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ORIGINAL PAPERS

EXOGENOUS ATYPICAL OPIOID PEPTIDES INHIBIT HYPERALGESIA ASSOCIATED WITH CARRAGEENAN PERIPHERAL INFLAMMATION

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Abstract. Studies examining the role of the endogenous opioid system in modulating edema and hyperalgesia in animal models of inflammatory pain are contradictory. The present study investigates whether exogenous atypical opioid peptides contribute to the modulation of localized inflammatory nociception. Inflammation was induced by intraplantar injection of carrageenan into the right hindpaw. Mechanical and thermal thresholds were determined respectively in order to determine the potential of the tested peptides in inhibiting hyperalgesia during inflammation. The evolution of the edema was monitored using a plethysmometer. Delta opioid agonist ([D-Ala2]deltorphin II) and miu opioid agonist 1-3 beta casomorphin were administered peripherally into the right hind paw. The exogenous opioid peptides tested induced a signifficant antihyperalgesic effect. The peptides were antihyperalgesic without significantly affecting edema, indicating that peripheral opioid receptors are not involved in edema formation due to acute inflammation. These results demonstrate the peripheral analgesic potential of atypical opioid peptides such as casomorphins and deltorphins. The differential effects of on mechanical versus thermal thresholds support the notion that distinct neuroanatomical or neurochemical mechanisms modulate the processing of thermal versus mechanical stimuli.
Keywords: deltophin, casomorphin, carrageenan, inflammation, edema, antihyperalgesia

Introduction

Today, the role of peripheral opioid receptors is well characterized in pain associated with inflammation. It is not known, however, whether antihyperalgesia due to agonism of peripheral opioid receptors is secondary to a reduction of edema. [4,12,14]

Endogenoues opioid peptides from both typical and atypical groups are expressed at the level of nervous sensorial terminations and immune cells under inflammation conditions. [3,6,9,15,16]

Studies examining the role of the endogenous opioid system in modulating edema and hyperalgesia in animal models of inflammatory pain are contradictory, especially in regard to the opioid influence over the edema development. [1,2,5,8,10,11]

The present study investigates whether exogenous atypical opioid peptides from two different families – deltorphins and casomorphins, contribute to the modulation of localized inflammatory nociception.

Methods

All experimental procedures employed in the present study were strictly in accordance with European ethical guidelines. The animal breeding facility of the Central Drug Testing Laboratory, "Gr. T. Popa" University of Medicine and Pharmacy, Iasi, supplied adult male Wistar rats with an average weight of 275–300 g. The animals were housed in a temperature-controlled room (21°C± 2°) 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.

Drugs administration

All reagents were aquired from Sigma-Aldrich (Sigma-Aldrich Chemie GmbH, Austria): delta opi-

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oid agonist [D-Ala2]deltorphin II and miu opioid agonist 1-3 beta casomorphin. The opioid peptide agonists were administered in a dose of 0.7 mg/rat, intraplantar (i.pl.) in a volume of 0.1ml/rat of 0.9% saline. The opioid peptides were administered 2 hours and 40 minutes after carrageenan injection (to investigate the effect over an established hyperalgesia).

In order to confirm the role of the endogenous opioid system in the antinociceptive effect observed, 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 peptides-treated groups, animals treated with appropriate drug vehicle (saline) were included in each experiment. The volume of administration and all other experimental procedures and conditions for vehicle and compound-treated rats were identical.

Model of inflammatory hyperalgesia and edema

Inflammation was induced by intraplantar injection of 0.1 carrageenan (Sigma-Aldrich Chemie GmbH, Austria) 1% diluted in 0.9% saline into the right hindpaw.

Mechanical and thermal hind paw withdrawal thresholds (PWTs) were determined respectively in order to determine the potential of the tested peptides in inhibiting hyperalgesia during inflammation. *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.

The evolution of the edema was monitored 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 casomorphin or deltorphin II 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 analisys

The antihyperalgesic effect was presented as percent maximum possible effect (% MPE), or percent inhibition of the response latency to nociceptive stimuli, and calculated accordingly to the following formula:

$$\%MPE = [(TX - T0)/(TM - T0)] \times 100$$

where $T0$ is response latency measured before the administration of test substances (base line latency), TX represents the latency measured at different time intervals consequently to substance administration and TM is *cut-off time* (maximum

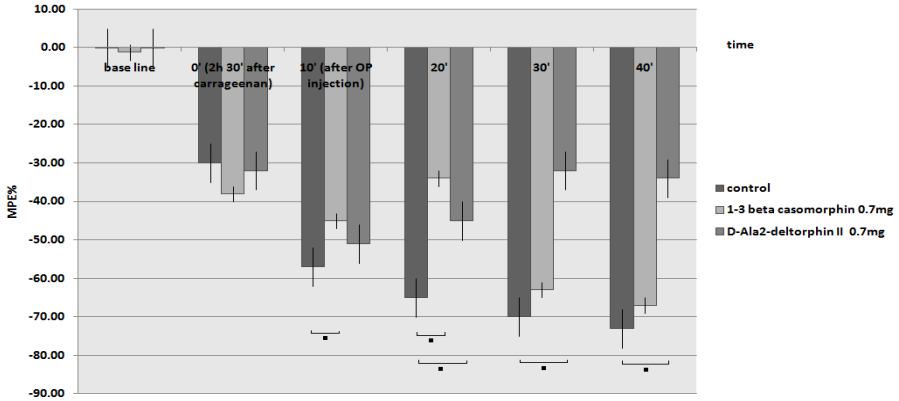

Figure 1. Plantar test, MPE% PWLs. Effect of opioid peptides injected intraplantar: [D-Ala2]deltorphin II and 1-3 beta casomorphin (0.7 mg/rat i.pl.), on a carrageenan 1% prolonged inflammation model; ■) marks a significant antihyperalgesic effect ($p<0.05$)

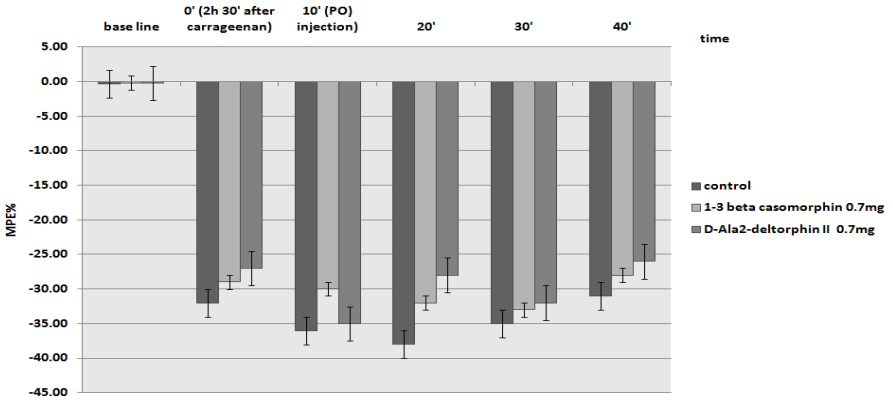

Figure 2. Algesimetric test, MPE% PWLs. Effect of opioid peptides injected intraplantar: [D-Ala2]deltorphin II and 1-3 beta casomorphin (0.7 mg/rat i.pl.) on a carrageenan 1% prolonged inflammation model; ■) marks a significant antihyperalgesic effect ($p<0.05$)

time allowed so no injuries could aflict the animal). Cut-off time was 20 seconds for the plantar test and 15 force units for the algesimetric test. 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 less than 0.05 were used to indicate a significant difference for all tests.

Results

Intraplantar injection of carrageenan produced a significant reduction of PWT at 2 hours and 30 minutes after injection, for both tests measuring nociception, proving a hiperalgesic effect: post-carrageenan levels for plantar test and algesimetric test, compared with pre-carrageenan levels. Carrageenan injection also produced a significant increase in paw volume 2 hours and 30 minutes later when compared with pre-carrageenan levels.

The exogenous opioid peptides tested, intraplantar administered 2 hours and 40 minutes after carrageenan, induced a significant antihyperalgesic effect. PWT in the plantar test increased significantly starting with 20 minutes after the administration of peptides, and reached maximum antihyperalgesic inhibition at 20-30 minutes after injection. The antihyperalgesic effect of the [D-Ala^2] deltorphin is significantly longer when compared with casomorphin 1-3, persisting until the end of the observed period of time. This is due to the fact that [D-Ala2] deltorphins are enzymatically stable, amphibian heptapeptides. The nonselective opioid antagonist naloxone reverts the antihyperalgesic effect. (Figure 1)

The results obtained in the algesimetric test did not show a significant encrease in PWT for either peptide (Figure 2).

The paw volumes measured by pletismometric test did not varied significantly as result of opioid peptides injection into the paw. (Figure 3)

Altogether, these peptides were antihyperalgesic without significantly affecting edema, indicating that peripheral opioid receptors are not involved in edema formation due to acute inflammation.

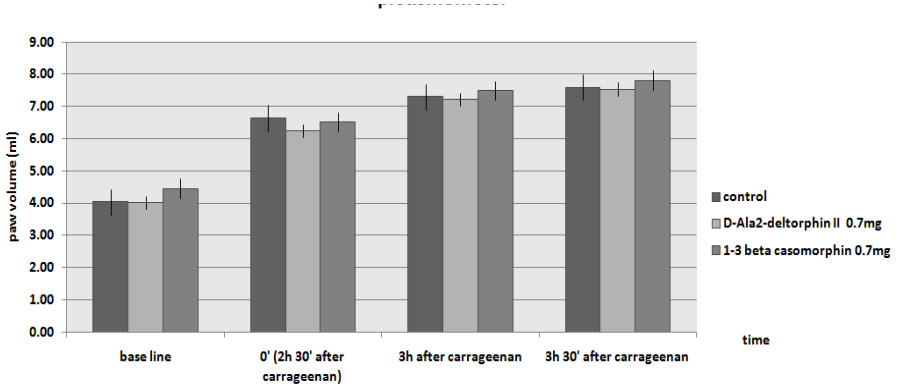

Figure 3. Pletismometer. Paw volume. Effect of opioid peptides injected intraplantar: [D-Ala2]deltorphin II and 1-3 beta casomorphin (0.7 mg/rat i.pl.) on a carrageenan 1% prolonged inflammation model; ■) marks a significant decrease in paw volume ($p<0.05$)

Conclusion

These results demonstrate the peripheral analgesic potential of atypical opioid peptides such as casomorphins and deltorphins. The differential effects of on mechanical versus thermal thresholds support the notion that distinct neuroanatomical or neurochemical mechanisms modulate the processing of thermal versus mechanical stimuli.

Both treatments were antihyperalgesic without affecting edema.

Other groups that investigated the action of local opioids on inflammatory edema (the effects of a single dose of opioid injected into the inflamed paw) obtained similar results and observed no effect on paw edema. These authors concluded that neither preemptive nor curative administration of opioids affects paw circumference. [7,8,13]

Our results are consistent with these observations and support the conclusion that peripheral miu opioid receptors, at the site of inflammation and on immune cells, are not involved in edema formation due to acute inflammation.

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