurada et al., 1999b), thermal hyperalgesia in the hot-plate and tail-flick tests (Minami et al., 1997, 2000; Hara et al., 1997; Sakurada et al., 1999a), hyperalgesia in the formalin test (Nakano et al., 2000), facilitation of the nociceptive flexor reflex (Xu et al., 1996, 1999) and allodynia (Minami et al., 1997, 2000; Hara et al., 1997). At higher doses, it shows analgesic actions in the tail-flick test (King et al., 1997; Wang et al., 1999b; Tian et al., 1997), formalin test (Nakano et al., 2000; Erb et al., 1997; Wang et al., 1999a; Yamamoto et al., 1997), on the nociceptive flexor reflex (Xu et al., 1996), and anti-allodynic actions (Hao et al., 1998).

Bidirectional actions have also been found in electrophysiological *in vivo* studies. In extracellular recordings from single spinal dorsal horn neurones, nociceptin/orphanin FQ at lower doses sometimes showed facilitation of C-fibre-evoked responses instead of the inhibition consistently seen at higher doses (Stanfa et al., 1996). And while nociceptin/orphanin FQ mostly depressed the responses to exogenously applied NMDA in trigeminal dorsal horn neurones measured by extracellular single neurone recording, in some cells, facilitation or a biphasic response were observed (Wang et al., 1996).

Previous *in vitro* studies, in contrast, failed to detect any bidirectional effects of nociceptin/orphanin FQ in spinal cord. Polysynaptic A- and C-fibre-evoked ventral root potentials measured in the neonatal hemisected spinal cord preparation were dose-dependently depressed but not potentiated (Faber et al., 1996). Likewise, presumably monosynaptic excitatory postsynaptic currents (EPSCs) evoked by stimulation of unidentified primary afferent fibres in substantia gelatinosa neurones recorded by the patch-clamp technique in spinal cord slices were also dose-dependently depressed but not potentiated by nociceptin/orphanin FQ (Liebel et al., 1997; Lai et al., 1997).

The present spinal cord–dorsal root slice preparation enabled us to record postsynaptically evoked potentials that are triggered by stimulation of Aδ-fibres as identified by their characteristic conduction velocities. Mono- and polysynaptic Aδ-fibre-evoked potentials were recorded simultaneously and their modulation by nociceptin/orphanin FQ could therefore be compared within the same experiment. We found bidirectional effects of nociceptin/orphanin FQ on the monosynaptic Aδ-fibre-evoked potential with potentiation at low doses and dose-dependent depression at higher doses. This fits well with the behavioural data and demonstrates that synaptic transmission from Aδ-fibres, many of which are nociceptive fibres, is modulated by nociceptin/orphanin FQ. The polysynaptic Aδ-fibre-evoked field potential, in contrast, showed no potentiation but only a dose-dependent depression. This is consistent with the data from ventral root recordings (Faber et al., 1996) and in line with our earlier observation that monosynaptic primary afferent synaptic transmission in superficial spinal dorsal horn can be modulated qualitatively differently from polysynaptic transmission (Ruscheweyh and Sandkühler, 2000). The potency of nociceptin/orphanin FQ to depress Aδ-fibre-evoked responses in this study was comparable to the potencies found in previous studies (Liebel et al., 1997; Faber et al., 1996). The maximal average inhibition by nociceptin/orphanin FQ was 24% for the monosynaptic potential (37–51% in other studies: Liebel et al., 1997; Lai et al., 1997) and 68% for the polysynaptic potential (70% for ventral root potentials: Faber et al., 1996).

Field potentials show very little variation of amplitudes over the time. Perhaps this is the reason why the small and transient potentiation of the monosynaptic Aδ-fibre-evoked potential could be detected in this study but not in patch-clamp studies (Lai et al., 1997; Liebel et al., 1997). Another explanation would be that the cell dialysis occurring during whole-cell patch-clamp recordings prevents postsynaptic effects of nociceptin/orphanin FQ. Indeed, the inhibition of EPSCs by nociceptin/orphanin FQ in substantia gelatinosa neurones has been found to be purely presynaptic in a patch-clamp study (Liebel et al., 1997) while nociceptin/orphanin FQ depressed currents evoked by exogenous application of NMDA onto the recorded cell in extracellular single-unit recordings in trigeminal dorsal horn neurones (Wang et al., 1996), a clearly postsynaptic action of nociceptin/orphanin FQ. The present results suggest that nociceptin/orphanin FQ not only modulates transmission at the first sensory synapse of Aδ-fibre pathways but also affects synapses further downstream.

In some constellations, the actions of nociceptin/orphanin FQ have been antagonised by the unspecific opioid receptor antagonist naloxone (King et al., 1997; Rossi et al., 1996; Jhamandas et al., 1998), but in most studies, this was not the case (Xu et al., 1996; Yamamoto et al., 1997; Faber et al., 1996; Lai et al., 1997; Liebel et al., 1997; Wang et al., 1996; Erb et al., 1997). As ORL-1 receptors and μ-opioid receptors are not colocalised on the same neurones in spinal dorsal horn and other regions of the CNS (Monteillet-Agius et al., 1998), and virtually no cross-binding is seen (Henderson and McKnight, 1997), the two systems seem to represent independent circuitries involved in the modulation of nociceptive information. Consistently, application of the μ-opioid receptor agonist morphine under the same experimental conditions had no effect on the monosynaptic Aδ-fibre-evoked field potential (Ruscheweyh and Sandkühler, 2000) and thus showed qualitatively different results as compared with nociceptin/orphanin FQ, which had bidirectional effects on the same potential. In addition, in the present study, naloxone did not prevent the effects of nociceptin/orphanin

FQ, indicating that the observed effects were not mediated by classical opioid receptors.

The allodynia evoked by small doses of intrathecal nociceptin/orphanin FQ is thought to involve activation of NMDA receptors as it is antagonised by the NMDA receptor antagonist D-AP-5 and cannot be evoked in NMDA glutamate receptor ε1 knock-out mice (Minami et al., 2000; Hara et al., 1997). The concomitant hyperalgesia, in contrast, is not sensitive to D-AP-5 (Minami et al., 2000; Hara et al., 1997). In this study, bath application of D-AP-5 affected neither the initial potentiation nor the depression of Aδ-fibre-evoked potentials by nociceptin/orphanin FQ. This is consistent with the notion that mechanical allodynia is mediated by primary afferent Aβ-fibres and not Aδ-fibres.

The peptide nocistatin is another cleavage product of the nociceptin/orphanin FQ precursor peptide prepronociceptin but it does not bind to the ORL-1 receptor. It has been reported to block some of the effects of nociceptin/orphanin FQ such as the allodynia and hyperalgesia evoked by small doses of nociceptin/orphanin FQ (Okuda-Ashitaka et al., 1998) or the inhibition of glutamate release from spinal cord dorsal horn (Nicol et al., 1998) but the interaction seems to be complex and difficult to reproduce (Xu et al., 1999; Yamamoto and Sakashita, 1999). Nocistatin itself has been reported to act as an analgesic (Nakano et al., 2000; Yamamoto and Sakashita, 1999), to have no effect on pain thresholds (Okuda-Ashitaka et al., 1998) and to produce hyperalgesia (Zeilhofer et al., 2000; Xu et al., 1999). In substantia gelatinosa single neurone recordings, nocistatin has been shown to selectively inhibit inhibitory transmission (Zeilhofer et al., 2000). In the present study, nocistatin showed no effect on monosynaptic field potentials and produced a small but significant inhibition of polysynaptic potentials. This is consistent with the notion that the monosynaptic potential is purely excitatory while the polysynaptic potential is modulated by inhibitory interneurones (Ruscheweyh and Sandkühler, 2000). Nocistatin did not prevent or modify any of the actions of nociceptin/orphanin FQ. That could be explained by the finding that, in spinal dorsal horn, nociceptin/orphanin FQ and nocistatin act on two non-overlapping synaptic populations: nociceptin/orphanin FQ only on excitatory synapses and nocistatin only on inhibitory synapses (Zeilhofer et al., 2000).

CONCLUSION

This is the first *in vitro* study showing bidirectional effects of nociceptin/orphanin FQ on sensory processing in spinal cord. In line with previous behavioural studies nociceptin facilitated monosynaptic transmission in Aδ-fibres at low concentrations and depressed transmission at higher concentrations.

REFERENCES

Anton, B., Fein, J., To, T., Li, X., Silberstein, L., Evans, C.J., 1996. Immunohistochemical localization of ORL-1 in the central nervous system of the rat. J. Comp. Neurol. 368, 229–251.

Erb, K., Liebel, J.T., Tegeder, I., Zeilhofer, H.U., Brune, K., Geisslinger, G., 1997. Spinally delivered nociceptin/orphanin FQ reduces flinching behaviour in the rat formalin test. NeuroReport 8, 1967–1970.

Faber, E.S., Chambers, J.P., Evans, R.H., Henderson, G., 1996. Depression of glutamatergic transmission by nociceptin in the neonatal rat hemisected spinal cord preparation *in vitro*. Br. J. Pharmacol. 119, 189–190.

Hao, J.X., Xu, I.S., Wiesenfeld-Hallin, Z., Xu, X.J., 1998. Anti-hyperalgesic and anti-allodynic effects of intrathecal nociceptin/orphanin FQ in rats after spinal cord injury, peripheral nerve injury and inflammation. Pain 76, 385–393.

Hara, N., Minami, T., Okuda-Ashitaka, E., Sugimoto, T., Sakai, M., Onaka, M., Mori, H., Imanishi, T., Shingu, K., Ito, S., 1997. Characterization of nociceptin hyperalgesia and allodynia in conscious mice. Br. J. Pharmacol. 121, 401–408.

Henderson, G., McKnight, A.T., 1997. The orphan opioid receptor and its endogenous ligand nociceptin/orphanin FQ. Trends Pharmacol. Sci. 18, 293–300.

Inoue, M., Kobayashi, M., Kozaki, S., Zimmer, A., Ueda, H., 1998. Nociceptin/orphanin FQ-induced nociceptive responses through substance P release from peripheral nerve endings in mice. Proc. Natl. Acad. Sci. USA 95, 10949–10953.

Inoue, M., Shimohira, I., Yoshida, A., Zimmer, A., Takeshima, H., Sakurada, T., Ueda, H., 1999. Dose-related opposite modulation by nociceptin/orphanin FQ of substance P nociception in the nociceptors and spinal cord. J. Pharmacol. Exp. Ther. 291, 308–313.

Ito, S., Okuda-Ashitaka, E., Imanishi, T., Minami, T., 2000. Central roles of nociceptin/orphanin FQ and nocistatin: allodynia as a model of neural plasticity. In: Sandkühler, J., Bromm, B., Gebhart, G.F. (Eds.), Nervous System Plasticity and Chronic Pain, Progress in Brain Research, Vol. 129. Elsevier, Amsterdam, pp. 205–218.

Jhamandas, K.H., Sutak, M., Henderson, G., 1998. Antinociceptive and morphine modulatory actions of spinal orphanin FQ. Can. J. Physiol. Pharmacol. 76, 314–324.

King, M.A., Rossi, G.C., Chang, A.H., Williams, L., Pasternak, G.W., 1997. Spinal analgesic activity of orphanin FQ/nociceptin and its fragments. Neurosci. Lett. 223, 113–116.

Lai, C.C., Wu, S.Y., Dun, S.L., Dun, N.J., 1997. Nociceptin-like immunoreactivity in the rat dorsal horn and inhibition of substantia gelatinosa neurons. Neuroscience 81, 887–891.

Liebel, J.T., Swandulla, D., Zeilhofer, H.U., 1997. Modulation of excitatory synaptic transmission by nociceptin in superficial dorsal horn neurones of the neonatal rat spinal cord. Br. J. Pharmacol. 121, 425–432.

Meunier, J.C., Mollereau, C., Toll, L., Suaudeau, C., Moisand, C., Alvinerie, P., Butour, J.L., Guillemot, J.C., Ferrara, P., Monsarrat, B., 1995. Isolation and structure of the endogenous agonist of opioid receptor-like ORL1 receptor. Nature 377, 532–535.

Minami, T., Okuda-Ashitaka, E., Mori, H., Sakimura, K., Watanabe, M., Mishina, M., Ito, S., 2000. Characterization of nociceptin/orphanin FQ-induced pain responses in conscious mice: neonatal capsaicin treatment and *N*-methyl-D-aspartate receptor GluRε subunit knockout mice. Neuroscience 97, 133–142.

Minami, T., Okuda-Ashitaka, E., Nishizawa, M., Mori, H., Ito, S., 1997. Inhibition of nociceptin-induced allodynia in conscious mice by prostaglandin D2. Br. J. Pharmacol. 122, 605–610.

Mogil, J.S., Grisel, J.E., Zhangs, G., Belknap, J.K., Grandy, D.K., 1996. Functional antagonism of μ-, δ- and κ-opioid antinociception by orphanin FQ. Neurosci. Lett. 214, 131–134.

Monteillet-Agius, G., Fein, J., Anton, B., Evans, C.J., 1998. ORL-1 and μ-opioid receptor antisera label different fibers in areas involved in pain processing. J. Comp. Neurol. 399, 373–383.
Moore, K.A., Baba, H., Woolf, C.J., 2000. Synaptic transmission and plasticity in the superficial dorsal horn. In: Sandkühler, J., Bromm, B., Gebhart, G.F. (Eds.), Nervous System Plasticity and Chronic Pain, Progress in Brain Research, Vol. 129. Elsevier, Amsterdam, pp. 63–80.
Morgan, M.M., Grisel, J.E., Robbins, C.S., Grandy, D.K., 1997. Antinociception mediated by the periaqueductal gray is attenuated by orphanin FQ. NeuroReport 8, 3431–3434.
Nakano, H., Minami, T., Abe, K., Arai, T., Tokumura, M., Ibii, N., Okuda-Ashitaka, E., Mori, H., Ito, S., 2000. Effect of intrathecal nocistatin on the formalin-induced pain in mice versus that of nociceptin/orphanin FQ. J. Pharmacol. Exp. Ther. 292, 331–336.
Nicol, B., Lambert, D.G., Rowbotham, D.J., Okuda-Ashitaka, E., Ito, S., Smart, D., McKnight, A.T., 1998. Nocistatin reverses nociceptin inhibition of glutamate release from rat brain slices. Eur. J. Pharmacol. 356, R1–R3.
Okuda-Ashitaka, E., Minami, T., Tachibana, S., Yoshihara, Y., Nishiuchi, Y., Kimura, T., Ito, S., 1998. Nocistatin, a peptide that blocks nociceptin action in pain transmission. Nature 392, 286–289.
Reinscheid, R.K., Nothacker, H.P., Bourson, A., Ardati, A., Henningsen, R.A., Bunzow, J.R., Grandy, D.K., Langen, H., Monsma, F.J.J., Civelli, O., 1995. Orphanin FQ: a neuropeptide that activates an opioidlike G protein-coupled receptor. Science 270, 792–794.
Riedl, M., Shuster, S., Vulchanova, L., Wang, J., Loh, H.H., Elde, R., 1996. Orphanin FQ/nociceptin-immunoreactive nerve fibers parallel those containing endogenous opioids in rat spinal cord. NeuroReport 7, 1369–1372.
Rossi, G.C., Leventhal, L., Pasternak, G.W., 1996. Naloxone sensitive orphanin FQ-induced analgesia in mice. Eur. J. Pharmacol. 311, R7–R8.
Ruscheweyh, R., Sandkühler, J., 1999. Differential action of spinal analgesics on mono- and polysynaptic Aδ-fibre-evoked field potentials in spinal dorsal horn *in vitro*. In: Abstracts of the 9th World Congress on Pain. IASP, Seattle, WA, p. 416.
Ruscheweyh, R., Sandkühler, J., 2000. Differential action of spinal analgesics on mono- versus polysynaptic Aδ-fibre-evoked field potentials in superficial spinal dorsal horn *in vitro*. Pain 88, 97–108.
Sakurada, C., Sakurada, S., Katsuyama, S., Sasaki, J., Tan-No, K., Sakurada, T., 1999a. Involvement of tachykinin NK1 receptors in nociceptin-induced hyperalgesia in mice. Brain Res. 841, 85–92.
Sakurada, T., Katsuyama, S., Sakurada, S., Inoue, M., Tan-No, K., Kisara, K., Sakurada, C., Ueda, H., Sasaki, J., 1999b. Nociceptin-induced scratching, biting and licking in mice: involvement of spinal NK1 receptors. Br. J. Pharmacol. 127, 1712–1718.
Sandkühler, J., 2000. Learning and memory in pain pathways. Pain 88, 113–118.
Schulz, S., Schreff, M., Nuss, D., Gramsch, C., Hollt, V., 1996. Nociceptin/orphanin FQ and opioid peptides show overlapping distribution but not co-localization in pain-modulatory brain regions. NeuroReport 7, 3021–3025.
Stanfa, L.C., Chapman, V., Kerr, N., Dickenson, A.H., 1996. Inhibitory action of nociceptin on spinal dorsal horn neurones of the rat, *in vivo*. Br. J. Pharmacol. 118, 1875–1877.
Tian, J.H., Xu, W., Fang, Y., Mogil, J.S., Grisel, J.E., Grandy, D.K., Han, J.S., 1997. Bidirectional modulatory effect of orphanin FQ on morphine-induced analgesia: antagonism in brain and potentiation in spinal cord of the rat. Br. J. Pharmacol. 120, 676–680.
Wang, J.L., Zhu, C.B., Cao, X.D., Wu, G.C., 1999a. Distinct effect of intracerebroventricular and intrathecal injections of nociceptin/orphanin FQ in the rat formalin test. Regul. Pept. 79, 159–163.
Wang, X.M., Zhang, K.M., Mokha, S.S., 1996. Nociceptin (orphanin FQ), an endogenous ligand for the ORL1 (opioid-receptor-like 1) receptor, modulates responses of trigeminal neurons evoked by excitatory amino acids and somatosensory stimuli. J. Neurophysiol. 76, 3568–3572.
Wang, Y.Q., Zhu, C.B., Wu, G.C., Cao, X.D., Wang, Y., Cui, D.F., 1999b. Effects of orphanin FQ on endomorphin-1 induced analgesia. Brain Res. 835, 241–246.
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. 27, 37–44.
Xu, I.S., Hashemi, M., Calo, G., Regoli, D., Wiesenfeld-Hallin, Z., Xu, X.J., 1999. Effects of intrathecal nocistatin on the flexor reflex and its interaction with orphanin FQ nociceptin. NeuroReport 10, 3681–3684.
Xu, X.J., Hao, J.X., Wiesenfeld-Hallin, Z., 1996. Nociceptin or antinociceptin: potent spinal antinociceptive effect of orphanin FQ/nociceptin in the rat. NeuroReport 7, 2092–2094.
Yamamoto, T., Nozaki-Taguchi, N., Kimura, S., 1997. Analgesic effect of intrathecally administered nociceptin, an opioid receptor-like 1 receptor agonist, in the rat formalin test. Neuroscience 81, 249–254.
Yamamoto, T., Sakashita, Y., 1999. Effect of nocistatin and its interaction with nociceptin/orphanin FQ on the rat formalin test. Neurosci. Lett. 262, 179–182.
Zeilhofer, H.U., Selbach, U.M., Guhring, H., Erb, K., Ahmadi, S., 2000. Selective suppression of inhibitory synaptic transmission by nocistatin in the rat spinal cord dorsal horn. J. Neurosci. 20, 4922–4929.

(*Accepted* 29 *May* 2001)