Effects of nociceptin and nocistatin on antidromic vasodilatation in hairless skin of the rat hindlimb *in vivo*

*,[1]H.-J. Häbler, [1]L. Timmermann, [1]J.-U. Stegmann & [1]W. Jänig

[1]Physiologisches Institut, Christian-Albrechts-Universität, Olshausenstrasse 40, 24098 Kiel, Germany;

1 We tested whether nociceptin (NCE), the endogenous ligand of the opioid receptor-like 1 (ORL1) receptor, and nocistatin (NST), which reverses central NCE effects when applied intrathecally (i.t.), affect small-diameter afferent fibre-mediated vasodilatation in rat hairless skin.

2 Female Wistar rats were vagotomized. Ongoing sympathetic vasoconstrictor activity was abolished by bilateral section of the lumbar sympathetic trunk between ganglia L2 and L3. Sensory axons were selectively stimulated in the dorsal root L5 by 20 electrical impulses supramaximal for activating C-fibres at 1 Hz. Blood flow was measured on the plantar skin of the left hind paw in the L5 dermatome using laser Doppler flowmetry.

3 NCE injected intravenously (i.v.) as single boluses (1, 10 and 100 nmol kg^{-1}) 7–8 min before dorsal root stimulation ($n=6$) dose-dependently decreased blood pressure and local vascular resistance and suppressed antidromic vasodilatation maximally by 47% ($P<0.01$). When NCE was injected 2 min before stimulation ($n=3$), antidromic vasodilatation was reduced by 64% after NCE (1 nmol kg^{-1}) and totally, or almost totally, abolished after the two higher doses.

4 NST (1–100 nmol kg^{-1} i.v., $n=6$) was without significant effect on blood pressure and cutaneous vascular resistance. Applied 5 ($n=6$) or 2 min ($n=3$) before stimulation it also did not affect antidromic vasodilatation. NST (100 nmol kg^{-1} i.v.) applied shortly before an equimolar dose of NCE did not antagonize NCE effects on vascular resistance, blood pressure and antidromic vasodilatation ($n=4$).

5 In conclusion, NCE inhibits antidromic vasodilatation, a component of neurogenic inflammation, in rat skin while NST is without effect. NST, at the small-diameter sensory ending, is not an effective antagonist of NCE.

Keywords: Nociceptin; orphanin FQ; nocistatin; opioid receptor-like 1; sensory neurones; neurogenic inflammation; sensory neuropeptides; antidromic vasodilatation; skin blood flow; laser Doppler

Abbreviations: DR, dorsal root; LST, lumbar sympathetic trunk; MAP, mean arterial pressure; NCE, nociceptin; NST, nocistatin; ORL1, opioid receptor-like 1

Introduction

Nociceptin (NCE), also called orphanin FQ, has structural similarities to dynorphin A and is an endogenous ligand of the opioid receptor-like 1 (ORL1) receptor which is a member of the opioid receptor family (Meunier *et al.*, 1995; Reinscheid *et al.*, 1995). The ORL1 receptor does not bind the known opioids with high affinity and the effects of NCE are not antagonized by naloxone (Darland *et al.*, 1998). NCE seems to have, as yet only in part well-defined, effects on a number of biological systems, e.g. on the nociceptive system and on the cardiovascular system (Darland *et al.*, 1998). Thus, when injected intracerebroventricularly (i.c.v.) or intrathecally (i.t.), it elicits hyperalgesia and allodynia in mice (Meunier *et al.*, 1995; Reinscheid *et al.*, 1995; Hara *et al.*, 1997) and reverses opioid-mediated antinociception (Mogil *et al.*, 1996). In contrast, in the spinal dorsal horn NCE rather seems to have an inhibitory effect on nociceptive transmission (Lai *et al.*, 1997; Liebel *et al.*, 1997; Yamamoto *et al.*, 1997a,b). NCE also evokes decreases in systemic blood pressure, apparently by influencing transmitter release from autonomic neurones supplying heart and resistance vessels (Giuliani *et al.*, 1997). Nocistatin (NST) is a neuropeptide which is derived from the same precursor molecule as NCE (Okuda-Ashitaka *et al.*, 1998). Administered i.t. in mice it antagonizes the hyperalgesia and allodynia generated by NCE (Minami *et al.*, 1998; Okuda-

Ashitaka *et al.*, 1998), although it does not bind to the ORL1 receptor and is not coupled to the same second messenger cascade (Okuda-Ashitaka *et al.*, 1998).

Small-diameter primary afferent neurones express opioid receptors (Smith & Buchan, 1984; Lundberg, 1996). Although opioids may not necessarily decrease the excitability of the nociceptive ending to physiological stimuli (Shakhanbeh & Lynn, 1993) they reduce the release of neuropeptides from afferent endings resulting in a suppression of neurogenic inflammation, i.e. antidromic vasodilatation (Lembeck & Donnerer, 1985; Holzer *et al.*, 1991; Shakhanbeh & Lynn, 1993; Holzer, 1998) and plasma extravasation (Lembeck & Donnerer, 1985; Holzer *et al.*, 1991; Towler & Brain, 1998). NCE is also capable of inhibiting neurogenic plasma extravasation in skin (Helyes *et al.*, 1997). In the isolated guinea-pig renal pelvis and the rat trachea NCE inhibits the release of neuropeptides (Giuliani & Maggi, 1996; Nemeth *et al.*, 1998). Neurogenic plasma extravasation is mainly mediated by neurokinins whereas small-diameter afferent fibre-induced vasodilatation is predominantly mediated by calcitonin gene-related peptide (CGRP) (Lundberg, 1996; Holzer, 1998; Häbler *et al.*, 1999a). Therefore, the inhibition of plasma extravasation by NCE does not necessarily imply that antidromic vasodilatation is also reduced by this neuropeptide. Whether NST affects neurogenic inflammation is unknown.

The purpose of this study was to test (i) whether NCE inhibits antidromic vasodilatation in hairless skin, (ii) whether

*Author for correspondence;
E-mail: j.haebler@physiologie.uni-kiel.de

NST has any effect on antidromic vasodilatation and (iii) whether NST can reverse the effects elicited by NCE. Preliminary results have been published recently (Häbler *et al.*, 1999b).

Methods

Experimental procedures have been described in detail recently (Häbler *et al.*, 1999a). Briefly, female Wistar rats (Charles River GmbH, Sulzfeld, Germany) (230–290 g) were anaesthetized with pentobarbital sodium i.p. (Nembutal®, 60 mg kg^{-1}, additional doses 10 mg kg^{-1} every hour i.v.).

The adequacy of anaesthesia was judged from the absence of withdrawal reflexes, gross fluctuations of blood pressure and heart rate and the absence of longlasting blood pressure effects upon brief noxious stimuli applied to the skin. Before starting experimental protocols animals were paralyzed (Pancuronium, Organon, 1 mg kg^{-1} initially, maintenance with 0.4 mg kg^{-1} when necessary) and artificially ventilated with O$_2$-enriched room air (ventilation-pump RUS-1300, FMI, Egelsbach, Germany). At intervals we let muscular paralysis wear off and assured that withdrawal reflexes were absent. Blood gases and blood acid-base status were measured at intervals (ABL 5, Radiometer, Copenhagen, Denmark). Arterial blood pressure was continuously recorded in the ventral caudal artery. Rectal
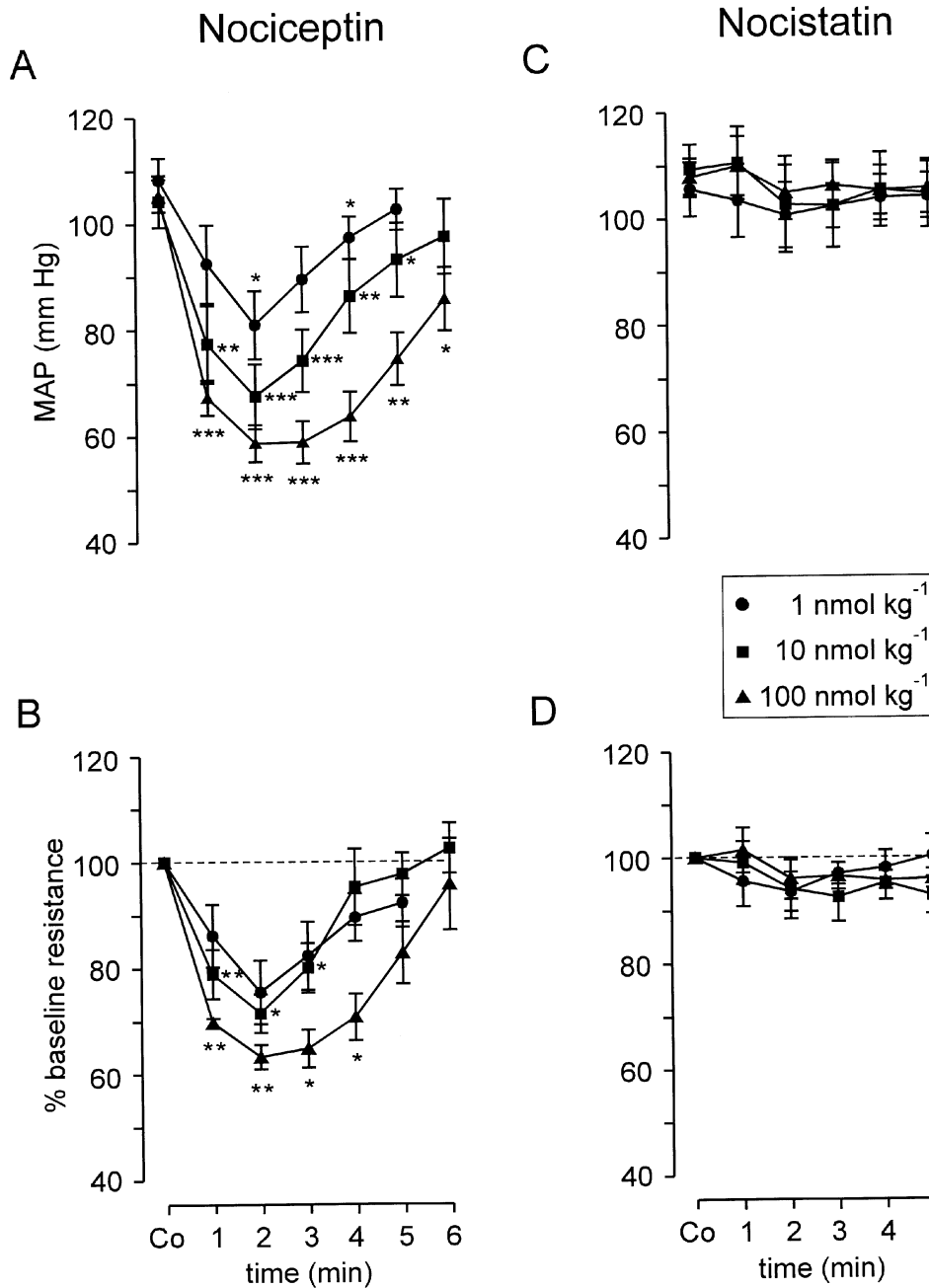
Figure 1 Cardiovascular effects elicited by single i.v. bolus injections of NCE (A, B) and NST (C, D). NCE (A) but not NST (C) dose-dependently and transiently decreased mean arterial blood pressure (MAP). Vascular resistance in hairless skin (calculated from MAP and laser Doppler flow; baseline denoted by broken line) was dose-dependently reduced by NCE (B) but not affected by NST (D). A vagal contribution to MAP responses was excluded by bilateral vagotomy. The decrease of vascular resistance in (B) was non-neurogenic because the LST was cut bilaterally. Co on abscissa indicates control. Data (means±s.e.mean) from $n = 6$ animals in both groups, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ (paired t-test).

temperature was kept constant close to 37°C by means of a servo-controlled heating blanket.

All efforts were made to minimize animal suffering and to reduce the number of animals used. At the end of the experiments rats were killed under deep anaesthesia by i.v. injection of a saturated solution of potassium chloride. All experiments had been approved by the local animal care committee of the state administration and were conducted in accordance with German Federal Law.

All rats were bilaterally vagotomized. Using a retro-peritoneal approach, both lumbar sympathetic trunks (LST) together with the white rami L3 were then cut caudally to ganglion L2 before starting experimental protocols to abolish on-going vasoconstrictor activity to the hindlimb which was

verified by a rise in skin temperature and blood flow after the intervention (Häbler *et al.*, 1997). After a lumbosacral laminectomy the left dorsal root (DR) L5 was identified, cut proximally and stimulated electrically with a train of 20 pulses supramaximal for activating C-fibres (20 V, pulse width 0.5 ms) at a frequency of 1 Hz to induce antidromic vasodilatation. A pool was formed from skin flaps and the exposed tissue was covered with warm mineral oil.

Superficial blood flow was measured within the L5 innervation territory on central plantar glabrous skin (proximal to the pads) of the left hindpaw using a laser Doppler flowmeter device (MBF3D, Moor Instruments, Axminster, Devon, U.K.). Flux signals were low pass filtered with the time constant set to 3 s.
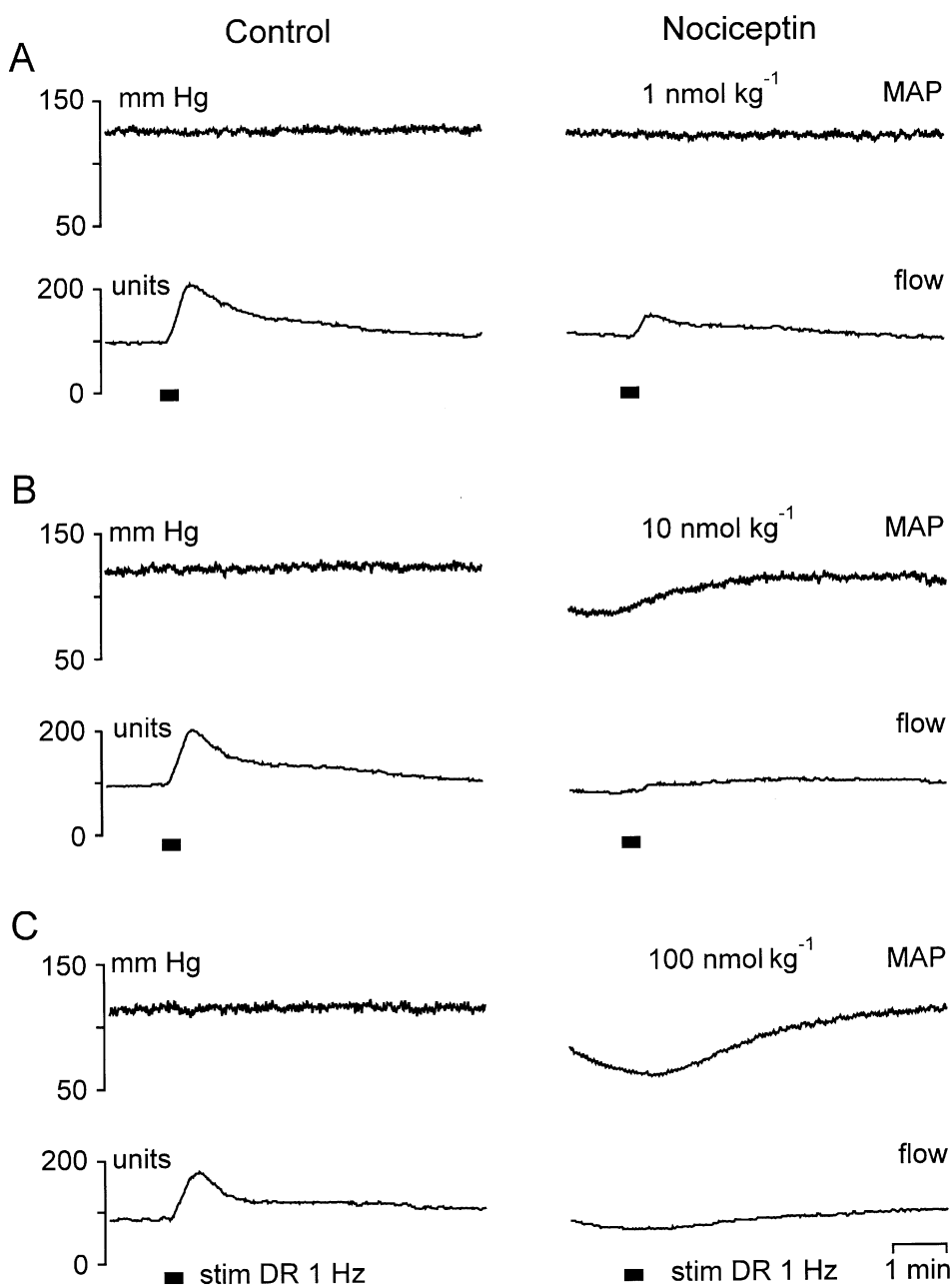
Figure 2 Strong suppression of antidromic vasodilatation by NCE injected 2 min prior to dorsal root L5 stimulation (20 s, 1 Hz, supramaximal for C-fibres; stim DR 1 Hz). Compared with control (left panel) NCE substantially inhibited stimulation-induced vasodilatation at the lowest dose tested (A). At the two higher doses, antidromic vasodilatation was almost totally suppressed (B) and completely abolished (C). Note that test stimulations were performed during NCE-induced decreases of MAP and cutaneous vascular resistance. The flow increases after DR stimulation in (B) and (C) (right panels) largely parallel the increase of MAP towards baseline and therefore are not neurogenic.

NCE and NST (Bachem, Bubendorf, Switzerland) were dissolved in physiological saline containing 1 mg ml^{-1} bovine serum albumin to prevent adsorption of the peptides and given as single i.v. boluses (0.2 ml) of 1, 10 and 100 nmol kg^{-1} in ascending order. In six rats, after 7–8 min following NCE injection, in order to allow blood pressure and flow to return to baseline, and 5 min after NST injection, antidromic vasodilatation was evoked electrically. In another three rats, DR stimulation was performed 2 min after NCE (1–100 nmol kg^{-1}) and NST (100 nmol kg^{-1}). Control and test vasodilatations were performed strictly in a paired manner for each dose of neuropeptide. The test vasodilatation was elicited 10–15 min after the control stimulation. Between two successive doses of neuropeptide at least 30 min were allowed and at least 1 h between the last dose of NCE and the first of NST, respectively. In four experiments, stimulation-induced vasodilatation was tested after a 100 nmol kg^{-1} dose of NST injected 30 s before an equimolar dose of NCE.

Antidromic vasodilatation was quantified by determining the peak increase of blood flow following stimulation. Integrated flow responses (area under the curve) were determined over 1, 2 and 3 min after stimulation. Vascular resistance in hairless skin was calculated in arbitrary units from mean arterial pressure (MAP) and blood flow. All responses were expressed as percentage changes of control flow and resistance, respectively, averaged within a time period of 60 s before stimulation. Data are expressed as means±s.e.-mean or means±s.d. as indicated. Statistical significance was assessed using two-tailed paired *t*-test and *t*-test as appropriate.

Results

NCE induced a dose-dependent transient decrease of systemic blood pressure (Figure 1A) which returned to baseline within 6–8 min after injection of the neuropeptide. In parallel, there was a significant decrease of cutaneous vascular resistance which was also dose-dependent (Figure 1B). Because both LST were cut this was not due to changes in sympathetic vasoconstrictor activity.

Stimulation-induced antidromic vasodilatation was inhibited by NCE given i.v. as a single bolus. The inhibition increased with each logarithmic increment in the dose of the neuropeptide (Figures 2–4) without changing the overall shape of antidromic vasodilatation. When the DR was stimulated 2 min after NCE application ($n=3$), i.e. while NCE-induced reductions of MAP and local vascular resistance were near to maximal, antidromic vasodilatation was totally abolished at the highest dose and almost totally suppressed after 10 nmol kg^{-1} (Figure 2B and C). At the lowest dose tested, stimulation-induced vasodilatation was substantially and significantly reduced (Figures 2A and 4, Table 1).

When antidromic vasodilatation was evoked 7–8 min after NCE injection, i.e. after MAP and vascular resistance had returned to baseline, peak vasodilatation and the flow responses integrated over 1–3 min after stimulation (area under the curve) were still significantly reduced compared with the controls after the two higher doses of NCE (Figures 3B, C and 4, Table 1). The reduction of stimulation-induced vasodilatation was no longer significant after the lowest dose of NCE (Figures 3A and 4, Table 1). About 20 min after the 100 nmol kg^{-1} dose of NCE, antidromic vasodilatation had fully recovered to control magnitude.

NST in any dose tested did not significantly change MAP nor vascular resistance in hairless skin (Figure 1C and D). When elicited 5 min after NST application, antidromic
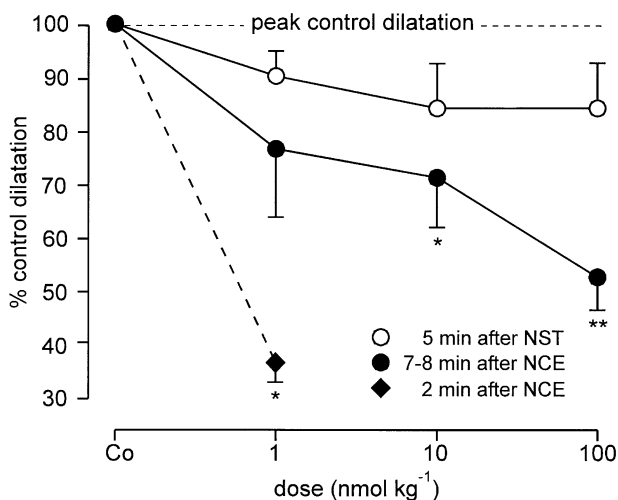
Table 1 Effects of nociceptin and nocistatin on antidromic vasodilatation (AVD)

	Dose (nmol kg^{-1})	Time after drug (min)	n	Peak AVD (% increase above baseline)		Flow integrated over 1 min after stim (% increase above baseline)		Flow integrated over 2 min after stim (% increase above baseline)		Flow integrated over 3 min after stim (% increase above baseline)	
				Control	Test	Control	Test	Control	Test	Control	Test
NCE	1	2	3	104.8±38.5	38.8±19*	62±24	19±13*	53±23	17±12*	43±20	14±9
NCE	1	7–8	6	74.8±20.1	56.5±29.3	50±15	37±20	41±15	32±19	34±16	27±19
NCE	10	7–8	6	85.5±25.6	59.7±28.3*	56±18	35±17*	43±17	26±12	34±17	22±8
NCE	100	7–8	6	80.8±18.2	42.5±15.2**	53±14	24±7**	41±15	18±6**	32±16	16±6*
NST	100	2	3	100.8±38.9	103.2±34.2	62±26	67±29	51±25	54±25	39±19	42±21
NST	1	5	6	84.5±15.5	76.0±16.5	54±10	47±13	42±9	36±13	35±9	28±13
NST	10	5	6	79.7±11.1	67.5±19.0	51±7	43±15	40±7	33±15	33±7	27±14
NST	100	5	5	76.2±13.2	64.7±19.9	49±10	41±15	38±9	31±15	32±9	25±15

Dorsal root stimulation was performed at different intervals after injection of NCE and NST, respectively ('time after drug'). Data expressed as mean±s.d. *P<0.05, **P<0.01 paired *t*-test. Baseline flow (in arbitrary units [mean±s.d.]) from which paired control and test antidromic vasodilatations were performed in nine rats was: 96±6, 94±5, 88±4 ($n=6$ stimulations) and 168±26, 117±12, 80±3, 62±3, 142±23, 76±4 ($n=12$ stimulations).

Control   ## Nociceptin
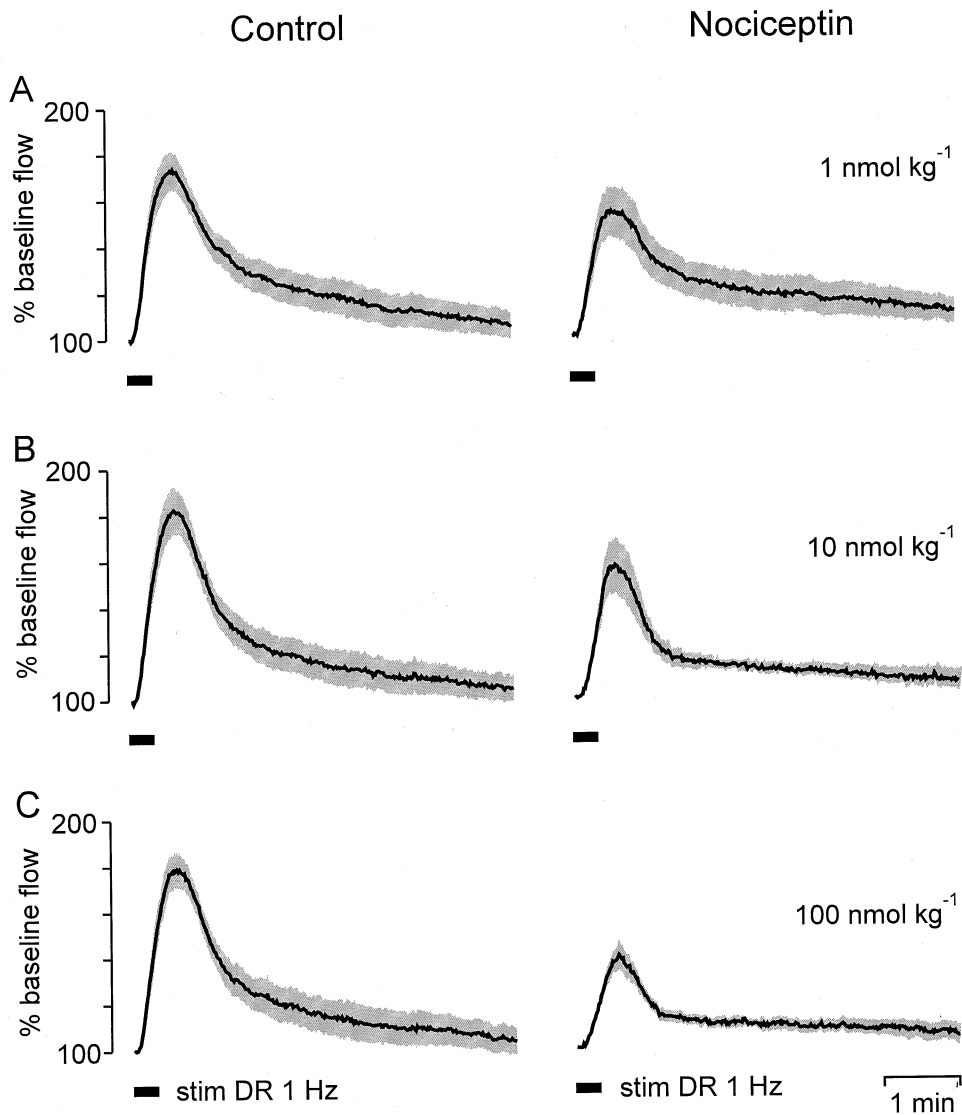
Figure 3 Dose-dependent suppression of antidromic vasodilatation by NCE injected i.v. The dorsal root L5 was stimulated for 20 s at a frequency of 1 Hz (stim DR 1 Hz) 7–8 min after applying NCE when blood pressure and skin blood flow had returned to baseline. Stimulation-induced (bar) vasodilator responses from six animals were normalized and averaged (shaded areas are means ± s.e.mean). Compared with the control dilatations (left panels) NCE decreased the magnitude but did not change the shape of antidromic vasodilatation (right panels).

vasodilatation was somewhat reduced but not significantly so and not in a dose-dependent manner (Figures 4 and 5, Table 1). Peak vasodilatation and integrated flow responses over 1–3 min after stimulation were not significantly smaller than controls (Table 1). NST (100 nmol kg^{-1}), injected 2 min before DR stimulation also did not reduce the resulting vasodilatation (Table 1).

A 100 nmol kg^{-1} bolus of NST, applied shortly before an equimolar dose of NCE, failed to reverse the inhibition of stimulation-induced antidromic vasodilatation elicited by NCE. Thus, peak vasodilatation (Figure 6A), maximum decrease of cutaneous vascular resistance (Figure 6B) and maximum decrease of MAP (Figure 6C) after combined

Figure 4 Reduction of the peak amplitude of antidromic vasodilatation by NCE and NST (applied 7–8 min and 5 min, respectively, before DR L5 stimulation). NCE dose-dependently reduced peak vasodilatation, at the highest dose almost by 50%. The inhibition by

NST was small and non-significant. Co indicates control. Data (means ± s.e.mean) from $n = 6$ animals in both groups. For comparison, the much stronger effect of the lowest NCE dose applied 2 min before DR stimulation is also shown (data from three rats). $*P < 0.05$, $**P < 0.01$ (paired t-test).
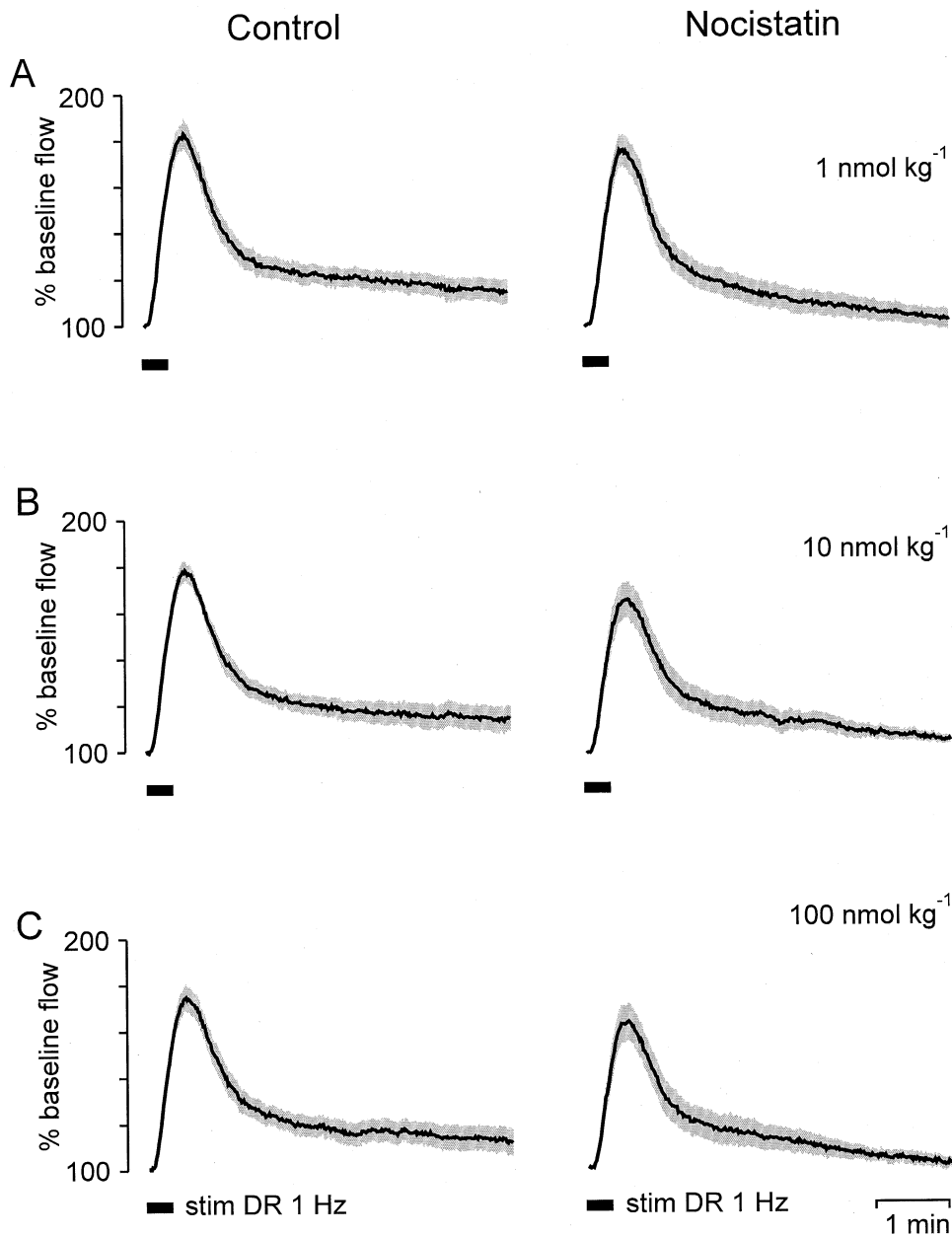
Figure 5 Lack of a significant effect on antidromic vasodilatation of NST. Stimulation-induced vasodilator responses from six animals were normalized and averaged (shaded areas are means±s.e.mean). The dorsal root L5 was stimulated for 20 s at a frequency of 1 Hz (stim DR 1 Hz) 5 min after applying NST. Compared with the control dilatations (left panels) NST reduced the magnitude of antidromic vasodilatation only little (right panels).

administration of both peptides were similar to those evoked by NCE (100 nmol kg^{-1}) alone.

Discussion

The results of the present study show that NCE (orphanin FQ), the endogenous ligand of the ORL1-receptor, applied i.v. inhibits small-diameter afferent fibre-mediated antidromic vasodilatation in rat hairless skin and relaxes cutaneous blood vessels by a non-neurogenic mechanism. However, the profound inhibition of antidromic vasodilatation 2 min after NCE injection was greatly attenuated 5 min later indicating that the NCE effects are rather short-lived. In contrast, NST, which when administered i.t. has been shown to be a functional antagonist of NCE because it was capable of reversing a number of central NCE effects (Minami *et al.*, 1998; Nicol *et*

al., 1998; Okuda-Ashitaka *et al.*, 1998), produced only a small, non-significant, inhibition of antidromic vasodilatation and had no significant effects on blood pressure and vascular resistance in the cutaneous bed. Furthermore, NST was not capable of antagonizing the NCE effects observed in the present study. These observations suggest that (i) NCE acting *via* ORL1 receptors on the peripheral endings of thin afferents has considerable anti-inflammatory potency and might be useful for the treatment of inflammation, (ii) NCE effects on antidromic vasodilatation, systemic blood pressure and blood vessels are similar to those exerted by opioids (Lembeck & Donnerer, 1985; Holzer *et al.*, 1991; Shakhanbeh & Lynn, 1993; Champion *et al.*, 1997b; Czapla *et al.*, 1998; Holzer, 1998) and (iii) NST, at the level of the peripheral afferent ending, is not an effective antagonist of NCE. Although NCE had a considerable potency at low doses, it is doubtful whether the effects of NCE observed in the present study play any role
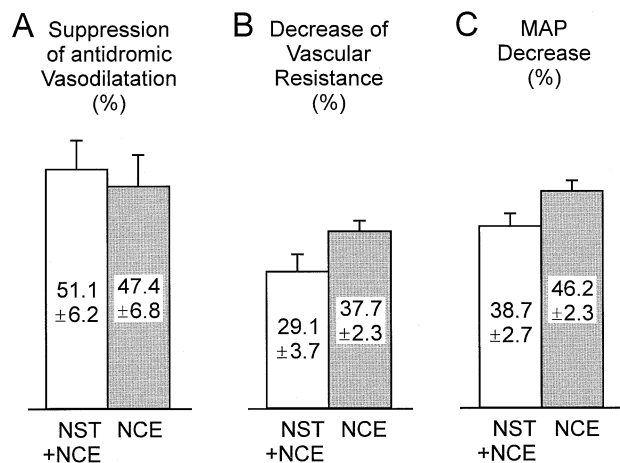
Figure 6 Effect of NST on NCE-induced changes of antidromic vasodilatation, skin vascular resistance and blood pressure. DR L5 was stimulated 7–8 min after injection of NCE. NST (100 nmol kg^{-1}) applied 30 s before NCE (100 nmol kg^{-1}) did not significantly affect NCE-mediated suppression of stimulation-induced vasodilatation (expressed relative to the controls, $P>0.05$, t-test) (A) nor the maximum decrease of vascular resistance (relative to baseline) after NCE ($P>0.05$) (B) nor the decrease of systemic blood pressure (relative to baseline pressure) due to NCE ($P>0.05$) (C). Data (means ± s.e.mean) from $n=4$ animals receiving NST and NCE (open columns) and $n=6$ animals (shaded columns) receiving only NCE.

under physiological conditions. In humans, NCE appears in the plasma but only at a concentration of about 10 pg ml^{-1} (Brooks *et al.*, 1998). However, during peripheral inflammation NCE is upregulated in related dorsal root ganglia (Andoh *et al.*, 1997). Under this condition NCE, after axonal transport into the periphery, may be released in the vicinity of nociceptor endings and inhibit neurogenic vasodilatation.

These results complement those reported by Helyes *et al.* (1997) who found that NCE inhibited neurogenic plasma extravasation, the other component of neurogenic inflammation, in rat skin *in vivo*. However, although substance P (SP) and/or neurokinin A and calcitonin gene-related peptide (CGRP) are involved in both plasma extravasation and antidromic vasodilatation (Häbler *et al.*, 1999a) it is predominantly SP which mediates plasma extravasation whereas CGRP plays the major role in antidromic vasodilatation (Lundberg, 1996; Holzer, 1998). The present results together with those of Helyes *et al.* (1997) therefore suggest that NCE suppresses neurokinin and CGRP release from small-diameter afferent endings in skin. This is consistent with previous evidence from *in vitro* experiments showing that NCE inhibits the release of both SP and CGRP from capsaicin sensitive nerve fibres supplying guinea-pig and rat airways (Fischer *et al.*, 1998; Nemeth *et al.*, 1998; Shah *et al.*, 1998) and guinea-pig atrium and renal pelvis (Giuliani & Maggi, 1996; 1997). Thus NCE in general appears to attenuate the efferent function of small-diameter afferent nerve fibres, at least when they are activated electrically. There is evidence that NCE does not attenuate neuropeptide release due to the activation of capsaicin receptors (Shah *et al.*, 1998) indicating that voltage dependent Ca^{2+} channels, but not the capsaicin-controlled cation channel may be the target of NCE (Connor *et al.*, 1996; Knoflach *et al.*, 1996). Our and previous results, which indicate inhibition of neuropeptide release by NCE, are in contrast to a recent report presenting evidence that NCE injected into the plantar skin of mice induced the release of

SP. This in turn elicited a flexor reflex, which was interpreted as indicative of pain (Inoue *et al.*, 1998).

NST which was capable of reversing the hyperalgesia due to NCE applied i.c.v. or i.t. in mice (Okuda-Ashitaka *et al.*, 1998) was not an effective antagonist of NCE in preventing the inhibition of antidromic vasodilatation. This is in line with the observation that NST did not bind to the ORL1 receptor nor did it affect the NCE-induced decrease of intracellular cyclic AMP (Okuda-Ashitaka *et al.*, 1998). Thus it appears that NST does not interfere with small-diameter afferents in the periphery.

Our results confirm earlier findings showing that NCE has profound cardiovascular effects (Champion & Kadowitz, 1997; Champion *et al.*, 1997a; Czapla *et al.*, 1997b; Giuliani *et al.*, 1997). It has been shown that the decrease in systemic arterial pressure is the consequence of two component responses, (i) a bradycardia mediated by both the vagus and the cardiac sympathetic nerves and (ii) a decrease of peripheral vascular resistance probably mediated by inhibition of transmitter release from sympathetic vasoconstrictor fibres (Giuliani *et al.*, 1997; Bucher, 1998). Elimination of the neurogenic influence by vagotomy and intravenous guanethidine abolished the NCE-induced MAP decrease (Giuliani *et al.*, 1997). Since NCE has been localized to brainstem regions which are involved in the regulation of central autonomic functions (Darland *et al.*, 1998), NCE may also play a role during on-going regulation of blood pressure. Thus, micro-injection of NCE into the rostral ventolateral medulla (Chu *et al.*, 1998), where 'pre-sympathetic' neurones are located, decreased blood pressure and heart rate in rats. However, since NCE applied i.v. is unlikely to cross the blood brain barrier this mechanism does not account for the MAP decrease in the present study.

We studied whether in addition to the effects brought about by the autonomic nervous system there is also a non-neurogenic vasodilator effect by NCE in the cutaneous vascular bed. To this end we eliminated on-going sympathetic vasoconstrictor activity by sectioning the LST bilaterally. Despite sympathetic denervation we saw a considerable dose-dependent decrease of vascular resistance suggesting that NCE has also a direct vasorelaxant effect in blood vessels supplying hairless skin. This observation is consistent with previous reports showing that NCE induced vasodilatation in the denervated rat hindlimb (Czapla *et al.*, 1997a) and relaxation of phenylephrine-precontracted rings of various cat arteries *in vitro* (Gumusel *et al.*, 1997). This direct relaxant effect of NCE may be mediated by the blockade of voltage-dependent Ca^{2+} channels in vascular smooth muscle, in analogy to the blockade of similar channels in neurones. Another possibility may be that the vascular endothelium expresses ORL1 receptors whose activation may increase the production of nitric oxide (NO) resulting in vasodilatation. There is evidence that this mechanism is used by opioids (Stefano *et al.*, 1995; Champion & Kadowitz, 1998; Wilderman & Armstead, 1998). However, recent evidence suggests that NCE is a vasodilator independent of endothelial NO (Champion *et al.*, 1998).

In conclusion, the present study shows that NCE is an effective inhibitor of small-diameter afferent fibre-mediated vasodilatation and may thus be useful as an anti-inflammatory agent. NCE has also profound depressant cardiovascular effects part of which seem to be mediated by a non-neurogenic action on smooth muscle. NST, which was shown recently to block some anti-opioid effects of NCE was almost totally ineffective in influencing antidromic vasodilatation and blood pressure. It also did not reverse NCE effects on antidromic

vasodilatation, blood pressure and local vascular resistance and thus does not seem to be a functional antagonist of NCE neither at the peripheral endings of small-diameter primary afferents nor at cutaneous blood vessels.

References

ANDOH, T., ITOH, M. & KURAISHI, Y. (1997). Nociceptin gene expression in rat dorsal root ganglia induced by peripheral inflammation. *Neuroreport,* **8,** 2793–2796.

BROOKS, H., ELTON, C.D., SMART, D., ROWBOTHAM, D.J., MCKNIGHT, A.T. & LAMBERT, D.G. (1998). Identification of nociceptin in human cerebrospinal fluid: comparison of levels in pain and non-pain states. *Pain,* **78,** 71–73.

BUCHER, B. (1998). ORL1 receptor-mediated inhibition by nociceptin of noradrenaline release from perivascular sympathetic nerve endings of the rat tail artery. *Naunyn Schmied. Arch. Pharmacol.,* **358,** 682–685.

CHAMPION, H.C., BIVALACQUA, T.J., FRIEDMAN, D.E., ZADINA, J.E., KASTIN, A.J. & KADOWITZ, P.J. (1998). Nitric oxide release mediates vasodilator responses to endomorphin 1 but not nociceptin/OFQ in the hindquarters vascular bed of the rat. *Peptides,* **19,** 1595–1602.

CHAMPION, H.C., CZAPLA, M.A. & KADOWITZ, P.J. (1997a). Nociceptin, an endogenous ligand for the ORL$_1$ receptor, decreases cardiac output and total peripheral resistance in the rat. *Peptides,* **18,** 729–732.

CHAMPION, H.C. & KADOWITZ, P.J. (1997). Nociceptin, an endogenous ligand for the ORL$_1$ receptor, has novel hypotensive activity in the rat. *Life Sci.,* **60,** PL241–PL245.

CHAMPION, H.C. & KADOWITZ, P.J. (1998). D-[Ala2]endomorphin 2 and endomorphin 2 have nitric oxide-dependent vasodilator activity in rats. *Am. J. Physiol.,* **274,** H1690–H1697.

CHAMPION, H.C., ZADINA, J.E., KASTIN, A.J., HACKLER, L., GE, L.J. & KADOWITZ, P.J. (1997b). Endomorphin 1 and 2, endogenous ligands for the mu-opioid receptor, decrease cardiac output, and total peripheral resistance in the rat. *Peptides,* **18,** 1393–1397.

CHU, X., XU, N., LI, P. & WANG, J.Q. (1998). Profound inhibition of cardiomotor neurons in the rat rostral ventrolateral medulla by nociceptin (orphanin FQ). *Neuroreport,* **9,** 1081–1084.

CONNOR, M., YEO, A. & HENDERSON, G. (1996). The effect of nociceptin on Ca^{++} channel current and intracellular Ca^{++} in the SH-SY5Y human neuroblastoma cell line. *Br. J. Pharmacol.,* **118,** 205–207.

CZAPLA, M.A., CHAMPION, H.C. & KADOWITZ, P.J. (1997a). Nociceptin, an endogenous ligand for the ORL$_1$ receptor, has vasodilator activity in the hindquarters vascular bed of the rat. *Peptides,* **18,** 793–795.

CZAPLA, M.A., CHAMPION, H.C. & KADOWITZ, P.J. (1997b). Decreases in systemic arterial and hindquarters perfusion pressure in response to nociceptin are not inhibited by naloxone in the rat. *Peptides,* **18,** 1197–1200.

CZAPLA, M.A., CHAMPION, H.C., ZADINA, J.E., KASTIN, A.J., HACKLER, L., GE, L.J. & KADOWITZ, P.J. (1998). Endomorphin 1 and 2, endogenous mu-opioid agonists, decrease systemic arterial pressure in the rat. *Life Sci.,* **62,** PL175–PL179.

DARLAND, T., HEINRICHER, M.M. & GRANDY, D.K. (1998). Orphanin FQ/nociceptin: a role in pain and analgesia, but so much more. *Trends Neurosci.,* **21,** 215–221.

FISCHER, A., FORSSMANN, W.G. & UNDEM, B.J. (1998). Nociceptin-induced inhibition of tachykinergic neurotransmission in guinea pig bronchus. *J. Pharmacol. Exp. Ther.,* **285,** 902–907.

GIULIANI, S. & MAGGI, C.A. (1996). Inhibition of tachykinin release from peripheral endings of sensory nerves by nociceptin, a novel opioid peptide. *Br. J. Pharmacol.,* **118,** 1567–1569.

GIULIANI, S. & MAGGI, C.A. (1997). Prejunctional modulation by nociceptin of nerve-mediated inotropic responses in guinea-pig left atrium. *Eur. J. Pharmacol.,* **332,** 231–236.

GIULIANI, S., TRAMONTANA, M., LECCI, A. & MAGGI, C.A. (1997). Effect of nociceptin on heart rate and blood pressure in anaesthetized rats. *Eur. J. Pharmacol.,* **333,** 177–179.

GUMUSEL, B., HAO, Q., HYMAN, A., CHANG, J.K., KAPUSTA, D.R. & LIPPTON, H. (1997). Nociceptin: an endogenous agonist for central opioid like1 (ORL1) receptors possesses systemic vasorelaxant properties. *Life Sci.,* **60,** PL141–PL145.

HÄBLER, H.-J., TIMMERMANN, L., STEGMANN, J.-U. & JÄNIG, W. (1999a). Involvement of neurokinins in antidromic vasodilatation in hairy and hairless skin of the rat hindlimb. *Neuroscience,* **89,** 1259–1268.

HÄBLER, H.-J., TIMMERMANN, L., STEGMANN, J.-U., JÄNIG, W. (1999b). Effects of nociceptin on antidromic vasodilatation in rat skin. *Pflügers Arch.,* **437** (suppl), R133 (Abstract).

HÄBLER, H.-J., WASNER, G. & JÄNIG, W. (1997). Interaction of sympathetic vasoconstriction and antidromic vasodilation in the control of skin blood flow. *Exp. Brain Res.,* **113,** 402–410.

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.

HELYES, Z., NEMETH, J., PINTER, E. & SZOLCSANYI, J. (1997). Inhibition by nociceptin of neurogenic inflammation and the release of SP and CGRP from sensory nerve terminals. *Br. J. Pharmacol.,* **121,** 613–615.

HOLZER, P. (1998). Neurogenic vasodilatation and plasma leakage in the skin. *Gen. Pharmacol.,* **30,** 5–11.

HOLZER, P., LIVINGSTON, E.H. & GUTH, P.H. (1991). Sensory neurons signal for an increase in rat gastric mucosal blood flow in the face of pending acid injury. *Gastroenterology,* **101,** 416–423.

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. U.S.A.,* **95,** 10949–10953.

KNOFLACH, F., REINSCHEID, R.K., CIVELLI, O. & KEMP, J.A. (1996). Modulation of voltage-gated calcium channels by orphanin FQ in freshly dissociated hippocampal neurons. *J. Neurosci.,* **16,** 6657–6664.

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.

LEMBECK, F. & DONNERER, J. (1985). Opioid control of the function of primary afferent substance P fibres. *Eur. J. Pharmacol.,* **114,** 241–246.

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.

LUNDBERG, J.M. (1996). Pharmacology of cotransmission in the autonomic nervous system: Integrative aspects on amines, neuropeptides, adenosine triphosphate, amino acids and nitric oxide. *Pharmacol. Rev.,* **48,** 113–178.

MEUNIER, J.-C., MOLLEREAU, C., TOLL, L., SUAUDEAU, C., MOISAND, C., ALVINERIE, P., BUTOUR, J.L., GUILLEMOT, J.-C., FERRARA, P., MONSARRAT, B., MAZARGUIL, H., VASSART, G., PARMENTIER, M. & COSTENTIN, J. (1995). Isolation and structure of the endogenous agonist of opioid receptor-like ORL$_1$ receptor. *Nature,* **377,** 532–535.

MINAMI, T., OKUDA-ASHITAKA, E., NISHIUCHI, Y., KIMURA, T., TACHIBANA, S., MORI, H. & ITO, S. (1998). Anti-nociceptive responses produced by human putative counterpart of nocistatin. *Br. J. Pharmacol.,* **124,** 1016–1018.

MOGIL, J.S., GRISEL, J.E., REINSCHEID, R.K., CIVELLI, O., BELKNAP, J.K. & GRANDY, D.K. (1996). Orphanin FQ is a functional anti-opioid peptide. *Neuroscience,* **75,** 333–337.

NEMETH, J., HELYES, Z., OROSZI, G., THAN, M., PINTER, E. & SZOLCSANYI, J. (1998). Inhibition of nociceptin on sensory neuropeptide release and mast cell-mediated plasma extravasation in rats. *Eur. J. Pharmacol.,* **347,** 101 – 104.

NICOL, B., LAMBERT, D.G., ROWBOTHAM, D.J., OKUDA-ASHITA-KA, 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 JR., F.J. & CIVELLI, O. (1995). Orphanin FQ: a neuropeptide that activates an opioidlike G protein-coupled receptor. *Science,* **270,** 792 – 794.

SHAH, S., PAGE, C.P. & SPINA, D. (1998). Nociceptin inhibits non-adrenergic non-cholinergic contraction of guinea-pig airway. *Br. J. Pharmacol.,* **125,** 510 – 516.

SHAKHANBEH, J. & LYNN, B. (1993). Morphine inhibits antidromic vasodilatation without affecting the excitability of C-polymodal nociceptors in the skin of the rat. *Brain Res.,* **607,** 314 – 318.

SMITH, T.W. & BUCHAN, P. (1984). Peripheral opioid receptors located on the rat saphenous nerve. *Neuropeptides,* **5,** 217 – 220.

STEFANO, G.B., HARTMAN, A., BILFINGER, T.V., MAGAZINE, H.I., LIU, Y., CASARES, F. & GOLIGORSKY, M.S. (1995). Presence of mu3 opiate receptor in endothelial cells. Coupling to nitric oxide production and vasodilation. *J. Biol. Chem.,* **270,** 30290 – 30293.

TOWLER, P.K. & BRAIN, S.D. (1998). Activity of tachykinin NK_1 and bradykinin B_2 receptor antagonists, and an opioid ligand at different stimulation parameters in neurogenic inflammation in the rat. *Neurosci. Lett.,* **257,** 5 – 8.

WILDERMAN, M.J. & ARMSTEAD, W.M. (1998). Role of endothelial nitric oxide synthase in hypoxia-induced pial artery dilatation. *J. Cereb. Blood Flow Metab.,* **18,** 531 – 538.

YAMAMOTO, T., NOZAKI-TAGUCHI, N. & KIMURA, S. (1997a). Analgesic effect of intrathecally administered nociceptin, an opioid receptor-like1 receptor agonist, in the rat formalin test. *Neuroscience,* **81,** 249 – 254.

YAMAMOTO, T., NOZAKI-TAGUCHI, N. & KIMURA, S. (1997b). Effects of intrathecally administered nociceptin, an opioid receptor-like1 (ORL1) receptor agonist, on the thermal hyperalgesia induced by carageenan injection into the rat paw. *Brain Res.,* **754,** 329 – 332.