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Modulation of excitatory synaptic transmission by nociceptin in superficial dorsal horn neurones of the neonatal rat spinal cord

Jörg T. Liebel, Dieter Swandulla & [1]Hanns Ulrich Zeilhofer

Institut für Experimentelle und Klinische Pharmakologie und Toxikologie, Universität Erlangen-Nürnberg, Universitätstrasse 22, D-91054 Erlangen, Germany

1 The modulatory actions of nociceptin/orphanin FQ on excitatory synaptic transmission were studied in superficial dorsal horn neurones in transverse slices from 7 to 14 day old rats.

2 Glutamatergic excitatory postsynaptic currents (e.p.s.cs) were recorded from the somata of the neurones in the whole-cell patch-clamp configuration. E.p.s.cs were evoked by extracellular electrical stimulation (100 μs, 3–10 V) of the ipsilateral dorsal root entry zone by use of a glass electrode. E.p.s.cs with constant short latency (<2.3 ms) and with no failures upon stimulation were assumed to be monosynaptic. These e.p.s.cs occurred with an average latency of 1.72 ± 0.098 ms and exhibited a fast decay with a time constant, τ, of 4.8 ± 0.53 ms ($n=30$).

3 Nociceptin reversibly reduced the amplitudes of e.p.s.cs in a concentration-dependent manner in 25 out of 27 cells tested. Average maximum inhibition was $51.6 \pm 5.7\%$ (mean $\pm$ s.e.mean; $n=9$), at concentrations >3 μM. EC_{50} was 485 ± 47 nM and the Hill coefficient was 1.29 ± 0.09.

4 Inhibition of synaptic transmission by nociceptin (10 μM) was insensitive to the non-specific opioid receptor antagonist naloxone (10 μM) indicating that nociceptin did not act via classical opioid receptors.

5 In order to determine the site of action of nociceptin spontaneous miniature e.p.s.cs (m-e.p.s.cs) were recorded. Nociceptin reduced the frequency of m-e.p.s.cs in 6 out of 7 cells but had no effect on their amplitude distribution or on their time course. These findings suggest a pre- rather than a postsynaptic modulatory site of action. This is in line with the finding that current responses elicited by extracellular application of L-glutamate (10 μM) were not affected by nociceptin (10 μM; $n=7$).

6 No positive correlation was found between the degree of inhibition by nociceptin (10 μM) and by the mixed δ- and μ-receptor agonist methionine-enkephalin (10 μM). This suggests that both neuropeptides acted on different but perhaps overlapping populations of synaptic connections.

7 Our results indicate that nociceptin inhibits excitatory synaptic transmission in the superficial layers of the rat dorsal horn by acting on presynaptic, presumably ORL_1 receptors. This may be an important mechanism for spinal sensory information processing including nociception.

Keywords: Nociceptin; orphanin FQ; methionine-enkephalin; synaptic transmission; ORL_1 receptor; spinal cord; nociception; hyperalgesia; analgesia; pain

Introduction

Nociceptin, also called orphanin FQ, is a heptadekapeptide recently identified as a putative endogenous ligand of an 'orphan' opioid-like receptor (ORL_1) (Meunier *et al*., 1995; Reinscheid *et al*., 1995). Nociceptin has negligible affinity to μ, δ and κ opioid receptors but exhibits high affinity binding to the ORL_1 receptors (Reinscheid *et al*., 1995). The ORL_1 receptor belongs to the seven transmembrane receptor family and has about 50–60% homology with μ, δ and κ opioid receptors (Bunzow *et al*., 1994; Chen *et al*., 1994; Fukuda *et al*., 1994; Mollereau *et al*., 1994; Wang *et al*., 1994; Wick *et al*., 1994). Like opioid receptors the ORL_1 receptor is negatively coupled to adenylate cyclase activity via G proteins (Meunier *et al*., 1995; Reinscheid *et al*., 1995; Sim *et al*., 1996). While classical opioidergic peptides are well known modulators of excitatory synaptic transmission in the dorsal horn of the rat spinal cord (Go & Yaksh, 1987; Chen & Huang, 1991; Collin *et al*., 1991; Kangrga & Randic, 1991; Rusin & Randic, 1991; Hori *et al*., 1992; Glaum *et al*., 1994; Kolaj & Randic, 1996), little is known about the effects of nociceptin on synaptic transmission.

In Chinese hamster ovary (CHO) cells transfected with the gene encoding for ORL_1 receptors nociceptin inhibits forskolin-induced accumulation of adenosine 3′:5′-cyclic monophosphate (cyclic AMP) (Reinscheid *et al*., 1995). It activates an inwardly rectifying K^+ conductance in dorsal raphe nucleus neurones (Vaughan & Christie, 1996) and inhibits Ca^{2+} channel currents in the human neuroblastoma cell line SH-SY5Y, where it also augments carbachol-induced Ca^{2+} release (Connor *et al*., 1996).

It has been shown that nociceptin exhibits both pro- and antinociceptive activity. Pronociceptive actions have been found after intrathecal and intracerebroventricular injection of nociceptin in the mouse hot-plate and tail-flick assay (Reinscheid *et al*., 1995; Meunier *et al*., 1995). Antinociceptive effects have been observed after local application of nociceptin to the lumbar spinal cord (Erb *et al*., unpublished result; Stanfa *et al*., 1996; Xu *et al*., 1996). However, the cellular mechanisms of these effects are not fully understood. One of the major sites of the antinociceptive action of classical opioid peptides is synaptic transmission within the dorsal horn of the spinal cord. Several studies have shown that nociceptin as well as ORL_1 receptors are expressed in the grey matter of the spinal cord dorsal horn (Lachowicz *et al*., 1995; Anton *et al*., 1996; Houtani *et al*., 1996). Especially the substantia gelatinosa shows dense staining for ORL_1 receptors (Anton *et al*., 1996). In the present study we have investigated the effects of nociceptin on excitatory synaptic transmission in the rat spinal cord dorsal horn. We have found that nociceptin reduces the amplitudes of evoked e.p.s.cs most likely through activation of presynaptic ORL_1 receptors. These findings may explain the antinociceptive activity of nociceptin.

[1] Author for correspondence.

Methods

Slice preparation

Sprague-Dawley rats, 7 to 14 days old, were anaesthetized with ether and killed by decapitation. The lumbar segments of the spinal cord were excised, kept in ice-cold standard external solution, which contained (in mM): NaCl 125, $NaHCO_3$ 26, NaH_2PO_4 1.25, KCl 2.5, $CaCl_2$ 2, $MgCl_2$ 1 and glucose 10 (pH 7.30, 315 mosmol l^{-1}). The dorsal roots were cut near the entry zone and the ventral side of the spinal cord was glued onto a gelatine block and 250 μm thick transverse slices were cut by a vibratome (Campden, Loughborough, U.K.). Before the electrophysiological experiments slices were incubated in standard external solution bubbled with 95% O_2/5% CO_2 (carbogene) at 37°C for 1 to 8 h after preparation.

Electrophysiological recordings

Electrophysiological recordings were made from neurones of the superficial dorsal horn (located about 100–120 μm from the dorsal surface of the slice) under visual control by use of the infrared gradient contrast technique coupled to a video microscopy system (Infracontrast, Luigs & Neumann, Ratingen, Germany; Dodt, 1992; Stuart *et al*., 1993; Dodt & Zieglgänsberger, 1994). A nylon mesh was placed on top of the slice and held in position by a platinum wire loop. Slices were completely submerged and continuously superfused with standard external solution bubbled with 95% O_2, 5% CO_2 at a rate of 1–2 ml min^{-1} at room temperature (air conditioned room; 19–21°C). A new slice was used for every recording. An upright microscope (Zeiss Axioskop FS, Jena, Germany) was used equipped with 40 × water immersion lenses (Zeiss, Jena, Germany) and a standard video camera (CF-8, Kappa, Gleichen, Germany). Patch pipettes pulled from borosilicate glass (Kimax 51, Kimble, U.S.A.) and fire polished typically had resistances of 2–3 MΩ when filled with standard internal solution containing (in mM): K-gluconate 130, KCl 20, $MgCl_2$ 2, EGTA 0.05, Na-ATP 3, Na-GTP 0.1 and Na-HEPES 10 (pH 7.30). QX-314 (5 mM) was added to the internal solution to block voltage-activated sodium currents in the neurone recorded from. Picrotoxin (50 μM) and strychnine (2 μM) were added to the bathing solution to block inhibitory postsynaptic currents (i.p.s.cs). Excitatory postsynaptic currents (e.p.s.cs) were elicited by ipsilateral extracellular electrical stimulation (100 μs, 3–10 V; AM Systems, Everett, WA, U.S.A.) of the dorsal root entry zone by a glass electrode (cathode; KIMAX 51, inner tip diameter about 1 μm) filled with 1 M NaCl. The anode was placed about 2 mm from the dorsal surface of the spinal cord in the recording chamber. In most cases attached dorsal roots were too short to classify the afferent fibres according to their conduction velocity and to stimulate defined classes of afferent fibres reliably. E.p.s.cs were

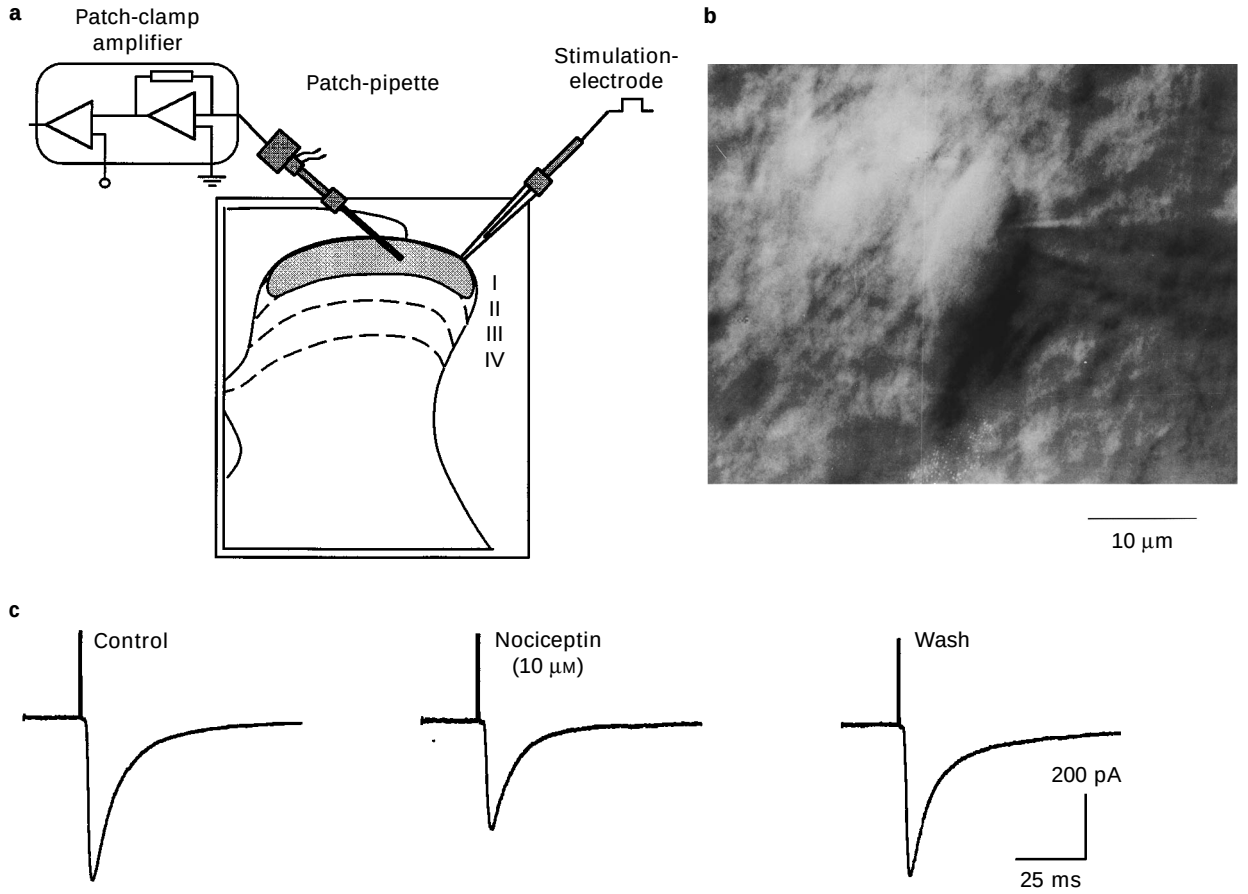

Figure 1 Modulation by nociceptin of monosynaptic e.p.s.cs from visually identified neurones of the superficial dorsal horn of the spinal cord. (a) Experimental set-up. Neurones in the superficial layers (laminae I and II) of the dorsal horn were visually identified. E.p.s.cs were elicited every 15 s by electrical stimulation of the dorsal root entry zone and recorded in the whole-cell configuration of the patch-clamp technique at a holding potential of −80 mV. Glycine and $GABA_A$ receptor mediated i.p.s.cs were blocked by adding strychnine (2 μM) and picrotoxin (50 μM) to the extracellular solution. (b) Superficial dorsal horn neurone with a patch-pipette attached visualized with the IR gradient contrast technique by 40 × water immersion lenses and a 4 × magnification lens in front of the video camera. Top: Dorsal surface of the spinal cord. (c) Averages of 20 traces recorded in a superficial dorsal horn neurone immediately before application of nociceptin, after a steady state of inhibition had been reached (5 to 10 min after application of nociceptin) and after wash out of nociceptin (10 to 15 min after switching back to control solution).

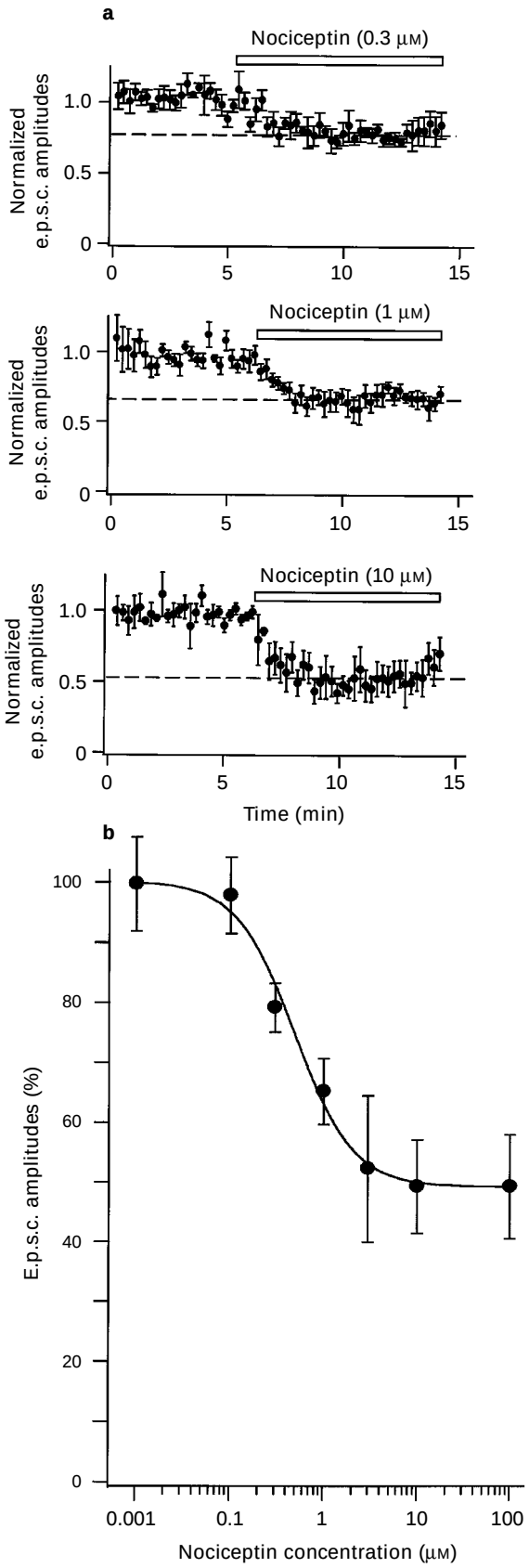

Figure 2 Time course and concentration-dependence of the inhibition of excitatory synaptic transmission by nociceptin. (a) Time course of inhibition of excitatory synaptic transmission by different concentrations of nociceptin. Normalized e.p.s.c. amplitudes (●) were plotted versus time. Steady state inhibition was reached for all concentrations tested within 3 to 5 min after application of nociceptin. (b) Concentration-effect curve. Ordinate scale: Effect of nociceptin (●) on e.p.s.cs expressed as percentage of reduction of e.p.s.c. amplitudes. Abscissa scale: nociceptin concentration on a logarithmic scale. In (a) and (b) points show means and vertical lines s.e.mean. Cells which exhibited no e.p.s.c. inhibition in the presence of 0.1 and 0.01 μM nociceptin were afterwards challenged with a higher concentration to ensure that the synaptic connection of this experiment was sensitive to nociceptin. Only cells with e.p.s.cs which responded were then included. Data points were fitted to the equation $y = y_{max} - [(y_{max} - y_{min})/(1 + (EC_{50}/C)^{n_H})]$ by use of Jandel Sigma Plot 3.0 software (Jandel GmbH, Erkrath, Germany); y_{max} is the normalized e.p.s.c. amplitude under control conditions, y_{min} the relative e.p.s.c. amplitude in the presence of saturating concentrations of nociceptin, C is the nociceptin concentration, EC_{50} is the half maximum effective concentration of nociceptin and n_H is the Hill coefficient. Average maximum inhibition was 51.6%, EC_{50} was 485 ± 47 nM and the Hill coefficient 1.29 ± 0.09. The number of experiments was 3, 8, 5, 6, 5, 6 and 3 for 0.01, 0.1, 0.3, 1.0, 3.0, 10 and 100 μM nociceptin, respectively. Inhibition was statistically significant ($P \leq 0.05$ to $P \leq 0.005$) for all concentrations ≥ 300 nM.

recorded in the whole-cell configuration of the patch-clamp technique with an EPC-7 patch-clamp amplifier (List Elektronik, Pfungstadt, Germany) and the Pulse Programme (HEKA Electronik, Lambrecht, Germany) running on a Macintosh Quadra 800 computer. Only cells with leakage currents < 100 pA, access resistances < 10 MΩ and with changes in the access resistance of $< 10\%$ during the recording time were included into the analysis. Spontaneous miniature excitatory postsynaptic currents (m-e.p.s.cs) were recorded in the presence of tetrodotoxin (TTX, 1 μM). In these experiments the extracellular recording solution contained 10 instead of 2.5 mM KCl to increase the baseline frequency of e.p.s.cs. Currents were sampled at 2 kHz and analysed by a custom made programme running under the IgorPro 3.0 programme (Wavemetrics, Lake Oswego, Oregon, U.S.A.). Drug solutions (nociceptin, nociceptin and naloxone, and methionine-enkephalin) were applied by bath perfusion at a rate of $1-2$ ml min^{-1}.

Chemicals

Nociceptin (purity $> 95\%$) was purchased from Neosystem Laboratoire (Strasbourg, France) and from Prof. C.-M. Becker (Institut für Biochemie, Universität Erlangen, Germany). Nociceptin was dissolved in distilled water and stored in aliquots (1 mM) at -20°C. Fresh dilutions were made with standard external solution on the day of the experiment.

All inorganic salts were from Merck, Darmstadt, Germany. L-glutamate, naloxone, tetrodotoxin (TTX), N-[2-hydroxyethyl]piperazine-N′-[2-ethanesulphonic acid (HEPES), ethylene glycol-*bis*(β-aminoethyl ether) N,N,N′,N′-tetraacetic acid (EGTA), ATP, picrotoxin, strychnine, methionine-enkephalin, and D-Ala^2, N-Me-Phe^4, Gly^5-ol-enkephalin (DAMGO) were from Sigma Chemie (Deisenhofen, Germany). 6-Nitro-7-sulphamoylbenzo[f]quinoxaline-2,3-dione (NBQX) and D(−)-2-amino-5-phosphonovaleric acid (D-APV) were from Tocris Cookson (Bristol, U.K.). Lidocaine N-ethyl bromid (QX-314) was from RBI (Natick, MA, U.S.A.).

Statistics

Data are given as mean $\pm$ s.e.mean. Statistical analysis was performed by the Wilcoxon rank test. Concentration-response curves were fitted with the Marquardt-Levenberg algorithm (Marquardt, 1963) running under Jandel Sigma Plot 3.0 software (Jandel GmbH, Erkrath, Germany).

Results

Effect of nociceptin on evoked e.p.s.cs in superficial layer rat dorsal horn neurones

E.p.s.cs were recorded in the whole-cell configuration of the patch-clamp technique. After being ruptured, the patch neu-

rones were recorded in the current-clamp mode to determine their resting membrane potential. Superficial dorsal horn neurones of newborn rats had average resting membrane potentials of -68 ± 1.9 mV with the solutions used. After being switched to the voltage-clamp mode hyperpolarizing voltage steps were applied every minute to control input membrane resistances and access resistances. The average input membrane resistance of the cells under study was 512 ± 33 MΩ at -80 mV and the access resistances were <10 MΩ. E.p.s.cs were evoked by extracellular electrical stimulation of the dorsal root entry zone every 15 s and recorded from the somata of superficial layer neurones (Figure 1a). Figure 1b shows an example of such a neurone visualized by the infrared gradient contrast technique (Dodt & Zieglgänsberger, 1994). E.p.s.cs were recorded in isolation by blocking i.p.s.cs with strychnine (2 μM) and picrotoxin (50 μM). The remaining e.p.s.cs were completely blocked by NBQX (10 μM) and D-APV (50 μM) indicating that they were mediated by ionotropic L-glutamate receptors of the α-amino-3-hydroxy-5-methyl-4-isoxazoleproprionic acid (AMPA) and/or N-methyl-D-aspartate (NMDA) type. Most e.p.s.cs occurred with short latencies (<2.6 ms) and showed no failures upon stimulation and little variability in synaptic latency ($<\pm 0.25$ ms) with increasing stimulus intensities. These e.p.s.cs were assumed to represent mainly monosynaptic events and only those were used for experiments with nociceptin. On average, postsynaptic current responses occurred with a latency of 1.72 ± 0.098 ms and were fast inactivating with a time constant of $\tau_{fast} = 4.8 \pm 0.53$ ms ($n = 27$). In about 50% of the cells e.p.s.cs exhibited a second slower current decay phase with a time constant of $\tau_{slow} = 33 \pm 5.6$ ms ($n = 17$).

Extracellular application of nociceptin/orphanin FQ (10 μM) produced a reversible decrease in the amplitudes of e.p.s.cs within minutes, without affecting the time course of the currents (Figure 1b). Steady state levels of inhibition were reached within less than 5 min after drug application for all concentrations tested. This indicates that the concentration of nociceptin equilibrated within a few minutes between the bath solution and the solution surrounding the synaptic connection investigated (Figure 2a). As illustrated in Figure 2b the inhibitory action of nociceptin occurred in a concentration-dependent manner with a maximum inhibition of $51.6 \pm 5.7\%$ (mean ± s.e.mean; $n = 9$) at concentrations exceeding 3 μM. An EC_{50} value of 485 ± 47 nM and a Hill coefficient of 1.29 ± 0.09 was determined.

Lack of effect of naloxone on nociceptin-induced inhibition of e.p.s.cs

To exclude the possibility that the inhibitory action of nociceptin was mediated via classical opioid receptors, we tested whether nociceptin-induced reduction of e.p.s.c. amplitudes could be reversed by application of naloxone (10 μM), a rather non-specific antagonist at μ, δ and κ opioid receptors. Reduction of e.p.s.c. amplitudes by nociceptin remained completely unaffected in the presence of naloxone (Figure 3a,b), whereas the inhibition by the μ opioid receptor selective agonist DAMGO (10 μM) was completely reversed by naloxone (10 μM). In a separate set of five experiments, inhibition by nociceptin (10 μM) alone was compared to inhibition by a combination of nociceptin and naloxone (each 10 μM) in the same cells. No significant difference in inhibition was observed

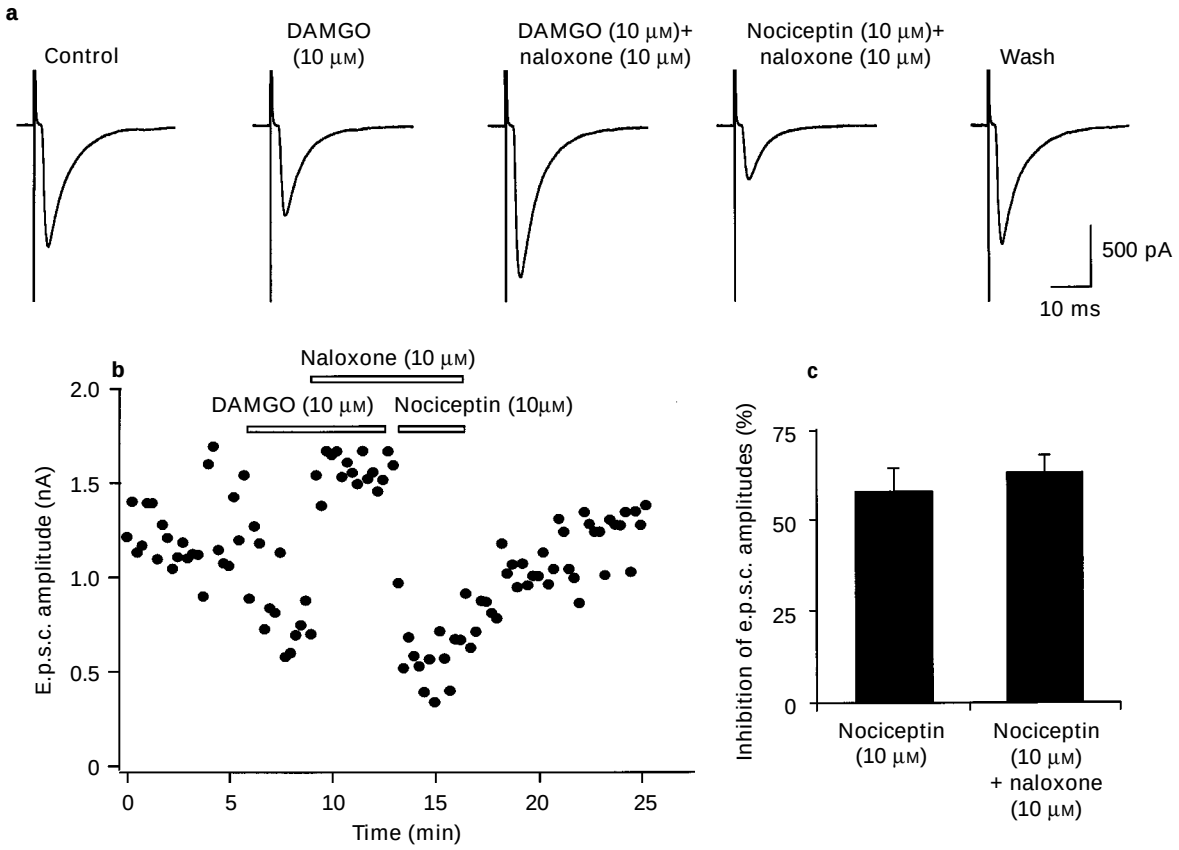

Figure 3 Inhibition by nociceptin was insensitive to naloxone. (a) Average e.p.s.cs recorded under control conditions, in the presence of the μ-receptor-selective agonist DAMGO (10 μM), in the presence of a combination of either DAMGO (10 μM) or nociceptin (10 μM) with the rather non-selective opioid receptor antagonist naloxone (10 μM), and after washout of both agents. Average e.p.s.c. traces were calculated from 10 e.p.s.cs each directly preceding the solution exchange. Note that naloxone reversibly increased e.p.s.c. amplitudes beyond the control level in this experiment. (b) E.p.s.c. amplitudes (●) plotted versus time for the complete time course of this experiment. (c) A summary of a set of five experiments, in which the effects of nociceptin (10 μM) applied alone and in combination with naloxone (10 μM) were compared. Means ± s.e.mean are shown, $n = 5$. No significant effects of naloxone on nociceptin-induced inhibition were found ($P > 0.4$, Wilcoxon rank test).

in these experiments ($n=5$; $P>0.4$, Wilcoxon rank test; Figure 3). This suggests that the observed inhibition was mediated via ORL_1 receptors rather than via classical opioid receptors.

Effects of nociceptin on spontaneous miniature e.p.s.cs

To determine whether nociceptin exerts its modulatory effect on excitatory synaptic transmission via a pre- or a postsynaptic site of action, two different approaches were chosen. In one series of experiments we analysed the frequency, amplitudes and time course of spontaneously occurring m-e.p.s.cs. In these experiments action potential-evoked transmitter release was prevented by adding tetrodotoxin (TTX, 1 μM) to the extracellular solution. Figure 4a shows averaged m-e.p.s.cs recorded under control conditions and in the presence of nociceptin (10 μM). Neither the amplitudes nor the time course of these m-e.p.s.cs were affected by nociceptin. The m-e.p.s.c. amplitudes recorded from the neurone shown in Figure 4 were 38 (24–52) pA (median; 25–75% interval) under control conditions, 35 (21–47) pA in the presence of nociceptin (Figure 4bi,ii) and 36 (24–50) pA after washout of nociceptin. The cumulative amplitude histograms did not differ significantly (Figure 4c). However, the frequency of m-e.p.s.cs was reversibly reduced by 45% in the presence of nociceptin (10 μM) in this cell (Figure 4d). The average reduction was 41 ± 8% in 6 out of 7 cells (Figure 4e). This suggests a presynaptic site of action for nociceptin.

Effect of nociceptin on currents elicited by exogenous L-glutamate application

To demonstrate further that nociceptin-induced inhibition was not produced on the postsynaptic site, we analysed the effects of nociceptin on inward currents elicited by external application of L-glutamate (L-Glu; 10 μM). A glass pipette (inner tip diameter about 0.5 μm) was positioned about 30 μm from the soma of the neurone recorded from. TTX (1 μM) was added to both the bathing solution and the L-Glu containing solution to prevent action potential generation and subsequent transmitter release from neighbouring neurones. When pressure-applied to the soma of neurones in the superficial layer L-Glu (10 μM) induced inward currents in the range of 100 pA, which were largely suppressed by the competitive L-Glu receptor antagonists NBQX (10 μM) and D-APV (50 μM) (Figure 5). As expected for a presynaptic site of action, amplitudes and time course of these currents were not significantly affected by nociceptin (10 μM). In addition, nociceptin had no effect on the holding current even at concentrations as high as 100 μM in the spinal cord neurones investigated here.

Comparison of nociceptin- and methionine-enkephalin-induced inhibition of e.p.s.cs

In order to compare the effects of nociceptin and of a classical opioid receptor agonist on e.p.s.cs we performed

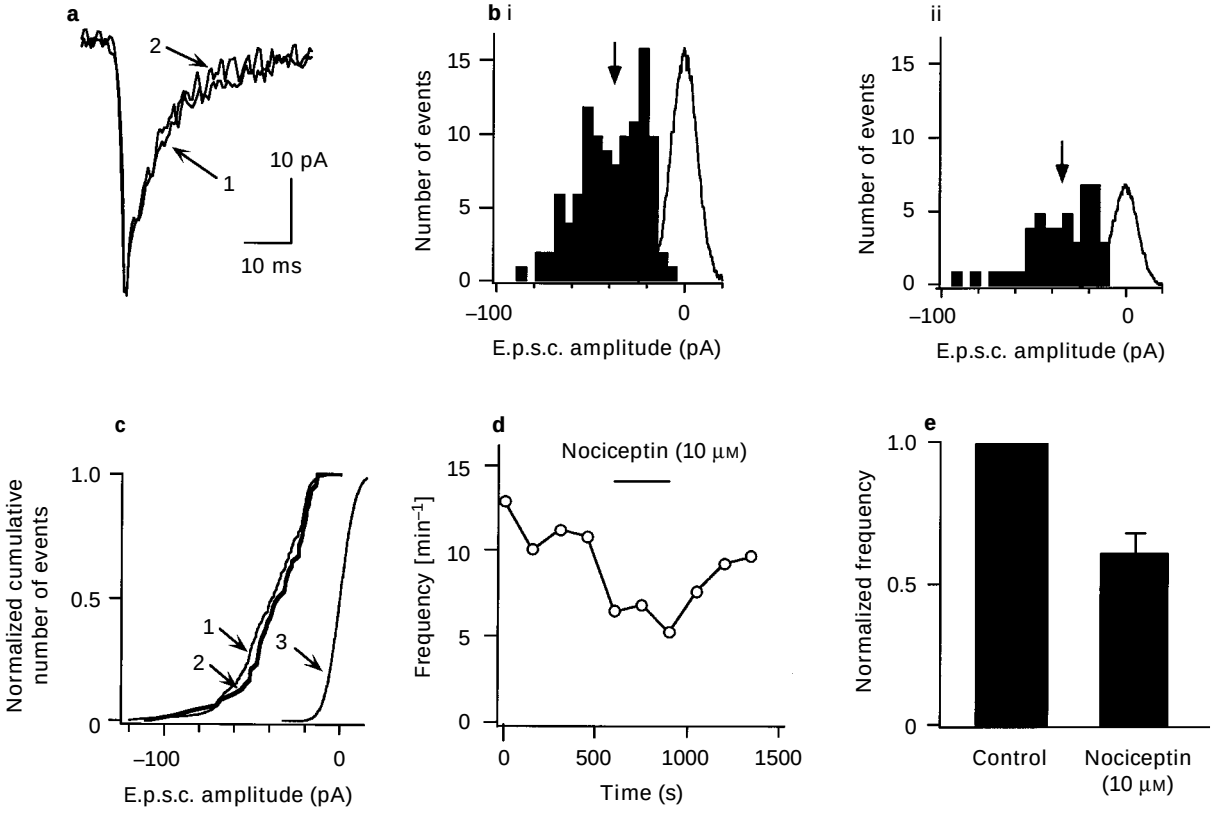

Figure 4 Analysis of spontaneous miniature e.p.s.cs revealed a presynaptic site of action. m-E.p.s.cs were recorded in the presence of TTX (1 μM) to block action potential evoked release and of strychnine (2 μM) and picrotoxin (50 μM) to block glycine and $GABA_A$ receptor-mediated m-i.p.s.cs. Nociceptin (10 μM) was applied for 7.5 min. (a) Average m-e.p.s.cs recorded under control conditions (10 min; $n=89$; arrow 1) and in the presence of 10 μM nociceptin (7.5 min; $n=39$; arrow 2). Neither the average amplitude nor the time course of the m-e.p.s.cs was affected by nociceptin. (b) Amplitude distributions (solid columns) under control conditions (i) and in the presence of nociceptin 10 μM (ii) were well separated from the current noise (solid line), indicating that most miniature events were detected. Arrows indicate median amplitudes, which were 38 pA and 35 pA under control conditions and in the presence of nociceptin, respectively. The plots showing the current noise are scaled amplitude histograms determined from current traces with no detectable m-e.p.s.cs. (c) Cumulative amplitude histograms of control events (1) and in the presence of nociceptin (2, bold line) also demonstrate that nociceptin does not affect the amplitudes of m-e.p.s.cs. (3) The cumulative current noise. (d) Frequency of m-e.p.s.cs (○) plotted versus time. Nociceptin led to a reversible decrease in the frequency of m-e.p.s.cs by about 45% in this cell. (e) Nociceptin (10 μM) reduced the frequency of m-e.p.s.cs in six out of the seven cells tested ($P\leqslant0.05$, Wilcoxon rank test) by 41 ± 8% on average. In one cell m-e.p.s.c. frequency remained constant in the presence of nociceptin (10 μM). Data shown are means$\pm$s.e.mean, $n=6$.

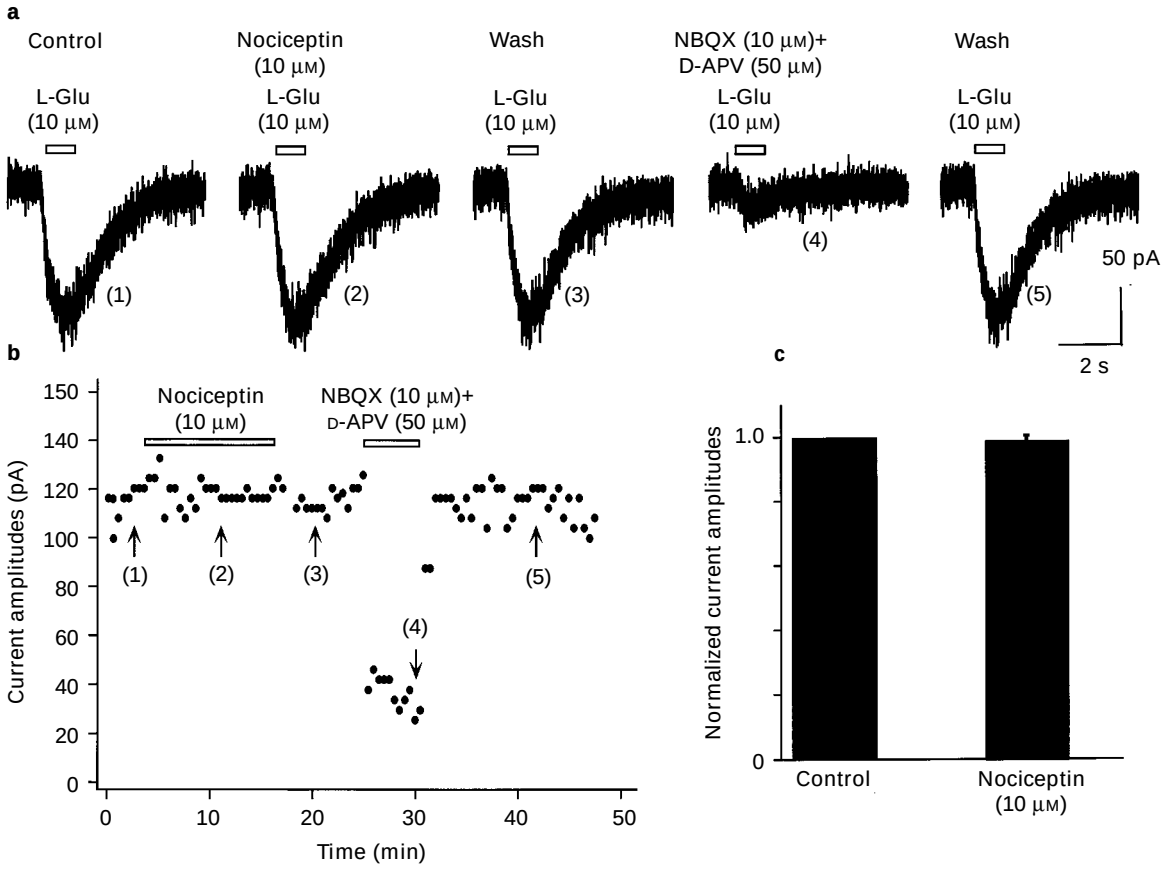

Figure 5 Currents elicited by extracellular application of L-glutamate (10 μM) were not affected by nociceptin (10 μM). Neurones were clamped at -80 mV and L-glutamate (L-Glu; 10 μM) was pressure-applied to the somata of these neurones every 30 s in the presence of TTX (1 μM). (a) Under these conditions L-Glu elicited inward currents in the range of 100 pA. Neither the amplitude nor the time course of these currents was significantly affected when nociceptin (10 μM) was bath-applied for 10 min. L-Glu-induced currents were reversibly blocked by a combination of the competitive L-Glu receptor antagonists NBQX (10 μM) and D-APV (50 μM) (b) Complete time course of the experiment. Peak amplitudes of L-Glu-induced currents (●) were plotted versus time. Current responses shown in (a) are labelled (1), (2), (3), (4) and (5) in (b). (c) Statistical analysis: normalized amplitudes of L-Glu-induced currents remained unaffected in all seven cells tested.

experiments in which we analysed the effects of nociceptin and of methionine-enkephalin, an endogenous agonist at μ and δ opioid receptors. Although reduction of evoked e.p.s.cs was about 50% on average at 10 μM for both neuropeptides, we found that the degree of inhibition by each agonist did not correlate positively between the neurones investigated (Figure 6). In 8 out of the 10 cells tested both neuropeptides were effective, while the other two neurones responded only to either nociceptin or methionine-enkephalin.

Discussion

Recent studies have demonstrated antinociceptive effects of nociceptin in rat spinal cord preparations (Erb *et al.*, unpublished observation; Faber *et al.*, 1996; Stanfa *et al.*, 1996; Xu *et al.*, 1996). In the present study, we have focused on the effect of nociceptin on excitatory synaptic transmission in the superficial layers (lamina I and II) of the spinal cord, where mainly thin and unmyelinated nociceptive afferents terminate (e.g. Light & Perl, 1979; Yoshimura & North, 1983; Yoshimura & Jessell, 1989). The main finding of the present study is that nociceptin inhibits excitatory synaptic transmission in the rat spinal cord at concentrations >100 nM, with an average maximum inhibition of about 50% at concentrations above 3 μM. This modulatory effect is mediated by presynaptic receptors and is insensitive to naloxone.

Site and mechanism of action

Classical opioid receptor agonists modulate excitatory synaptic transmission through both presynaptic (Go & Yaksh, 1987; Collin *et al.*, 1991; Kangrga & Randic, 1991; Hori *et al.*, 1992; Glaum *et al.*, 1994) and postsynaptic mechanisms (Chen & Huang, 1991; Rusin & Randic, 1991; Kolaj & Randic, 1996; for a review see Zieglgänsberger & Tölle, 1993). To distinguish between the two sites we have analysed spontaneous m-e.p.s.cs and currents elicited by extracellular application of L-glutamate. Our findings suggest a presynaptic rather than a postsynaptic site of action of nociceptin at synapses of superficial rat dorsal horn neurones. These findings are in line with the observation that nociceptin inhibits voltage-activated N-type Ca^{2+} channels that are sensitive to the *Conus geographus* toxin ω-CgTxGVIA (Connor *et al.*, 1996). N-type Ca^{2+} channels together with ω-AgaIVA-sensitive P- and/or Q-type Ca^{2+} channels are criticlly involved in triggering transmitter release from the presynaptic terminals of central neurones (Turner *et al.*, 1992; Takahashi & Momiyama, 1993; Wheeler *et al.*, 1994; Zeilhofer *et al.*, 1996). Inhibition of excitatory synaptic transmission by nociceptin occurred with an EC_{50} of about 450 nM. This is about 10 times higher than the EC_{50} obtained for inhibition of N-type Ca^{2+} channel currents (Connor *et al.*, 1996). Similar shifts have been obtained for classical opioid receptor agonists and for N-type Ca^{2+} channel blockers. The μ-selective agonist (DAMGO) inhibits voltage-activated Ca^{2+} currents with an EC_{50} of about 20 nM (Rhim & Miller, 1994),

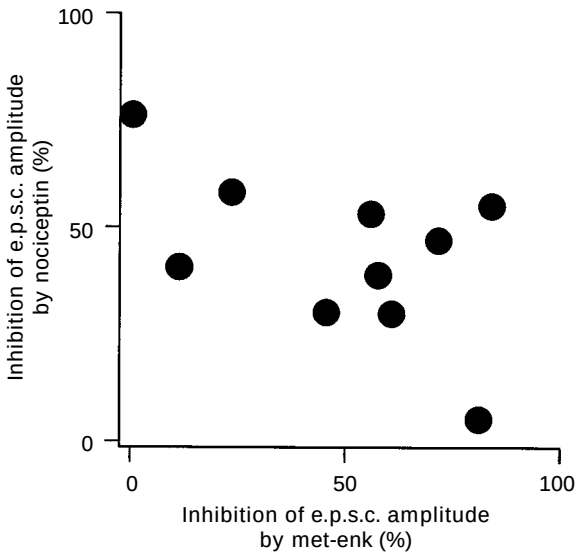

Figure 6 Inhibition of e.p.s.c. amplitudes by nociceptin did not correlate positively with inhibition by methionine-enkephalin (met-enk). Percentage inhibition by nociceptin (●) was plotted versus inhibition by met-enk. Each data point represents one neurone. Nociceptin (10 μM) and met-enk (10 μM) were applied alternately and inhibition of e.p.s.c. amplitudes was determined for each peptide. No positive correlation was observed for inhibition by the two agonists. Correlation coefficient $r = -0.53$; correlation coefficients $\geqslant +0.1$ can be rejected ($P \leqslant 0.05$, one-tailed t test after Fisher transformation of correlation coefficients).

but reduces excitatory synaptic transmission with an EC_{50} in the range of several hundred nM (Glaum *et al*., 1994). ω-CgTx GVIA inhibits Ba^{2+} currents through rat N-type Ca^{2+} channels with an EC_{50} of 0.1–0.7 nM (Boland *et al*., 1994), but reduces the amplitudes of evoked postsynaptic currents with an EC_{50} of 20 to 130 nM (Takahashi & Momiyama, 1993; Castillo *et al*., 1994). A possible explanation for these observations might be the existence of so called 'spare' Ca^{2+} channels in close vicinity to the release site. Block of one of several Ca^{2+} channels or partial inhibition of the Ca^{2+} channels would then produce only little or no effect on release, leading to a rightward shift in the concentration-effect curve (Dunlap *et al*., 1995). Other possible reasons may include a much lower receptor density in presynaptic terminals in the rat spinal cord as compared to that of transfected cells (see also Knoflach *et al*., 1996).

The maximum inhibition achieved with nociceptin (>3 μM) was about 50%. This may indicate that either inhibition of Ca^{2+} channel types involved in transmitter release is incomplete, which has been shown for N-type Ca^{2+} channels (Connor *et al*., 1996), or that only a subpopulation of the afferent terminals stimulated is sensitive to nociceptin. The latter would be in line with the abolition of monosynaptic reflexes by nociceptin (Faber *et al*., 1996).

Functional implications

The inhibitory effects of nociceptin on glutamatergic excitatory synaptic transmission in the superficial layers of the rat spinal cord demonstrated here are comparable to the presynaptic effects of classical opioid receptor agonists described in the literature (Hori *et al*., 1992; Glaum *et al*., 1994). This inhibitory modulation is a possible mechanism of the antinociceptive effects of opioids as well as of nociceptin. Despite the similarities of their effects on synaptic transmission significant differences in the modulatory actions have been observed in behavioural tests (Reinscheid *et al*., 1995; Meunier *et al*., 1995) and in electrophysiological experiments designed to investigate population potentials from ventral roots (Faber *et al*., 1996).

Our finding that the degrees of inhibition by the two agonists did not correlate positively between the neurones investigated opens the possibility that they modulate different, perhaps overlapping populations of excitatory synapses and implies different locations of ORL_1 and μ-/δ-receptors in the spinal cord. These differences might involve a differential expression of these receptors in synapses projecting to different cell types, like local inhibitory interneurones versus excitatory projection neurones, as well as in different types of afferent fibres.

In summary, the results presented here provide a cellular basis for the antinociceptive action of nociceptin at the spinal cord level and support the physiological role of nociceptin as an important inhibitory neuromodulator of sensory information processing in the rat spinal cord dorsal horn.

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