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European Journal of Pharmacology 317 (1996) 9–13

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Nociceptin stimulates locomotion and exploratory behaviour in mice

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Received 26 June 1996; revised 28 August 1996; accepted 30 August 1996

Abstract

The recently characterized heptadecapeptide nociceptin, the endogenous agonist of the orphan opioid receptor-like 1 (ORL1 receptor), has been tested for its effects on locomotion and exploratory behaviour in mice. I.c.v. administration of as little as 10 ng of nociceptin/animal stimulated locomotor activity. This effect was dose-dependent, increasing in intensity up to 100 ng and in duration for doses in the range of 1000–10000 ng. The stimulation of horizontal locomotion elicited by 100 ng nociceptin was accompanied by a stimulation of the vertical component of locomotion. These effects were not reversed by high doses (1.5 and 4.5 mg/kg s.c.) of the opioid receptor antagonist naloxone. Increasing doses of the dopamine D_2 receptor antagonist haloperidol (0.1–0.5 mg/kg i.p.) as well as of the dopamine D_1 receptor antagonist SCH 23390 [*R*(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine hydrochloride] (0.0075–0.03 mg/kg s.c.) reversed this effect, suggesting that nociceptin exerts its motor-stimulant actions by increasing central dopaminergic transmission. Nociceptin was also found to increase the number of head dips in the hole-board test, indicating that the peptide stimulates exploratory behaviour.

Keywords: Nociceptin; Locomotor activity; Exploratory behavior; Dopamine transmission

1. Introduction

Orphan receptor ORL1 is a novel member of the G-protein-coupled receptor family. The cDNA of ORL1 has been isolated from a human brain stem library and its sequence has some similarities with those of opioid receptors (Mollereau et al., 1994). ORL1 appears to be involved in the regulation of nociperception since inhibition of its expression in vivo, with an antisense oligonucleotide, increases the nociceptive threshold in mice (Meunier et al., 1995). The endogenous ligand of the ORL1 receptor was recently isolated from brain tissues and identified as a peptide of 17 amino acids (Meunier et al., 1995; Reinscheid et al., 1995) displaying clear structural homologies with another naturally occurring heptadecapeptide, the endomorphin dynorphin A. The novel peptide induces hyperalgesia upon i.c.v. injection in mice, hence, its designation nociceptin.

Preliminary behavioural screening suggested awakening and psycho-stimulant actions for nociceptin in mice. We decided to examine its effects on the horizontal and vertical components of locomotion as well as on exploratory behaviour. Since the ORL1 receptor resembles opioid receptors and nociceptin has some similarities with dynorphin A, we determined whether nociceptin-induced stimulation of locomotion in mice is sensitive to the opiate antagonist naloxone (Sawynock et al., 1979). In addition, we investigated whether the stimulant locomotor effect of the peptide involves dopamine transmission using antagonists of dopamine D1 or D2 receptors, because the latter participates frequently in psycho-stimulant effects (Costentin, 1996).

2. Materials and methods

2.1. Animals

Male Swiss albino mice (CD1, Charles River, Saint Aubin lès Elbeuf, France) weighing 20–25 g were used in this study. They were housed, 20/box (L 40 cm, W 25

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PII S0014-2999(96)00707-8

cm, H 18 cm), with unlimited access to standard semi-synthetic laboratory food and tap water, under controlled environmental conditions (temperature 22 ± 1°C; 07:00–19:00-h light-dark cycle). Experiments were carried out between 10:00 and 18:00 h. Each animal was used once.

2.2. Intracerebroventricular injections

Intracerebroventricular injections (10 μl) were given, in the left ventricle, in about 3 s with a microsyringe (Hamilton, 50 μl) connected to a needle (diameter 0.5 mm) of which the median part of the bevel protruded only 3.5 mm from a guard limiting its penetration into the brain of manually immobilized mice, according to the procedure of Haley and Mc Cormick (1957). The animals were routinely tested, starting 5 min after injection.

2.3. Locomotor activity

Locomotor activity was assessed using a Digiscan Animal Activity Monitor (Omnitech Electronics). This system consists of cages (L 20 cm, W 20 cm, H 30 cm) surrounded with two superimposed sets each of 8 IR beam sensors, the lower set for monitoring horizontal and the upper vertical displacements. The cages were placed in a dimly lit, sound-attenuated room. Horizontal activity was measured from the number of crossings of the lower set of beam sensors while vertical activity was determined using the upper set of beam sensors.

2.4. Hole-board test

The hole-board test, adapted from Boissier and Simon (1962), consists of a plastic square plate (40 × 40 cm, 1 cm thick) with 16 holes, 2 cm in diameter, evenly spaced, at 3.5 cm from the edges. The animals were placed in the center of the plate and the number of head dips was measured during 4 consecutive periods of 5 min each.

2.5. Drugs and solutions

Nociceptin was solid phase synthetized by Dr. H. Mazarguil (CNRS, Toulouse). Naloxone hydrochloride was from Endo and SCH 23390 [*R*(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine hydrochloride] from Schering. Haloperidol solutions were obtained by dilution in saline (NaCl 0.9%, w/v) of Haldol® (Janssen).

2.6. Statistical analysis

The data are expressed as the mean ± S.E.M. Differences between groups were assessed by two-way analysis of variance (ANOVA) and Student's *t*-test. $P < 0.05$ was taken as the significant level of difference.

3. Results

Fig. 1 shows that i.c.v. injection of a dose of nociceptin as low as 10 ng/mouse elicited an increase in horizontal

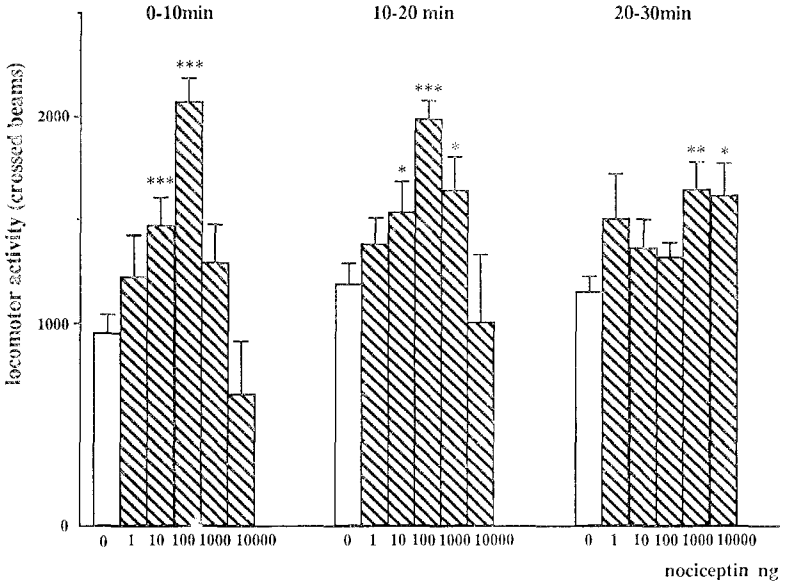

Fig. 1. Effects of increasing doses of nociceptin on horizontal locomotor activity in mice. Mice were i.c.v. injected with 0–10000 ng of nociceptin immediately before being introduced into the cages of the actimeter. Horizontal activity was measured during three consecutive periods of 10 min each, the first starting 5 min after introduction into the cage. Mean ± S.E.M. from 10 animals/group. Statistical comparisons (Student's *t*-test) refer to saline controls. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Table 1
Effects of increasing doses of nociceptin on vertical locomotor activity in mice

Nociceptin dose (ng/mouse)	Number of vertical movements during the periods (min)		
	0–10 min	10–20 min	20–30 min
0	96 ± 24.3	131 ± 29.4	143 ± 33
10	195 [b] ± 34	223 [a] ± 39	222 [a] ± 39
100	266 [d] ± 41	253 [c] ± 33.5	231 [a] ± 34.5
1000	239 [b] ± 56.2	301 [d] ± 36.6	295 [b] ± 50.5

Mice were i.c.v. injected with 0–1000 ng of nociceptin immediately before being introduced into the cages of the actimeter. The number of vertical movements was measured during three consecutive periods of 10 min each, the first one beginning 5 min after their introduction into the actimeter. Mean ± S.E.M. from 20 animals/group.
[a] Not significant.
[b] $P < 0.05$.
[c] $P < 0.01$.
[d] $P < 0.001$ compared to saline-injected controls.

locomotor activity. This effect apparently peaked at the dose of 100 ng. However, the stimulant action of the peptide lasted no more than 20 min. At higher doses (1000 and 10000 ng/mouse), nociceptin appeared to stimulate locomotion only after a delay of at least 20 min following introduction of the animal into the monitor. The peptide also increased vertical locomotor activity during the periods 0–10 and 10–20 min of observation and these increases were significant for the 100- and 1000-ng doses (Table 1).

Table 2
Effect of nociceptin on the exploratory bahaviour in mice

Treatment (i.c.v.)	Number of explored holes during the periods (min)			
	0–5	5–10	10–15	15–20
Saline	28.5 ± 5.7	39 ± 4.6	46.8 ± 4.3	40.7 ± 6.5
Nociceptin (100 ng/mouse)	50.4 [b] ± 6.8	64.5 [c] ± 7.3	62.4 [b] ± 5.6	58.6 [a] ± 6.6

Each mouse was i.c.v. injected with either saline (10 μl) or nociceptin (100 ng) and put in its home cage for 5 min. Then, the animal was put on the hole board (see Section 2) and the holes it explored were counted over 4 consecutive periods of 5 min each. Mean ± S.E.M. from 10 animals/group.
[a] Not significant.
[b] $P < 0.05$.
[c] $P < 0.01$ compared to saline-injected controls.

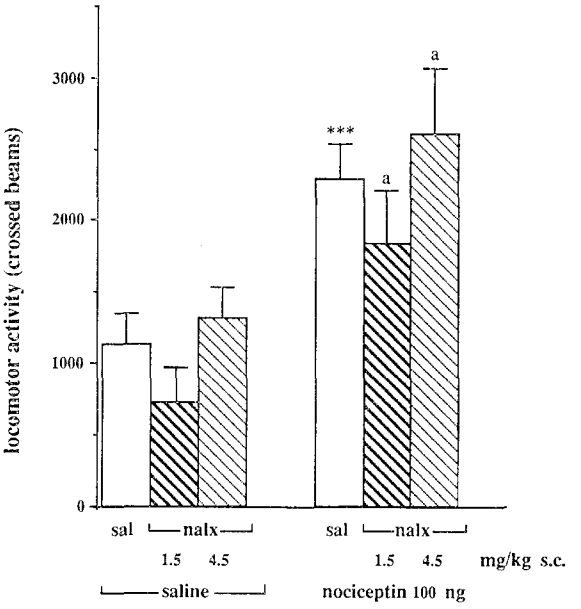

Fig. 2. Effects of naloxone on nociceptin-induced stimulation of horizontal locomotor activity in mice. Saline (sal) or increasing doses of naloxone (nalx) were s.c. injected 10 min before i.c.v. administration of either saline (10 μl) or nociceptin (100 ng/mouse). The animals were introduced immediately into the actimeter and horizontal activity was measured between min 5 and 15. Mean ± S.E.M. from 10 animals/group. *** $P < 0.001$ compared to saline s.c./saline i.c.v. controls and [a] $P < 0.05$ compared to naloxone controls.

In the hole-board test, nociceptin (100 ng/mouse i.c.v.) induced a significant increase in the number of explored holes (Table 2). During the 20-min period of observation, this number increased from 155 ± 15 in saline to 226 ± 18 in peptide-treated animals ($P < 0.01$). When the animals were injected with morphine (5 mg/kg s.c.) and tested 15 min later under the same conditions as nociceptin-treated animals, the number of explored holes was considerably reduced (150 ± 8 in saline vs. 24 ± 2 in morphine-treated groups, $P < 0.001$). All the morphine-treated mice maintained their tail vertically, a behaviour that corresponds to the so-called 'Straub tail' phenomenon whereas the nociceptin-treated mice did not differ in this respect from saline-injected controls.

Naloxone (1.5–4.5 mg/kg s.c.) injected 10 min before i.c.v. administration of 100 ng nociceptin did not suppress the motor-stimulant effect of the peptide observed during the first 10 min of testing (pre-treatment × treatment interaction $F(1,20) = 0.023$) (Fig. 2).

The dopamine D_1 receptor antagonist, SCH 23390 (schering) proved to be effective in antagonizing nociceptin stimulation of locomotor activity in mice (pre-treatment × treatment interaction $F(1,20) = 4.25$; $P < 0.01$) (Fig. 3). The lowest dose of SCH 23390 (0.0075 mg/kg) did not significantly decrease the spontaneous locomotor activity in controls, although a tendency was observed, but almost completely reversed the stimulant effect of nociceptin 100 ng. A complete antagonism was observed for

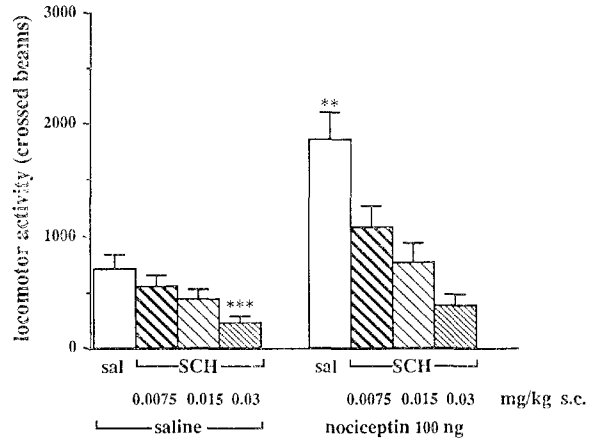

Fig. 3. Effects of increasing doses of SCH 23390 on nociceptin-induced stimulation of horizontal locomotor activity in mice. Saline (sal) or increasing doses of SCH 23390 (SCH) were s.c. injected 20 min before i.c.v. administration of either saline (10 μl) or nociceptin (100 ng/mouse). The animals were introduced immediately into the actimeter and horizontal activity was measured between min 5 and 15. Mean ± S.E.M. from 10 animals/group. ** $P < 0.01$ *** $P < 0.001$ compared to saline s.c./saline i.c.v. controls.

higher doses of SCH 23390 (0.015 and 0.03 mg/kg), but these doses dramatically reduced the spontaneous locomotor activity in controls. Similarly, the dopamine D_2 receptor antagonist, haloperidol, antagonized the nociceptin-induced stimulation of locomotor activity (pre-treatment × treatment interaction $F(1,20) = 3.2$; $P < 0.05$) (Fig. 4). The lowest tested dose of haloperidol (0.05 mg/kg) did not significantly antagonize the nociceptin-induced stimulation of locomotion. The 0.1- and 0.25-mg/kg doses of haloperidol did not significantly decrease the spontaneous locomotor activity in controls although a tendency was

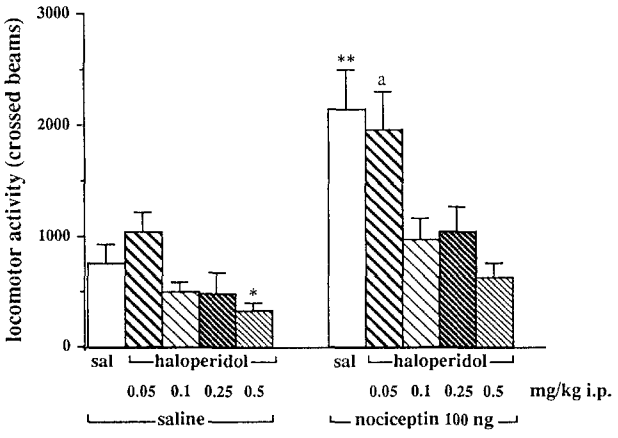

Fig. 4. Effects of increasing doses of haloperidol on the nociceptin-induced stimulation of horizontal locomotor activity in mice. Saline (sal) or increasing doses of haloperidol were i.p. injected 30 min before i.c.v. administration of either saline (10 μl) or nociceptin (100 ng/mouse). The animals were introduced immediately into the actimeter and horizontal activity was measured between min 5 and 15. Mean ± S.E.M. from 10 animals/group. * $P < 0.05$, ** $P < 0.01$ compared to saline i.p./saline i.c.v. controls and [a] $P < 0.05$ compared to haloperidol controls.

observed but almost completely reversed the stimulant effect of nociceptin 100 ng. For highest tested dose of haloperidol 0.5 mg/kg, a marked reduction of locomotion was observed in controls.

4. Discussion

The recently discovered neuropeptide nociceptin (Meunier et al., 1995) stimulated both the horizontal and vertical components of locomotion in mice. This effect was readily seen at the low dose of 10 ng/mouse and seemed to peak at 100 ng. Regardless of the dose, nociceptin's action appeared to be of short duration, suggesting a rapid inactivation of the peptide. At the highest tested dose (10000 ng), stimulation of horizontal locomotor activity by nociceptin did not show up until at least 25 min following i.c.v. injection, as if it exerted, during this period an effect opposing that of low doses of the peptide. Since nociceptin has some structural similarities with dynorphin A, which itself and other agonists of κ-opioid receptors depress locomotion (Chaillet et al., 1983; Marçais-Collado et al., 1983), it is possible that such an opposing action of high doses of nociceptin might be a consequence of the aspecific stimulation of these opioid receptors.

Reinscheid et al. (1995) have reported that i.c.v. administered 'orphanin FQ' (nociceptin) induces a decrease in horizontal and vertical motor activities in mice. The discrepancy between their results and ours may be accounted for by differences in experimental design. In particular, those authors used comparatively large doses of the peptide (10 nmol i.e. 18 μg/mouse) and monitored the animals for 10 min immediately following injection. Since it was not determined whether or not naloxone opposes the observed motor-depressant effect of orphanin FQ, the question that this effect may have involved κ-opioid receptors remains open.

Various data argue against the involvement of μ-opioid receptors in the stimulant locomotor effect of nociceptin: (1) the locomotor effect induced by nociceptin includes a stimulation of vertical activity, in contrast to the so-called 'running fit' behaviour elicited by morphine (Marçais-Collado et al., 1983; Michael-Titus et al., 1989); (2) nociceptin did not induce the Straub tail reaction, characteristic of μ-opioid receptor agonist (Aceto et al., 1969); (3) nociceptin, instead of being analgesic, appears to be hyperalgesic (Meunier et al., 1995; Reinscheid et al., 1995); and (4) its stimulatory action on locomotion is not antagonized by the opioid receptor antagonist naloxone. This opioid receptor antagonist, even at the 4.5-mg/kg dose, had no effect on the stimulant activity of nociceptin. This dose of naloxone appears not only able to block μ-opioid receptors but also δ-opioid receptors, since it inhibits the stimulation of locomotion elicited in mice by the enkephalinase inhibitor acetorphan (Michael-Titus et al., 1988) and also

κ-opioid receptors, since it antagonizes the inhibition of vertical locomotor activity elicited by ketocyclazocine (Marçais-Collado et al., 1983). Another important difference is that nociceptin stimulates exploratory behaviour while morphine strongly decreases it (see the hole-board test). δ-Opioid receptor agonists are known to stimulate both the horizontal and vertical components of locomotion; this effect is reversed by naloxone and is accompanied by a naxolone-reversible analgesia in the hot-plate test (Blumberg et al., 1966; Michael-Titus et al., 1990; Bousselmame et al., 1991). Hence, the motor-stimulant effect of nociceptin is unlikely to involve δ-opioid receptors. Taken together, these data argue against the notion that the stimulant/awakening actions of nociceptin in mice involve opioid receptors.

Most psycho-stimulant agents operate through an increase in central dopaminergic transmission (Pulvirenti and Koob, 1994; Costentin, 1996). To test this hypothesis, we considered the interaction of nociceptin with the dopamine D_2 receptor antagonist haloperidol (Cox et al., 1980) and the dopamine D_1 receptor antagonist SCH 23390 (Iorio et al., 1983). Antagonism was observed in each case, indicating that dopamine neurons, probably mesolimbic, are involved in the nociceptin-elicited stimulation of locomotion. However, the effective doses of haloperidol (0.5 mg/kg) or SCH 23390 (0.03 mg/kg) had an intrinsic tendency to decrease spontaneous locomotor activity. This observation complicates interpretation of the nociceptin antagonism experiment and thereby the involvement of dopamine transmission in this phenomenom is not unambiguous.

In conclusion, the recently discovered neuropeptide nociceptin, the endogenous agonist of opioid receptor-like ORL 1 receptor, stimulates locomotor and exploratory behaviours in mice by mechanisms that are independent of μ-, δ- and κ-opioid receptors and which could involve an increase in dopaminergic transmission.

Acknowledgements

This work is supported, in part, by the DRET (Contrat 95-167 to J.C.), the ARC (Contrat 1078 to J.-C.M.) and the MENESR (ACC-SV5 to J.-C.M. and J.C.).

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