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Carrageenan-Induced Hyperalgesia Is Partially Alleviated by Endomorphin-1 Locally Delivered into Inflamed Paws in Rat

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This study was performed to test whether endomorphin-1 has analgesic effect, when locally administrated into inflamed peripheral tissue. Carrageenan suspension (0.5%) was injected intraplantarly into the right paw of Sprague-Dawley male rats, and the rats were subjected to a' series of mechanical stimuli with von Frei filaments before and after the injection. Carrageenan-injected rats showed typical inflammatory hyperalgesic signs and decrease of withdrawal threshold, peaked at 3 to 6 hours after the injection and lasted more than 3 days. Endomorphin-1 was intraplantarly injected with carrageenan, simultaneously or 3~4 hours after carrageenan. Simultaneous injection of endomorphin-1 with carrageenan significantly reduced hyperalgesia and thd analgesic effect was prolonged up to 8 hours. The delivery of endomorphin-1 (50 μg) into the inflamed area after 3 to 4 hours of carrageenan injection significantly increased the threshold of hyperalgesic mechanical withdrawal response, but only partially. Intrathecal treatment of endomorphin-1 completely reversed carrageenan-induced hyperalgesia. This report is the first to show that peripherally delivered endomorphin-1 relieved inflammatory hyperalgesia. But a control through peripheral μ-opioid receptors appears to be not sufficient for complete pain treatment.

Key Words: Endomorphin-1, μ-Opioid receptors, Inflammation, Hyperalgesia, Pain

INTRODUCTION

Endomorphin-1 is an innate novel peptide consisted of 4 amino acids and has high affinity and specificity for μ-opioid receptor (Zadina et al, 1997). It produces analgesia as strong as morphine, when administered into spinal cavity and its effect is blocked by naloxone and other specific μ-opioid receptor blockers (Stone et al, 1997; Zadina et al, 1997). Through μ-opioid receptors, endomorphin-1, like other opioid agonists, inhibits Ca^{2+}-channel currents (Higashida et al, 1998; Harrison et al, 1999), activates K^{+}-channel current (Hayar & Guyenet, 1998; Gong et al, 1999) and inhibits adenylate cyclase activity (Gong et al, 1999; Harrison et al, 1999) through G_i/G_o proteins (Zollner et al, 2003). In the physiological state, they may be involved in pain pathway and innate analgesic pathways, such as acupunctural analgesia and descending nonspecific inhibitory pathway (Han et al, 1999; Hao et al, 2000; Krzanowska et al, 2000; Ohsawa et al, 2000).

Presently, there is no direct evidence on the local analgesic effect of endomorphi-1. However histological studies confirmed that small numbers of opioid receptors are present in the peripheral tissues and they are rapidly increased when an inflammation occurs (Hassan et al, 1993; Stein et al, 1996). Several studies showed that injection of other μ-opioid receptor agonists into inflamed tissue relieved inflammatory hyperalgesia (Levine & Taiwo, 1989; Stein et al, 1991; Stein et al, 1997; Whitford et al, 1997; DeHaven-Hudkins et al, 1999; Nozaki-Taguchi & Yaksh, 1999; Perrot et al, 1999), however, others could not observe such effects (Gupta et al, 1993; Rossland et al, 1999; Motamed et al, 2000). These conflicting results have made the interpretation of the analgesic effect of peripherally administrated opioids confusing.

Local administration of opioid drugs into painful areas seems to have distinct benefits for pain treatment: Small amount of drug usage may decrease the risks of side effects, and avoidance of tolerance which can result from systematic treatments may prolong the period of effective drug treatment (Stein et al, 1996; Tokuyama et al, 1998). However, there is no direct evidence until now to show analgesic effect of endomorphin-1 in the peripheral inflamed tissues. Therefore, we undertook to clarify whether endomorphin-1 has any analgesic effect when locally administrated into inflamed tissue.

METHODS

Experimental procedure

For development of inflammatory pain, 50 μl of 0.5% or 2% carrageenan (Sigma, St. Louis, MO, USA) solution in normal saline was intraplantarly injected into hind paws of Sprague-Dawley male rats (200~250 g). Pain-like

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ABBREVIATIONS: DAMGO, [D-Ala^2,N-Me-Phe^4,Gly^5-ol] enkephalin; EM-1, endomorphin-1.

behavior was tested by up-and-down trials with a series of von Frei filaments (0.25, 0.65, 1.05, 1.56, 2.60, 4.99, 6.16, 8.40, 15.25, 21.75 in gram force; always starting with 6.16 g) to measure 50% withdrawal threshold (Chaplan et al, 1994) before and after carrageenan injection. To investigate the effect of endomorphin-1 (purchased from Tocris, U.S.A.) at the initial stage of inflammation, two different amounts (10 and 50 μg) of endomorphin-1 dissolved in saline and mixed with 0.5% carrageenan solution were injected into inflammatory area. And, for the analgesic effect of endomorphin-1 after hyperalgesia developed, endomorphin-1 was injected at 3~4 hrs after carrageenan injection. For comparing the spinal analgesic effect of endomorphin-1, 5 or 10 μg of endomorphin-1 was injected into spinal cavity at the L1 to 4 level through previously implanted PE-10 tubing 3~4 hrs after carrageenan injection. PE-10 tubing implanted animals were allowed for a week to recover, before carrageenan injection. All animals showing neurological deficits were excluded from experiments. All injections were performed under enflurane anesthesia (2%) to avoid stress and further injury from the movement. The experimental protocols and surgical preparations were approved by the Animal Care Committee of School of Medicine, Hanyang University, and followed guidelines on ethical standards for investigations on experimental pain in animals (Zimmerman, 1983).

Statistical analysis

All data are expressed as mean±S.E.M. (standard error of the mean). The statistical significance between data from time points in each experimental groups and between experimental groups at each time points was determined by one-way ANOVA and supplemented with independent Student's *t*-test. *P* values less than 0.05 are considered statistically significant.

RESULTS

Inflammatory hyperalgesia by carrageenan injection

Carrageenan-injected rats exhibited hyperalgesic signs such as holding up, guarding and licking, and their thresholds of 50% withdrawal responses to mechanical stimuli declined within 3 hours and continued for several days. The thresholds were not much different between 0.5% and 2% carrageenan-injected groups (Fig. 1). But, the paws of 2% carrageenan-injected rats showed severe edema, and they were always lifting and guarding the inflamed paws during the experiment. Therefore, application of von Frei filaments on their plantar was very difficult and, even when it was possible, the filaments were frequently slipped away on the plantar surfaces. Moreover, in some cases, rats injected with 2% carrageenan solution showed increased threshold for 50% withdrawal responses in the inflamed area in spite of severe edema and guarding behavior. Consequently, we discontinued the use of 2% carrageenan, and used 0.5% carrageenan solution was used to induce inflammatory hyperalgesia for further experiments.

Effect of endomorphin-1 on the development of hyperalgesia

High dose (50 μg) of endomorphin-1 with carrageenan increased withdrawal threshold to the mechanical stimulation, compared with the same amount of saline with carrageenan, however, low dose (10 μg) did not. Analgesic effect of endomorphin-1 was prominent for the first hour after the injection, and the effect was prolonged up to 8 hrs after the injection. Naltrexone (100 μg), a specific μ-opioid receptor antagonist, almost completely blocked the analgesic effect of endomorphin-1 during the first hour, but did not affect on the prolonged (4 to 8 hrs after) analgesic effect (Fig. 2).

Effect of endomorphin-1 on fully developed hyperalgesia

Endomorphin-1 injection into inflamed paws at the time

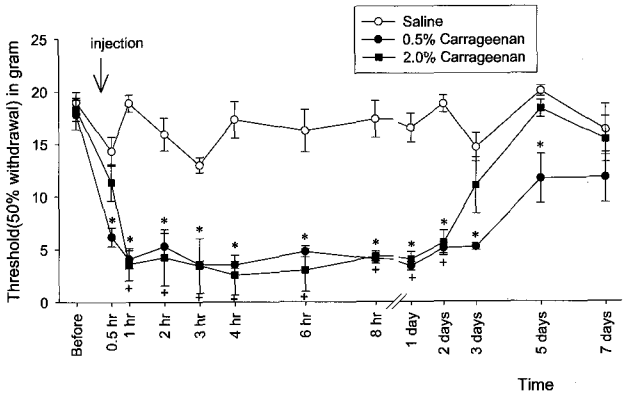

Fig. 1. Hyperalgesia induced by carrageenan injection into rat hind paws. Every rats were injected with 50 μl of solutions, saline, 0.5% or 2% carrageenan solution. Notice similarity of patterns and intensity of inflammatory hyperalgesia between 0.5% and 2% carrageenan-injected groups. * and + indicate the statistical difference from saline treated group.

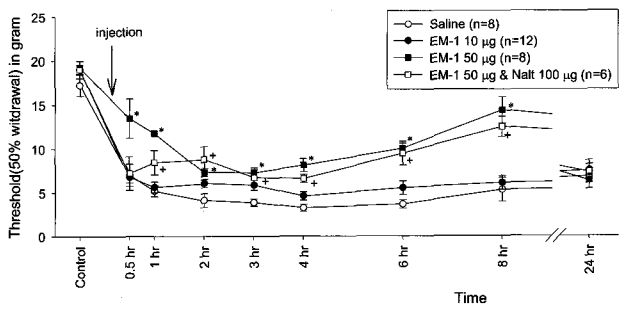

Fig. 2. Effect of endomorphin-1 at the initial stage of inflammation. Drugs (arrow) were injected at the same time of carrageenan injecton. Endomorphin-1 produced pain-relieving effect at initial stage, and its analgesic effect was prolonged to several hours after injection. Naltrexone completely blocked analgesic effect of endomorphin-1. * and + indicate the statistical difference from carrageenan only treated group.

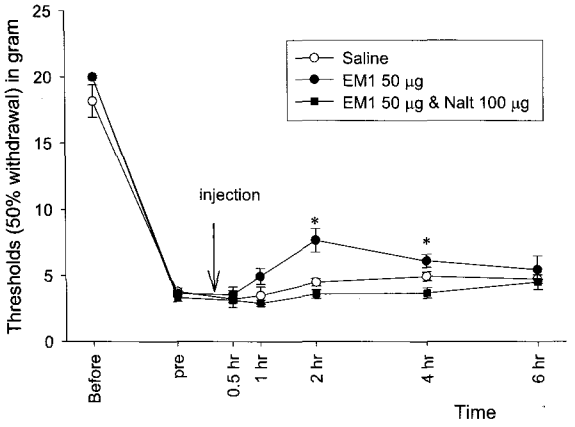

Fig. 3. Analgesic effect of peripherally treated endomorphin-1. Drugs (arrow) were injected between 3 to 4 hrs after carrageenan injection. Fifty μg of endomorphin-1 locally treated partially relieved inflammatory hyperalgesia, but not completely. Naltrexone completely blocked analgesic effect of endomorphin-1. *represents statistical difference from saline treated group.

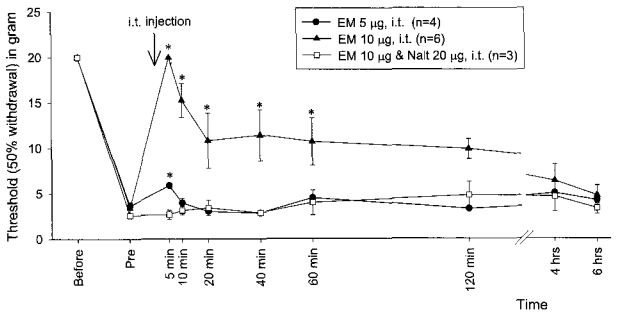

Fig. 4. Analgesic effect of endomorphin-1 injected into spinal cavity. After hyperalgesia developed, endomorphin-1 was intrathecally injected at the lumbar level. Endomorphin-1 immediately reduced pain-like behavior evoked by mechanical stimulation. Co-treatment of naltrexone completely blocked analgesic effect of endomorphin-1. *indicates statistical difference from saline injected group.

of 3 to 4 hrs after carrageenan injection statistically significantly increased withdrawal threshold, but only slightly and temporarily. This analgesic effect was almost completely blocked by μ-opioid blocker, naltrexone (100 μg)(Fig. 3). This effect of endomorphin-1 was not due to central or systematic influence, because administration of equal or 4 times (200 μg) of the amount into the abdominal cavity did not show any pain-alleviating effect (data not showing).

Effect of endomorphin-1 on the spinal cord

Endomorphin-1 (10 μg) injection into spinal cavity of inflamed rats 3 to 4 hours after carrageenan injection completely abolished the hyperalgesic response to mechanical stimuli, and its effect was completely blocked by 20 μg of naltrexone (Fig. 4).

DISCUSSION

The results in this study showed that the endomorphin-1 locally delivered with carrageenan into hind paw prevented development of hyperalgesia for 8 hours after the injection. This prolonged effect seems to be due to anti-inflammatory effects of μ-opioid receptor. Mazone et al. (1990) showed that morphine inhibited human granulocyte aggregation and secretion of inflammatory mediators, and Hong & Abbott (1995) reported that DAMGO depressed pain behavior and extravasation in the formalin-injected rats. Furthermore, locally administrated endomorphin-1 reduced vasodilation, plasma extravasation and paw edema (Khalil et al, 1999). It is, therefore, highly likely that less extent of inflammation may lead to low degree of inflammatory hyperalgesia, and that the disturbance of inflammation development may result in prolonged delay of inflammation and hyperalgesia. Jin et al. (1999) showed that simultaneous injection of endomorphin-1 with carrageenan lessens the increase of c-Fos expression in the spinal cord after carrageenan injection, in agreement with our result.

Then, what is the mechanism of endomorphin-1 on the inhibition of inflammation? Normally, locally injected μ-opioids show little analgesic effects, because tight intercellular contacts at the innermost layer of the perineurium act as a diffusion barrier for high molecular weight or hydrophilic substances such as peptides (Olsson, 1990). However, in inflammatory conditions, the perineurial barrier seems to be disrupted, and this disruption enhances the passage of opioids to sensory neurons (Olsson, 1990; Antonijevic et al, 1995). Through the processes, endomorphin-1 could reach the opioid receptors and block the release of substance P from peripheral nerve endings (Horvath. 2000). However, naltrexone, a potent μ-opioid receptor antagonist, did not completely reverse the effect of endomorphin-1 in this study. The possibility that another types of opioid receptors on the peripheral sensory terminals and other tissue cells might be involved in the action of endomorphin-1 cannot be excluded. Although might be weak, endomorphin-1 seems to act through other types of opioid receptors, possibly κ-opoid receptor.

After inflammatory hyperalgesia was fully developed, endomorphin-1 injected into inflamed tissue increased withdrawal threshold to von Frei stimuli slightly but statistically significantly, greatly differing from others. Most of the previous studies dealing with animal models for inflammatory pain reported strong analgesic effect of morphine and μ-opioid receptor agonists (Levine & Taiwo, 1989; Stein et al, 1989; Parsons et al, 1990; Perrot et al, 1999, 2001). However there are some distinguishable differences between their studies and ours. Perrot et al. (1999, 2001) tested response to pressure on dorsal surface of paw, and reported that intraplantar application of 150 μg of morphine almost completely relieved inflammatory pain, but not 50 μg. Coggeshall et al. (1997) injected an amount of DAMGO similar to this study, but they used frequency of withdrawal response with a filament (39.2 mN, about 4 g force). It can be seen in Fig. 2 that 50 μg of endomorphin-1 almost increased the threshold of 50% withdrawal response from 3.6 g to 7.7 g. Therefore, if we had tried withdrawal frequency with a filament with a force of 39.2 mN, the frequency must have been reduced to prominently. We didn't use dose higher than 50 μg, because it is still high for μ-opioid receptor [Zadina et al. (1997) reported that K_i value of endomorphin-1 for μ-opioid receptor was 0.36 nM]. If endomorphin-1 in the micromolar

range were used, it should act not only through μ-type, but also through another types of opioid receptors. Since antagonists have similar patterns of binding properties onto each individual opioid receptors, antagonists might also act like specific antagonist. However we do not know actual concentration of agonists or antagonists nearly around opioid receptors in the tissues. Furthermore, the time of treatment seems to be important for the effect of locally delivered opioid analogs. Recently, Dionne et al. (2001) reported that peripheral opioid analgesia could be evoked in a model of chronic, but not acute, inflammatory pain in the human. It appears to be valid, because the increase of opioid receptors in the peripheral tissues requires a certain period of time for anterograde transport from cell bodies in the dorsal root ganglia.

In conclusion, a single local delivery of endomorphin-1 into peripheral tissue could alleviate inflammatory hyperalgesia induced by carrageenan, in both at the developmental and fully developed inflammation. μ-opioid receptors in the peripheral tissues could be targeted for pain treatment strategy, however, other strategies seem to be needed, like drug combination, for the complete pain relief.

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