

Trifluoperazine, an orally available clinically used drug, disrupts opioid antinociceptive tolerance

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Abstract

Calcium/calmodulin dependent protein kinase II (CaMKII) has been shown to play an important role in the generation and maintenance of opioid tolerance. In this study, trifluoperazine was studied for its effect on morphine tolerance in mice. Acute treatment with trifluoperazine (0.5 mg/kg, i.p.) completely reversed the established antinociceptive tolerance to morphine. Pretreatment with trifluoperazine also significantly attenuated the development of antinociceptive tolerance ($p < 0.01$). Morphine induced a significant up-regulation of supraspinal and spinal CaMKII activity in tolerant mice, which was abolished after the pretreatment or acute treatment with trifluoperazine. These data suggested that trifluoperazine was capable of suppressing opioid tolerance, possibly by the mechanism of inhibiting CaMKII. Since trifluoperazine has been safely used as an antipsychotic drug, we propose that the drug should be studied in humans for the prevention and treatment of opioid tolerance and addiction. © 2005 Elsevier Ireland Ltd. All rights reserved.

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Opioids are used primarily as analgesics. Repeated administration of opioid drugs leads to loss of effectiveness as tolerance develops. The mechanism underlying opioid tolerance remains unclear. A number of studies have suggested the role of phosphorylation by several protein kinases in opioid tolerance [1,4,9,13,19,21,26].

Ca²⁺/calmodulin dependent protein kinase II (CaMKII) is a multifunctional serine/threonine protein kinase that is co-localized with the mu opioid receptor in spinal cord and dorsal root sensory neurons [2]. Ca²⁺ and calmodulin, two required elements for the activation of CaMKII [6], are regulated by opioids. Opioids increase intracellular Ca²⁺ levels and calmodulin activity [14–16,18]. On the other hand, cellular opioid receptor desensitization is enhanced when a constitutively active CaMKII is also expressed [7,10]. In rodent models of opioid tolerance and dependence, it has been reported that CaMKII inhibitors attenuate opioid tolerance either directly [23] or through learning/memory pathways [3]. These data have led to our hypothesis that CaMKII plays a

critical role in the development and maintenance of opioid tolerance.

The antipsychotic drug trifluoperazine has been known for some time to be a potent calmodulin antagonist, suppressing calmodulin and CaMKII activity [17]. Therefore, trifluoperazine not only presents a unique opportunity to test our hypothesis, but more importantly may provide a drug candidate for the attenuation of opioid tolerance. In the current study, we tested the effect of trifluoperazine in a mouse model of acute morphine tolerance.

All experiments were performed in accordance with the NIH guidelines and after approval by the Animal Care and Use Committee of the University of Illinois. Male ICR mice (20–25 g, Harlan, Indianapolis, IN) used in these studies were housed under a 12:12 h light/dark cycle with access to food and water ad libitum. Trifluoperazine (Sigma, MO) and morphine sulfate (Abbott Laboratories, North Chicago, IL) were prepared in normal saline. For each experiment, differences among all groups were first analyzed by ANOVA. When a statistical significance was detected, Student's *t*-test was used to determine the statistical difference between a testing group and its corresponding control group. Statistical significance was established at 95%.

We first tested if trifluoperazine itself produced antinociception or affected the antinociceptive effect of morphine.

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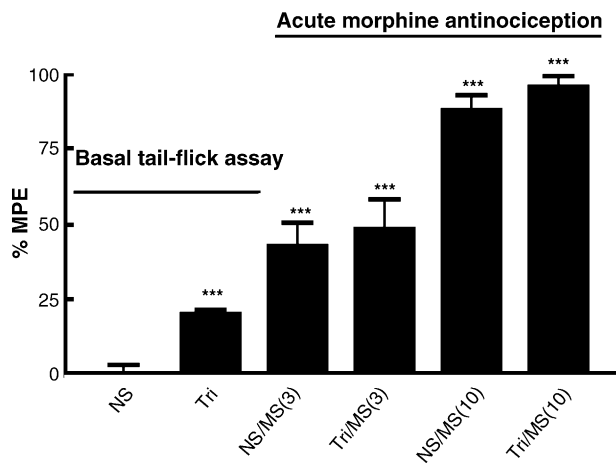


Fig. 1. Effect of trifluoperazine on basal nociception and acute morphine antinociception. To test the effect of trifluoperazine on basal thermal nociception, mice were treated with trifluoperazine (0.5 mg/kg, i.p. “Tri”) or normal saline (“NS”) 30 min before the tail-flick test. To investigate the effect of trifluoperazine on acute morphine antinociception, trifluoperazine (0.5 mg/kg, i.p., “Tri/MS(dose)” group) or normal saline (“NS/MS(dose)” group) was given to mice 30 min before the test dose of morphine (3 or 10 mg/kg, s.c.). Results are presented in “%MPE” as defined as “ $100 \times (\text{test} - \text{control}) / (\text{cut-off} - \text{control})$ ”, and expressed as mean \pm S.E.M ($n \geq 6$ for each group). Trifluoperazine produced slight antinociception by itself, but did not affect morphine induced antinociception ($p > 0.05$). *** $p < 0.001$ compared with the normal saline group, Student’s *t*-test.

Trifluoperazine (0.5 mg/kg, i.p.) produced slight antinociception ($20.4 \pm 1.2\%$ MPE) in a warm water (52°C) tail-flick test when given to naive mice (Fig. 1).

To test antinociception, the latencies of tail flick responses were measured before and 30 min after the administration of morphine (s.c.). A cut-off of 12 s was applied to prevent tissue damage. Results were presented in “%MPE” (maximal possible effect) as defined by the formula: $\%MPE = 100 \times (\text{test} - \text{control}) / (\text{cut-off} - \text{control})$. When trifluoperazine was given 30 min before the administration of morphine, it did not enhance morphine (10 mg/kg, s.c.) antinociceptive response. To rule out a potential ceiling effect, another experiment was performed using a lower dose of morphine (3 mg/kg, s.c.). Similarly, trifluoperazine did not alter the antinociceptive effect of morphine (3 mg/kg).

To induce tolerance, mice were treated with morphine sulfate (100 mg/kg, s.c.) as previously described [1]. Control mice received an equal volume of saline (s.c.). Mice were tested for the presence of opioid tolerance, 5 h later, by monitoring the antinociception produced by a test dose of morphine (10 mg/kg, s.c.) [1]. In order to test the effect of trifluoperazine on morphine tolerance, mice were given trifluoperazine (0.5 mg/kg, i.p.) 4 h after the treatment with morphine (100 mg/kg, s.c.) or saline (i.e., 30 min before the test dose of morphine). Morphine (10 mg/kg, s.c.) produced $88.2 \pm 4.7\%$ MPE in saline-treated mice (Fig. 1), which was not different from its antinociceptive effect in naive untreated mice (data not shown). However, the same test dose of morphine produced significantly lower antinociception ($30.7 \pm 3.6\%$ MPE, $p < 0.001$) in morphine (100 mg/kg)-treated mice, indicative of the development of morphine antinociceptive tolerance (Fig. 2). When trifluop-

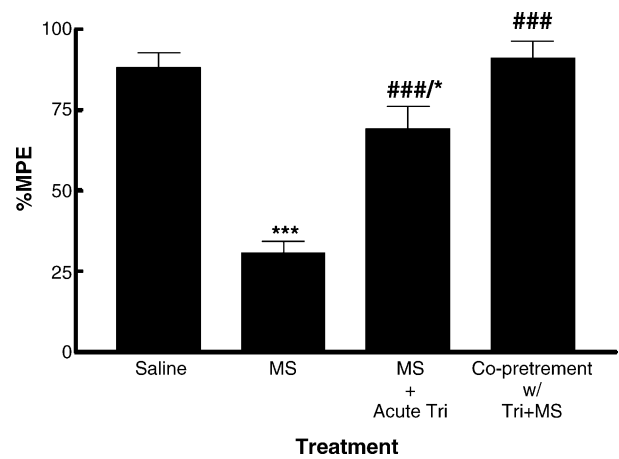


Fig. 2. Effect of trifluoperazine on antinociceptive tolerance to morphine. Male ICR mice were injected with morphine (100 mg/kg, s.c.) to induce tolerance. Control mice received the same volume of normal saline. Five hours later, the antinociception produced by a test dose of morphine (10 mg/kg, s.c.) was evaluated using a 52°C warm-water tail-flick assay. A cut-off of 12 s was used to prevent tissue damage. Data are expressed as mean \pm S.E.M ($n = 8$ for each group). Morphine (100 mg/kg, s.c.) induced opioid antinociceptive tolerance (“MS” group), as evidenced by the significantly decreased antinociception. Trifluoperazine (0.5 mg/kg, i.p.) given 30 min before the test dose of morphine (“MS+ acute Tri” group) was able to reverse the established tolerance. Mice co-pretreated with trifluoperazine (0.5 mg/kg, i.p.) and morphine (100 mg/kg, s.c.) (“co-pretreatment w/Tri+MS” group) developed significantly less tolerance. * $p < 0.05$, *** $p < 0.001$ compared with the normal saline group; ### $p < 0.001$ compared with the “MS” group, Student’s *t*-test.

erazine was given 30 min before the test dose of morphine, morphine antinociceptive tolerance was completely abolished in morphine (100 mg/kg)-treated (i.e., tolerant) mice ($p < 0.001$ compared with morphine group; not significantly different from saline group; Fig. 2). These data suggested the trifluoperazine reversed the established acute morphine antinociceptive tolerance.

We further tested if pretreatment with trifluoperazine could prevent the development of morphine antinociceptive tolerance. In these experiments, mice were injected with trifluoperazine (0.5 mg/kg, i.p.) immediately before the administration of morphine (100 mg/kg, s.c.). Compared with mice that received morphine alone, mice co-treated with trifluoperazine and morphine showed significantly reduced antinociceptive tolerance to morphine ($p < 0.001$; Fig. 1). Co-treated mice still exhibited some tolerance when compared to those that received saline ($p < 0.05$). These data suggested that trifluoperazine was also effective in preventing the development of morphine antinociceptive tolerance. The incomplete prevention of morphine tolerance may be due to relatively short duration of action of trifluoperazine, since its peak plasma level occurs less than 3 h following oral administration in humans [11].

Since trifluoperazine did not alter acute morphine antinociception (Fig. 1), its effect on morphine tolerance could not be due to directly enhancing acute morphine antinociception. To correlate the behavioral effect of trifluoperazine with its cellular inhibitory effect on CaMKII activity, we examined the CaMKII activity in mice treated with morphine and/or trifluoperazine. Brain and spinal CaMKII activities were determined using

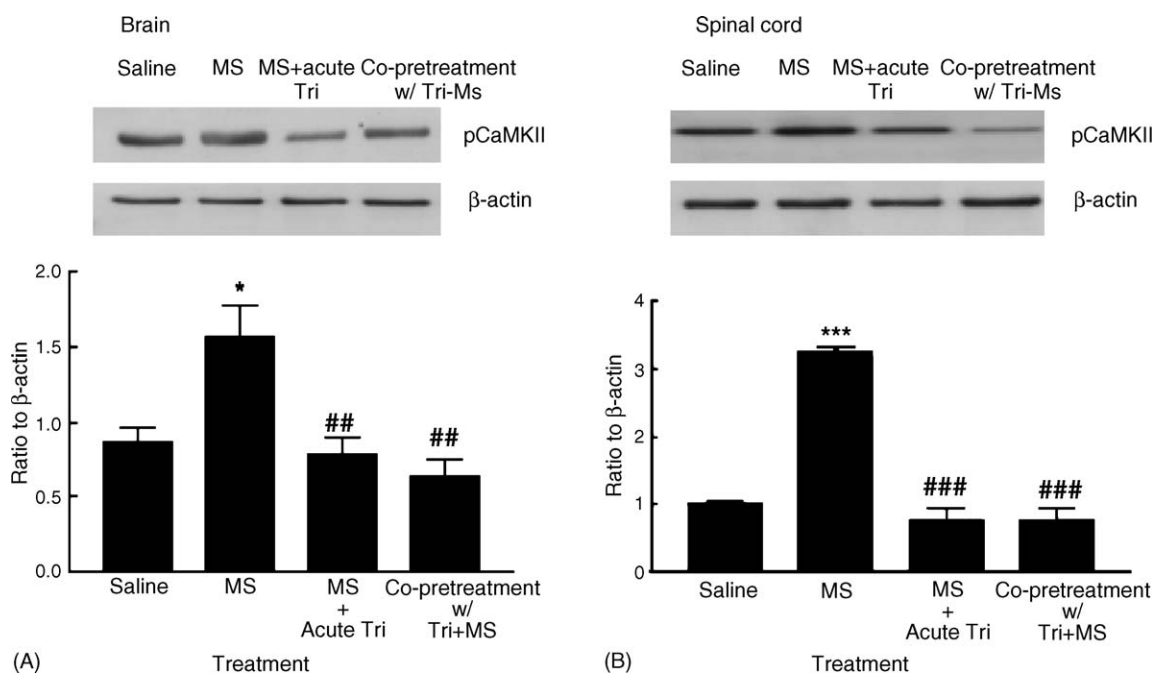


Fig. 3. Effect of trifluoperazine on superspinal (A) and spinal (B) CaMKII activity. Solubilized brain and spinal tissue samples were subjected to 10% polyacrylamide gel electrophoresis and transferred onto PVDF membranes, which were then incubated with anti-pCaMKII and HRP-conjugate anti-rabbit secondary antibody. Ratios of the optical densities of pCaMKII to that of β-actin were calculated for each sample. Data are expressed as mean ± S.E.M ($n = 3$ for each group). Both supraspinal and spinal CaMKII activity was significantly up-regulated in tolerant mice (“MS” group), which was reduced by the pretreatment (“co-pretreatment w/Tri+MS” group) or acute treatment (“MS+ acute Tri” group) with trifluoperazine. * $p < 0.05$, *** $p < 0.001$ compared with the “saline” group; ## $p < 0.01$, ### $p < 0.001$ compared with the “MS” group, Student’s t -test.

the western blotting method [20,22]. Solubilized tissue samples were subjected to 10% polyacrylamide gel electrophoresis and transferred onto PVDF membranes, which were then probed with a rabbit antibody recognizing the activated form of CaMKII (anti-pCaMKII antibody, 1/1000, Promega, Madison, WI), followed by the incubation with HRP-conjugated donkey anti-rabbit secondary antibody (1/1000, Amersham, Piscataway, NJ). The membranes were developed using an enhanced chemiluminescence (ECL) detection system (Amersham). ECL-signals were captured by a ChemiDoc imaging system and analyzed using Quantity One program (Bio-Rad, Hercules, CA). The membranes were then stripped and re-probed with a mouse anti-β-actin antibody (1/10,000, Sigma), then an anti-mouse HRP-conjugated secondary antibody (1/10,000, Amersham), and developed as above. CaMKII activity was significantly increased in the brain (81% increase, $p < 0.05$) and spinal cord (222% increase, $p < 0.001$) of mice tolerant to morphine compared with saline treated mice (Fig. 3). The enhanced CaMKII activity was completely abolished in mice pretreated with both morphine and trifluoperazine or acutely treated with trifluoperazine (Fig. 3).

In this study, we found that trifluoperazine effectively reversed and significantly prevented the development of acute antinociceptive tolerance to morphine. These results are in agreement with our hypothesis that CaMKII is essential for the development and maintenance of opioid tolerance. Biochemical analyses indicated that trifluoperazine inhibited morphine-induced CaMKII activity, confirming that trifluoperazine could act as a potent CaMKII inhibitor [17]. It has also been shown

recently that trifluoperazine significantly suppressed morphine-induced place preference and attenuated naloxone-precipitated withdrawal symptoms in morphine-dependent animals [8,25]. Future studies will test if trifluoperazine is also effective in attenuating opioid tolerance and dependence in other experimental models. A relatively low dose of trifluoperazine was used in this study to reverse and prevent morphine tolerance. Since higher doses of trifluoperazine are required to produce antipsychotic effects [5,12,24], it is expected that CaMKII activity can be inhibited by trifluoperazine at the doses that are used to treat psychotic disorders in humans. Although trifluoperazine may not be as specific as antisense or other experimental inhibitors in inhibiting CaMKII, it has been safely used in humans for many years and can be taken orally. Therefore, we propose that trifluoperazine should be tested for the prevention and treatment of opioid tolerance and addiction in humans. Additionally, it may be used as an adjunct to opioids for treatment of pain, since it would potentiate the efficacy of opioids by preventing opioid tolerance. If proven to be effective, trifluoperazine will provide the first clinically available treatment for opioid tolerance and dependence based on the CaMKII pathway.

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