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SPECIAL REPORT

Nociceptin (1–7) antagonizes nociceptin-induced hyperalgesia in mice

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Nociceptin and its N-terminal fragment, nociceptin (1–7), were administered intrathecally (i.t.) into conscious mice. Nociceptin (3.0 fmol) produced a significant reduction in the nociceptive thermal threshold (hyperalgesia) measured as the tail-flick and paw-withdrawal responses. Nociceptin (1–7), injected i.t., at 150–1200 fmol had no significant effect. However, when nociceptin (1–7) (150–1200 fmol) was injected simultaneously with nociceptin (3.0 fmol), nociceptin-induced hyperalgesia was significantly reduced. Analgesia induced by a high dose (1200 pmol) of nociceptin was not antagonized by co-administration of nociceptin (1–7) (1200 fmol). These results suggest that N-terminal fragments of nociceptin formed endogenously could modulate the hyperalgesic action of nociceptin in the spinal cord.

Keywords: Nociceptin; nociceptin (1–7); intrathecal injection; hyperalgesia; spinal cord

Abbreviations: CSF, cerebrospinal fluid; i.c.v., intracerebroventricular; i.t., intrathecal(ly); ORL_1, opioid receptor-like 1

Introduction Nociceptin, also referred to as orphanin FQ, is thought to be a putative endogenous ligand for the orphan opioid-like receptor (ORL_1) (Meunier *et al*., 1995; Reinscheid *et al*., 1995). Nociceptin is a 17 amino acid peptide (Phe-Gly-Gly- Phe -Thr-Gly -Ala -Arg -Lys -Ser -Ala -Arg -Lys -Leu -Ala-Asn-Gln) with some sequence homology to the endogenous opioid peptide dynorphin A (Mollereau *et al*., 1994). Behavioural studies have shown that nociceptin exhibits both hyperalgesic and analgesic activities. Hyperalgesic effects have been observed after intracerebroventricular (i.c.v.) and intrathecal (i.t.) injection of nociceptin in the mouse hot-plate and tail-flick assays (Meunier *et al*., 1995; Reinscheid *et al*., 1995; Hara *et al*., 1997). Analgesic actions have been found after i.c.v. and i.t. injection of nociceptin in mice and rats (Xu *et al*., 1996; King *et al*., 1997; Rossi *et al*., 1997; Tian *et al*., 1997). Nociceptin has also been reported to act as an anti-opioid peptide at the supraspinal but not at the spinal level in the rat (Grisel *et al*., 1996). The complex pharmacology of nociceptin suggests that some of the observed effects may be exerted by shorter fragments of nociceptin. Recently, Montiel *et al*. (1997) have showed that nociceptin (1–7), a N-terminal fragment, is one of the main metabolites of nociceptin in mouse brain cortical slices. The aim of the present study was to investigate whether nociceptin (1–7) is a bioactive metabolite of nociceptin at the spinal cord level.

Methods Male mice of ddY strain (20–22 g) were utilized throughout the study. On test days, animals were transported to the test environment and behavioural testing began 1 h later. All testing was conducted between 10:00 h and 16:00 h in a sound attenuated room, which was used only for these experiments. All compounds or artificial cerebrospinal fluid (CSF) was administered i.t. in a volume of 5 μl in unanaesthetized mice, essentially as described by Hylden & Wilcox (1980). A 28-gauge needle connected to a Hamilton microsyringe (50 μl) was directly inserted between the L5–L6 segment in mice. For i.t. injections, nociceptin and nociceptin (1–7) were dissolved in sterile artificial CSF containing (mM): NaCl 126.6, KCl 2.5, $MgCl_2$ 2.0 and $CaCl_2$ 1.3. Nociceptin or nociceptin (1–7) alone was given in a volume of 5 μl. Nociceptin in combination with nociceptin (1–7) was also co-administered i.t. in a total volume of 5 μl.

Hyperalgesia and analgesia were assessed by measuring nociceptive thermal thresholds with an automated tail-flick unit (BM kiki, Tokyo, Japan). In the tail-flick test, the heat stimulus intensity was measured as the reaction time to removal of the tail from beneath a source of noxious radiant heat. The intensity of the light beam was adjusted so that baseline reaction time was 8–9 s. The light beam was focused on the same spot, about 1.0 cm from the tip of the tail. The average threshold of the control response prior to injections of nociceptin or in combination with nociceptin (1–7) was determined by a total of two consecutive measurements each separated by 10 min. Mice were also examined for latency to withdraw their hind paw from a noxious thermal stimulus using the tail-flick unit. The radiant heat source was positioned under the glass floor directly beneath the hind paw. Values were recorded twice for the right hind paw and the mean calculated for each animal before i.t. injection. Mice not responding within 20 s were assigned a maximal score. Antinociception was expressed as per cent analgesia = 100 × (test latency − control latency)/(20 − control latency).

Nociceptin and nociceptin (1–7) were a generous gift from Dr Jun Sasaki of Asahi Glass Co., Yokohama, Japan. These peptides were synthesized by solid-phase peptide methodology and purified by high performance liquid chromatography. Data are presented as mean ± standard error of the mean (s.e.mean).

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Results Figure 1 illustrates the time course of the hyperalgesic effect induced by a most effective dose (3.0 fmol) of i.t. nociceptin in the tail-flick test. The i.t. administration of nociceptin produced a significant decrease in reaction time to removal of the tail from the noxious heat stimulus. The hyperalgesic effect of nociceptin was evoked at 10 min, reached a maximum at 15 min and then declined until cessation at 30 min. Three fmol of nociceptin caused a 32.2% reduction from a mean control value of 8.7 ± 0.5 s to a mean value of 5.9 ± 0.3 s at 15 min post-injection. The reaction time remained relatively unaffected by i.t. administration artificial CSF. Figure 2a,b illustrates the relation between the doses and the decrease in reaction time at 15 min post-injection in the tail flick and paw-withdrawal tests. The dose-dependency of nociceptin-induced hyperalgesia showed a bell-shaped pattern in the dose-range (0.375–30.0 fmol). The maximum effect was observed at 3.0 fmol in both tests. In further experiments, 3.0 fmol of nociceptin was therefore used, in combination with nociceptin (1–7). As shown in Figure 1, the nociceptin-induced decrease in reaction time observed 10, 15 and 20 min after i.t. injection was reduced significantly when nociceptin (1–7) (1200 fmol) was co-administered i.t. in the tail-flick test. Co-injection of nociceptin (1–7) in the lower dose range (150–600 fmol) also blocked nociceptin-induced hyperalgesia at 15 min post-injection in both tests (Figure 3a,b). Nociceptin (1–7) alone at doses ranging from 150–1200 fmol had no effect on the nociceptive thermal threshold (Table 1). High doses of i.t. nociceptin (300–1200 pmol) resulted in a significant and dose-dependent analgesia with a peak effect at 15 min (data not shown), similar to that seen in the hyperalgesia paradigm as assayed by the tail-flick and paw-withdrawal tests (Table 2). Analgesia induced by i.t. nociceptin (1200 pmol) was not affected by co-injection of nociceptin (1–7) (1200 fmol).

Discussion This study is the first to demonstrate that nociceptin-induced hyperalgesia is reduced by simultaneous i.t. injection of nociceptin (1–7) in the tail-flick and paw-withdrawal tests. Our results are in keeping with those from behavioural studies demonstrating that i.c.v. injection of nociceptin produces a hyperalgesic response measured by the hot-plate or tail-flick test in mice (Meunier *et al*., 1995; Reinscheid *et al*., 1995), and nociceptin-induced hyperalgesia is evoked 10 min after i.t. injection and the maximum effect is observed at 15 min in mice in the hot-plate test (Hara *et al*., 1997). It seems probable that small doses (fmol orders) of nociceptin given i.t. produce hyperalgesia (Hara *et al*., 1997) as well as scratching, biting and licking response (Sakurada *et al*., 1999) in mice. Conversely, analgesia can be induced in mice by relatively high doses (pmol or nmol orders) of i.t. nociceptin (Rossi *et al*., 1997; King *et al*., 1997). In agreement with these previous studies, i.t. nociceptin in high doses ranging from 300 to 1200 pmol reduced the tail-flick and paw-withdrawal responses (analgesia), as opposed to the hyperalgesia at lower doses (1.5–30.0 fmol). Given supraspinally, nociceptin N-terminal fragments, nociceptin (1–11) and nociceptin (1–7), produce an analgesic response (Rossi *et al*., 1997). However, at the spinal level, the two N-terminal fragments induce no hyperalgesia and far weaker analgesia than nociceptin (King *et al*., 1997). This is consistent with our data that i.t. injection of nociceptin (1–7) alone gave no effect on the nociceptive thermal responses.

In the present study, we find that hyperalgesia but not analgesia induced by nociceptin was reduced significantly by co-administration of nociceptin (1–7). These results reveal distinct mechanisms for nociceptin-induced hyperalgesia and analgesia. In the mechanism underlying the hyperalgesic effect of nociceptin, it seems evident that there is a possible functional

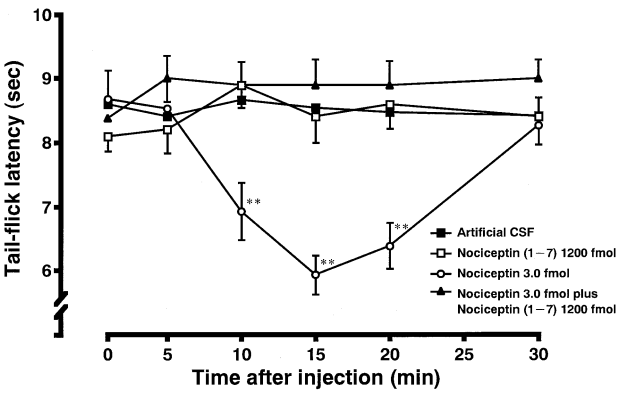

Figure 1 Effect of nociceptin (1–7) on nociceptin-induced hyperalgesia in the tail-flick test. Mice were injected i.t. with nociceptin, nociceptin (1–7) or artificial CSF, or were injected i.t. simultaneously with nociceptin and nociceptin (1–7). Each point represents the mean and vertical lines show s.e.mean ($n=10$). $^{**}P<0.01$, compared with the CSF-injected group at the respective time point.

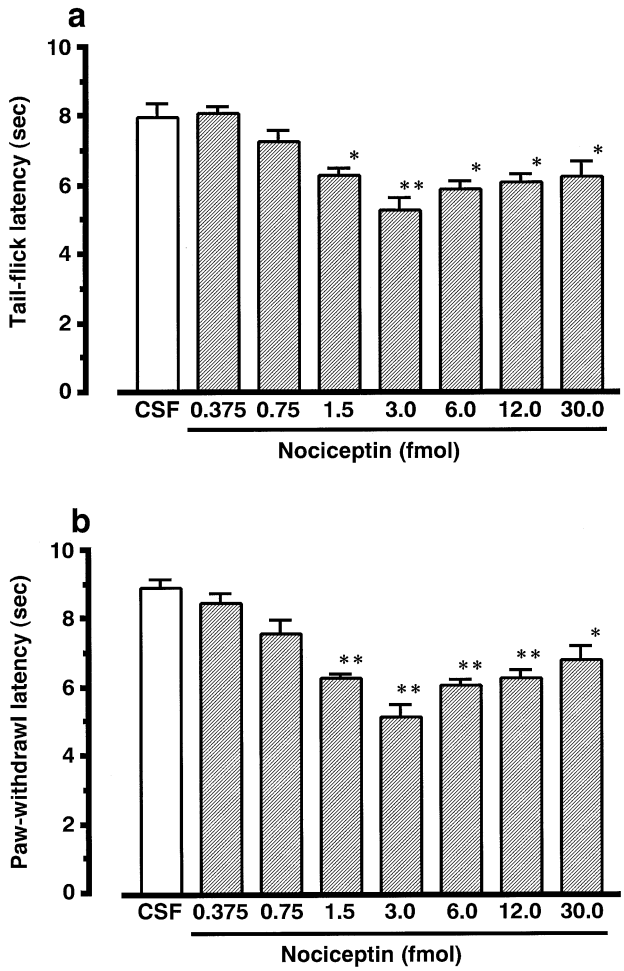

Figure 2 Hyperalgesia elicited by varying doses of nociceptin in the tail-flick (a) and paw-withdrawal (b) tests. Mice were injected i.t. with nociceptin in doses ranging from 0.375 to 30.0 fmol. Assessment of thermal latencies was made at 15 min following i.t. injection. Each column (mean ± s.e.mean) represents the time until the mice showed the tail-flick (a) or paw-withdrawal (b) response ($n=10$). $^{*}P<0.05$, $^{**}P<0.01$, compared with the CSF-injected group.

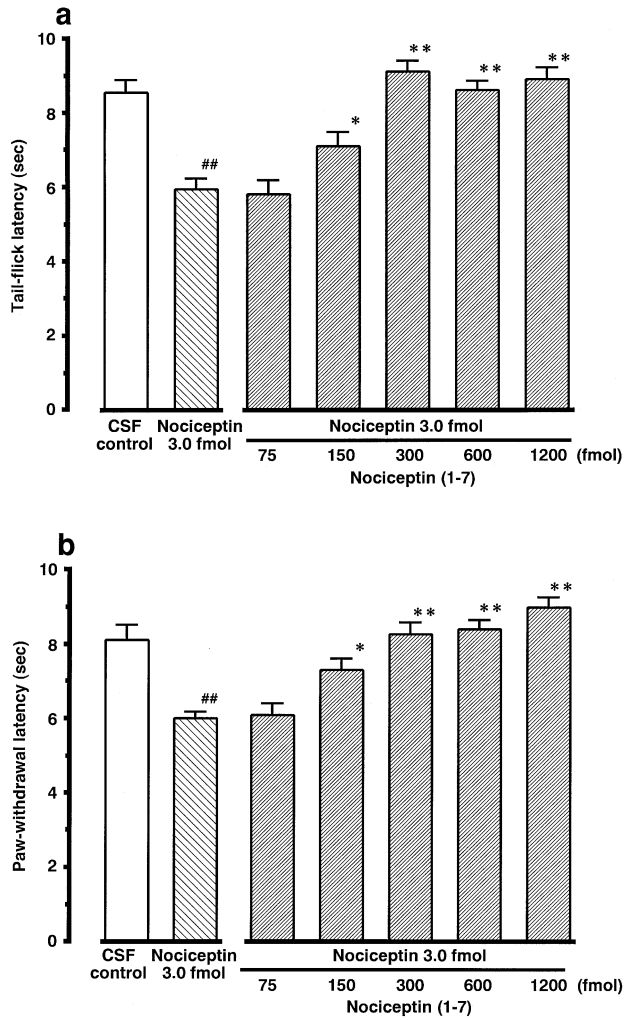

Figure 3 Effect of varying doses of nociceptin (1–7) on nociceptin-induced hyperalgesia in the tail-flick (a) and paw-withdrawal (b) tests. Mice were injected i.t. with 3.0 fmol of nociceptin alone, or in combination with varying doses of nociceptin (1–7). Assessment of thermal latencies was made at 15 min following i.t. injection. Each column (mean ± s.e.mean) represents the time until the mice showed the tail-flick (a) or paw withdrawal (b) response ($n=10$). ##$P<0.01$, when compared with the CSF-injected group. *$P<0.05$, **$P<0.01$, compared with the nociceptin (3.0 fmol)-injected group.

Table 1 Effect of nociceptin (1–7) on thermal latencies in the tail-flick and paw-withdrawal tests

Method	*Treatment*	*Latencies* (s)
Tail-flick test	CSF-control	8.5 ± 0.3
	Nociceptin (1–7) 150 fmol	8.4 ± 0.2
	300 fmol	8.3 ± 0.2
	600 fmol	8.2 ± 0.1
	1200 fmol	8.4 ± 0.4
Paw-withdrawal test	CSF-control	8.1 ± 0.4
	Nociceptin (1–7) 150 fmol	7.9 ± 0.1
	300 fmol	8.5 ± 0.2
	600 fmol	8.5 ± 0.2
	1200 fmol	8.7 ± 0.2

Mice were injected i.t. with nociceptin (1–7) or artificial CSF. Assessment of thermal latencies was made at 15 min following i.t. injection. Each value represents the mean and s.e.mean ($n=10$).

Table 2 Effect of nociceptin (1–7) on nociceptin-induced analgesia in the tail-flick and paw withdrawal tests

Method	*Treatmemt*	*% Analgesia*
Tail-flick test	CSF-control	−1.2 ± 0.1
	Nociceptin 300 pmol	40.9 ± 10.7
	600 pmol	74.2 ± 6.8
	1200 pmol	94.7 ± 2.2
	Nociceptin 1200 pmol plus nociceptin (1–7) 1200 fmol	92.5 ± 2.9
Paw-withdrawal test	CSF-control	−0.1 ± 0.0
	Nociceptin 300 pmol	28.4 ± 8.7
	600 pmol	37.4 ± 6.9
	1200 pmol	40.1 ± 2.5
	Nociceptin 1200 pmol plus nociceptin (1–7) 1200 fmol	42.4 ± 2.3

Mice were injected i.t. with nociceptin alone or in combination with nociceptin (1–7). Assessment of thermal latencies was made at 15 min following i.t. injection. The reaction time to tail-flick and paw-withdrawal under control conditions was 8–9 s. Each value represents the mean and s.e.mean ($n=10$).

antagonism between nociceptin and the N-terminal fragments in the spinal cord. However, it is unlikely that nociceptin (1–7) is a competitive antagonist against nociceptin, since nociceptin (1–7) has negligible affinity for orphanin FQ receptors (Butour *et al*., 1997). Furthermore, nociceptin (1–7) has not been reported to have affinity for traditional opioid receptors (Mathis *et al*., 1997), ruling out an opioid receptor interaction. Whatever the underlying mechanism, the data suggest that nociceptin (1–7) has an antagonizing effect on nociceptin-induced hyperalgesia without affecting analgesic action of nociceptin.

Recent findings suggest that nociceptin (1–7) is a principal metabolite of nociceptin (Montiel *et al*., 1997). It is rapidly and abundantly produced when nociceptin is incubated with mouse brain cortical slices. It is a major cleavage fragment produced by endopeptidase 24.15, an enzyme thought to play a major role in degrading endogenous nociceptin. Regulation of peptide action may, besides metabolic inactivation, occur *via* enzymatic conversion to fragments with antagonistic properties against nociceptin-induced hyperalgesia.

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