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LOCAL VERSUS SYSTEMIC ENDOMORPHIN 2 IN A CARRAGEENAN MODEL OF INFLAMMATORY HYPERALGESIA

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Abstract. Inflammation effectively increases the activation of opioid receptors on peripheral terminals of sensory neurons. The resulting inflammatory hyperalgesia responds to local treatment with opioid analgesics by decreasing its intensity. Comparatively, much less is known about the efficiency of opioid peptides administered peripherally at the inflammation site. The study examined the antihyperalgesia elicited by endomorphin 2, a miu agonist, when administered peripherally in a model of acute inflammation with carrageenan. The effects of locally administered endomorphin 2 in three different doses were investigated with behavioral assays. The local paw injection of endomorphin at the site of inflammation induced an antihyperalgesic effect. For confirmation of the peripheral mechanism of analgesia, the peptide was systemically administered (i.p.) and at the dosage we used, endomorphin 2 was not significantly antihyperalgesic. Endomorphin 2 was antihyperalgesic without significantly affecting edema. In conclusion, the antihyperalgesic effect of the endomorphin 2 is not secondary to a reduction of edema, since reduction of edema does not occur. These observations plead for an action at the level of opioid receptors on nerve terminals rather than on immune cells.
Keywords: endomorphine 2, carrageenan, inflammation, edema, antihyperalgesia

Opioid receptors have been identified on structures of the peripheral nervous system, specifically miu opioid receptors on the cell bodies of primary afferent sensory neurons located in the dorsal root ganglia [1, 2, 3].

Inflammation effectively increases the activation of opioid receptors on peripheral terminals of sensory neurons. In addition, under inflammatory conditions, endogenous opioid peptides are upregulated in resident immune cells within inflamed tissues and have a functional significance in pain control under hyperalgesic inflammatory conditions. The inflammatory hyperalgesia responds to local treatment with exogenous opioid analgesics by decreasing its intensity. Comparatively, much less is known about the efficiency of opioid peptides administered peripherally at the inflammation site. [4, 5, 6, 7]

Therefore, the role of peripheral miu opioid receptors in pain associated with inflammation is well established. Literature provides contradictory information regarding the importance of the edema reduction in the opioid mediated antihyperalgesia in inflamed tissues. [8, 9, 10, 11, 12]

This research intends to examine the antihyperalgesic effect of endomorphin 2 (EM2) in acute inflammation when acting at the level of peripherally located opioid receptors and its significance

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in edema reduction. We compared the effects of endomorphin 2 administered either systemically or locally.

Materials and methods

Animals. All experimental procedures employed in the present study were strictly in accordance with the international guidelines regarding ethics. Male Wistar rats (National Institute for Research and Development Victor Babeş, Bucharest), weighing 180 to 200 g at the start of experiments, were used. The animals were housed in a temperature-controlled room (21°C ± 2°C) with a 12 hours/12 hours light/dark cycle, 1 rat per cage, and allowed to acclimate for at least 24 hours before use, with free access to food and water.

Substances and Administration Procedures. All reagents were acquired from Sigma-Aldrich (Sigma-Aldrich Chemie GmbH, Austria). The opioid peptide agonist, endomorphin 2, was administered in doses of 0.3, 0.5 and 0.7 mg/rat. A dose of 0.5 mg/rat, was administered either systemicaly - intraperitoneal (i.p.) in a volume of 0.2 ml/rat of 0.9% saline, or intraplantar (i.pl.) in a volume of 0.1ml/rat of 0.9% saline.

In order to confirm the role of the endogenous opioid system in the observed antinociceptive effect, naloxone, an opioid non-selective antagonist (1 mg/kg), was administered s.c. in a volume of 0.2 ml/rat of 0.9% saline just prior to the injection of the opioid peptide.

For comparison with compound-treated groups, animals treated with appropriate drug vehicle were included in each experiment. The volume of administration and all other experimental procedures and conditions for vehicle and compound-treated rats were identical.

Carrageenan Model of Inflammatory Hyperalgesia and Edema

The rats received an intraplantar injection of 0.1 ml λ-carrageenan (Sigma-Aldrich Chemie GmbH, Austria) 1% diluted in 0.9% saline to the right hind paw.

The opioid peptide endomorphine 2 was administered 2 hours and 40 minutes after carrageenan injection (to investigate the effect on an established hyperalgesia). Rats received endomorphine 2, systemically (i.p.) or locally (i.pl.).

Inflammatory Hyperalgesia. For this assay, in order to evaluate the antinociceptive effect of the tested substances, hind paw withdrawal thresholds (PWTs) to a noxious mechanical (algesimetric test) or thermal (plantar test) stimulus were determined. *The algesimetric test* (*mechano-algesic test).* The test was performed on rats using an analgesymeter (model 7200; Ugo Basile, Varese, Italy). The analgesymeter applied a linearly increasing force (16g/s) to the hind paw, between the third and fourth metatarsals Cut-off time was set at 15 force units, and the endpoint was taken as complete paw withdrawal. *The plantar test.* The rats were habituated to the apparatus that consisted of six individual Perspex boxes on a glass table. A mobile radiant heat source was located under the table and focused onto the desired paw and paw withdrawal latencies (PWLs) were recorded. Cut-off time was set at 40 seconds, and the endpoint was recorded automatically at paw withdrawal. PWT was determined once for each rat at each time point. PWT was determined at baseline (pre-carrageenan PWT) and after 2 hours and 30 minutes following carrageenan injection. After peptides injection, PWTs were again measured at 10, 20, 30 and 40 minutes. (postdose PWT). Treatments that produced a significant increase in the nociceptive threshold were considered to be antinociceptive.

Inflammatory Edema. For this assay, hind paw volume was determined using a plethysmometer (model 7140; Ugo Basile). Paw volume was determined once for each rat at each time point. Paw volume was determined at baseline (pre-carrageenan paw volume) and after 2 hours and 30 minutes following carrageenan injection. Three hours after carrageenan injection, paw volume was again measured as described above (predose paw volume). Secondary to endomorphine 2 injection, paw volume was measured again, at 3 hours, and finally at 3 hours and 30 minutes after carrageenan injection (post dose paw volume).

Statistical Analysis. Data are shown as mean ± standard deviation for each measurement time. Differences between treatment groups were analyzed using ANOVA one-way method for comparison at each time point, followed by Bonferroni post-hoc tests. The p values of under 0.05 were used for indicating a significant difference for all tests. Mean values of the test groups were routinely compared with control values collected the same day.

Results

Carrageenan-Induced Hyperalgesia and Edema. In each experiment, injection of carrageenan produced a significant reduction of PWT at 2 hours and 30 minutes after injection, for both tests measuring nociception, proving a hyperalgesic effect: post-carrageenan levels for plantar test (range

5.75 ± 0.49 sec to 6.16 ± 0.59 sec) and algesimetric test (range 0.26 ± 0.05 to 0.31 ± 0.06 force units), compared with pre-carrageenan levels (range 10.65 ± 0.38 sec to 10.68 ± 0.54 sec for plantar test and 3.36 ± 0.16 to 3.73 ± 0.3 g for algesimetric test). Carrageenan injection also produced a significant increase in paw volume 2 hours and 30 minutes later (range 6.49 ± 0.46 ml to 6.84± 0.13 ml) compared with pre-carrageenan levels (range 4.06 ± 0.33 ml to 4.3± 0.23 ml).

Local Administration of Endomorphine 2. Endomorphine 2 (0.3, 0.5 and 0.7mg/rat i.pl.) administered 2 hours and 40 minutes after carrageenan, produced a significant inhibition of hyperalgesia. PWT in plantar test increased significantly starting with 20 minutes after injection. The maximum inhibition was reached at 40 minutes after injection, with values of the response latency of 7.3 ± 0.38 sec to 12.31±0.44. (fig. 1)

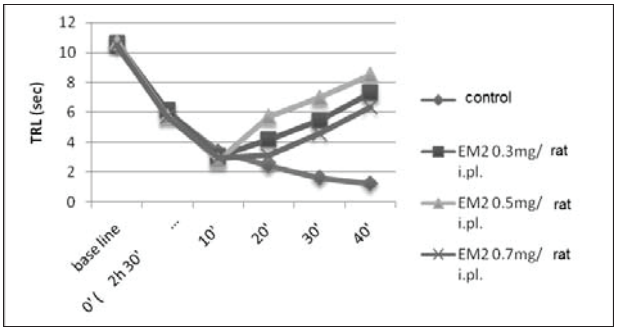

Figure 1. Plantar test. Effect of EM2 injected intraplantar: (0.3, 0.5 and 0.7mg/rat i.pl.) on a carrageenan 1% prolonged inflammation model; ■) marks a significant antihyperalgesic effect (p<0.05)

The results obtained in the algesimetric test did not show a significant increase in PWT (fig. 2).

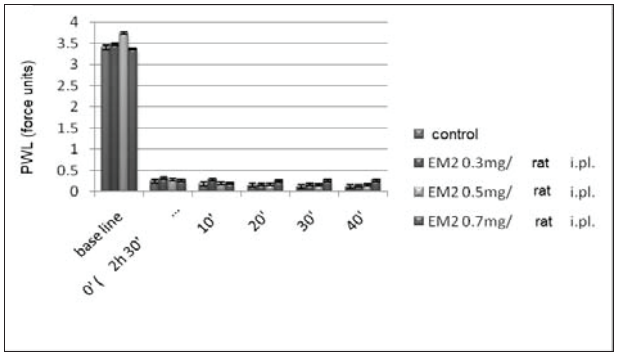

Figure 2. Algesimetric test. Effect of EM2 injected intraplantar: (0.3, 0.5 and 0.7mg/rat i.pl.) on a carrageenan 1% prolonged inflammation model; ■) marks a significant antihyperalgesic effect (p<0.05)

Endomorphin 2, administered 2 hours and 40 min after carrageenan, did not inhibit edema (fig. 3).

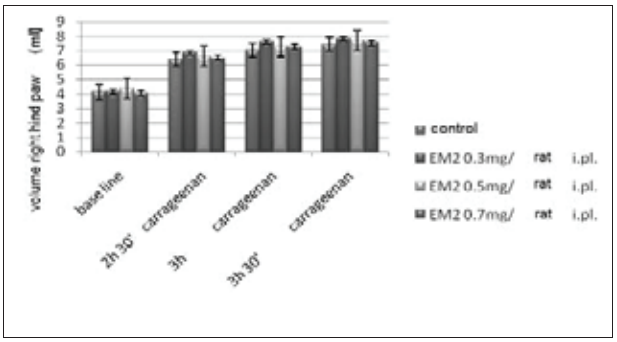

Figure 3. Pletismometer. Effect of EM2 injected intraplantar: (0.3, 0.5 and 0.7mg/rat i.pl.) on a carrageenan 1% prolonged inflammation model; ■) marks a significant decrease in paw volume (p<0.05)

Systemic Administration of Endomorphine 2. Endomorphine 2 (0.5 mg/rat i.p.), administered 2 hours and 40 minutes after carrageenan, did not result in a statistically significant inhibition of hyperalgesia in either of the plantar or algesimetric tests. (fig. 4)

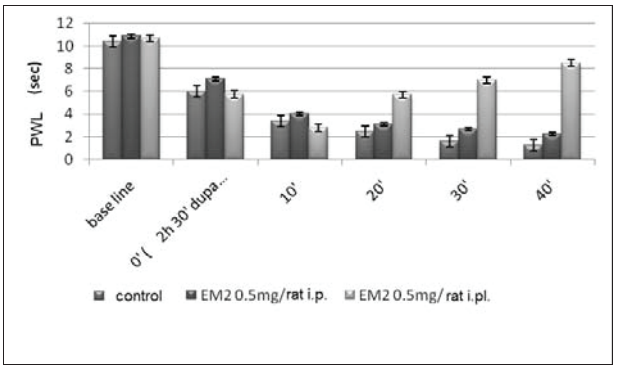

Figure 4. Plantar test. Effect of EM2 injected intraperitoneal: (0.5 mg/rat i.pl.) on a carrageenan 1% prolonged inflammation model; ■) marks a significant antihyperalgesic effect (p<0.05)

Endomorphine 2 (0.5 mg/rat i.p.), administered systemically after carrageenan, was not efficacious in inhibiting edema.

Discussion

Activation of opioid receptors in the brain results in analgesia, whereas activation of the same receptors in the periphery, after inflammation, results in antihyperalgesia. [9, 10, 11, 12]

Since endomorphin 2 does not penetrate the blood-brain barrier, both local and systemic administration of this opioid peptide resulted in activation of opioid receptors located in the periphery. Both treatments showed an antihyperalgesic tendency, yet at the dosage we used, only the direct

local administration at the site of inflammation was significantly antihyperalgesic. A single dose of endomorphin 2 was antihyperalgesic without significantly affecting edema.

In the current study, we sought to investigate the role of peripherally located miu opioid receptors in pain and edema caused by acute inflammation.

We activated opioid receptors located in the periphery by local administration of the miu opioid agonist endomorphin 2. All treatments were antihyperalgesic without affecting edema.

The reviewing of significant literature pointed only three other studies that have investigated the action of local opioids on edema with results similar to our own; Sacerdote et al. [9] assessed the effects of a single dose of morphine (10 μg) administered into the inflamed paw in a yeast model of acute inflammation and observed no effect on paw edema. Similarly, Perrot et al. [13] and Whiteside et al. [14] administered morphine locally (50–200 μg), both before and after intraplantar carrageenan. These authors concluded that neither preemptive nor curative administration of morphine affects paw circumference.

Our results are consistent with these observations and support the conclusion that peripheral miu opioid receptors are not involved in edema formation due to acute inflammation.

Also, the antihyperalgesic effects of the endomorphin 2 is not secondary to a reduction of edema and may indicate an action on miu receptors of nerve terminals rather than of immune cells; however, further experimentation would be required to demonstrate this.

An alternative explanation is that activation of opioid receptors can affect inflammation which is predominantly neurogenic in origin. Indeed, opioid agonists reduce plasma extravasation in the formalin model [15] a model that induces substantial neurogenic inflammation. In contrast, the carrageenan model has been described as inducing "non-neurogenic" inflammation [16].

Furthermore, the antihyperalgesic effect of endomorphin 2 was reversible by opioid antagonist naloxone. The antihyperalgesic effect: endomorphin 2 is effective only after 40 minutes since the injection. According to certain authors, endomorphin 2 is not directly effective. Endomorphin 2 binds to miu opioid receptors, leading to the release of another endogenous opioid peptide as dynorphin or metion-enkephalin. Furthermore, these opioid peptides released from immune system cells are the agonists stimulating the corresponding opioid receptor, with antihyperalgesic effect, which might explain the delayed response after the injection of endomorphin 2. [17,18,19]

Our results pointed out a difference between results obtained in a thermal versus a mechanical model of nociception. The simplest explanation for these conflicting observations may be that the role and importance of the peripheral opioid system varies among the groups of primary sensory neurons mobilized by different types of nociceptive stimuli.

Conclusions

These results confirm the role of peripheral miu opioid receptors in the pathology of pain associated with acute inflammation and argue against the involvement of these receptors in edema formation.

Naloxone completely blocked the analgesic activity of endomorphin 2, these findings showing that the antinociception was mediated through the opioid system.

The clinical implication of this work is that although peripheral miu opiod peptides agonists may prove useful in the treatment of pain associated with inflammation, they may not be as effective in reducing other manifestations of inflammation, such as edema.

Thus, although certain mechanisms by which these opiates work remain obscure, there is overwhelming evidence for a peripheral site of action of opiates in damaged tissue.

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