FORMALIN- AND CARRAGEENAN-INDUCED INFLAMMATION ATTENUATES PLACE PREFERENCES PRODUCED BY MORPHINE, METHAMPHETAMINE AND COCAINE

Tsutomu Suzuki, Yayoi Kishimoto and Miwa Misawa

Department of Pharmacology, School of Pharmacy, Hoshi University, Shinagawa-ku, Tokyo 142, Japan

(Received in final form August 30, 1996)

Summary

Morphine-, methamphetamine- and cocaine-induced place preferences under inflammation produced by unilateral injections of 2.5 % formalin (50 μl) and 1 % carrageenan (100 μl) into the hind paw were examined in rats. Formalin and carrageenan reduced the paw pressure threshold, and this hyperalgesia lasted for 9 and 13 days, respectively. Morphine-, cocaine- and methamphetamine-induced place preferences were significantly attenuated in inflamed groups as compared with the respective non-inflamed groups. However, indomethacin failed to produce a place preference in either group. Furthermore, the morphine-induced place preference in the inflamed group gradually recovered to the respective control level as the inflammation healed. These results suggest that the rewarding effects of morphine, cocaine and methamphetamine are attenuated in the presence of inflammatory nociception.

Key Words: inflammation, conditioned place preference, reward, morphine, methamphetamine, cocaine

The administration of the opioids to patients with persistent pain is controversial. Some clinical studies find rampant opioid abuse in chronic pain patients (1,2). A review of litigation involving prescription opioid addiction would tend to support the concept that psychological and physiological addiction must result from long term oral narcotic medication (3). Recent clinical studies, however, have demonstrated that when opiates are used to control pain, tolerance is not a major concern and patients rarely show withdrawal signs when the pain is relieved and the drug is withdrawn (4). Terminal cancer patients who self-medicate with large doses of opiates seldom exhibit the craving and purposive behaviors associated with opioid abusers. Furthermore, Lyness et al. (5) reported that the reinforcing effect of morphine was reduced in complete Freund's adjuvant (CFA)-induced arthritis, which is an animal model of persistent pain.

Several animal models of continuous nociception resembling human clinical conditions have been developed and extensively studied in recent years. One such model involves the administration of CFA (5), carrageenan (6) or formalin (7, 8) to the paw, joint or base of the tail to produce

All correspondence to Tsutomu Suzuki, Ph.D.
chronic inflammation. Under inflammatory nociception, suppression of the development of
tolerance to and physical dependence on morphine (7, 8), changes in the endogenous opioid system
(6), central sensitization of dorsal horn neurons (wind-up) and enhancement of extracellular
excitatory amino acids (9) have been observed. However, some findings in animal models of
chronic pain are not always consistent with clinical findings (10, 11).

Therefore, in the present study, we investigated the rewarding effects of morphine,
methamphetamine and cocaine under carrageenan- and formalin-induced inflammation using the
conditioned place preference paradigm. In addition, the rewarding effect of indomethacin, which
has an antinociceptive effect but no reinforcing properties, was also evaluated under the same
conditions.

**Methods**

The present studies were conducted in accordance with the Guide for Care and Use of
Laboratory Animals adopted by the Committee on Care and Use of Laboratory Animals of Hoshi
University which is accredited by the Ministry of Education, Science, Sports and Culture of Japan.

**Animals:** Male Sprague-Dawley rats (Tokyo Experimental Animal, Inc., Tokyo, Japan) weighing
160-200 g were housed in groups of 3-4 in a temperature-controlled room (22 ± 1 °C). They were
maintained on a 12-h light/dark cycle with laboratory rat chow and water available *ad libitum*.

**Induction and assessment of inflammation:** Formalin (2.5 %, 50 μl), carrageenan (1 %,
100 μl) or vehicle (saline, 100 μl) was injected into the plantar surface of the right rat paw. The
thickness of the paw was measured with vernier calipers (Mitutoyo, Tokyo, Japan) for 17 days.
The paw pressure threshold was measured by the Randall-Selitto apparatus as follows: each rat was
gently restrained and incremental pressure was applied via a wedge-shaped, blunt piston onto the
dorsal surface of one hind paw by an automated gauge (Ugo Basile, Comerio, Italy). The pressure
which elicited either paw withdrawal or a struggle response was determined. Results were
expressed as the mean ± S.E.M.

**Place conditioning:** Place conditioning was performed according to Suzuki et al. (12). The
apparatus consisted of a shuttlebox (30 x 60 x 30 cm: w x l x h) which was divided into two
compartments of equal size. One compartment was white with a textured floor and the other was
black with a smooth floor. For conditioning, rats were confined to one compartment after drug
injection and to the other compartment after saline injection. The orders of the injection (drug or
saline) and the compartment (white or black) were counterbalanced across the subjects.

Conditioning sessions (2 for drug: 2 for saline) were conducted once daily. In each session,
the animals were confined to each compartment for 50 min. On day 5, conditioning was tested as
follows: the partition which separated the two compartments was raised to 12 cm above the floor,
and a neutral platform was inserted along the seam separating the compartments. The time spent in
each compartment during a 900-s session was then measured automatically in a blinded fashion by
an infrared beam sensor (KN-80, Natsume Seisakusyo, Tokyo, Japan). The position of the rat was
defined by the position of its body. All sessions were conducted under conditions of dim
illumination and masking white noise.

**Effects of inflammation on morphine-, methamphetamine-, cocaine- and
indomethacin-induced place preferences:** Conditioning was started at the first day, the fifth
day and the fourteenth day after the induction of inflammation. Control rats were injected with
vehicle (saline: 1 ml/kg, i.p.) instead of drugs at each of the conditioning sessions; rats were
confined to one compartment on the first day and to the other compartment on the next day after
vehicle injection. This conditioning session was repeated twice. Either injection of vehicle was
randomly regarded as a substitute for the drug before the start of the experiments. Drugs (morphine
2-8 mg/kg, methamphetamine 1-4 mg/kg, cocaine 2-8 mg/kg and indomethacin 2.5-10.0 mg/kg)
and vehicle (1.0 ml/kg) were injected i.p. on alternate days. The rats were immediately confined to the respective compartment after the injection. After conditioning, the tests were performed and the time spent in each compartment was measured. Conditioning scores represented the time spent in the drug-paired place minus the time spent in the vehicle-paired place and are expressed as the mean ± S.E.M.

**Drugs:** The drugs used in the present study were formaldehyde solution (Wako Pure Chemical Industries, Ltd., Osaka, Japan), λ-carrageenan (Zushikagaku Laboratories, Zushi, Japan), morphine hydrochloride (Sankyo Co., Tokyo, Japan), cocaine hydrochloride (Takeda Pharmaceutical Industries, Inc., Osaka, Japan), methamphetamine hydrochloride (Dainippon Pharmaceutical Co. Ltd., Osaka, Japan) and indomethacin (Sigma Chemical Co., St. Louis, USA). Indomethacin and other drugs were dissolved in 0.2 M Na₂HPO₄ and saline, respectively.

**Data analysis:** Behavioral data were evaluated statistically with a one-way analysis of variance followed by Dunnett’s test and with a two-way analysis of variance using the Pharmacological Calculation System of Tallarida and Murray (13).

### Results

**Development of inflammation:** The formalin- and carrageenan-treated paws continued to swell for 11 and 15 days, respectively (not shown). Furthermore, a pronounced reduction in the formalin- and carrageenan-treated paw pressure thresholds was observed for 9 and 13 days, respectively (Fig. 1). The time course of swelling paralleled that of the paw pressure threshold. Therefore, in the present study, we performed the following experiments during the reduction in the paw pressure threshold produced by formalin and carrageenan.

![Fig. 1](image-url)

**Fig. 1**

Time course of the paw pressure threshold after the injection of carrageenan (A) or formalin (B). Vehicle (■), 1% carrageenan (●) or 2.5% formalin (▲) was injected into the plantar surface of the right paw. Each point represents the mean with S.E. of 15-19 animals. * P < 0.05, ** P < 0.01, *** P < 0.001; significantly different from the vehicle-treated by a one-way analysis of variance followed by Dunnett's test.

**Effects of inflammation on morphine-, methamphetamine-, cocaine- and indomethacin-induced place preferences:** Preference for the injection-associated place, which was regarded as a substitute for the drug, was calculated. As shown in Fig. 2, none of the vehicle-, formalin- or carrageenan-treated rats that received saline in conditioning sessions exhibited a significant preference for either compartment of the test box. In the vehicle-treated group,
Fig. 2

Effects of inflammation and the conditioning period on the development of the morphine-induced place preference. The ordinate represents the preference for the drug-paired place. Each column represents the mean with S.E. of 8-10 animals. The conditioning scores were analyzed with a one-way analysis of variance followed by Dunnett's test. * P < 0.05, ** P < 0.01; significant conditioning. # P < 0.05, ## P < 0.01; significantly different from the preference in the vehicle-treated group at the respective dose.

Fig. 3

Effects of inflammation and the conditioning period on the development of methamphetamine- and cocaine-induced place preference. The ordinate represents the preference for the drug-paired place. Each column represents the mean with S.E. of 8-10 animals. The conditioning scores were analyzed with a one-way analysis of variance followed by Dunnett's test. * P < 0.05, ** P < 0.01; significant conditioning. # P < 0.05, ## P < 0.01; significantly different from the preference in the vehicle-treated group at the respective dose.
Table 1

Effects of inflammations on the indomethacin-induced place preference.

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Indomethacin conditioning dose (mg/kg, i.p.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vehicle 2.5 5.0 10.0</td>
</tr>
<tr>
<td>Vehicle</td>
<td>-13.6 ± 47.25 41.0 ± 77.98 -11.5 ± 99.53</td>
</tr>
<tr>
<td>2.5% Formalin</td>
<td>10.8 ± 77.24 38.5 ± 98.20 -10.0 ± 110.7</td>
</tr>
<tr>
<td>1% Carrageenan</td>
<td>7.9 ± 89.53 44.8 ± 89.45 17.8 ± 70.73</td>
</tr>
</tbody>
</table>

Each score represents the mean with S.E. of 8-10 animals of the preference for the drug-paired place. Indomethacin failed to produce significant conditioning in either treatment group.

morphine (2-8 mg/kg), methamphetamine (1-4 mg/kg) and cocaine (2-8 mg/kg) produced a dose-related preference for the drug-associated place ($F(1,65) = 10.325, P < 0.01, F(1,61) = 13.782, P < 0.01, F(1,57) = 16.009, P < 0.01$, respectively); significant conditioning was observed at a morphine dose of 4 mg/kg ($P<0.01$), methamphetamine doses of 1 ($P<0.05$), 2 and 4 mg/kg ($P<0.01$) and cocaine doses of 4 and 8 mg/kg ($P<0.01$) (Figs. 2 and 3). The morphine-, cocaine- and methamphetamine-induced place preferences were significantly suppressed in formalin- and carrageenan-treated groups compared with the respective vehicle-treated group (Figs. 2 and 3).

The vehicle-control rats exhibited no preference for either place, and indomethacin (2.5-10.0 mg/kg) failed to produce preference for the drug-associated place (Table 1).

Morphine (8 mg/kg) produced a significant preference for the drug-associated place in the vehicle-treated group regardless of whether the conditioning started on the first, fifth or fourteenth day after the injection of vehicle into the paw, with a mean conditioning scores of 300.0 ± 60.0 s, 360.0 ± 21.6 s and 342.3 ± 65.5 s, respectively (each group: $P < 0.01$, Fig. 2). The morphine-induced place preference in the formalin- and carrageenan-treated groups gradually recovered to the level in the respective vehicle-treated control as the inflammation healed. Rats which began conditioning on the 14th day after the induction of inflammation showed a significant preference for the drug-associated place, and this place preference was similar to the morphine-induced place preference in vehicle-treated rats (Fig. 2).

Discussion

Inflammation is characterized by hyperalgesia and edema. In the present study, formalin- and carrageenan-induced hyperalgesia lasted for 9 days and 13 days, respectively. This hyperalgesia paralleled the edema. These prolonged periods of edema in formalin- and carrageenan-induced inflammations were consistent with previous findings (6, 14). Therefore, our data also suggest a correlation between hyperalgesia and edema in inflammation.

To our knowledge, this is the first study to show suppression of the morphine-induced place preference in a chronic pain model. It has been reported that the morphine-induced place preference is not attenuated in complete Freund's adjuvant (CFA)-induced arthritis (10, 11). The discrepancy between these findings may be due to differences between the models of inflammation and the number of conditioning sessions used in the studies. The inflammation induced by formalin and carrageenan in the present study developed more rapidly than that induced by CFA, and the
intradermal inoculation of CFA to the base of tail (so called polyarthritis) (5) produces greater inflammation than the injection of formalin and carrageenan into the unilateral hindlimb (10). Moreover, Satka (10) used 4 conditioning sessions each of drug and vehicle prior to testing instead of the 2 sessions used in the present study. However, in our preliminary experiment, morphine-induced place preference after 3 conditioning sessions was also significantly attenuated in inflamed group as compared with the respective non-inflamed group. In his report (10), an enhanced morphine place preference in the choice preference measure was observed in inflamed animals, suggesting that the enhancement manifests morphine’s negatively reinforcing effects, and that higher doses of morphine are required to produce the negatively reinforcing effects against CFA-induced inflammatory nociception than morphine doses that typically produce analgesic effects against acute nociceptive stimulation. In the present study, the negatively reinforcing effects of morphine were not observed because we did not measure the choice preference and the highest dose of morphine was 8 mg/kg. These discrepant observations are difficult to reconcile, and have different theoretical implications with regard to the development of opiate dependence, and different clinical implications in terms of the treatment of chronic pain.

Nevertheless, the suppression of morphine’s rewarding effect under the chronic pain is consistent with clinical findings in the morphine-administration for the severe of cancer patients (4). Furthermore, Lyness et al. showed that although they used only one dose of morphine, morphine self-injection is suppressed by CFA-induced arthritis (5). When the CFA-induced arthritis dissipated, arthritic rats rapidly began to increase morphine self-injection. These findings indicate that the presence of persistent pain apparently reduces the addictive properties of morphine. On the other hand, it is well known that indomethacin, a non-steroidal anti-inflammatory drug (NSAID), does not have a reinforcing effect. In the present study, indomethacin failed to produce either place preference or aversion with or without inflammation. These results confirm those in a previous report under CFA-induced arthritis (10), and suggest that the antinociceptive effect of indomethacin does not affect the suppression of the morphine-induced place preference under inflammation.

We also found that the suppression of the morphine-induced place preference corresponds to the intensity of inflammatory hyperalgesia on the 1st, 5th and 14th days after the induction of inflammation. According to a recent study, the change in the level of mRNA for substance P and calcitonin-gene related peptide, a possible nociceptive transmitter, may depend upon the time course and severity of CFA-induced inflammation (15). Therefore, the tonic nociceptive stimulation may produce sensitization of dorsal horn neurons (wind-up) and increase not only the level of dynorphin or preprodynorphin mRNA but also the release of glutamate and aspartate in the spinal cord (6, 9, 16). Suppression of the morphine-induced place preference may result from this complex alteration of multiplex neurons under inflammation. Our results also suggest that the progress of this alteration is related to the development and healing of inflammation.

In humans, a severe and persistent pain impairs sleep and appetite, thereby producing fatigue and reducing the availability of nutrients to organs. It has been suggested that tonic pain affects neural systems with tracts that pass through the central core of the brainstem and send impulses to the limbic system (4). These systems may control the emotional component of pain, producing feelings that might be described as wretched or terrified. They may also influence the response to such feelings. It has been reported that not only noradrenergic and serotonergic neurons but also dopaminergic neurons are altered under some types of stress (17). Indeed, morphine affects not only dopaminergic but also noradrenergic and serotonergic neurons through μ-opioid receptor (18). Methamphetamine and cocaine are psychostimulants which can facilitate dopaminergic transmission either by stimulating the release of dopamine or by inhibiting dopamine uptake. It is well known that the action sites of opiates and psychostimulants are the mesolimbic and nigrostriatum dopaminergic systems, and this action is closely related to their reinforcing effects (19, 20). In the present study, we found that the methamphetamine- and cocaine-induced place preferences were suppressed under chronic pain, as with morphine, suggesting that dopaminergic neurons may also be altered under formalin- and carrageenan-induced inflammation. On the other hand, it is also possible that the inflammation and associated pain is serving as the primary stimulus in these animals, and that a place-preference conditioning can not be obtained to morphine, cocaine and methamphetamine because the pain stimulus overshadows the drug
stimulus. More recently, however, we found that morphine-induced increase of dopamine turnover in the limbic forebrain was also significantly attenuated under inflammation, and that the attenuation of morphine-induced place preference and morphine-induced increase of dopamine turnover in the limbic forebrain under inflammation were blocked by pretreatment with κ opioid receptor antagonist nor-binaltorphimine but not with δ opioid receptor antagonist naltrindole (21). Furthermore, we previously found that the morphine- and cocaine-induced place preferences were blocked by κ opioid receptor agonist U-50,488H (22,23). These results suggest that endogenous κ opioid neuron may be activated by chronic inflammatory nociception; as a result, rewarding effects of these drugs may be suppressed by the inflammatory nociception.

In summary, the morphine-induced place preference was suppressed under formalin- and carrageenan-induced inflammation, while indomethacin failed to produce place preference or aversion in both control inflamed groups. These results suggest that the antinociceptive effect of morphine does not affect the suppression of the morphine-induced place preference under inflammation. These results are consistent with clinical findings. Furthermore, the suppression of the morphine-induced place preference paralleled the severity of inflammation. Moreover, we found that the methamphetamine- and cocaine-induced place preferences were also suppressed under the inflammation, suggesting that the activity of dopaminergic neurons may be attenuated under inflammatory nociception and that this attenuation may correspond to the strength of the nociceptive stimulation.

Acknowledgements

This work was supported in part by Grants-in-Aid from the Ministry of Education, Science, Sports and Culture of Japan (No. 08670125) and a Research Grant (5A-7) for Nervous and Mental Disorders and a Research Grant from the Ministry of Health and Welfare to T. Suzuki. We wish to thank Ms. Yoko Murakami for her technical assistance.

References