Inhibitory action of nociceptin/orphanin FQ on functionally different thalamic neurons in urethane-anaesthetized rats

*,[1]Doris Albrecht, [1]Roman Blühdorn, [1]Herbert Siegmund, [2]Hartmut Berger & [3]Girolamo Calo'

[1]Johannes-Müller-Institute of Physiology (Charité), Humboldt University Berlin, Germany; [2]Institute of Molecular Pharmacology, Berlin, Germany and [3]Department of Experimental and Clinical Medicine, Section of Pharmacology, University of Ferrara, Ferrara, Italy

1 In this study we administered nociceptin/orphanin FQ (NC) ionotophoretically onto neurons located in functionally distinct thalamic structures of urethane-anesthetized rats. Extracellular single unit recordings were made in the medial and lateral ventroposterior nucleus, posterior thalamic nucleus, zona incerta, lateral posterior nucleus, laterodorsal nucleus, ventrolateral nucleus and reticular nucleus.

2 NC decreased the firing rate in 60% of thalamic neurons. This decrease in firing rate was accompanied by a significant reduction in the number of high threshold bursts.

3 In about 20% of the neurons NC increased the firing rate. In most cells NC-induced increases in discharge rate could be blocked by the $GABA_A$ receptor antagonists bicuculline and SR 95531.

4 The NC receptor ligands [Phe$^1\Psi$(CH$_2$-NH)Gly^2] nociceptin(1-13)NH$_2$, Ac-RYYRIK-NH$_2$ and [Nphe^1]NC(1-13)NH$_2$ were also evaluated. All these peptides inhibited NC-induced changes in firing rate. In addition, in some neurons where NC inhibited firing, [Nphe^1]NC(1-13)NH$_2$ and Ac-RYYRIK-NH$_2$ elicited *per se* an increase in firing rate, suggesting the existence of tonic innervation of thalamic neurons by NC-containing fibres.

5 In NC-inhibited neurons nocistatin induced a significant increase in firing rate.

6 The present study demonstrated that NC regulates various thalamic nuclei related not only to somatosensory, but also to the visual and motor functions.
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Abbreviations: AcRNH$_2$, Acetyl-Arg-Tyr-Tyr-Arg-Ile-Lys-NH$_2$; F/G, [Phe$^1\Psi$(CH$_2$-NH)Gly^2]Nociceptin(1-13)NH$_2$; LD, laterodorsal nucleus; LP, lateral posterior nucleus; NC, nociceptin/orphanin FQ; Nphe, [Nphe^1]NC(1-13)NH$_2$; PO, posterior thalamic nucleus; Rt, reticular nucleus; VL, ventrolateral nucleus; VPL, lateral ventroposterior nucleus; VPM, medial ventroposterior nucleus; ZI, zona incerta

Introduction

Over the last few years, a new G-protein-coupled receptor (ORL1) that shows high homology with opioid receptors, but is not activated by the known opioid ligands, has been identified and cloned. In 1995 its endogenous ligand was simultaneously identified by two groups (Meunier *et al.*, 1995; Reinscheid *et al.*, 1995). Despite consistent inhibitory actions of NC at the cellular level in various brain sites (Moran *et al.*, 2000), the effect of NC on pain behaviour remains controversial.

The thalamus, especially the somatosensory thalamus, is involved in pain perception (Willis, 1997). Somatic sensory information is relayed to the cortex through major thalamic nuclei, the medial ventroposterior nucleus (VPM), the lateral ventroposterior nucleus (VPL), and the posterior thalamic nucleus (PO). In the rat the former two nuclei are also referred to as the ventrobasal complex. Whereas the VPL receives inputs from dorsal column nuclei and the spinal cord, the VPM receives information from trigeminal nuclei

(Price, 1995). The zona incerta (ZI) is also involved in somatosensory processing (Nicolelis *et al.*, 1992) in addition to other functions (Kim *et al.*, 1992; Spencer *et al.*, 1988). The lateral posterior nucleus (LP) as well as the laterodorsal nucleus (LD) receives fibres from several vision-related structures. The ventrolateral nucleus (VL) is thought to be a motor nucleus, relaying activity from the cerebellum (Price, 1995). Each of these nuclei are connected to a restricted sector of the reticular nucleus (Rt), which is composed of GABAergic cells that receive synapses from both thalamocortical and corticothalamic fibres (Jones, 1985). *In situ* hybridization analysis in rats has revealed that NC receptor mRNA is expressed in all these different thalamic nuclei (Neal *et al.*, 1999).

In the present study we have evaluated the effects of NC on spontaneous firing rates and burst patterns in these thalamic nuclei. Furthermore, we have attempted a pharmacological characterization of the receptor mediating the effects of NC using the ORL-1 receptor ligands [Phe$^1\Psi$(CH$_2$-NH)Gly^2]Nociceptin(1-13)NH$_2$ (F/G) (Guerrini *et al.*, 1998) and Acetyl-Arg-Tyr-Tyr-Arg-Ile-Lys-NH$_2$ (AcRNH$_2$) (Dooley *et al.*, 1997; Berger *et al.*, 1999; 2000a),

*Author for correspondence at: Institute of Physiology, Faculty of Medicine (Charité), Humboldt University Berlin, Tucholskystr. 2, 10117 Berlin, Germany; E-mail: doris.albrecht@charite.de

which have been shown to exert both agonist and antagonist activity in different *in vitro* and *in vivo* systems (Calo' *et al.*, 2000a), and the recently identified selective and pure ORL-1 receptor antagonist, [Nphe[1]]NC(1-13)NH$_2$ (Nphe) (Guerrini *et al.*, 2000). In addition, the actions of nocistatin, a biologically active peptide derived from the same precursor as NC (Okuda Ashitaka *et al.*, 1998) were evaluated on thalamic firing rate and compared with NC-induced effects.

Methods

Maintenance and preparation of animals

All procedures performed on adult male rats (Wistar, 300 – 400 g) were carried out according to the standards of animal welfare and were approved by the Berlin regional animal ethics committee (G 0056/97). Rats were housed five to a cage with food and water available *ad libitum* and on a diurnal light – dark cycle. All testing took place during daylight hours.

Rats were anaesthetized with urethane (1.2 g kg^{-1}, i.p.) and placed in a stereotaxic frame. Throughout the recording sessions, depth of anaesthesia was confirmed by the lack of withdrawal reflex to pinching of the hindlimbs and by the absence of corneal reflexes. Further injections of urethane were administered as required (for detail see Albrecht & Davidowa, 1989). Rectal temperature was maintained at 37 – 38°C using a heating pad. Electrocardiogram as well as EEG from the visual cortex was monitored. A small hole was drilled into the skull at a site 2.5 mm lateral to the midline suture and 5.0 mm anterior to the lambdoid suture. An electrode was lowered 3.5 to 6 mm with a microdrive through the hole to the level of the ventrobasal thalamus.

Recording

Glass microelectrodes for extracellular recording were filled with saturated Trypan blue solution (tip resistance 10 – 30 MΩ). Recorded action potentials displayed on an oscilloscope were analysed, after passing a window discriminator (World Precision Instruments, Sarasota, U.S.A.), with custom-made software (Spike2 from Cambridge Electronic Design, U.K.) running on a personal computer. Standardized pulses corresponding to individual action potentials from a single neuron were used for computing frequency time histograms, which were displayed on-line during sampling. Data were stored on disk for subsequent analysis.

Drugs and iontophoresis

Electrodes for iontophoresis were prepared from 5- or 7-barrel micropipettes (World Precision Instruments, Sarasota, U.S.A.) with a horizontal puller (P-87, Sutter Instr. Co.), and the tips were broken under microscopic visualization (tip diameter 5 – 7 μm). Micropipette assembly was glued to the recording electrode with a tip separation of 20 – 40 μm.

Drugs were ejected with positive currents by iontophoresis: NC (100 μM, pH 4; Bachem; ORL-1 agonist); F/G (1 mM, pH 4; Tocris; ORL-1 antagonist); AcRNH$_2$ (1 mM, pH 4; from our institutes; ORL-1 antagonist), and Nphe (3 mM;

pH 4; from our institutes; ORL-1 antagonist); nocistatin (100 μM, pH 4; Bachem); (−)-bicuculline methiodide (5 mM, pH 3; Research Biochemicals International, GABA$_A$ antagonist); SR 95531 (5 mM in 150 mM NaCl, pH 3.5; Tocris, GABA$_A$ antagonist), CGP 35348 (CGP, 1 mM, pH 3.0; a gift of Ciba-Geigy, GABA$_B$ antagonist). Retaining currents (4 – 10 nA) of opposite polarity were applied to the pipettes between drug ejections.

In a number of experiments a barrel filled with sodium chloride (165 mM, pH 4.0) was used for current balance. In control experiments no significant contribution from current or pH was detected.

Experimental protocols

Iontophoretic ejection of NC was used repeatedly during continuous recording of baseline activity. Following determination of NC-induced effects, the action of different receptor antagonists (in most cases 180 s before the co-administration with NC) was tested. To evaluate the possible involvement of GABAergic transmission in NC actions the GABA receptor antagonists bicuculline or SR95531 (GABA$_A$) and CGP 35348 (GABA$_B$) were tested against NC. Finally, we investigated the effect of nocistatin on neuronal firing rate. Since not all neurons received all drug combinations experimental numbers vary in the results section.

Analysis of neuronal responses

Drug responses were compared with control firing frequency recorded immediately before drug administration. From continuously recorded rate meter counts, the average discharge rate of each neuron was evaluated for 100 s prior to iontophoresis. This value (referred to as 'control') was subtracted from all subsequent changes in firing rate and results were expressed as '% change of control'. If the average change in discharge rate during the entire response time was larger than $\pm 40\%$, the neuron was considered as being sensitive to the applied substance. This criterion was used before co-application of NC with GABA receptor antagonists to divide the neurons into 'responders' and 'non-responders'.

In addition, we analysed the pattern of discharge (i) of spontaneous activity, (ii) during drug administration and (iii) during recovery. In addition to what extent spikes occurred singularly or in groups was quantified. Sequences of spikes with an interspike interval $\leqslant 4$ ms were regarded as bursts as recommended by Lo *et al.* (1991) and Lu *et al.* (1992) and low-threshold bursts were differentiated from high-threshold bursts (high threshold spike, HTS) by analysis of the pre-burst interval. The absolute requirement for hyperpolarization prior to the occurrence of a low-threshold Ca^{2+} spike (LTS) has the practical implication that these events may be detected using extracellular recordings as a high-frequency burst of action potentials that is preceded by a period of inhibition lasting longer than 100 ms (Lo *et al.*, 1991; Lu *et al.*, 1992; Guido & Weyand, 1995). As in our previous investigation (Davidowa *et al.*, 1995) several parameters were determined: the absolute number of bursts per 100 s, the percentage of spikes involved in bursts, and the number of spikes per burst.

Localization of recording sites

At the end of recording, a small amount of Trypan blue was iontophoretically deposited in the brain by passing a 10 μA negative current through the recording electrode for approximately 10 min. The rat was then killed with an overdose of urethane, decapitated and the brain fixed with 10% formaldehyde. Frozen frontal sections were stained with nuclear red and the location of blue spots within the thalamus was determined. The location of each neuron studied was mapped according to the atlas of Paxinos & Watson (1998).

Data analysis

Data are presented in the text and in Table 1 as the mean $\pm$ s.d., in the bar histograms as the mean $\pm$ s.e.mean of the number (n) of neurons. Wilcoxon Rank sum test (two tailed) or paired t-test was used to determine the predominating effect of a drug in a neuronal population. Mann–Whitney test and χ^2 test were used for statistical analysis of differences between two samples of neurons (software GraphPad Prism (San Diego, CA, U.S.A.) and SPSS).

Results

Effect of NC on firing rate in functionally different thalamic neurons

Extracellular recordings were made from a total of 171 spontaneously active neurons localized in functionally different thalamic nuclei. In almost all thalamic nuclei the responsiveness to NC was high (mean: 76%) (Table 1). In the whole sample of neurons, NC induced a significant decrease in firing rate ($P = 0.001$, Wilcoxon test; $n = 171$). For all neurons in the different thalamic nuclei examined, significant decreases in firing rate were found in LP ($P = 0.027$), LD ($P = 0.018$), ZI ($P = 0.043$) and Rt ($P = 0.013$). The dose-dependent inhibitory response of a representative example is illustrated in Figure 1A. Higher ejection currents caused greater decreases in firing rate of single neurons. A decrease in firing rate occurred in 54% of neurons (92/171), whereas an increase was obtained in 22% (38/171). The mean used iontophoretic current and the

duration of NC-ejection did not differ significantly in both samples (NC-inhibited neurons: 38 ± 9.5 nA and 73 ± 21 s versus NC-excited neurons: 39 ± 6 nA and 75 ± 15 s). Similar results were obtained concerning the mean duration of NC-responses (NC-inhibited neurons: 277 ± 181 s versus NC-excited neurons: 312 ± 179 s). Most of the NC-excited neurons were localized in the ventrobasal complex and the VL. In neurons excited by NC, these increases in firing rate were also dose-dependent (Figure 1B). Baseline activity was significantly higher in NC-inhibited neurons when compared to NC-excited neurons ($P = 0.043$, Mann–Whitney test, $n = 130$).

In this study, using urethane-anaesthetized rats, LTS bursts were most often found (152 out of the 171 thalamic neurons), whereas HTS bursts were only found in 77 out of 171 neurons. For baseline activity of the whole sample, there was a significant difference in the percentage of spikes involved in bursts of the LTS- and the HTS-type (LTS-type: mean $= 47.41 \pm 32.52\%$ versus HTS-type: mean $= 6.03 \pm 8.27\%$; $P = 0.0001$, Mann–Whitney test). With the exception of Rt (4–8 spikes within a burst), the bursts consisted of 2–3 spikes.
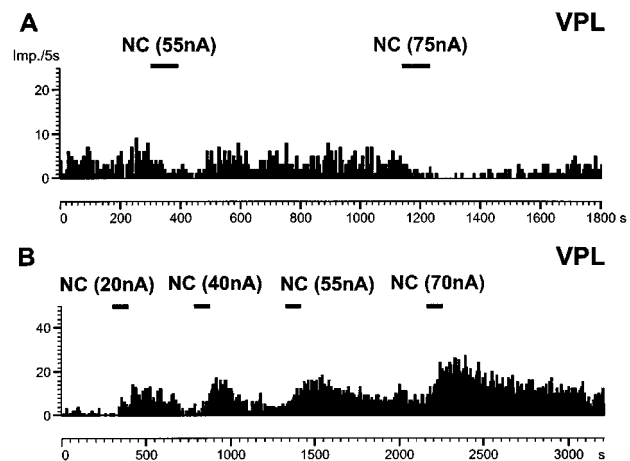

Figure 1 Effects of nociceptin (NC) on discharge rate. Frequency time histograms show dose-dependent decreases (A) or increases (B) in the firing rate of two neurons located in lateral ventroposterior nucleus (VPL). The y-axes indicate the number of spikes per 5 s, the x-axes indicate the time in seconds. The bars represent time and duration of ejection, the numbers show current intensity.

Table 1 Effect of nociceptin on firing rates (imp s^{-1} $\pm$ s.d.) and number (n) of affected neurons recorded in functionally different thalamic nuclei

Nuclei	Control	Inhibition NC	Recovery	n	Control	Excitation NC	Recovery	n	Control	No effect NC	Recovery	n	sum
PO	4.50 ± 3.12	$1.68 \pm 2.45^*$	4.64 ± 3.46	8	5.31 ± 4.01	13.20 ± 5.95	5.95 ± 5.21	4	4.73 ± 4.15	4.26 ± 4.41	3.91 ± 4.36	5	17
VPL	5.28 ± 7.80	$1.83 \pm 2.46^{***}$	5.78 ± 8.30	17	3.32 ± 3.00	$7.81 \pm 5.56^{**}$	3.06 ± 2.94	10	3.18 ± 2.03	2.78 ± 1.92	3.19 ± 2.00	5	32
VPM	4.60 ± 5.18	$2.01 \pm 2.09^{***}$	4.26 ± 3.75	22	2.29 ± 1.65	$8.54 \pm 3.90^{**}$	3.58 ± 3.30	11	5.95 ± 3.60	5.27 ± 3.29	6.35 ± 3.66	9	42
ZI	5.65 ± 5.69	$3.26 \pm 4.47^*$	5.20 ± 4.37	5				0				0	5
LP	5.80 ± 6.43	$1.75 \pm 2.52^*$	5.41 ± 6.00	6				0				0	6
LD	4.82 ± 5.63	$1.40 \pm 1.56^{**}$	6.44 ± 6.78	12	1.62 ± 1.15	3.84 ± 2.52	4.04 ± 2.39	4	3.65 ± 2.48	3.29 ± 2.01	3.53 ± 2.48	8	24
VL	6.87 ± 12.93	$4.59 \pm 11.46^{**}$	6.89 ± 12.79	14	2.46 ± 1.71	$5.96 \pm 3.43^{**}$	2.85 ± 2.53	9	3.57 ± 3.15	4.11 ± 3.23	3.59 ± 2.99	9	32
Rt	7.32 ± 10.44	$1.57 \pm 1.74^*$	8.89 ± 11.95	8				0	2.19 ± 2.13	1.80 ± 1.68	2.19 ± 2.15	5	13
sum				92				38				41	171

$^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

NC induced a significant increase in the percentage of spikes involved in bursts of the LTS-type in NC-excited neurons ($p = 0.0004$, Wilcoxon test). In NC-inhibited neurons no significant changes were observed (Figure 2A). As shown in Figure 2B, the percentage of spikes involved in bursts of the HTS-type was significantly increased in NC-excited neurons ($P = 0.0021$) and significantly decreased in NC-inhibited neurons ($P = 0.0132$). The number of spikes within a burst did not change significantly following NC ejection.

However, when considering the anatomical localization of functionally different thalamic nuclei, the distribution of neurons inhibited, excited or unaffected by NC did not differ significantly ($P > 0.05$; χ^2 test). Therefore, for detailed analysis of the effects of other drugs (GABA, ORL1 receptor antagonists and nocistatin) all thalamic neurons were considered regardless of their localization.

Involvement of GABAergic signalling in NC-induced effects

Low-ejection currents (29 ± 6.85 nA; $n = 41$) of the GABA$_A$ receptor antagonists caused frequently an increase in

A Percentage of spikes involved in bursts of NC-inhibited cells

B NC-excited cells

Figure 2 Effects of NC on appearance of burst discharges. Bar histogram shows the percentage of spikes involved in bursts of low threshold-type (LTS) and high threshold-type (HTS). Bars represent the percentage ($\pm$s.e.mean) before NC (control), during (NC) and after NC administration (recovery). The asterisks show significant differences (**$P < 0.001$, ***$P < 0.0001$); n = number of neurons.

discharge rate (for example see Figure 3A). In 34 neurons bicuculline methiodide was used, in seven neurons SR 95531. For NC-inhibited as well as for NC-excited neurons this
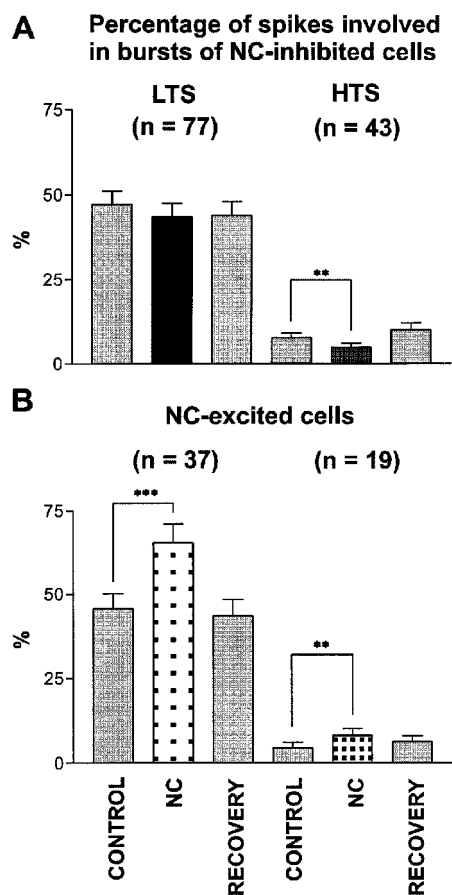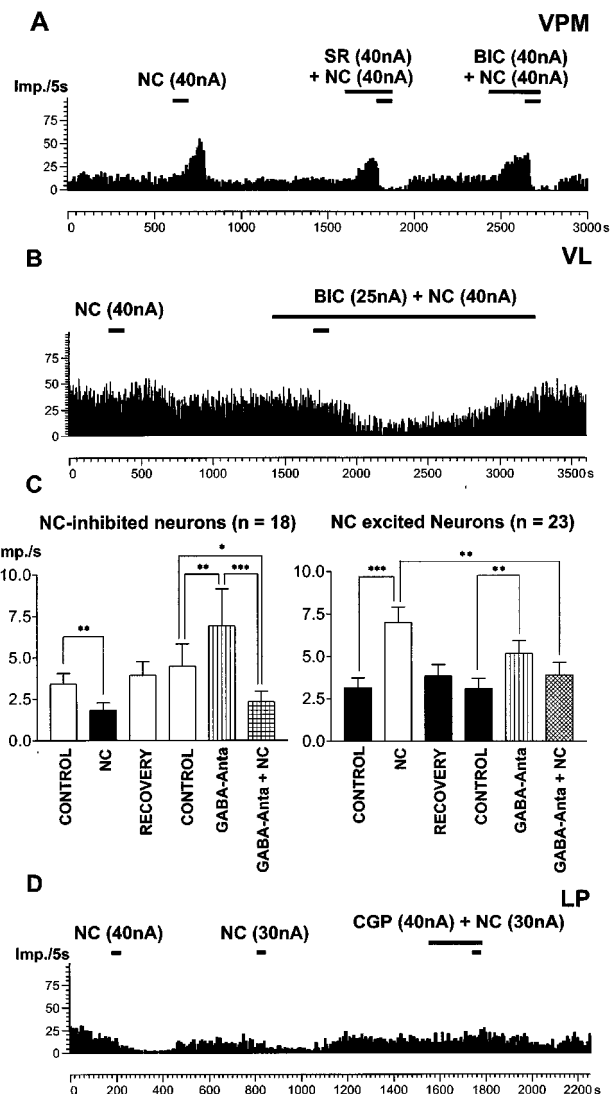
Figure 3 Effects of GABA receptor antagonists. (A, B) Frequency time histograms showing that SR 95531 (SR) and bicuculline (BIC) blocked NC-induced increases in firing rate in two neurons located in the lateral ventroposterior (VPM) and ventrolateral (VL) nuclei. With the exception of some neurons (B), bicuculline or SR 95531 alone induced strong increases in firing rate (A). (C) Histogram demonstrating the effect of co-administration of bicuculline or SR 95531 and NC on mean firing rate (Imp s^{-1} $\pm$s.e.mean). In both samples of neurons GABA$_A$ receptor antagonists (GABA-Anta) significantly enhanced activity (*$P < 0.05$, **$P < 0.001$, ***$P < 0.0001$). In NC-excited neurons NC-induced discharge rate differed significantly from that activity induced by the co-ejection of GABA$_A$ antagonists and NC. In contrast, in NC-inhibited neurons GABA$_A$ antagonists did not influence the inhibitory effect of NC. Co-administration of GABA$_A$ antagonists and NC did differ significantly from baseline recorded immediately prior to GABA$_A$ antagonists ejection (control) (n = number of neurons). (D) The GABA$_B$ antagonist CGP35348 reduced NC-induced decrease in firing rate in a neuron located in the lateral posterior nucleus (LP). In (A, B, D) the y-axes indicate the number of spikes per 5 s, the x-axes indicate the time in seconds. The bars represent time and duration of ejection and numbers show current intensity.

increase in firing rate was significant (Figure 3C). Whereas GABA$_A$ receptor antagonists increased the firing rate, they did not change significantly the percentage of spikes involved in high or low threshold bursts. In some cases, as shown in Figure 3B (a 'non-responder' neuron), a clear response to the iontophoretic ejection of NC was only observed when bicuculline was co-administered. These examples also show that the effects of co-administration of GABA$_A$ receptor antagonists and NC were not additive. It can be seen in Figure 3c that bicuculline or SR 95531 significantly reduced the excitatory effect of NC. A GABA$_A$ receptor antagonist-evoked reduction of the NC-induced excitation was also found in eight neurons located in the ventrobasal complex. The remaining neurons which were located in the ventrobasal complex and were investigated by co-administration of NC and GABA$_A$ antagonists responded with a decrease of activity in response to the application of NC. In contrast to the results obtained in NC-excited neurons, the NC-induced decrease in firing rate could not be blocked by GABA$_A$ receptor antagonists. In NC-inhibited neurons co-administration of bicuculline or SR 95531 together with NC did not significantly differ from the effect of NC alone on firing rate.

In 11 neurons the GABA$_B$ receptor antagonist CGP 35348 was tested. This antagonist did not affect NC-induced increases in firing rate ($n=6$). In neurons that were inhibited by NC, CGP 35348 was able to reduce the inhibitory effect of NC in four out of five neurons. An example is shown in Figure 3D.

Pharmacological characterization of the receptor mediating the effects of NC

In order to pharmacologically characterize the effects of NC on thalamic neurons several ORL-1 receptor ligands were used. In the first experiments, we used F/G (mean current: 37 ± 5 nA) to block NC-induced effects. An example of the pure antagonistic effect of F/G is shown in Figure 4A. However, F/G alone induced effects comparable to NC in 7/41 neurons as shown, for example, in Figure 4B. In the whole sample of neurons, co-administration of F/G and NC significantly reduced neuronal activity when compared to the ejection of F/G alone and to control drug free conditions in neurons which are inhibited by NC (Figure 5A). Nevertheless, F/G blocked the action of NC in 24 out of 41 neurons (59%).

AcRNH$_2$ was tested in 39 neurons (mean current: 57 ± 15 nA). Examples of the inhibitory effects of AcRNH$_2$ on NC-induced changes in discharge rate are shown in Figure 4C,E. As shown in Figure 5B for NC-inhibited neurons iontophoretic administration of AcRNH$_2$ alone significantly increased discharge rate. Co-administration of AcRNH$_2$ and NC did not significantly modify this activity when compared to control, either in NC-inhibited neurons or NC-excited neurons. Therefore, AcRNH$_2$ efficiently blocked the NC-induced effects on discharge rate considering the whole sample of neurons, although AcRNH$_2$ only blocked efficiently the NC-induced effects in 26 neurons (67%).

Using a concentration of Nphe 30 times higher than NC (mean current 64 ± 9 nA), NC-induced effects were blocked in nearly all neurons tested (51/58, 88%). Examples of the inhibitory action of Nphe on NC-induced decreases in firing
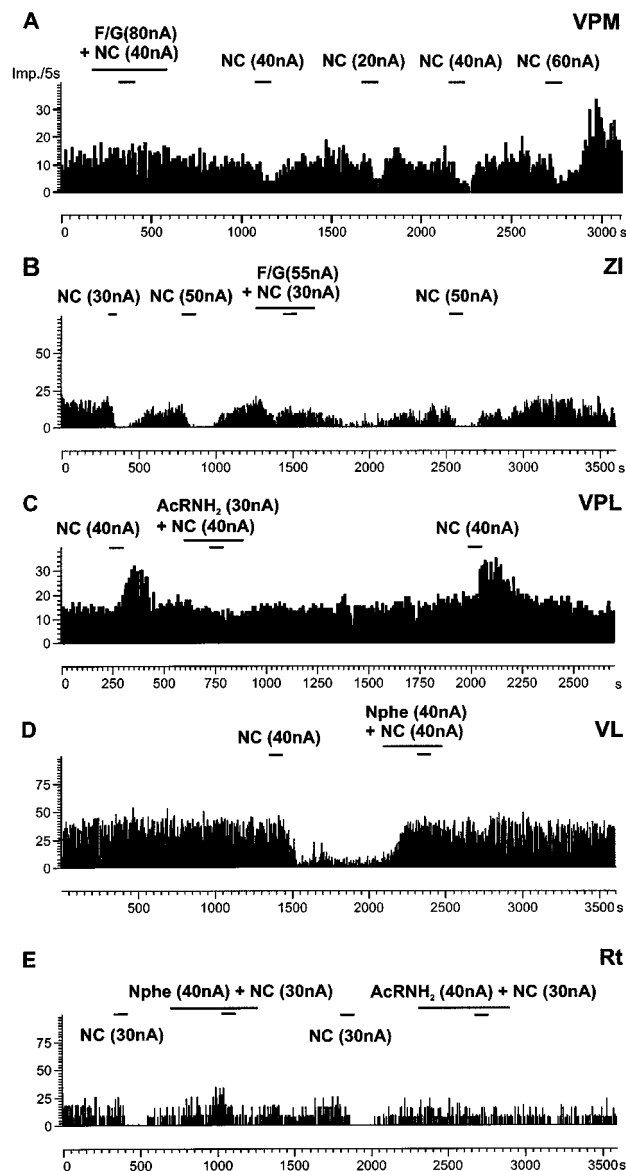

Figure 4 Representative examples of the action of NC receptor antagonists. Frequency time histograms show NC-induced decreases or increases in firing rate of different neurons located in the medial ventroposterior nucleus (VPM), the zona incerta (ZI), the lateral ventroposterior (VPL), the ventrolateral (VL) and the reticular nucleus (Rt). In (A) the NC receptor antagonist F/G blocked NC-induced inhibition. An example of an agonist effect of F/G is shown in (B). The NC receptor antagonists AcRNH$_2$ and Nphe effectively blocked NC-induced effects on discharge rate (C – E). The y-axes indicate the number of spikes per 5 s, the x-axes indicate the time in seconds. The bars represent time and duration of ejection and numbers show current intensity.

rate in single cells are shown in Figure 4D,E. In neurons inhibited by NC, the ejection of Nphe alone induced a significant increase in firing rate (Figure 5C) comparable to the effects of AcRNH$_2$.

Effects of nocistatin

Nocistatin (51 ± 17 nA, 75 ± 17 s) significantly increased the discharge rate of thalamic neurons ($P = 0.009$, $n = 35$,
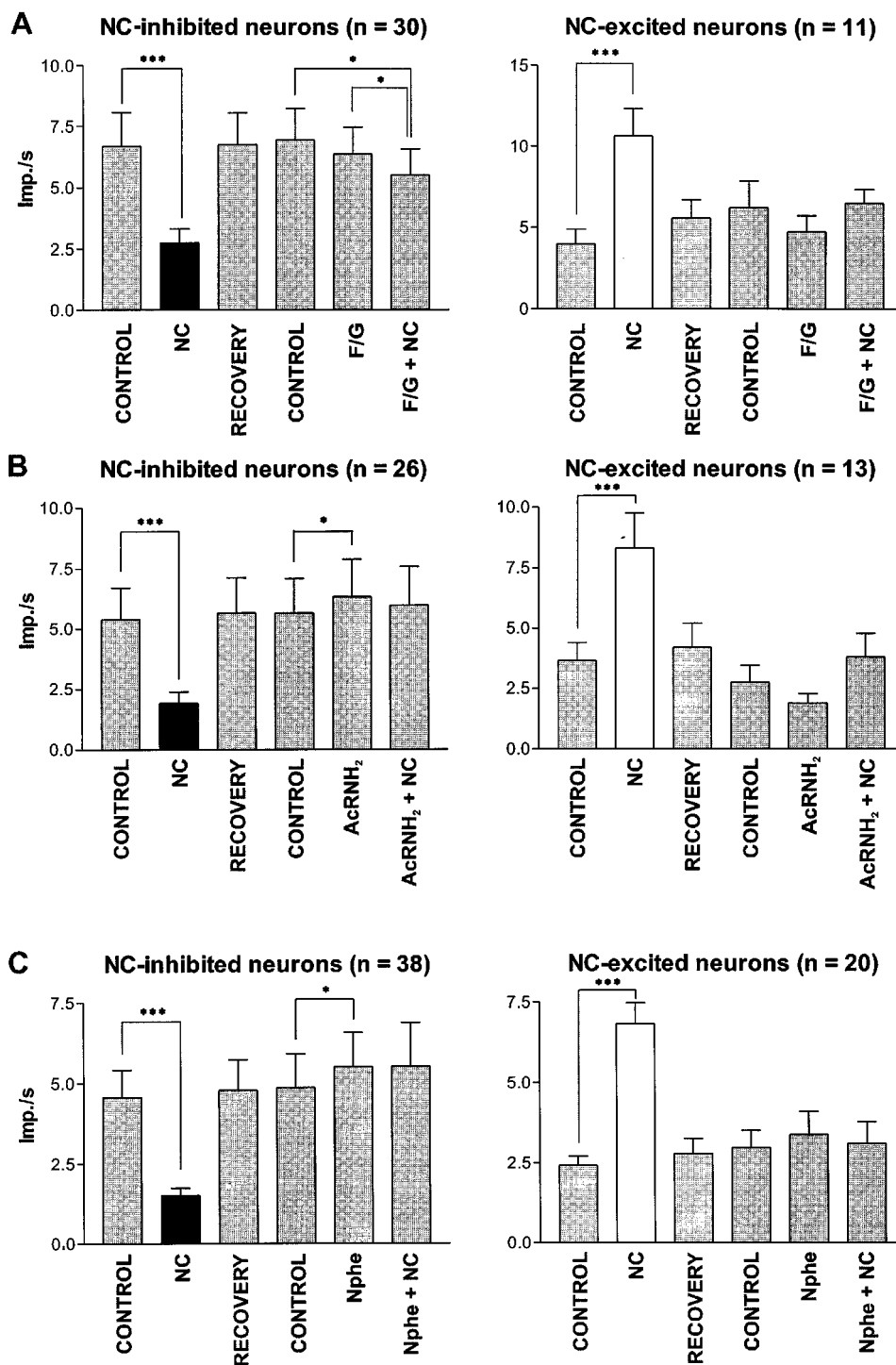
Figure 5 Effects of NC receptor antagonists. Bar histograms demonstrate the changes in mean firing rates ± s.e.mean induced by NC, by the NC-receptor antagonists (F/G, AcRNH$_2$, Nphe) and by co-administration of antagonists and NC. Co-administration of F/G and NC caused a significant decrease in firing rate when compared to baseline activity recorded immediately prior to F/G ejection (control) in the sample of NC-inhibited neurons. In addition, there was a significant difference between the firing rate during F/G ejection and that during co-administration of F/G and NC. AcRNH$_2$ and Nphe alone induced a significant increase in firing rate. Co-administration of NC antagonists and NC blocked the NC-induced effects. *$P<0.05$, ***$P<0.0001$, n = number of neurons.

Wilcoxon test). An example of the effect of nocistatin is shown in Figure 6C. No significant changes in burst firing were observed. Responsiveness of thalamic neurons to nocistatin (11/35 (40%)) was lower than that to NC (25/35 (71%)). In NC-inhibited neurons only ($n=16$), nocistatin induced a significant increase in firing rates (Figure 6A), whereas nocistatin did not significantly change firing rate in NC-excited neurons ($n=9$, Figure 6B).
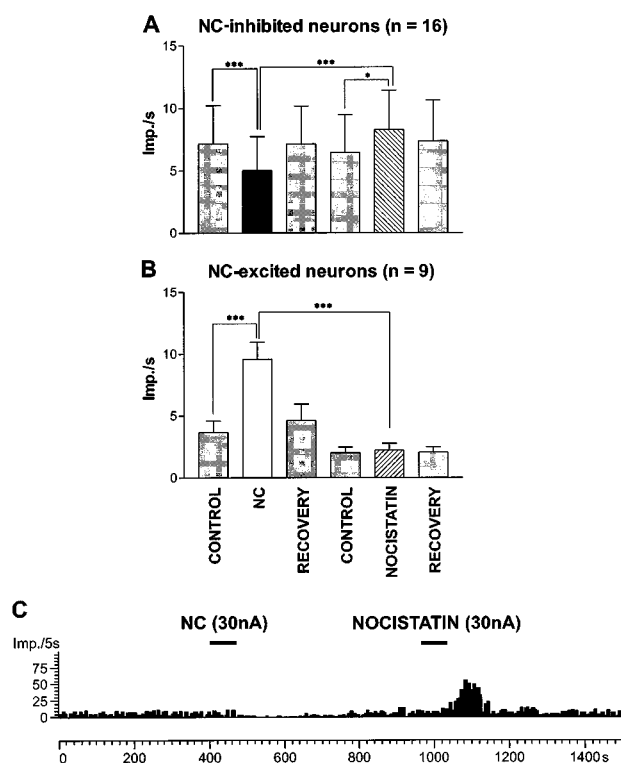
Figure 6 Effects of nocistatin on mean firing rate ($\pm$s.e.mean) in the sample of nociceptin-inhibited (A) and NC-excited neurons (B). Significant NC-induced decreases in firing rates were accompanied by significant increases in firing rate induced by nocistatin (A). There were no significant changes induced by nocistatin (B). *$P<0.05$, ***$P<0.0001$, n = number of neurons. (C) Frequency time histogram shows a representative example of a neuronal response to nocistatin.

Discussion

This study provides the first electrophysiological description of the actions of NC in the rat thalamus. These data showed that a strong responsiveness to NC was not only obtained in somatosensory neurons but also in visual- and motor-related thalamic nuclei. It has been shown that only a small fraction (10%) of neurons in the VPL, VPM and PO can be activated by noxious cutaneous stimuli (Willis, 1997). In contrast to these observations we obtained an 80% NC response rate in the thalamus. NC-responsible neurons were also found in visual and motor-related nuclei. Therefore, it seems unlikely that NC has actions only related to pain perception in the thalamus.

In all thalamic nuclei investigated NC mainly produced a decrease in firing rate. In line with these results NC profoundly inhibited spontaneous discharges of neurons in the rostral ventrolateral medulla (Chu et al., 1998), in the supraoptic nucleus (Doi et al., 1998) and in ventromedial hypothalamic neurons (Lee et al., 1997) of rat brain slices. The NC receptor has been shown to be coupled to a G-protein-activated inwardly rectifying K^+ (GIRK) conductance in several neuronal populations (Connor et al., 1996; Ikeda et al., 1997; Madamba et al., 1999). This conductance seems to be involved in the mediation of NC-induced effects in the ventrobasal thalamus and the Rt (S. Meis, personal communication). In addition, NC has been reported to inhibit low and high-threshold calcium currents in dorsal root ganglion neurons (Abdulla & Smith, 1997). In acutely dissociated rat periaqueductal grey neurons NC inhibited (predominantly) the N- and P/Q-type Ca^{2+} channel. L- and R-type currents were relatively unaffected (Connor & Christie, 1998). Similar results were described in the hippocampus (Knoflach et al., 1996). In agreement with these data we obtained a significant decrease in the number of bursts of the HTS-type following NC ejection in NC-inhibited neurons. In NC-excited neurons the percentage of spikes involved in bursts of both types significantly increased. Collectively, the inhibitory effect of NC in thalamic neurons might be due to either the opening of a potassium conductance and/or the closing of voltage sensitive Ca^{2+} channels. It is worthy of mention that we have shown that the appearance of burst discharges is higher in urethane-anaesthetized rats when compared to freely moving rats (Albrecht et al., 1998).

Our results also show that $GABA_A$ receptor antagonists blocked most of the NC-induced excitatory effects in the thalamus. Therefore, we suggest that GABAergic signalling is involved in the mediation of NC-induced increases in firing rate. In the hippocampus it has been shown that opioids excite pyramidal neurons indirectly by inhibition of neighbouring inhibitory interneurons (Zieglgänsberger et al., 1979; Nicoll & Alger, 1981). Because CGP 35348 did not influence NC-induced excitatory effects it can be concluded that NC-mediated excitation is exclusively via the $GABA_A$ receptor. In contrast to other thalamic relay nuclei, the rat ventrobasal complex can be characterized by a virtual absence of GABAergic interneurons (Harris & Hendrickson, 1987). Thus, with the exception of that complex (VPL, VPM) the excitatory effects of NC could be mediated by GABaergic interneurons. A NC-induced hyperpolarization of the local circuit neuron would cause a disinhibition of thalamic projection neurons. If NC receptors are localized presynaptically on GABAergic afferents, then a reduced release of GABA might depolarize thalamic projection neurons. A possible presynaptic location of NC receptors is also supported by our results with $GABA_A$ receptor antagonists in the ventrobasal complex. $GABA_A$ receptor antagonists also reduced the excitatory effect of NC in VPM and VPL neurons. It has been shown that GABAergic transmission is also involved in somatosensory processing within the ventrobasal complex by using either bicuculline methiodide (Salt, 1989; Vahle Hinz et al., 1994) or the $GABA_A$ antagonist SR95531 (Roberts et al., 1992). NC could increase the discharge rate via a presynaptic inhibition of afferents from the Rt. Such a presynaptic inhibitory action of NC has been reported in substantia gelatinosa (Lai et al., 1997; Liebel et al., 1997).

Blocking the $GABA_B$ receptor by CGP 35348 reduced the inhibitory effects of NC. It has been shown that the $GABA_B$ agonist, baclofen, acts on calcium channels (Connor & Christie, 1998) and also couples to increases in an inwardly rectifying K^+ conductance (Connor et al., 1996) similar to NC. Thus, these results can be interpreted assuming that ORL-1 and $GABA_B$ receptors act synergistically in reducing neuronal excitability, and the block of the $GABA_B$ reduces the response to ORL1 activation. However, this hypothesis needs further studies to be validated. In summary, our study favours the existence of both postsynaptic and presynaptic actions of NC within the thalamus.

The synthetic analogue of NC, F/G, was reported to fully antagonize the inhibitory effects of NC in amygdaloid (Meis & Pape, 1998) as well as ventrolateral medullary neurons (Chu et al., 1999). Moreover, F/G activated a small outward current and significantly reduced the amplitude of the NC-stimulated current in suprachiasmatic nucleus neurons (Allen et al., 1999). In contrast, in hypothalamic neurons F/G acted as a full agonist at the ORL-1 receptor (Yakimova & Pierau, 1999). In locus coeruleus and periaqueductal grey neurons, F/G exhibited partial agonist activity for inhibition of calcium channel currents and opening of K^+ channels (Chiou, 1999; Connor et al., 1999). In our study, the pseudopeptide did not effectively block NC-induced effects in all cells tested. In agreement with our results, F/G has also been shown to exert partial agonist activity, as well as NC antagonist actions in hippocampal CA1 neurons (Madamba et al., 1999). The number of receptors expressed on a given NC-responsive cell appears to be an important factor in determining the pharmacological behaviour of ORL-1 receptor ligands. In cells transfected with the human ORL1 receptor it was found that partial agonists demonstrated pure antagonistic activity at low levels of expression (Toll et al., 1998). The spectrum of activity of F/G in different thalamic neurons may possibly be explained by differences in coupling reserve between different neurons (Berger et al., 2000a). We have recently shown that $AcRNH_2$ and Nphe antagonized the NC stimulation of $[^{35}S]$-GTPγS binding to G proteins in membranes and sections of rat brain (Berger et al., 1999; 2000b). In the present study $AcRNH_2$ and Nphe completely blocked NC-induced effects in most cells tested. For the whole sample of neurons tested both $AcRNH_2$ and Nphe increased the discharge rate when administered alone. Therefore, it can be suggested that NC-containing fibres terminate in the thalamus and exert a tonic inhibitory influence on neuronal activity which was antagonized when $AcRNH_2$ or Nphe were ejected. A similar antagonistic effect of F/G might be hypothesized. However because of some residual agonist activity such receptor antagonist-induced increases in firing rate were missed. The data presented in our study support previous findings (Calo' et al., 2000a; Guerrini et al., 2000) that Nphe represents a pure ORL-1 receptor antagonist. However, a limitation of Nphe is represented by its low potency (pA_2 6.0 – 6.5 in different in vitro assays) and its chemical (peptide) nature. Therefore, further studies should involve new non-peptide ORL-1 receptor antagonist recently identified (Kawamoto et al., 1999).

We have shown that nocistatin per se has some biological activity. In contrast to the NC-induced decreases in firing rate, our results show that nocistatin mainly induced an increase in the discharge rate of thalamic neurons. However, the responsiveness of neurons to nocistatin was lower than that to NC. Nocistatin binds to membranes of mouse brain and of spinal cord in vitro (Okuda Ashitaka et al., 1998). However, nocistatin does not displace bound $[^3H]$-NC and does not prevent NC mediated inhibition of forskolin-induced cyclic AMP accumulation in Chinese hamster ovary cells transfected with the ORL1 receptor (Okuda Ashitaka et al., 1998). We did not find any significant changes in the appearance of burst discharges and this result is in agreement with the findings of Connor et al. (1999) in locus coeruleus neurons where nocistatin did not affect the calcium conductance. A possible mechanism might be a nocistatin-mediated reduction of GABAergic activity in the thalamus as Zeilhofer et al. (2000) have recently shown that nocistatin reduces inhibitory glycinergic and GABAergic transmission.

In summary, NC has been implicated in many behavioural and physiological processes, including nociception, allodynia, locomotion, learning, stress responses, sexual behaviour, feeding, cardiovascular control, and sodium balance (Darland et al., 1998; Taylor & Dickenson, 1998; Calo' et al., 2000b). Although the main effect of NC within functionally different structures in the thalamus is inhibitory, with excitatory effects mediated by an involvement of GABAergic neurons, the functional role of NC needs to be analysed in further experiments using sensory stimulation.

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