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Up-regulation of ORL-1 receptors in spinal tissue of allodynic rats after sciatic nerve injury

Luca Briscini [1], Laura Corradini, Ennio Ongini [2], Rosalia Bertorelli*

Schering-Plough Research Institute, San Raffaele Science Park, Via Olgettina, 58, 20132-Milan, Italy

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Abstract

Nociceptin, acting through the opioid receptor-like 1 (ORL1) receptor, produces anti-nociception in several models of neuropathy. We examined the involvement of the ORL1 receptor system in the allodynia developed after sciatic nerve ligation. Allodynic rats were selected by the von Frey hair and treated intrathecally with nociceptin or morphine. The peptide induced dose-dependent anti-allodynic activities, while morphine was effective at the higher dose only. By the semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR) assay, the two described ORL1 receptor isoforms were up-regulated in the allodynic animals, but unmodified in non-allodynic rats. Both short and long ORL1 receptor mRNA isoforms increased in the ipsilateral lumbar enlargement (by 50% and 100%, respectively), while 50% and 60% increases were found in the ipsilateral L5–L6 dorsal root ganglia, respectively. No significant changes were observed for either the nociceptin precursor or μ-opioid receptor expression. Thus, the ORL1 receptor system seems to regulate the mechano-allodynia that developed after nerve damage, suggesting its potential role in the treatment of neuropathic pain. © 2002 Published by Elsevier Science B.V.

Keywords: Allodynia; Neuropathic pain; Nociceptin/orphanin FQ; ORL1 receptor splice variant

1. Introduction

Nociceptin, also called orphanin FQ, has been previously described as the endogenous ligand of the opioid receptor-like 1 (ORL1) receptor. Nociceptin and its receptor are localized in various regions of the central nervous system, which are associated with nociception, such as cerebral cortex, thalamus, periaqueductal gray and dorsal horns of the spinal cord. Similarly, using immunoreactivity approaches the receptor protein has been detected in fiber processes of different brain regions and in the gray matter of the spinal cord, particularly in the superficial layer II of the lumbar dorsal horn and in the dorsal root ganglia. A similar pattern of distribution exists for the nociceptin precursor (prepronociceptin) mRNA and nociceptin peptide (see Harrison and Grandy, 2000 and Calo' et al., 2000, for reviews).

Two alternatively spliced isoforms of the rat ORL1 receptor mRNA have been identified, which differ in an insertion in the region encoding the second extracellular loop of the receptor (Wang et al., 1994). Although they co-exist in the tissues examined so far, the short and long ORL1 receptor transcripts are expressed in different ratios in several tissues. In particular, in the rat brain and dorsal root ganglia the short form was found to be more abundant than the long one, while in sympathetic superior cervical ganglia and lumbar sympathetic ganglia the long form is more abundant (Xie et al., 1999).

Intrathecal (i.t.) administration of nociceptin can antagonize the mechanical and cold allodynia that results from photochemically induced ischemia of both spinal cord and sciatic nerve in rats (Hao et al., 1998). By the same administration route, nociceptin is also effective against hyperalgesia resulting from either chronic constriction injury of the sciatic nerve (Yamamoto et al., 1997) or partial nerve injury in rats (Yamamoto and Nozaki-Taguchi, 1997). These anti-nociceptive responses, together with the ORL1 receptor expression in spinal cord and dorsal root ganglia, suggest an important role for this peptide system in the spinal modulation of nociceptive signals.

Neuropathic pain arising from peripheral nerve injury is a clinical disorder characterized by a combination of spontaneous burning pain, sensory loss, hyperalgesia and allodynia. A central hyperactive state resulting from the neuronal

* Corresponding author. Tel.:+39-02-21219216; fax: +39-02-21219253.
E-mail address: rosalia.bertorelli@spcorp.com (R. Bertorelli).
[1] Present address: Sandwich Laboratories, Pfizer Limited, Sandwich, Kent CT13 9NJ, UK.
[2] Present address: Nicox Research Institute, Via Ariosto, 21, 20091 Bresso, Milan, Italy.

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plastic changes within both spinal cord and dorsal root ganglia may play a critical role in the nociception associated with nerve injury and inflammation. The underlying mechanisms of neuropathic pain are poorly understood, but changes in primary sensory neurons may be involved. One consequence of such injuries is the appearance of adaptive changes in the expression of a variety of receptors, ion channels, and enzymes in both dorsal root ganglia and spinal cord (Sugimoto et al., 1995; Goff et al., 1998; Blackburn-Munro and Fleetwood-Walker, 1999; Dib-Hajj et al., 1999; Siddall et al., 1999). For example, changes in spinal opioid receptor expression are suggested to contribute to the hyperalgesic state caused after nerve damage (Besse et al., 1992; Goff et al., 1998).

Although many behavioral studies have been performed in animal models of neuropathy, there is limited information as to the expression changes which take place for the nociceptin system in the spinal cord and dorsal root ganglia following nerve injury. To better understand the mechanisms that give rise to neuropathic pain, we examined the molecular changes that occur in the lumbar enlargement and L5–L6 dorsal root ganglia of rats, which are the entry zone of sciatic nerve afferents, following chronic constriction injury of the sciatic nerve (Bennett and Xie, 1988), a model relevant to the human neuropathic condition. Thus, we analyzed the expression of the two described splice isoforms of ORL1 receptor, i.e. short and long, during the development of neuropathy, using the reverse transcription-polymerase chain reaction (RT-PCR) assay. Furthermore, we analyzed the mRNA expression of the μ-opioid receptor and the prepronociceptin. Changes occurring at molecular level were compared with anti-nociceptive effects produced by administering either nociceptin or morphine spinally.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (Charles River, Calco, Italy) weighing 175–200 g were housed with food and water available ad libitum, with a 12-h day/night cycle at constant room temperature of 22 °C. Procedures involving animals and their care were conducted in conformity with the institutional guidelines, in compliance with the European Community Council Directive (OJ L 358, 1, December 12, 1987).

2.2. Chronic constriction injury rat model

The surgical procedure was first described by Bennett and Xie (1988). Rats were anesthetized with chloral hydrate (400 mg/kg, i.p.) and the common sciatic nerve was exposed at the level of the mid-thigh. At about 1 cm, proximally to the nerve trifurcation, four ligatures (4/0 silk) were tied loosely around the nerve and leaving 1-mm space between them. Ligatures were tied such that the circulation through the superficial epineural vasculature was retarded but did not arrest. The incision was then closed in layers. On the contralateral side an identical dissection was performed except that the sciatic nerve was not ligated.

2.3. Intrathecal (i.t.) injection technique

Immediately after the nerve injury, a chronic i.t. catheter was implanted into the spinal cord of rats (modified by Størkson et al., 1996). A PE10 tube (17 cm of length) was inserted into the space between the T13-L1 vertebrae, and gently advanced 2 cm to the lumbar enlargement. The external end of the tube was passed sub-dermally and secured to the back of the neck where an incision had been made to allow exit. After behavioral experiments, to confirm the catheter was in the correct position, 10 μl of lidocaine (50 mg/ml) was administered. Lidocaine induces a transient paralysis of hind paws when injected into the lumbar enlargement; if paralysis did not occur within 5 min the animal was excluded from the study.

2.4. Measurement of mechanical sensitivity

The mechanical sensitivity of animals was measured at various days post surgery. The plantar surface of the hind paws was stimulated by a series of calibrated von Frey hair (bending force ranging from 0.4 to 447 g). Each filament was applied five times before moving to the next one, in an ascending order. When a clear pain-like response (paw withdrawal, shaking or licking) was observed, the filament corresponding force was recorded.

2.5. Semiquantitative RT-PCR

Total cytoplasmic RNA was isolated from the lumbar enlargement of the spinal cord and the L5–L6 dorsal root ganglia of control (not operated) and neuropathic animals at 3, 7 and 15 days post surgery using Trizol reagent (Life Technologies, Rockville, MD, USA). One microgram of total RNA was digested with DNase RNase-free enzyme (Promega, Madison, WI, USA) to eliminate genomic DNA, and then converted to complementary DNA (cDNA) using 200 U Moloney murine leukemia virus reverse transcriptase (Promega, Madison, WI, USA) in 20 μl of the buffer containing 0.4 mM deoxy-nucleotide triphosphates, 2 U/ml RNase inhibitor, and 0.8 μg oligo(deoxythymidine)$_{15}$ primer. As control, a sample without reverse transcriptase was incubated in every experiment for each cDNA. Oligonucleotide primers used for RT-PCR amplification were designed according to the published sequences. The ORL1 receptor oligonucleotides span the insert region of the ORL1 receptor so they can distinguish the long and the short splice form in the same amplification reaction. Specific primers for the ORL1 receptor cDNA were 5′-CAGGCTGTTAATG-TGGCCATATG-3′ and 5′-GAGCCTGAAAGCAGACGG-

ACACC-3′, which anneal to bases 493–515 and 743–721 for the ORL1 receptor-short and 827–805 for the ORL1 receptor-long isoforms (Xie et al., 1999). Primers for the μ-opioid receptor cDNA were 5′-ACCTGGCTCCTGGCTCAACTT-3′ and 5′-TGGACCCCTGCCTGTATTTTG-3′, which anneal to bases 284–304 and 832–852 (Buzas and Cox, 1997). The primers for amplifying the prepronociceptin cDNA were 5′-GTGACTCTGAGCAGCTCAGC-3′ and 5′-TTCTGGTTGGCCAACTTCCG-3′, which anneal to bases 224–243 and 460–479 (Andoh et al., 1997). The housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression was used as an internal control. It is widely utilized for the PCR reaction and did not change in rat dorsal root ganglia and spinal cord after chronic constriction injury of sciatic nerve (Dib-Hajj et al., 1999; Levy and Zochodne, 2000; Rausch et al., 2000). In order to prevent the inhibition of the amplification sequences by excess GAPDH templates, cDNA for the ORL1 receptor, μ-opioid receptor and prepronociceptin were co-amplified with the GAPDH cDNA using the "primer dropping" method (Wang et al., 1994), by adding the two primer sets to the reaction tube at different cycling steps (28 cycles for ORL1 receptor, μ-opioid receptor and prepronociceptin, while 16 cycles for GAPDH). The PCR conditions for all cDNAs were as follows: denaturation at 94 °C for 30 s, annealing at 60 °C for 30 s and elongation at 72 °C for 30 s with truncated *Thermus aquaticus* DNA polymerase (Promega). Then, 5 μl of the PCR products (25 μl) was separated by agarose gel electrophoresis and revealed by ethidium bromide staining. The number of cycles for the semiquantitative RT-PCR assay and the reaction temperature conditions was estimated to be optimal to provide a linear relationship between the amount of input template and the amount of PCR product generated over a wide concentration range, from 0.5 to 5 μg of total RNA (data not shown).

2.6. Densitometric and data analysis

RT-PCR products were run on agarose gel. Gels were semiquantitatively analysed by using the Scion Image software (Release, Beta 3b) for Windows (NIH image). The amplified mRNA levels were expressed as the ratio between its signal intensity and GAPDH signal intensity. To normalize the densitometer differences among experiments, data were corrected for background and expressed as a percentage of a specific reference value taken as one within each experiment. If the density of GAPDH of a given sample deviated by >20 % from the mean density of the samples in gels, it was removed from the analysis.

2.7. Drugs

Nociceptin (Sigma, St. Louis, MO, USA) and morphine (Salars, Como, Italy) were dissolved in saline (0.16 M NaCl). Nociceptin was stored at −20 °C as 2 mM stock solution, and diluted before use.

2.8. Statistical analysis

The force elicited by the von Frey filaments on the plantar surface of the rat paw was expressed in grams (g) and the mechanical pain-like threshold is the geometric mean ± 95% confidence limits (95% c.l.) for each group. Statistical test was performed by the analysis of variance for repeated measures, after logarithmic transformation of data. Data reported in the RT-PCR experiments are the mean values ± S.E.M. of three independent determinations. The comparisons were made using two-tail Student's *t*-test; and a *P* value of <0.05 vs. controls was considered significant.

3. Results

3.1. Behavioral studies

After surgery the animals appeared well groomed and gained weight normally. The toes of the affected paw were held together and slightly ventroflexed. While walking it was evident that the animals were reducing the amount of weight put on the neuropathic paw and when in a sitting or standing position the animals often kept the paw off the ground in a guarded position. Neuropathic animals were often seen licking the injured paw, suggesting the perception of spontaneous nociceptive behavior; no autotomy was observed in any of the animals.

3.2. Development of mechanical sensitivity in nerve injured rats

Unilateral nerve injured rats were tested by the von Frey hair to evaluate the development of mechanical sensitivity for a maximal time period of 30 days post surgery (Fig. 1). The mean mechanical threshold of naive rats [30.2 g (12.7–72.0)] was not significantly different from that displayed from the contralateral paw of neuropathic rats during the time course recording [range: 24.5 g (14.3–42.3) to 46.1 g

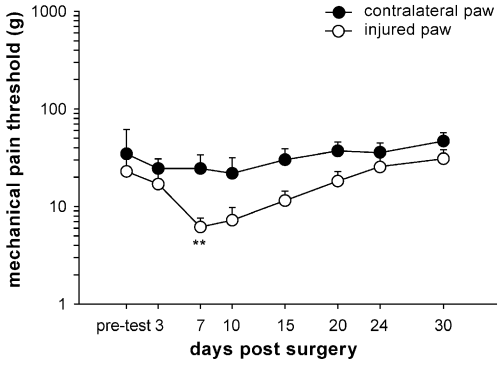

Fig. 1. Development of mechanical sensitivity in the chronic constriction injury rat model. Data are geometric means ± 95% c.l. of 20 rats. ** $P<0.01$ vs. contralateral sham operated side (analysis of variance for repeated measures after logarithmic transformation of data).

(30.3–72.2)]. After unilateral nerve injury, all rats developed a decrease in the mechanical pain threshold of the ipsilateral hind paw. Three days after surgery, the nerve injured paw displayed about 30% reduction in the mechanical threshold compared to the contralateral and prior surgery threshold paws [17.0 g (7.52–38.4) vs. 24.5 g (14.3–42.3) and 25.7 g (11.4–58.1), respectively]. This hypersensitivity was more consistent between 7 and 10 days post surgery when the decreasing of pain threshold reached about 75% compared to the pre-injured value of ipsilateral paw [6.2 g (4.0–9.5) and 7.2 g (3.6–14.7) at day 7 and 10 after surgery, respectively]. The pain threshold in the ipsilateral paw of nerve injured rats showed 55% and 20% of reduction compared to the basal value at days 15 and 20 post surgery, respectively, while, starting from day 24, a recovery was observed. Animals walked correctly, placing completely the plantar surface of ipsilateral paw on the floor and their mechanical pain threshold was very similar to that recorded prior to nerve ligation [25.1 g (15.4–41.0) and 30.9 g (20.0–47.7)]. In our experimental conditions, the maximal decrease in the pain threshold to mechanical stimulus was observed a week after surgery, when about 60% of the operated animals displayed a pain threshold below 4 g (Corradini et al., 2001). Thus, nerve injured rats were chosen and divided into two groups according to the threshold: allodynic rats, which responded to innocuous stimuli (≤ 4 g; Hunter et al., 1997), and non-allodynic rats, which react to noxious stimuli (>4 g).

Therefore, at day 7 post surgery we selected allodynic animals for pharmacological studies and at different time post surgery (3, 7 and 15 days), allodynic and non-allodynic rats for molecular studies.

3.3. Effects of nociceptin and morphine

Given i.t. nociceptin produced effects which were compared with those elicited by morphine. The pre-selected allodynic rats received spinally either morphine or nociceptin (0.2, 2 and 20 nmol) and were tested at 5, 15 and 30 min post drug administration (Fig. 2). Both compounds significantly increased the mechanical pain threshold of allodynic rats. However, morphine produced a long-lasting effect (60 min; data not shown) significant at 20 nmol only [91.2 g (14.4–578.6) vs. 3.7 g (1.8–7.5) of control group at 5 min; $p < 0.01$], while nociceptin dose-dependently reversed the allodynic-like behavior of neuropathic rats, starting from 2

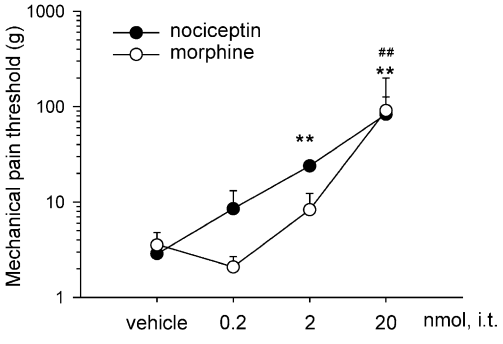

Fig. 2. Anti-allodynic activity of nociceptin and morphine in neuropathic rats. Both compounds were injected i.t. at the doses of 0.2, 2 and 20 nmol. Graph shows the mechanical pain threshold of the injured paw 5 min post-administration. Data are geometric means ± 95% c.l. of 9–10 rats. ** $P < 0.01$ vs. vehicle (analysis of variance for repeated measures after logarithmic transformation of data).

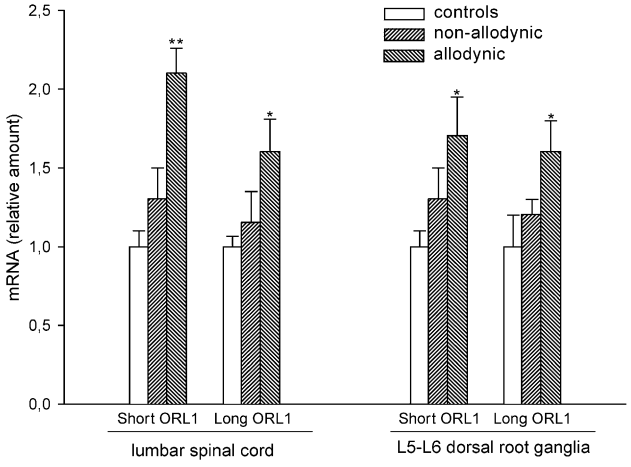

Fig. 3. Semiquantitative RT-PCR analysis of the [long ORL1 receptor]/[GAPDH] and [short ORL1 receptor]/[GAPDH] mRNA ratios in both lumbar spinal cord and L5–L6 dorsal root ganglia ipsilateral to sciatic nerve injury. Control, allodynic and non-allodynic rats were studied 7 days after chronic constriction injury of sciatic nerve. Bars represent the mean values ± S.E.M. of three independent experiments ($n = 6$). The values for control rats were assumed as 1. * $P < 0.05$ and ** $P < 0.01$ vs. control (Student t-test).

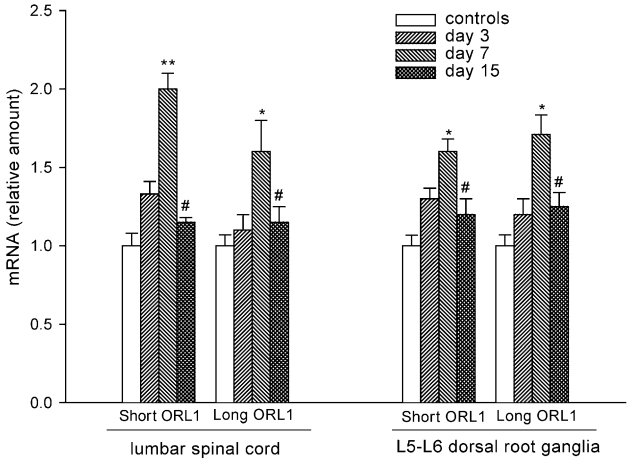

Fig. 4. Semiquantitative RT-PCR analysis of the [long ORL1 receptor]/[GAPDH] and [short ORL1 receptor]/[GAPDH] mRNA ratios in both lumbar spinal cord and L5–L6 dorsal root ganglia ipsilateral to sciatic nerve injury during the developments of neuropathic pain. Rats at days 3, 7 and 15 were tested before tissue withdrawal and displayed non-allodynic, allodynic and non-allodynic mechanical pain threshold, respectively. Bars represent the mean values ± S.E.M. of three independent experiments ($n = 6$). The values for control rats were assumed as 1. * $P < 0.05$ and ** $P < 0.01$ vs. control, $^{\#}P < 0.05$ vs. day 7 value (Student t-test).

nmol [23.9 g (18.3–31.5) vs. 2.9 g (1.9–4.4) of saline treated group at 5 min; $p < 0.01$].

3.4. ORL1 receptor mRNA in the spinal cord and dorsal root ganglia

Both ORL1 receptor mRNA splice variants were found in the lumbar spinal cord and L5–L6 dorsal root ganglia of control (not operated) rats, and the expression of the shorter ORL1 receptor mRNA was about five times more than the longer splice isoform (data not shown). Seven days after the chronic constriction injury of sciatic nerve, we observed a significant up-regulation of the two ORL1 receptor splice variants in the ipsilateral spinal tissue of allodynic animals. Specifically, the short form of ORL1 receptor increased by 100% ($p < 0.01$), while the long form by 50% over control ($p < 0.05$) in the ipsilateral lumbar enlargement (Fig. 3). On the contrary, non-allodynic rats did not show any modulation in the ORL1 receptor mRNA expression. Similar results were obtained in the ipsilateral L5–L6 dorsal root ganglia rats where the short form of ORL1 receptor was increased by 60% ($p < 0.05$), while the long form by 50% over control ($p < 0.05$) only in the tissue of allodynic animals (Fig. 3).

This up-regulation was associated to the mechanical pain threshold of rats. Indeed, non-allodynic animals at 3 and 15 (Fig. 4) or 7 (Fig. 3) days after surgery [16.6 g (10.7–25.6), 15.5 g (10.0–23.9) and 14.1 g (5.9–33.7), respectively] showed ORL1 receptor mRNA levels comparable to those of control group, for both splice variants in the ipsilateral lumbar tissue. In the contralateral side of the spinal cord and L5–L6 dorsal root ganglia no changes were recorded at any time, as in control animals.

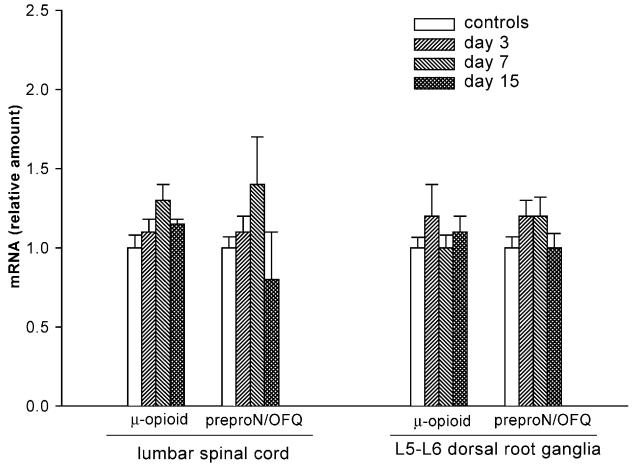

Fig. 5. Semiquantitative RT-PCR analysis of the [μ-opioid receptor]/[GAPDH] and [prepronociceptin]/[GAPDH] mRNA ratios in both lumbar spinal cord and L5–L6 dorsal root ganglia ipsilateral to sciatic nerve injury during the developments of the allodynic-like behavior after chronic constriction injury. Rats at days 3, 7 and 15 were tested before tissue withdrawal and displayed non-allodynic, allodynic and non-allodynic mechanical pain threshold, respectively. Bars represent the mean values ± S.E.M. of three independent experiments ($n = 6$). The values for control rats are assumed as 1. No significant value was recorded (Student *t*-test).

3.5. Prepronociceptin and μ-opioid receptor mRNA expression

No significant changes were recorded for the amplification products of the prepronociceptin and μ-opioid receptor either in the ipsilateral or in the contralateral side of both lumbar enlargement of the spinal cord and L5–L6 dorsal root ganglia in rats, at any time after the chronic constriction injury of the sciatic nerve (Fig. 5).

4. Discussion

Nociceptin and its receptor appear to be involved in nociception at least at the spinal cord level (Abdull and Smith, 1998; Hao et al., 1998; Yamamoto et al., 2000). In this study, we have demonstrated that nociceptin acts as an anti-nociceptive peptide when injected i.t. in a model of neuropathic pain. Indeed, in the chronic constriction injury rat model, 2 nmol of nociceptin was sufficient to reverse the mechanical allodynia displayed from neuropathic rat. These findings are in line with previous papers, where cumulative doses of 1.7 and 5.7 nmol (Hao et al., 1998) or 17 nmol (Yamamoto et al., 1997; Yamamoto and Nozaki-Taguchi, 1997) of nociceptin produced anti-nociceptive effects in models of neuropathy, such as the partial ligation and the chronic constriction injury of the sciatic nerve and the photochemically induced peripheral nerve injury. The anti-nociceptive effect of nociceptin in the chronic constriction injury rat model was reversed by a selective ORL1 receptor antagonist (Corradini et al., 2001) indicating that it is mediated through ORL1 receptors. Compared with nociceptin, the anti-allodynic effects of morphine were noticeable and statistically significant only starting from the dose of 20 nmol. This is not surprising since the activity of μ-opioid receptor agonists such as morphine in models of neuropathy is quite controversial. There is no explanation as yet for the fact that opiates are only moderately effective in neuropathic pain, both in experimental and clinical studies. However, experimental data obtained mainly in rats with chronic constriction injury of the sciatic nerve suggest the potential efficacy of opiates (Ollat and Cesaro, 1995).

Sciatic nerve injury in rats is known to cause modulation of neurotrasmitters, proteins and neuropeptides within the spinal cord, such as for the μ-opioid receptor (Goff et al., 1998), *c-fos* (Siddall et al., 1999), α and β subunits of the sodium channel (Blackburn-Munro and Fleetwood-Walker, 1999; Dib-Hajj et al., 1999), galanin (Zhang et al., 1998b). All such molecular changes may well account for behavioral syndromes that appear during peripheral nerve injury. With the present study, we suggest that the nociceptin system could play an important role in the modulation of nociceptive signals at the spinal level during the neuropathic pain

condition due to chronic constriction injury of the sciatic nerve. In agreement with previous findings (Xie et al., 1999), we have detected two ORL1 receptor mRNA splice variants, a long and a short isoform, in both dorsal root ganglia (L5–L6) and lumbar enlargement of the spinal cord. We have also confirmed that in our experimental conditions, expression of the shorter ORL1 receptor mRNA is predominant. We selected allodynic rats at 7 days after surgery and we found an up-regulation of both isoforms, correlated to the development of mechanical allodynia. In fact, at the same time, non-allodynic animals did not show any modulation of ORL1 receptor mRNA expression. Moreover, this up-regulation observed in allodynic rats returns to normal values 15 days after surgery when neuropathic animals were recovered showing higher mechanical pain thresholds (i.e. non-allodynic rats). These results suggest that the ORL1 receptors, either in the spinal cord or dorsal root ganglia, are up-regulated only during the allodynic-like condition, when animals displayed pain responses to innocuous stimuli. Further studies are required to confirm that the ORL1 receptor mRNA modulation in the allodynic state of neuropathic rats is associated with an increase of ORL1 receptor protein expression. At this time no evidence is reported.

These results and the growing body of data implicating the opioid system as potential neuromodulator of nociception at the spinal level provide further support to a possible function of the ORL1 receptor in the nociceptive processing which leads to neuropathic pain. These data strengthen the suggestion by Yamamoto et al. (2000) that in the chronic constriction injury rat model the development and maintenance of thermal hyperalgesia seems to be mediated by an ORL1 receptor-dependent spinal facilitation.

There is evidence suggesting the important role for nociceptin system in the modulation of persistent nociception. It has been previously reported that the increased prepronociceptin mRNA expression occurs following carrageenan-elicited inflammation in primary sensory neurons peaking within 30 min, when nociceptive hypersensitivity is not developed yet (Andoh et al., 1997). Furthermore, Jia et al. (1998) showed an increase in nociceptin binding sites 4 days after injection of complete Freund's adjuvant, a model of persistent peripheral inflammation. However, despite this evidence, in our model of neuropathy, we observed only a small non-significant change in the prepronociceptin mRNA expression in allodynic animals.

It has been demonstrated that the neuropathic pain state is also characterized by regulation of the μ-opioid receptor gene (Goff et al., 1998), but results concerning the receptor protein and binding sites are still controversial. Indeed, both protein and receptor increase in the dorsal horn of animals after chronic constriction injury (Goff et al., 1998), but decline in other animal models of neuropathy (Stevens et al., 1991; Zhang et al., 1998a; Yu et al., 1999). In our experimental conditions, there are no μ-opioid receptor mRNA expression changes either in lumbar enlargement or in L5–L6 dorsal root ganglia of neuropathic rats. This discrepancy with the μ-opioid receptor up-regulation described by Goff et al. (1998) may be due, at least in part, to the different techniques used (immunohistochemistry assay vs. RT-PCR).

In conclusion, our results are the first molecular evidence suggesting that the ORL1 receptor could play an important role in the modulation of nociceptive signals at the spinal level, therefore showing its involvement during the neuropathic pain condition. These receptor changes, which could represent a compensatory mechanism, suggest that the nociceptin system is involved in regulating the sensation and perception of nociception, at least in neuropathic conditions. Efforts made over the last few years have led to the discovery of small molecular weight molecules which interact with the ORL1 receptor either as agonists or antagonists (Calo' et al., 2000). Initial studies have shown that ORL1 receptor agonists may not be effective in pain models (Jenck et al., 2000) while antagonists appear to have some activities (Ozaki et al., 2000; Shinkai et al., 2000). However, thus far no information is available as to their pharmacological activity in models of neuropathic pain. Understanding the function of ORL1 receptor reorganization after constriction of the sciatic nerve may provide further information regarding both spinal cord plasticity and discovery of more effective treatments for neuropathic pain.

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