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Supraspinal Nitric Oxide Synthesis Inhibition Enhanced Antinociception of Morphine in Morphine Tolerant Rats

Ho-Kyung Song, M.D., and **Yeon Jang,** M.D.

Department of Anesthesiology, College of Medicine, The Catholic University of Korea, Seoul, Korea

= **Abstract** =

Background: Opioids such as morphine are widely used in the treatment for pain, but chronic treatment with morphine can be complicated by the development of tolerance. The mechnisms of tolerance were still not completely understood, but recently it has been reported that NOS inhibitors can prevent development of morphine tolerance in animals. The present study accessed the possible role of supraspinal NO on antinociceptive effect of morphine in morphine tolerance using a highly specific inhibitor of the neuronal isoform of NOS, 1-(2-trifluoromethylphenyl) imidazole (TRIM).

Methods: Thirty two male SD rats (300 g) were prepared with intracerebroventricular (icv) and IV cannulae. We administrated IV morphine, 3 mg/kg, daily for 4 days, resulting in tolerance. On the fifth day, a challenge dose of morphine, 3 mg/kg, was administered following pretreatment with icv TRIM, 10 μg. We also evaluated the antinociceptive effect of icv TRIM alone and the effect on a single dose of morphine (3 mg/kg) in morphine nave rats. Antinociception from morphine was determined by response to intraplantar injection of 5% formalin 100 μl was qualified as the number of flinches in the first 0−10 min (first phase), 10−40 min Phase IIa, and 40−60 min (Phase IIb).

Results: Pretreatment with icv TRIM significantly enhanced the antinociceptive effects of systemically administered morphine in morphine tolerant rats. The antinociceptive effect of morphine in opioid nave rats was also significantly increased by pretreatment with icv TRIM.

Conclusions: Our results further support the hypothesis that supraspinal NO modulates morphine-sensitive nociceptive process in morphine tolerance due to chronic intravenous administration.

Key Words: Analgesics, Morphine tolerance, Intracerebroventricular, Nitric oxide, Pain

INTRODUCTION

Opioids such as morphine are widely used in the treatment for pain, but chronic treatment with morphine can be complicated by the development of tolerance and dependence, limiting its clinical use. The phenomenon of opioid tolerance has been investigated for many years, but its mechanism is still not completely understood.

Opioid tolerance may reflect actions on cellular/biochemical cascades in the central nervous system. For example, non-competitive N-methyl-D-aspartate (NMDA) receptor antagonists attenuate the development of tolerance to analgesia from morphine, indicating a role for the NMDA receptor in the development of opiod tolerance.[1] These NMDA actions occur in a consequence of NO

Corresponding to : Ho-Kyung Song, Department of Anesthesiology, Our Lady of Mercy Hospital, College of Medicine, The Catholic University of Korea, #665 Pupyung-dong, Pupyung-gu, Inchon 403-720, Korea
Tel: 82-32-510-5518, 5665, Fax: 82-32-518-2718
E-mail: song@olmh.cuk.ac.kr

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production, followed by sustained NO production from activation of constitutive neuronal NO synthase (NOS) which is linked to the NMDA complex.[2,3] Although it has been proposed that spinal NO can, under some circumstances, mediate the antinociceptive effect from systemically administered morphine,[4] other studies a positive role of NO in transmission of nociceptive information.[5] For example, inhibition of NO synthesis by NOS inhibitors, administered systemically or intrathecally (i.t.), produces opioid-independent antinociception in animals.[6,7] Furthermore, NOS inhibitors enhance antinociceptive actions of morphine and prevent development of morphine tolerance.[1,8]

In most previous studies regarding the role of NO in morphine tolerance, NOS inhibitors were administered by systemic or i.t. routes. However, these studies do not adequately differentiate between spinal and supraspinal sites of action of NOS inhibitors.[7,9] The current study specifically test the hypothesis that production at supraspinal sites plays a key role in morphine tolerance. For this purpose, we utilized a highly specific inhibitor of the neuronal isoform of NOS, TRIM.

METHODS

After approval by the Animal Care and Use Committee, icv cannula (for administration of NOS inhibitor) and jugular vein catheters (for administration morphine) were inserted in anesthetized male Sprague-Dawley rats (250−300 g).

Icv injection of the NOS inhibitor was made directly into the lateral ventricle according to the modified method of Haley and McCormick.[10] A stainless steel cannula was inserted into a position 1.5 mm lateral and 1 mm caudal to the bregma at a depth of 4.5−5.0 mm, and fixed with dental cement. Location of the cannula tip was confirmed in some, but not all, animals by post mortem dissection. At least 3 days elapsed after recovery from anesthesia before study, and animals with no evidence of neurologic deficit after insertion of cannula were studied. For production of morphine tolerance, the right external jugular vein was cannulated with PE-50 tubing, fixed on the dorsal skin of the neck, for daily IV administration of morphine.

The antinociceptive effect of morphine was determined by intraplantar injection of formalin. Rats were placed in an open plexiglas observation chamber for 30 min to allow them to accommodate to their surroundings, then they were removed for formalin administration. The right hind paw of the rat was injected with 100 μl of dilute formalin (5%), using a 30-gauge needle. The animal was then returned to the chamber for observation. A mirror was placed behind the chamber to enable unhindered observation of the formalin-injected paw. The rats were observed for nociceptive behavior immediately after formalin injection for 1 min at 5 min intervals until 60 min after injection. Nociceptive behavior was quantified as the number of flinches in the first 0−10 min (first phase), 10−40 min Phase IIa, and 40−60 min (Phase IIb). Animals were then killed by cervical dislocation.

Antinociception from iv single dose of morphine, 3 mg/kg, icv TRIM, 10 μg and iv saline as control were determined initially. Next, 1-(2-trifluormethylphenyl) imidazole (TRIM)[6] as a neuronal NOS specific-inhibitor was examined. In one set of experiments, animals received iv bolus injections of morphine, 3 mg/kg, in a volume of 0.5 ml over every 24 hrs for 4 days. On the fifth day, a challenge dose of morphine, 3 mg/kg, was administered following either icv TRIM 10 μg or saline with 5 minutes interval. Antinociception was evaluated in response to intraplantar injection of formalin 5 min after morphine administration. In other aninmals, the effect of icv TRIM on the antinociception of 3 mg/kg of morphine was evaluated in opioid nave rats. There were eight animals in each experiment, and the investigator was not blinded to the icv injected drug. Animals showing respiratory complications were eliminated from the experiments. Drug given by icv injection was dissolved in normal saline and administered in a volume of 10 μl followed by 2 μl flush with normal saline. TRIM was obtained from Research Biomedicals International (St. Louis, MO, USA) and morphine-HCl was obtained from Myung-Moon (Korea).

Data are presented as mean ± SD. Differences between groups were statistically analyzed by repeated measures

ANOVA on Ranks followed by Turkey's test. A probability value $P < 0.05$ was considered statistically significant.

RESULTS

In all cases examined, the tip of the icv cannula resided in the lateral ventricle space. Repeated morphine exposure, resulted in antinociceptive tolerance, as demonstrated following a challenge dose of morphine on the fifth day. Studies were directed at determining whether supraspinal NO was involved in the development of morphine tolerance.

The single challenge dose of iv morphine (3 mg/kg) showed less increase in number of flinches compared to iv saline control ($P < 0.05$) after intraplantar injection of formalin (5%, 100 μl). Repeated administration of tolerance inducing dose of iv morphine (3 mg/kg) resulted in a higher increase in the number of flinches than single challenge dose of iv morphine (Fig. 1)($P < 0.05$).

In morphine tolerant rats, the antinociceptive effects of iv morphine followed by pretreatment with icv TRIM or icv saline was quantified as the number of flinches in the first and second phase. As icv TRIM pretreatment resulted in a smaller increase in the number of flinches than saline pretreated rats, the antinociceptive effects of morphine

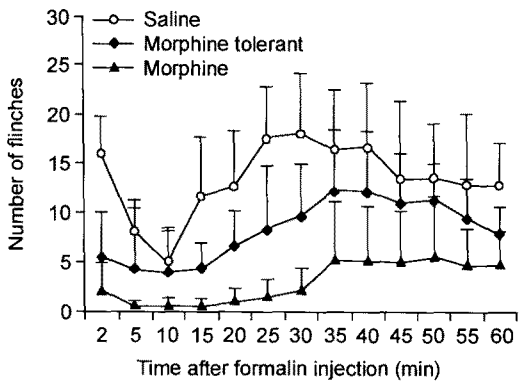

Fig. 1. Mean number of flinches per minute plotted as a time after intraplantar injection of formalin (5%, 100 μl). The single challenge dose of morphine (3 mg/kg) shows less increase in number of flinches compared to saline control ($P < 0.05$). Repeated administration of tolerance inducing dose of IV morphine (3 mg/kg × 4 days) resulted in a higher increase in the number of flinches than single challenge dose ($P < 0.05$).

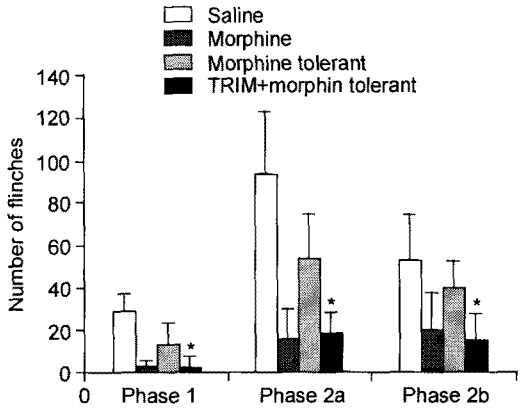

Fig. 2. Potentiation of the antinociceptive effect (number of flinches) of intravenously administered morphine (3 mg/kg) by intracranioventricular injection of the specific neuronal NO synthase inhibibitor 1-(2-trifluoromethylphenyl) imidazole (TRIM, 10 μg) after intraplantar formaline injection (5%, 100 μl) in morphine tolerant rats. Values represent the mean ± SD. *: $P < 0.05$, morphine tolerant vs. TRIM + morphine tolerant group.

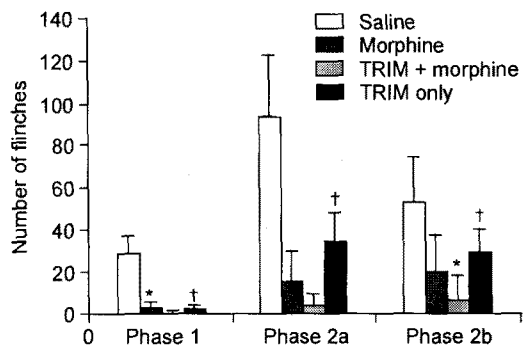

Fig. 3. Antinociceptive effect (number of flinches) of intracranioventricular injection of the specific neuronal NO synthase inhibibitor, 1-(2-trifluoromethylphenyl) imidazole (TRIM, 10 μg), on intraplantar formaline injection (5%, 100 μl) and potentiation of the antinociceptive effect of intravenously administered morphine (3 mg/kg) in morphine nave rats. Values represent the mean ± SD. *: $P < 0.05$, TRIM + morphine vs. morphine. †: $P < 0.05$, TRIM only vs. saline.

were significantly enhanced by pretreatment of icv TRIM compared to the rats that were injected with saline (Fig. 2)($P < 0.05$).

Icv TRIM showed less increase in number of flinches compared to saline control ($P < 0.05$) and pretreatment of icv TRIM also increased the antinociceptive effect of a single dose of morphine in opioid nave rats. Iv morphine followed by icv TRIM has produced lesser increase in number of flinches in the first and second phase (A and B), respectively (Fig. 3) ($P < 0.05$).

DISCUSSION

The present study demonstrates that supraspinal administration of the NOS inhibitor, TRIM, potentiates antinociception from morphine in both of opioid tolerant and opioid nave rats. Although the mechanisms underlying tolerance to the antinociceptive actions of opioids are unclear, several lines of evidence suggest the participation of NMDA receptors and NO.[11,12]

It has been demonstrated that various NOS inhibitors have opioid-independent antinociceptive activity themselves in mice and rats when they are administered intraperitoneally,[6,13] i.t.,[7,14] icv,[5,15] peripherally[16] or orally.[11] In such studies, the antinociceptive activity of NOS inhibitors was demonstrated in formalin-induced paw licking as well as acetic acid-induced writhing and hot-plate tests. We have used TRIM, a relatively potent inhibitor of neuronal NOS, which has been used for the investigation of biological roles of NO within the central nervous system. It also exhibits dose-related and opioid-independent antinociceptive activity, which is assessed as inhibition of the late phase formalin-induced hindpaw licking behavior.[6]

It is well known that the formalin-induced first- and second-phase nociceptive response are related mainly to neuronal and inflammatory mechanisms, respectively, and NO is an important factor in the induction of the second-phase nociception.[17] Therefore, systemic administration of NOS inhibitors reduce first and second phases response of this test, possibly due to central effects.[5] In addition, NO has been accepted as a modulator of the synaptic transfer of excitatory neurotransmission and as a mediator of nociceptive events in the peripheral and central nervous system. NOS from rat brain has been cloned, sequenced, and expressed, and it has been reported that long term treatment of NOSI progressively decreases NOS activity in both cerebellum and brain stem, two regions shown to have high levels of NOS.[16] However, the role of NO in spinal nociceptive processing[4,9,18] and peripheral mechanisms[16,17] have been reported as complex, either inhibitory or excitatory for nociception. In contrast, supraspinal inhibition of NO production results in opioid-independent antinociceptive effects in the mouse.[6] These findings may suggest an excitatory role of the NO-cyclic GMP system in supraspinal transmission of nociceptive information.[5,17] Therefore, NO may still have different roles depending on the nociceptive stimuli and the type of primary sensory neurons involved.

Previous studies regarding the role of NO in opioid analgesia and tolerance are inconsistent. For example, the analgesic effect of morphine is increased by NOS inhibitors, whereas an NO donor decreases this effect in a dose-dependent manner.[7,19] Therefore, inhibition of NO production may contribute to morphine tolerance and its development after chronic administration of morphine. There are many reports supporting this positive role of NOS inhibitors on morphine tolerance.[1,8,20] However, studies by others concur this antinociceptive effect of NOS inhibitor. For example, the NOS inhibitor, L-NAME, has little effect on morphine tolerance spinally[21] and further diminishes morphine-elicited analgesia. Moreover, it is reported that NOS inhibitors, given systemically with morphine, may not affect morphine analgesia despite their ability to block and reverse morphine tolerance.[20]

Given the discrepant results, it is not clear whether differences in species or strains of experimental animals, different activities of NO synthase inhibitors, or different routes of administration are responsible.[3] The magnitude of opioid tolerance appears to be affected by the route of dministration.[22] Icv or it administration of opioid produces a larger magnitude of tolerance compared to systemic administration. Perhaps this reflects a relatively higher potency of these routes compared to systemic administra-

tion, since both supraspinal and spinal receptors are activated, resulting in multiplicative interactions.[23] These complex interactions are important in the interpretation of nociception in the central nervous system.[16] There is relatively consistent evidence that NO at supraspinal but not the spinal sites plays an important role in the mediation of morphine antinociceptive tolerance.

As regards opioid tolerance, the current study supports the view that tolerance is attenuated after inhibition of supraspinal NO production, confirming the role of NO in morphine tolerance. It is likely that the enhancement of morphine antinociception by a NOS inhibitor may result from the possible additive or synergistic actions between the antinociceptive effects of NOS inhibitors and morphine.[12]

In summary, we have shown that the inhibition of supraspinal NO synthesis potentiates morphine-induced antinociception in morphine tolerant and nave rats. Our results further support the hypothesis that supraspinal NO modulates morphine-sensitive nociceptive process involved in the morphine tolerance due to chronic intravenous administration.

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