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Pharmacological characterization of the nociceptin receptor mediating hyperalgesia in the mouse tail withdrawal assay

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1 The newly discovered neuropeptide nociceptin (NC) has recently been reported to be the endogenous ligand of the opioid-like orphan receptor. Despite its structural similarity to opioids, when injected intracerebroventricularly (i.c.v.) in the mouse, NC exerts a direct hyperalgesic effect and reverses opioid-induced analgesia. In the present investigation, these two effects of NC were evaluated under the same experimental conditions; in addition, a pharmacological characterization of the receptor mediating these central effects of NC was attempted.

2 NC caused a dose dependent (0.1–10 nmol/mouse), naloxone-insensitive reduction of tail withdrawal latency with a maximal effect of about 50% of the reaction time observed in saline injected mice. In the same range of doses, NC inhibited morphine (1 nmol/mouse) induced analgesia.

3 The effects of the natural peptide were mimicked by $NCNH_2$ and $NC(1-13)NH_2$ (all tested at 1 nmol/mouse) while 1 nmol $NC(1-9)NH_2$ was found to be inactive either in reducing tail withdrawal latency or in preventing morphine analgesia.

4 $[Phe^1\psi(CH_2\text{-}NH)Gly^2]NC(1-13)NH_2$ ($[F/G]NC(1-13)NH_2$), which has been shown to antagonize NC effects in the mouse vas deferens, acted as an agonist, mimicking NC effects in both the experimental paradigms. In addition, when NC and $[F/G]NC(1-13)NH_2$ were given together, their effects were additive.

5 These results demonstrate that both the direct hyperalgesic action and the anti-morphine effect of NC can be studied under the same experimental conditions in the mouse tail withdrawal assay. Moreover, the pharmacological characterization of the NC functional site responsible for these actions compared with the peripherally active site, indicates the existence of important differences between peripheral and central NC receptors.

Keywords: nociceptin; nociceptin receptor agonists; nociceptin receptor antagonist; morphine analgesia; mouse; tail withdrawal assay

Introduction

The newly discovered neuropeptide nociceptin (NC) has recently been reported to be the endogenous ligand of the opioid-like orphan receptor (Meunier *et al*., 1995; Reinscheid *et al*., 1995). High structural homologies have been demonstrated between NC and its receptor with peptides and receptors of the opioid family. Moreover, the effects of NC at the peripheral and the spinal cord level are similar to those of opioids, as indicated by the inhibition of neurotransmitter release from sympathetic (Berzetei-Gurske *et al*., 1996; Calo *et al*., 1996; Giuliani & Maggi, 1997; Zhang *et al*., 1997) or parasympathetic (Calo *et al*., 1997; Patel *et al*., 1997; Zhang *et al*., 1997) nerve terminals, the inhibition of neuropeptide release from primary sensory neurons (Giuliani & Maggi, 1996; 1997; Helyes *et al*., 1997; Rizzi *et al*., 1997), the induction of diuresis (Kapusta *et al*., 1996) and hypotension (Champion & Kadowitz, 1997; Giuliani *et al*., 1997) in the rat *in vivo*, and the analgesic affect of NC applied intrathecally (Stanfa *et al*., 1996; Xu *et al*., 1996; Yamamoto *et al*., 1997a,b). Furthermore, similar to opioids, the activation of the NC receptor has been reported to inhibit cyclic AMP accumulation at the cellular level (Meunier *et al*., 1995; Reinscheid *et al*., 1995). A completely different picture emerges from the analysis of the main biological effect of NC in the brain; in fact NC reduces the pain threshold both in rodents (Meunier *et al*., 1995; Nishi *et al*., 1997; Reinscheid *et al*., 1995) and in invertebrates (Kavaliers & Perrotsinal, 1996). Moreover, NC is able to reverse the opioid induced analgesia (Grisel *et al*., 1996; Mogil *et al*., 1996a,b).

Pharmacologically, the NC system is distinct from opioids. In fact, all the above mentioned biological actions mediated by NC were not modified either by naloxone or by more selective opioid receptor antagonists and were therefore considered to be due to the activation of a specific NC receptor. However, the lack of selective NC receptor antagonists prevented a definitive pharmacological characterization of the receptor(s) mediating the central and peripheral effects of the peptide. In a recent paper published in this journal, we reported the identification of a NC related peptide, $[Phe^1\psi(CH_2\text{-}NH)Gly^2]NC(1-13)NH_2$ ($[F/G]NC(1-13)NH_2$), which has been found to act as a selective NC receptor antagonist in peripheral tissues of the mouse (pA_2 6.75) and the guinea-pig (pA_2 7.02) (Guerrini *et al*., 1998). This and other compounds discovered in our laboratory have been used in the present study to evaluate, under the same experimental conditions, both the direct hyperalgesic and the anti-morphine effects of NC in the mouse tail withdrawal assay. We attempted to pharmacologically characterize the NC receptor(s) which mediates these effects, using some NC receptor agonists (NC, $NCNH_2$, $NC(1-13)NH_2$ and $NC(1-9)NH_2$) and the newly discovered NC receptor antagonist $[F/G]NC(1-13)NH_2$. The results of this study are compared with, and discussed in relation to those obtained in bioassay studies performed on the

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mouse vas deferens (Calo *et al*., 1996, 1997; Guerrini *et al*., 1997, 1998) in order to find out similarities or differences between central and peripheral NC functional sites.

Methods

Animals and experimental protocol

Male Swiss mice weighing 20–25 g were used. They were housed in standard conditions (22°C, 12 h light–dark cycle) with food and water *ad libitum*. Animals were used only once. All experiments were started at 10.00 h. Nociception was assessed using the tail withdrawal (TW) assay: the animals were placed in a holder and the distal half of the tail was immersed in water at 48°C; the withdrawal latency time was measured by an experienced observer blind to drug treatment. A cut off time of 20 s was chosen to avoid tissue damage. Five mice were randomly assigned to each experimental group. TW time was determined immediately before and at 5, 15, 30 and 60 min after i.c.v. injection of 2 μl of saline (control) or of various treatments. I.c.v. injections were made directly into the right lateral ventricle. When required, naloxone was administered subcutaneously (s.c.) 5 min before i.c.v. injections of NC or morphine.

Chemicals

The peptides used in this study were prepared by solid phase synthesis and purified by high pressure liquid chromatography according to Guerrini *et al*. (1997). Morphine was from Salars (Como, Italy), naloxone from Tocris Cookson (Bristol, U.K.), all reagents and salts were from Sigma Chemical Co. (St. Louis, MO, U.S.A.) or from Merck (Darmstadt, Germany). Stock solutions (5 mM) were made in saline and kept at −20°C until use.

Data analysis and statistics

The time course of TW latency measured in animals submitted to different treatments are shown in the figures. The raw data from each animal were converted to the area under the time × TW latency curve (AUC min s^{-1}), as described by Grisel *et al*. (1996). We calculated the AUC data for the interval of time in which nociceptin induced a clear change of TW latencies, namely the 5–30 min period. AUC values obtained under the various treatment were statistically compared *via* a software package (Tallarida & Murray, 1987) by means of one-way ANOVA followed by the Dunnett test for multiple comparisons: differences were considered to be statistically significant when P values were lower than 0.05.

Results

Results summarized in Figure 1 indicate that TW reaction time of saline injected mice were stable at values around 5–6 s over the time course of the experiment. No significant differences were found in the TW reaction time between saline injected mice and uninjected mice (see Table 1). As expected, i.c.v. injection of 1 nmol morphine significantly increased TW latency (Figure 1 and Table 1) displaying a maximal analgesic effect at 30 min. On the contrary, NC 1 nmol significantly reduced TW latency, its maximal effect (about 50% of reduction of the TW latency) was measured 5 min after the injection; then the effect of the peptide decreased progressively during the experiment and disappeared 60 min after the injection. Pretreating the animal with a dose of naloxone of 3 mg/Kg s.c. did not modify *per se* the TW latency but fully prevented the analgesic effect of morphine. On the other hand, pretreatment with naloxone did not interfere with the effect of NC (Figure 1 and Table 1).

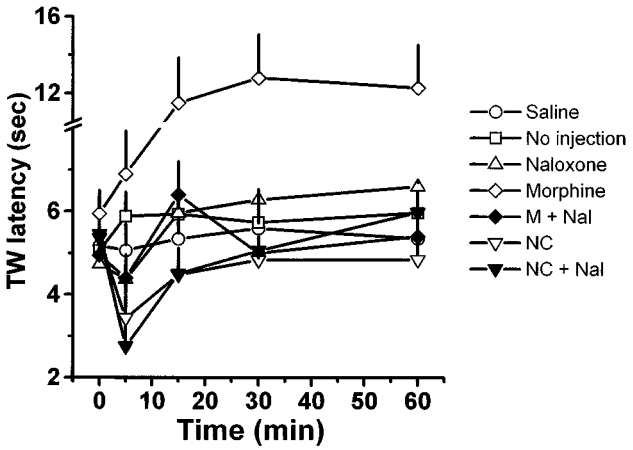

Figure 1 Effects of different treatments on TW latency in the mouse. Saline and uninjected mice did not show statistically significant differences. Morphine (M, 1 nmol, i.c.v.) induced analgesia, while nociceptin (NC, 1 nmol, i.c.v.) induced hyperalgesia. Naloxone (Nal, 3 mg/Kg s.c.) was inactive *per se* and *vs* nociceptin while it fully prevented the analgesic action of morphine. All these data, converted to the area under the curve (AUC, see Methods) and statistically analysed by one-way ANOVA followed by the Dunnett test for multiple comparisons, are presented in Table 1.

Table 1 Effects of i.c.v. injection of nociceptin or morphine on tail withdrawal latencies in mice pretreated subcutaneously with saline or naloxane

Treatment	*Saline (s.c.)*	*Naloxone (3 mg/kg s.c.)*
No injection	146±15	n.d.
Control (saline)	134±10	143±13
Morphine 1 nmol	274±30*	139±13
NC 1 nmol	109±7*	108±9*

The data, mean±s.e.m. of at least five experiments, were expressed as area under the curve (see Methods). n.d. = not determined. Naxolone or saline were administered subcutaneously (s.c.) 5 min before i.c.v. injections of NC or morphine. *$P<0.05$ *versus* control according to one-way ANOVA followed by the Dunnett test for multiple comparison.

The effects of NC were carefully quantified by applying three doses (0.1, 1 and 10 nmol) of the peptide. I.c.v. injections of NC at 0.1 and 1 nmol did not induce any evident effect on the general behaviour; on the contrary, mice injected with 10 nmol NC showed decrease in locomotor activity and muscular tone (especially in the hindpaws), ataxia and loss of the righting reflex. These effects were indeed very similar to those firstly described by Reinscheid *et al*. (1995) and then confirmed both in the mouse and in the rat by other authors (Noble & Roques, 1997; Nishi *et al*., 1997; Devine *et al*., 1996).

As for the nociceptive effects of NC, the results illustrated in Figure 2 (left panel), indicate that a small decrease of TW reaction time occurs already with the minimum dose of 0.1 nmol NC and became more evident (in fact statistically significant, see Table 2) with NC 1 nmol. The highest dose of NC (i.e. 10 nmol) did not further reduce the TW latency, but

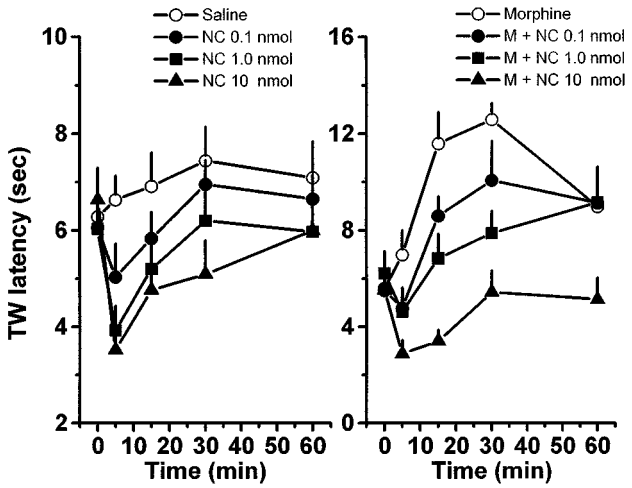

Figure 2 Left panel: nociceptin (NC) dose dependently reduced TW latency in mice. Data are means ± s.e.m. of at least six experiments. Right panel: NC dose dependently counteracted morphine (M, 1 nmol, i.c.v.) induced analgesia. Data are means ± s.e.m. of at least five experiments; All these data, converted into AUC and statistically analysed by one-way ANOVA followed by the Dunnett test for multiple comparisons, are presented in Table 1.

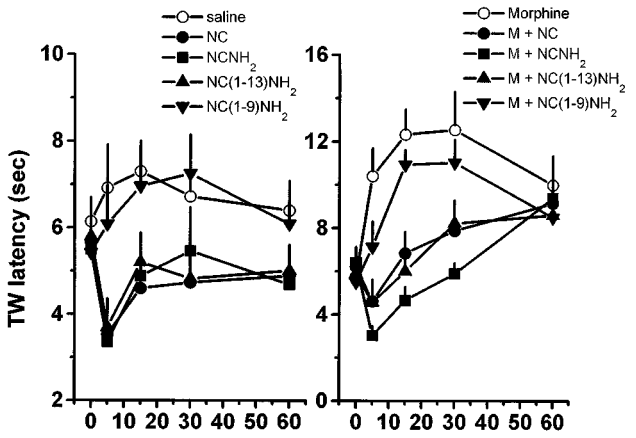

Figure 3 Left panel: Effects of nociceptin (NC, 1 nmol, i.c.v.) and NC related peptides (all administered at 1 nmol, i.c.v.) on TW latency in mice. Data are means ± s.e.m. of at least six experiments. Right panel: Effects of nociceptin (NC, 1 nmol, i.c.v.) and NC related peptides (all administered at 1 nmol, i.c.v.) on morphine (M, 1 nmol, i.c.v.) induced analgesia. Data are means ± s.e.m. of at least five experiments. All these data, converted into AUC and statistically analysed by one-way ANOVA followed by the Dunnett test for multiple comparisons, are presented in Table 1.

Table 2 Effects of i.c.v. injection of nociceptin and nociceptin related peptides on tail withdrawal latencies in saline- and morphine-treated mice

Treatment	*Saline*	*Morphine 1 nmol*
Control	175 ± 5	274 ± 25
NC 0.1 nmol	150 ± 16	206 ± 21
NC 1 nmol	131 ± 10*	168 ± 15*
NC 10 nmol	115 ± 12*	98 ± 10*
Control	175 ± 16	300 ± 33
NC 1 nmol	111 ± 9*	167 ± 15*
$NCNH_2$ 1 nmol	118 ± 12*	117 ± 15*
$NC(1-13)NH_2$ 1 nmol	120 ± 9*	159 ± 13*
$NC(1-9)NH_2$ 1 nmol	172 ± 13	255 ± 27
Control	138 ± 12	236 ± 22
NC 1 nmol	97 ± 10*	174 ± 19*
$[F/G]NC(1-13)NH_2$ 1 nmol	102 ± 8*	157 ± 13*
NC + $[F/G]NC(1-13)NH_2$	83 ± 9*	125 ± 13*

The data, mean ± s.e.m. of at least five experiments, were expressed as area under the curve (see Methods). *$P<0.05$ *versus* control according to one-way ANOVA followed by the Dunnett test for multiple comparison.

prolonged the effect of the peptide. NC, in the same range of doses (0.1 – 10 nmol), was also applied simultaneously with 1 nmol morphine to test its ability to interfere with the alkaloid analgesic effect. Results illustrated in Figure 2 (right panel), indicate that, in the presence of NC, the analgesic effect of morphine was decreased and the reduction was dependent on the dose of the peptide. A statistically significant inhibition of morphine effect was obtained with 1 nmol of NC (Table 2). The high dose of NC (10 nmol) not only blocked the analgesic effect of morphine but also caused a reduction above the baseline of the TW reaction time, mimicking the effect of the peptide alone.

Other experiments were carried out to measure the effects of some NC related peptides, $NCNH_2$, $NC(1-13)NH_2$ and $NC(1-9)NH_2$. As shown in Figure 3 (left panel), when applied i.c.v. at the dose of 1 nmol, NC, $NCNH_2$ and $NC(1-13)NH_2$ all exerted a statistically significant reduction of TW latency as compared to controls (Table 2). On the contrary, $NC(1-9)NH_2$ was found to be completely inactive. The three peptides which induced hyperalgesia (NC, $NCNH_2$ and $NC(1-13)NH_2$) and the inert peptide $NC(1-9)NH_2$, were also tested against the effect of 1 nmol morphine (Figure 3, right panel). NC, $NCNH_2$ and $NC(1-13)NH_2$ significantly inhibited the morphine induced analgesia (Table 2), the effect of $NCNH_2$ being longer lasting than that of the other two compounds. $NC(1-9)NH_2$ did not exert any statistically significant effect against morphine.

A final series of experiments was performed to evaluate the *in vivo* effect of a new compound, $[F/G]NC(1-13)NH_2$, which has been recently described as a competitive antagonist of the NC receptor in *in vitro* isolated preparations (Guerrini *et al*., 1998). The new compound was tested at 1 nmol alone and *versus* 1 nmol NC. The results presented in Figure 4 (left panel) indicate that $[F/G]NC(1-13)NH_2$ is acting in a similar way as NC, reducing in a statistically significant manner the TW latency as compared to controls (Table 2); moreover, the coadministration of NC and $[F/G]NC(1-13)NH_2$ further decreased the TW latency. As shown in Figure 4 (right panel) $[F/G]NC(1-13)NH_2$ was also able to significantly inhibit the analgesic effect of morphine, with the same intensity as NC. The simultaneous administration of NC, and $[F/G]NC(1-13)NH_2$ *vs* morphine leads to a complete block of morphine evoked analgesia and to a reduction below the baseline of the TW reaction time, similar to what was obtained with NC alone at 10 nmol.

Discussion

The results presented above indicate that, under the experimental conditions described in the Method section, NC exerts two distinct measurable effects in awake mice: induction of the hyperalgesia and inhibition of morphine-evoked analgesia. Hyperalgesia induced by NC in the mouse has been demonstrated by Meunier *et al*. (1995) using the hot plate test

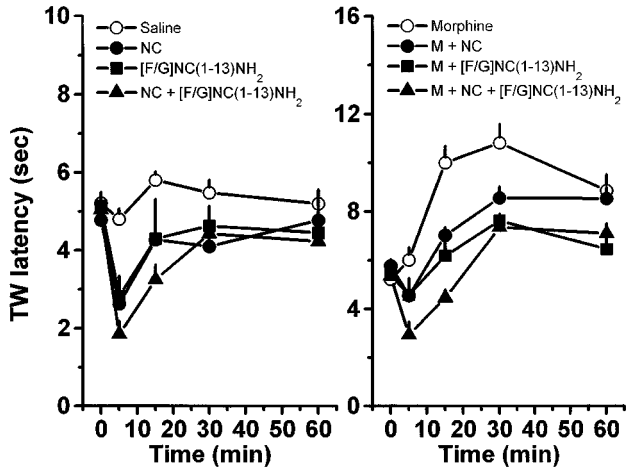

Figure 4 Left panel: Effects of nociceptin (NC, 1 nmol, i.c.v.) and [Phe$^1\psi$(CH$_2$-NH)Gly^2]NC(1–13)NH$_2$ ([F/G]NC(1–13)NH$_2$) (1 nmol, i.c.v.) on TW latency in mice. Data are means ± s.e.m of at least six experiments. Right panel: Effects of nociceptin (NC, 1 nmol, i.c.v.) and [F/G]NC(1–13)NH$_2$ (1 nmol, i.c.v.) on morphine (M, 1 nmol, i.c.v.) induced analgesia. Data are means ± s.e.m of at least five experiments. All these data, converted into AUC and statistically analysed by one-way ANOVA followed by the Dunnett test for multiple comparisons, are presented in Table 1.

and by Reinscheid *et al*. (1995) using the tail flick assay. The results obtained with the TW test utilized in the present experiments are similar to those already described both in terms of dose-dependency (0.1–10 nmol range), or of potency of the peptide (1 nmol being the first dose of NC inducing a statistically significant effect) and in terms of length of effect (about 15 min). The hyperalgesic effect of NC appears to be due to the activation of a specific NC receptor since (i) it can be evoked by various NC related peptides; (ii) it is not affected by naloxone; (iii) it can be prevented by i.c.v. injections of antisense oligonucleotide complementary to NC receptor (Zhu *et al*., 1997); (iv) it can be induced in wild-type mice but not in mice lacking the nociceptin receptor gene (Nishi *et al*., 1997). Other investigators did not observe hyperalgesia after i.c.v. application of NC in the mouse; however, they showed that NC reversed the stress induced antinociception (mediated by the endogenous opioid system) associated with the i.c.v. injection procedure (Mogil *et al*., 1996a). Under the experimental conditions utilized in the present study, stress induced antinociception did not occur since (i) naloxone was completely inactive on baseline TW latencies and (ii) no statistically significant differences between saline-injected mice and uninjected mice were found. Thus, NC induced hyperalgesia measured with the TW assay can be attributed to a decrease of pain threshold independently from the endogenous opioid system. NC dose-dependently inhibited the analgesic effect of morphine: these findings confirmed previous observations in the mouse (Grisel *et al*., 1996; Mogil *et al*., 1996a) and in the rat (Tian *et al*., 1997a; Zhu *et al*., 1997). NC was also shown to antagonize analgesia induced by selective opioid receptor agonists in the mouse (Mogil *et al*., 1996b) or by electropuncture in the rat (Tian *et al*., 1997b). When injected at high doses (i.e. 10 nmol) in the mouse, NC not only prevented 1 nmol morphine-induced analgesia, but induced a statistically significant hyperalgesic effect, as did the peptide given alone at 1 nmol. This indicates that NC and morphine have (on a molar basis) similar potencies, although the direction of their effects on pain threshold is opposite. The effects of NC and morphine are mediated by distinct receptors as demonstrated by naloxone which blocks morphine and does not affect NC. The inhibition of morphine induced analgesia by NC results, therefore, from a functional antagonism. As to the mechanism(s) of such antagonism, the findings by Morgan *et al*. (1997) are worthy of note: NC not only inhibited the analgesia induced by morphine when microinjected into the ventral periaqueductal gray, but also inhibited that induced by kainic acid (an agonist of ionotropic glutamate receptors). Therefore, NC receptors seem to be present on those neurons which control the output of the periaqueductal gray area downstream from opioid sensitive neurons. In line with this hypothesis, Vaughan *et al*. (1997) demonstrated that NC inhibited all periaqueductal gray neurons including those that did not respond to opioids. Inhibition by nociceptin of periaqueductal gray output neurons was, therefore, proposed by Morgan *et al*. (1997) as an effective mechanism to explain the pronociceptive actions of this peptide.

The specific NC functional sites mediating pronociceptive effects in the mouse were characterized by the use of a few NC related peptides to compare the pharmacological profiles of the central NC receptor with the peripheral receptor which has been already characterized (Calo *et al*., 1997; Guerrini *et al*., 1997). The present results indicate that (i) the effects of NCNH$_2$ are very similar to those of NC, the naturally occurring peptide; (ii) the minimum NC sequence that maintains full activity is NC(1–13)NH$_2$; (iii) NC(1–9)NH$_2$ is completely inactive. Thus, the spectrum of activities of these NC receptor agonists in the mouse central nervous system (CNS) is: NC = NCNH$_2$ = NC(1–3)NH$_2$ > > > NC(1–9)NH$_2$ which corresponds to the order of potency of agonists which has been determined in the peripheral NC receptor of the mouse vas deferens (Calo *et al*., 1997; Guerrini *et al*., 1997). Similar results were also obtained with the receptor binding approach in displacement studies performed on rat (Dooley & Houghten, 1996) or mouse (Calo *et al*., 1998) brain membranes. Taken together these findings demonstrate that NC receptor agonists do not discriminate between peripheral and central NC functional sites.

An important difference has however been found between the peripheral and the central NC receptors with [F/G]NC(1–13)NH$_2$ which acts as an antagonist in the periphery (Guerrini *et al*., 1998) and as an agonist in the CNS (present results). In fact, [F/G]NC(1–13)NH$_2$ has exactly the same effects as NC in the two experimental paradigms analysed in the present study: it induces hyperalgesia and it inhibits the morphine evoked analgesia. Moreover when NC and [F/G]NC(1–13)NH$_2$ were applied together, the evoked effect was higher (although not significantly) than that of the single treatments, suggesting that the effects of the two peptides are additive. To interpret these findings, several explanations can be proposed. Firstly, the effect of NC in the CNS and in the periphery may be mediated by different receptors; however, no data to corroborate this view are presently available in the literature and the results of *in vivo* experiments may not be sufficient to prove the two receptor hypothesis. Secondly, different splicing variants of the same NC receptor gene could account for the different pharmacological profiles of [F/G]NC(1–13)NH$_2$; in this regard it is worthy of note that, using the reverse transcription-polymerase chain reaction approach, Wang *et al*. (1994) found to different mRNA species of the rat NC receptor in several tissues. This is in line with the findings by Mathis *et al*. (1997) and by Adapa & Toll (1997) who detected two different NC binding sites in the mouse brain membranes and in intact CHO cells transfected with the mouse NC receptor cDNA. Thirdly, different products of [F/G]NC(1–

13)NH_2 metabolism could originate at the CNS and the peripheral level; the involvement of these metabolites in the different action of the molecule can not be ruled out. Independent from the molecular mechanism responsible for the dual behaviour of [F/G]NC(1–13)NH_2, the present findings clearly indicate that, using [F/G]NC(1–13)NH_2, it is now possible to pharmacologically discriminate between NC actions at the peripheral (which are prevented by this compound) and central (which are mimicked by this compound) level. Furthermore, these findings underlie the need of new agents which should be able to block the effects of NC in the CNS.

In conclusion, in the present report, we were able to evaluate (for the first time under the same experimental conditions) both the direct hyperalgesic and the anti-morphine effects of NC in the mouse, using the tail withdrawal assay. We also attempted a pharmacological characterization of the NC functional site responsible for these actions: the comparison of the present results with those obtained with the same compounds in a peripheral tissue of the mouse point to the existence of important differences between the NC functional sites mediating the central and the peripheral effects of the peptide. Further studies and the identification of compounds which should be able to antagonize NC central actions are needed to support and validate this intriguing hypothesis.

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References

ADAPA, I.D. & TOLL, L. (1997). Relationship between binding affinity and functional activity of nociceptin/orphanin FQ. *Neuropeptides,* **31,** 403–408.

BERZETEI-GURSKE, I.P., SCHWARTZ, R.W. & TOLL, L. (1996). Determination of activity for nociceptin in the mouse vas deferens. *Eur. J. Pharmacol.,* **302,** R1–R2.

CALO, G., RIZZI, A., BODIN, M., NEUGEBAUER, W., SALVADORI, S., GUERRINI, R., BIANCHI, C. & REGOLI, D. (1997). Pharmacological characterization of nociceptin receptor: an in vitro study. *Can. J. Physiol. Pharmacol.,* **75,** 713–718.

CALO, G., RIZZI, A., BOGONI, G., NEUGEBAUER, V., SALVADORI, S., GUERRINI, R., BIANCHI, C. & REGOLI, D. (1996). The mouse vas deferens: a pharmacological preparation sensitive to nociceptin. *Eur. J. Pharmacol.,* **311,** R3–R5.

CALO, G., VARANI, K., RIZZI, A., MERIGHI, S., TOTH, G., GUERRINI, R., SALVADORI, S., REGOLI, D. & BOREA, P.A. (1998). [^{3}H]-$NCNH_2$ binding to mouse forebrain membranes. In: *8th Annual Meeting of the European Neuropeptide Club*. Gent, Belgium.

CHAMPION, H.C. & KADOWITZ, P.J. (1997). (Tyr1)-nociceptin, a novel nociceptin analog, decreases systemic arterial pressure by a naloxone-insensitive mechanism in the rat. *Biochem. Biophys. Res. Commun.,* **234,** 309–312.

DEVINE, D.P., REINSCHEID, R.K., MONSMA, F.J., CIVELLI, O. & AKIL, H. (1996). The novel neuropeptide orphanin FQ fails to produce conditioned place preference or aversion. *Brain Res.,* **727,** 225–229.

DOOLEY, C.T. & HOUGHTEN, R.A. (1996). Orphanin FQ: receptor binding and analog structure activity relationships in rat brain. *Life Sci.,* **59,** 23–29.

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.

GRISEL, J.E., MOGIL, J.S., BELKNAP, J.K. & GRANDY, D.K. (1996). Orphanin FQ acts as a supraspinal, but not a spinal, anti-opioid peptide. *Neuroreport,* **7,** 2125–2129.

GUERRINI, R., CALO, G., RIZZI, A., BIANCHI, C., LAZARUS, L.H., SALVADORI, S., TEMUSSI, P.A. & REGOLI, D. (1997). Address and message sequences for the nociceptin receptor–A structure-activity study of nociceptin(1–13) amide. *J. Med. Chem.,* **40,** 1789–1793.

GUERRINI, R., CALO, G., RIZZI, A., BIGONI, R., BIANCHI, C., SALVADORI, S. & REGOLI, D. (1988). A new selective antagonist of the nociceptin receptor. *Br. J. Pharmacol.,* **123,** 163–165.

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.

KAPUSTA, D.R., SEZEN, S.F., CHANG, J.K., LIPPTON, H. & KENIGS, V.A. (1996). Diuretic and antinatriuretic responses produced by the endogenous opioid-like peptide, nociceptin (orphanin FQ). *Life Sci.,* **60,** PL15–PL21.

KAVALIERS, M. & PERROTSINAL, T.S. (1996). Pronociceptive effects of the neuropeptide, nociceptin, in the land snail, Cepaea nemoralis. *Peptides,* **17,** 763–768.

MATHIS, J.P., RYAN-MORO, J., CHANG, A., HOM, J.S., SCHEINBERG, D.A. & PASTERNAK, G.W. (1997). Biochemical evidence for orphanin FQ/nociceptin receptor heterogeneity in mouse brain. *Biochem. Biophys. Res. Commun.,* **230,** 462–465.

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

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

MOGIL, J.S., GRISEL, J.E., ZHANGS, G., BELKNAP, J.K. & GRANDY, D.K. (1996b). Functional antagonism of mu-, delta- and kappa-opioid antinociception by orphanin FQ. *Neurosci. Lett.,* **214,** 131–134.

MORGAN, M.M., GRISEL, J.E., ROBBINS, C.S. & GRANDY, D.K. (1997). Antinociception mediated by the periaqueductal gray is attenuated by orphanin FQ. *Neuroreport,* **8,** 3431–3434.

NISHI, M., HOUTANI, T., NODA, Y., MAMIYA, T., SATO, K., DOI, T., KUNO, J., TAKESHIMA, H., NUKADA, T., NABESHIMA, T., YAMASHITA, T., NODA, T. & SUGIMOTO, T. (1997). Unrestrained nociceptive response and disregulation of hearing ability in mice lacking the nociceptin/orphaninFQ receptor. *The EMBO Journal,* **16,** 1858–1864.

NOBLE, F. & ROQUES, B.P. (1997). Association of aminopeptidase N and endopeptidase 24.15 inhibitors potentiate behavioral effects mediated by nociceptin/orphanin FQ in mice. *FEBS,* **401,** 227–229.

PATEL, H.J., GIEMBYCZ, M.A., SPICUZZA, L., BARNES, P.J. & BELVISI, M.G. (1997). Naloxone-insensitive inhibition of acetylcholine release from parasympathetic nerves innervating guinea-pig trachea by the novel opioid, nociceptin. *Br. J. Pharmacol.,* **120,** 735–736.

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.

RIZZI, A., RADO, W., CALO, G., GEPPETTI, P., FABBRI, L. & REGOLI, D. (1997). Nociceptin inhibits neuropeptide release from sensory nerves of the guinea pig bronchus. In: *7th Annual Meeting of the European Neuropeptide Club*. Marburg, Germany.

STANFA, L.C., CHAPMAN, V., KERR, N. & DICKENSON, A.H. (1996). Inhibitory action of nociceptin on spinal dorsal horn neurones of the rat, *in vivo*. *Br. J. Pharmacol.,* **118,** 1875–1877.

TALLARIDA, R.J. & MURRAY, R.B. (1987). *Manual of pharmacologic calculations with computer programs*. New York: Springer Verlag.

TIAN, J.H., XU, W., FANG, Y., MOGIL, J.S., GRISEL, J.E., GRANDY, D.K. & HAN, J.S. (1997a). Bidirectional modulatory effect of orphanin FQ on morphine-induced analgesia: antagonism in brain and potentiation in spinal cord of the rat. *Br. J. Pharmacol.,* **120,** 676–680.

TIAN, J.H., XU, W., ZHANG, W., FANG, Y., GRISEL, J.E., MOGIL, J.S., GRANDY, D.K. & HAN, J.S. (1997b). Involvement of endogenous orphanin FQ in electroacupuncture-induced analgesia. *Neuro report,* **8,** 497–500.

VAUGHAN, C.W., INGRAM, S.L. & CHRISTIE, M.J. (1997). Actions of the ORL1 receptor ligand nociceptin on membrane properties of rat periaqueductal gray neurons *in vitro*. *J. Neurosci.,* **17,** 996–1003.

WANG, J.B., JOHNSON, P.S., IMAI, Y., PERSICO, A.M., OZENBERGER, B.A., EPPLER, C.M. & UHL, G.R. (1994). cDNA cloning of an orphan opiate receptor gene family member and its splice variant. *FEBS,* **348,** 75–79.

XU, X.J., HAO, J.X. & WIESENFELD-HALLIN, Z. (1996). Nociceptin or antinociceptin: potent spinal antinociceptive effect of orphanin FQ/nociceptin in the rat. *Neuroreport,* **7,** 2092–2094.

YAMAMOTO, T., NOZAKI-TAGUCHI, N. & KIMURA, S. (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.

ZHANG, G., MURRAY, T.F. & GRANDY, D.K. (1997). Orphanin FQ has an inhibitory effect on the guinea pig ileum and the mouse vas deferens. *Brain Res.,* **772,** 102–106.

ZHU, C.B., CAO, X.D., XU, S.F. & WU, G.C. (1997). Orphanin FQ potentiates formalin-induced pain behavior and antagonizes morphine analgesia in rats. *Neurosci. Lett.,* **235,** 37–40.

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