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Effects of intrathecal orphanin FQ on a flexor reflex in the rat after inflammation or peripheral nerve section

Isabella S. Xu, Stefan Grass, Zsuzsanna Wiesenfeld-Hallin, Xiao-Jun Xu *

Department of Medical Laboratory Sciences and Technology, Division of Clinical Neurophysiology, Karolinska Institute, Huddinge University Hospital, S-141 86 Huddinge, Sweden

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Abstract

We examined the effects of intrathecal orphanin FQ, the endogenous ligand for the orphan opioid-like receptor, on the hamstring nociceptive flexor reflex in decerebrate, spinalized, unanesthetized rats after carrageenan-induced inflammation or unilateral sciatic nerve transection. As described previously [Xu, X.-J., Hao, J.-X., Wiesenfeld-Hallin, Z., 1996. Orphanin FQ or antiorphanin FQ: potent spinal antinociceptive effect of orphanin FQ/orphanin FQ in the rat. NeuroReport 7, 2092–2094.], intrathecal orphanin FQ induced a dose-dependent depression of the flexor reflex with a ED_{50} of 965 ng. Initial reflex facilitation was noted in some experiments at lower doses (10 or 100 ng). A similar bi-phasic response pattern to intrathecal orphanin FQ was observed in experiments conducted in inflamed or axotomized rats. However, the magnitude of the initial reflex facilitation was significantly increased in inflamed rats compared to normals whereas the duration of reflex depression was significantly shortened. The ED_{50} for reflex depression was 2.4 μg for inflamed rats. In contrast, axotomy did not significantly alter the facilitatory and depressive effect of orphanin FQ with ED_{50} for reflex depression being 374 ng. These results confirmed an inhibitory action of orphanin FQ on spinal nociception in rats. It is suggested that the effect of orphanin FQ may be modulated by inflammation and nerve injury. In particular, unlike morphine, there seems to be no reduction in the effect of spinal orphanin FQ in inducing antinociception after peripheral nerve axotomy.

Keywords: Antinociception; Nerve injury; Neuropathic pain; Opioid; Spinal cord

1. Introduction

Orphanin FQ is a heptadecapeptide which is an endogenous ligand for the orphan opioid receptor-like G-protein coupled receptor (ORL_1) (Meunier et al., 1995; Reinscheid et al., 1995). Although the sequence of orphanin FQ has similarity with other endogenous opioid peptides, it does not bind with significant affinity to any subtypes of known opioid receptors (Meunier et al., 1995; Reinscheid et al., 1995). ORL_1 receptors (Bunzow et al., 1994; Wick et al., 1994; Anton et al., 1996) and orphanin FQ-like immunoreactivity (Riedl et al., 1996; Schulz et al., 1996) are widely distributed in the central nervous system. The orphanin FQ system may have important roles in numerous physiological functions (see Henderson and McKnight, 1997; Meunier, 1997; Darland et al., 1998 for review).

In the spinal cord, immunohistochemical studies have demonstrated a dense plexus of orphanin FQ-like immunoreactive fibers and terminals in the superficial dorsal horn, lateral spinal nucleus and near the central canal (Riedl et al., 1996; Schulz et al., 1996; Lai et al., 1997). These fibers are not primary afferents, as the orphanin FQ-like immunoreactivity is not influenced by dorsal rhizotomy (Riedl et al., 1996). These findings, together with the fact that ORL_1 receptors are also expressed in dorsal spinal cord (Bunzow et al., 1994; Wick et al., 1994; Anton et al., 1996), suggest a possible role for this new peptide in the modulation of nociceptive signals at spinal level.

Conflicting results have, however, been reported concerning the effects of orphanin FQ on nociception, particularly after intracerebroventricular (i.c.v.) administration (see Henderson and McKnight, 1997; Darland et al., 1998 for review). At spinal level, most behavioral and electrophysiological studies have shown an inhibitory effect of orphanin FQ (Faber et al., 1996; Stanfa et al., 1996; Wang

* Corresponding author. Tel.: +46-8-58582213; Fax: +46-8-7748856; E-mail: xiao-jun.xu@neurophys.hs.sll.se

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et al., 1996; Xu et al., 1996; Henderson and McKnight, 1997; Darland et al., 1998 for review), although a few studies have shown a hyperalgesic effect after intrathecal (i.t.) orphanin FQ (Hara et al., 1997).

The spinal nociceptive system undergoes major anatomical, biochemical and functional alterations in response to peripheral nerve injury or inflammation and such plasticity may have an important role in the generation and maintenance of chronic pain states of neuropathic and inflammatory origin (Hökfelt et al., 1997). Changes in the orphanin FQ system (the peptide or its receptor) after nerve injury or inflammation have not, however, been studied in detail. The present study was conducted to examine the effects of i.t. orphanin FQ in depressing the nociceptive flexor reflex in rats after inflammation induced by subcutaneous carrageenan or after sciatic nerve section and to compare the effects with that observed in normal rats.

2. Methods

Female Sprague–Dawley rats weighing 200–250 g (B&K Universal, Sollentuna, Sweden) were used. The experimental protocol was approved by the local animal research ethics committee. Eight normal, untreated rats were used. In another 9 rats inflammation was induced under methohexital anesthesia (Brietal, Lilly, Indianapolis, USA, 70 mg/kg) by subcutaneous injection of 0.1 ml of 2% λ-carrageenan (Sigma) into the plantar skin of the left hindpaw. The rats were allowed to recover and the acute experiments were conducted 24–72 h after carrageenan injection, when the signs of peripheral inflammation were at their peak. In another 9 rats the left sciatic nerve was transected under methohexital anesthesia 10–16 d prior to the acute experiments. The common sciatic nerve was exposed at the level of the trifurcation and the tibial, peroneal and sural branches of the nerve were tightly ligated and transected distally to the ligation. The ligation and transection of the sural nerve was performed as distally as possible so that the sural nerve could be stimulated in subsequent electrophysiological experiments. The wound was closed in layers and the rats were returned to their cages. Autotomy was observed in some rats after nerve section. These rats were promptly used in electrophysiological experiments at least 10 d after axotomy. No other overt behavioral abnormalities were observed in axotomized rats and they groomed themselves and had no weight loss.

In the electrophysiological experiments the rats were briefly anesthetized with methohexital, ventilated and decerebrated by aspiration of the forebrain and midbrain. The spinal cord was exposed by a laminectomy at mid-thoracic level and sectioned at Th8-9. An i.t. catheter (PE 10) was implanted caudal to the transection with its tip on the lumbar spinal cord (L4-5). In normal or inflamed rats, the left sural nerve was dissected free distal to the trifurcation and stimulated with a pair of silver hook electrodes. In axotomized rats, the wound was reopened and the sural nerve was identified and stimulated proximal to the transection.

The flexor reflex was elicited by supramaximal electric shocks applied to the sural nerve (0.5 ms, 10 mA, 1/min) that activated A- and C-fibers. The flexor reflex was recorded as electromyogram (EMG) activity via stainless steel needle electrodes inserted into the ipsilateral posterior biceps femoris/semitendinosus muscles and was integrated over 2 s (see Xu et al., 1992 for details). The experiments usually commenced at least 1 h after spinalization and in our paradigm, spinal shock was rarely observed. During the experiment the heart rate was monitored (usually between 360–480 beats/min) and rectal

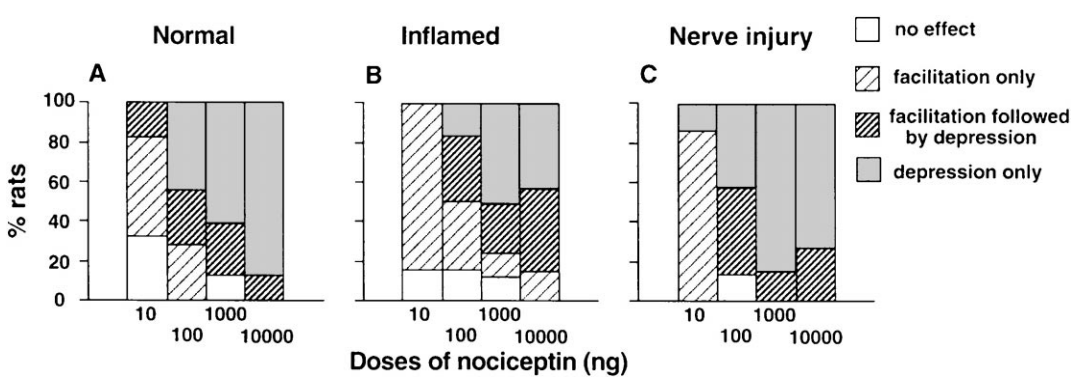

Fig. 1. Percentage of rats showing different types of responses to i.t. orphanin FQ in normal (A), inflamed (B) and nerve injured rats. Six to eight rats were included in each group. The different responses are shown in the figures. The classification is based on the changes in reflex magnitude after i.t. orphanin FQ. In case of facilitation, peak increase in reflex magnitude of more than 20% within 5 min of orphanin FQ application is included and in case of depression, peak decrease in reflex magnitude of more than 20% within 30 min of orphanin FQ application is included. The χ^2 test did not reveal any significant differences among the three groups.

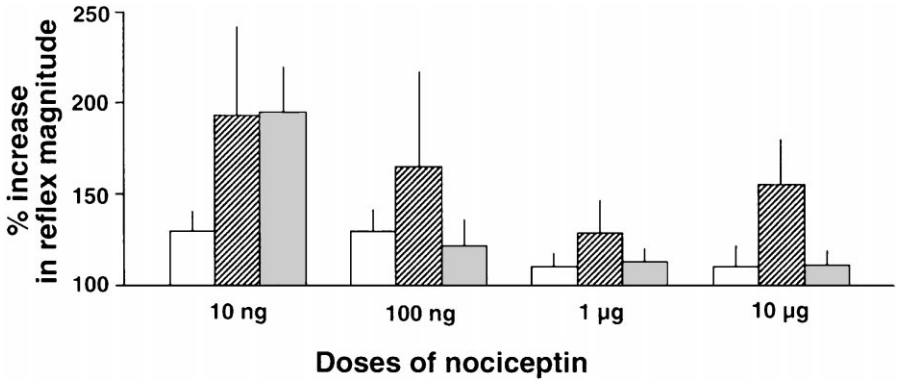

Fig. 2. Magnitude of peak reflex facilitation after various doses of orphanin FQ in normal (open columns), inflamed (hatched columns) and nerve injured (grey columns) rats. The percent of reflex magnitude related to baseline, which is defined as 100%, is shown as mean ± S.E.M. and 6 to 8 experiments were included in each group. The data was collected within 5 min after orphanin FQ application. Two-way ANOVA indicated a significant overall dose effect ($F_{3,71} = 3.3$, $P < 0.05$). No significant overall difference was seen among the three different groups with ANOVA ($F_{2,71} = 3.1$, $P > 0.05$), although the Fisher PLSD test indicated a significant difference between normal and inflamed rats ($P < 0.05$).

temperature of the rat was maintained between 36–37°C with a heating pad.

Orphanin FQ was obtained from Peninsula Laboratories (Merseyside, UK), dissolved in 0.9% saline and injected i.t. in a volume of 10 µl followed by 10 µl saline to flush the catheter. Multiple injections were made in single experiments, always in a 10- or 100-fold increment dosing regime. The interval between injections was at least 20 min and when higher doses of orphanin FQ were administered, the subsequent injection was made when the reflex had returned to baseline level. Not all rats received all four doses of orphanin FQ.

A stable baseline reflex magnitude was established for at least 20 min before each i.t. injection. The effects of i.t. orphanin FQ on the flexor reflex were expressed as percent change in reflex magnitude compared to baseline, which

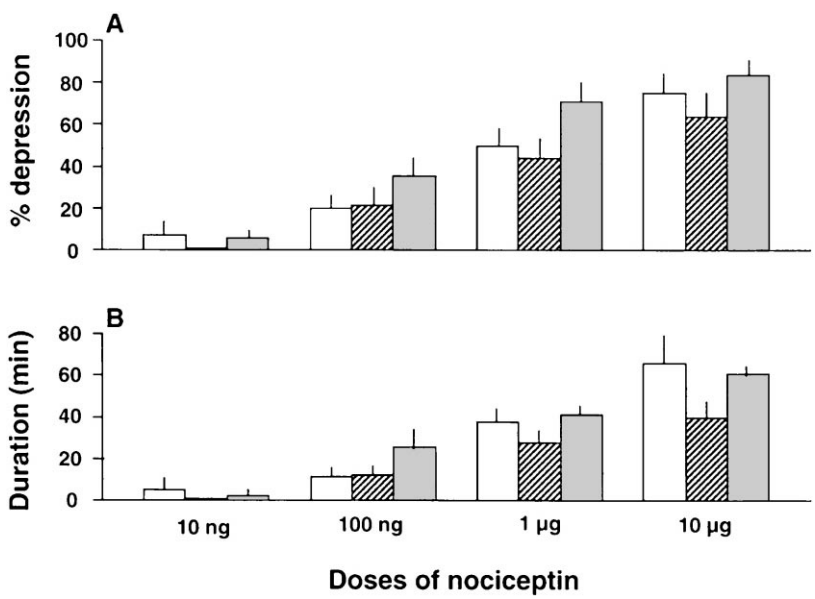

Fig. 3. Magnitude (A) and duration (B) of peak reflex depression after various doses of i.t. orphanin FQ in normal (open columns), inflamed (hatched columns) and nerve injured (grey columns) rats. The peak percent reduction in reflex magnitude over baseline (which usually occurred within 30 min after the application of orphanin FQ) and the duration of depression in minutes (defined as time from orphanin FQ injection to the time when reflex magnitude returned to previous baseline level) are shown as mean ± S.E.M. and 6 to 8 experiments were included in each group. Two-way ANOVA indicated a significant overall dose effect for both the magnitude and duration of depression ($F_{3,71} = 36.4$ and 33.0 respectively, $P < 0.0001$). Significant overall difference was also seen among the three different groups with ANOVA for both the magnitude and duration of depression ($F_{2,71} = 3.3$ and 3.7 respectively, $P < 0.05$), The Fisher PLSD test indicated that for magnitude of depression, there is a significant difference between inflamed and nerve injured rats ($P < 0.05$) and for the duration of depression there were significant differences between normal and inflamed, as well as between nerve injured and inflamed rats ($P < 0.05$).

was defined as 100%. The data are expressed as mean ± S.E.M and were analysed with the χ^2 test or analysis of variance (ANOVA) followed by Fisher Protected Least Significant Difference (PLSD) test using a statistical software (StatView 4.51 for Macintosh). $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. Normal rats

I.t. administration of orphanin FQ at low doses (10 and 100 ng) briefly facilitated the flexor reflex in about half of the experiment (Figs. 1 and 2). The reflex facilitation was usually maximal 1 or 2 min after i.t. administration. Ten ng orphanin FQ did not depress the flexor reflex whereas at 100 ng it caused some depression of the reflex 5–10 min after administration in 4 of 7 experiments (Figs. 1 and 3). Orphanin FQ rarely facilitated the flexor reflex when administered i.t. at high doses (1 and 10 μg) (Figs. 1 and 2). Furthermore, these two doses of orphanin FQ reversibly depressed the flexor reflex (Figs. 1 and 3). Regression analysis indicated that the depressive effect of orphanin FQ in normal rats was dose-dependent and had an ED_{50} value of 965 ng (398 ng–3.3 μg, 95% confidence intervals).

3.2. Inflamed rats

The flexor reflex discharges elicited by single electric shocks to the sural nerve were similar in normal rats and 1–3 d after carrageenan injection, although in inflamed rats the reflex discharge had longer duration. In inflamed rats orphanin FQ at 10 ng and 100 ng induced facilitation of the flexor reflex in the majority of the experiment (Figs. 1 and 2). At 100 ng, orphanin FQ depressed the flexor reflex in 3 of 6 experiments (Figs. 1 and 3). I.t. orphanin FQ also induced initial reflex facilitation in about half of the experiments after the two higher doses (Figs. 1 and 2) and reflex depression was also frequently observed at these doses (Fig. 1). Two-way ANOVA indicated an overall significant difference between normal and inflamed rats in the magnitude of reflex facilitation. There was a significant difference between normal and inflamed rats in the duration, but not magnitude, of reflex depression. Regression analysis indicated that the depressive effect of orphanin FQ in inflamed rats was also dose-dependent and had an ED_{50} value of 2.4 μg (95% confidence interval 750 ng–15.8 μg, Fig. 4).

3.3. Axotomized rats

Stimulation of the proximal end of the axotomized sural nerve 10–16 d after nerve injury elicited flexor reflex discharges similar to that observed in normal rats. Ten ng orphanin FQ elicited an initial reflex facilitation in the majority of experiments (Figs. 1 and 2). The overall pattern of reflex facilitation was otherwise similar to that seen in normal rats and there was no significant overall difference between normal and axotomized rats regarding the magnitude of reflex facilitation (Fig. 2).

Reflex depression was observed after 10 ng i.t. orphanin FQ in axotomized rats and the magnitude and duration of the depression increased after higher doses of orphanin FQ (Figs. 3 and 4). The overall magnitude and duration of reflex depression induced by i.t. orphanin FQ was significantly greater for axotomized than for inflamed rats, but no difference was noted between normal and axotomized rats (Fig. 3). Regression analysis indicated that the depressive effect of orphanin FQ in axotomized rats was dose-depen-

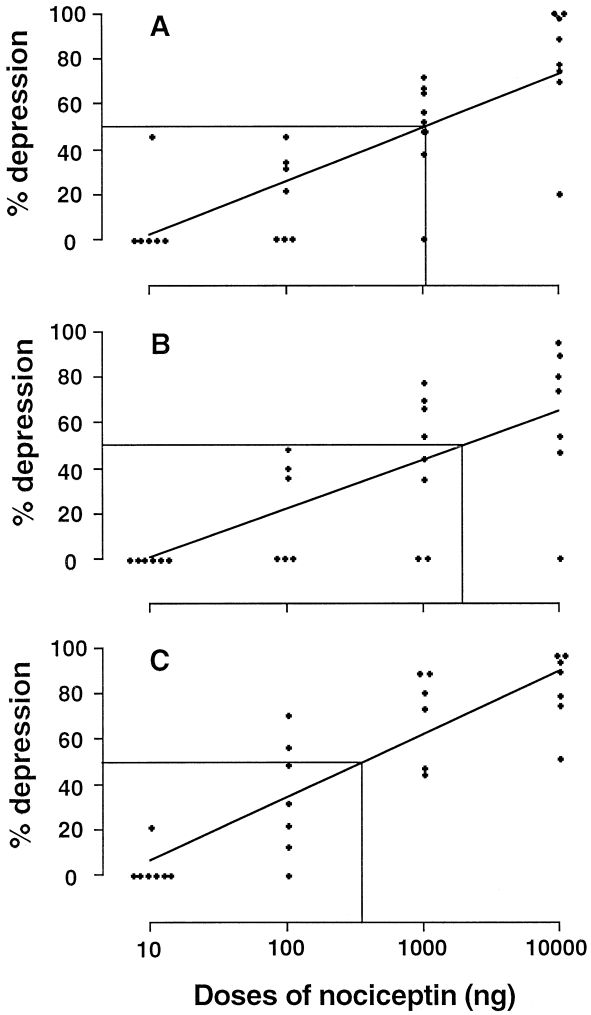

Fig. 4. Regression analysis of the dose-dependent depression of the flexor reflex induced by i.t. orphanin FQ in normal (A), inflamed (B) and nerve injured (C) rats. Each point represents one experiment and the doses of orphanin FQ are shown in a log scale on the x-axis. The regression lines are drawn according to the formula $y = 24.0x - 21.6$ for normals, $y = 21.2x - 21.1$ for inflamed and $y = 27.5x - 20.7$ for nerve injured rats. ANOVA indicated that all three regressions are highly significant. The ED_{50}s are calculated based on the regression lines and are indicated.

dent and had an ED_{50} value of 374.2 ng (95% confidence interval, 200 ng–794 ng, Fig. 4).

4. Discussion

In the present study we observed that i.t. orphanin FQ dose-dependently facilitated and depressed the flexor reflex in normal rats and after peripheral inflammation or nerve section. In normals, orphanin FQ produced reflex facilitation in some experiments at low doses (10 and 100 ng). The mechanisms for the facilitatory effect of orphanin FQ are unclear. Studies of single cell activity have shown primarily an inhibitory effect of orphanin FQ in the spinal cord (Wang et al., 1996; Lai et al., 1997; Liebel et al., 1997). Thus, it is possible that the reflex facilitatory effect of orphanin FQ is due to disinhibition. Alternatively, low doses of morphine have been shown to increase the release of excitatory neurotransmitters from the spinal cord, possibly via a direct excitatory effect on sensory neurons (Crain and Shen, 1990; Suarez-Roca and Maixner, 1995). Orphanin FQ may have a similar effect. It is unclear whether the facilitatory effect of orphanin FQ seen in our decerebrate spinalized preparation can be manifested as behavioral hyperalgesia, as reported by some authors after i.t. administration (Hara et al., 1997).

The predominant effect of i.t. orphanin FQ on the flexor reflex is a dose-dependent depression, which is likely to be mediated by its inhibitory effect on dorsal horn neurons (Stanfa et al., 1996; Wang et al., 1996; Lai et al., 1997; Liebel et al., 1997). Orphanin FQ may also have a presynaptic inhibitory effect (Abdulla and Smith, 1997). Although it can be argued that the reflex depression by i.t. orphanin FQ is due to the fact that we have used a spinalized preparation, this is unlikely to be the case as depressive effect of orphanin FQ has been seen in intact preparations (Stanfa et al., 1996; Wang et al., 1996) as well as in behavioral experiments (Xu et al., 1996; Henderson and McKnight, 1997). It is unclear, however, what is the net effect of systemic ORL_1 receptor agonists as the supraspinal effect of orphanin FQ appears to be hyperalgesic or anti-opioid in nature (Darland et al., 1998).

We have shown in the present study that i.t. orphanin FQ exerts depression on spinal nociceptive input during peripheral inflammation and nerve injury. These results are in agreement with recent behavioral studies showing anti-allodynic and anti-hyperalgesic effect of i.t. orphanin FQ after inflammation or partial peripheral nerve injury (Yamamoto et al., 1997a,b; Hao et al., 1998). The potency of the depressive effect of orphanin FQ on the flexor reflex is somewhat decreased in inflamed rats and increased marginally in nerve injured rats. Similarly, we have recently found that the efficacy of the anti-hyperalgesic effect of i.t. orphanin FQ is reduced in inflamed rats (Hao et al., 1998). It is interesting to note that changes in sensitivity for the depressive effect of orphanin FQ during inflammation and nerve injury are opposite to that of morphine. Thus, peripheral inflammation is associated with increased sensitivity to spinal opioids (Stanfa and Dickenson, 1995) whereas spinal morphine has no or reduced effect after nerve injury (Xu and Wiesenfeld-Hallin, 1991; Dickenson, 1994; Mao et al., 1995).

Many factors may account for the differential sensitivity of morphine in alleviating pain-related behaviors of various origins, but two major mechanisms are likely to be in operation. These involve plasticity of both the endogenous opioid system, particularly opioid receptors (Hökfelt et al., 1997), and in anti-opioid systems (Wiesenfeld-Hallin and Xu, 1996). Peripheral inflammation and nerve injury may regulate endogenous orphanin FQ and its receptors in ways that are different or opposite to the classical opioid systems. Moreover, the effect of orphanin FQ may not be modulated by anti-opioid substances, such as cholecystokinin (Wiesenfeld-Hallin and Xu, 1996). Little is known about the effects of peripheral inflammation or nerve injury on spinal orphanin FQ systems. A recent study showed that carrageenan induced a rapid increase in the synthesis of orphanin FQ in sensory neurons (Andoh et al., 1997). This change was, however, brief and orphanin FQ levels returned to normal within 6 h. The relevance of this study to our results is therefore unclear. Moreover, the efficacy and potency of exogenously applied orphanin FQ should be primarily determined by the status of ORL_1 receptors, rather than peptide level. Thus, studies examining the effects of inflammation or nerve injury on orphanin FQ receptors are required to address the mechanisms of action of orphanin FQ in these two pathological painful conditions.

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