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Rapid communication

Reversal of morphine antinociceptive tolerance by acute spinal inhibition of Ca^{2+}/calmodulin-dependent protein kinase II

Zaijie Jim Wang*, Lei Tang, Lili Xin

Department of Biopharmaceutical Sciences, University of Illinois, 833 South Woods Street, Chicago, IL 60612, USA

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Abstract

It has been reported that Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) can modulate opioid tolerance via its action on learning and memory. In this study, we examine if CaMKII can directly affect opioid tolerance. We found that spinal CaMKII activity was increased in rats tolerant to morphine. In these rats, acute spinal administration of 2-[*N*-(2-hydroxyethyl)]-*N*-(4-methoxybenzenesulfonyl)]amino-*N*-(4-chlorocinnamyl)-*N*-methylbenzylamine) (KN93), a CaMKII inhibitor, was able to reverse the already-established antinociceptive tolerance. These results suggest that CaMKII may directly promote opioid tolerance.

Keywords: Opioid receptor; CaMKII; KN93

Prolonged use of opioid drugs leads to the development of tolerance, which limits their effectiveness as analgesics. The molecular mechanism underlying opioid tolerance is not entirely understood. *N*-methyl-D-aspartate (NMDA) receptor is a key component in promoting opioid tolerance. NMDA receptor activity is phosphorylated and regulated by Ca^{2+}-dependent protein kinases including protein kinase C and Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII). Studies have started to elucidate modulatory roles of protein kinase C in opioid tolerance; no evidence, however, directly links CaMKII with opioid tolerance.

Both NMDA receptor and CaMKII are essential for the generation of long-term potentiation and learning and memory process in hippocampus. Recently, hippocampal, but not striatal, CaMKII was found to modulate opioid tolerance and dependence, suggesting the importance of learning/memory pathways (Fan et al., 1999; Lou et al., 1999). Chronic, not acute, hippocampal CaMKII inhibition was able to attenuate morphine tolerance (Fan et al., 1999). However, given the intimate cross-regulation between NMDA receptor and CaMKII, it is possible that CaMKII may directly modulate NMDA receptor activity and opioid tolerance, without affecting learning and memory. Activation of NMDA receptor leads to the influx of Ca^{2+}, which can activate CaMKII. The latter's activation, in turn, phosphorylates and activates NMDA receptor.

To test the hypothesis that CaMKII can be a critical and direct step in promoting opioid tolerance, acute spinal inhibition of CaMKII was studied in a rat model of morphine antinociceptive 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. Groups of male Sprague–Dawley rats (220–250 g) were implanted with intrathecal (i.th.) catheters according to the method described previously (Vanderah et al., 2000). To induce tolerance, rats were implanted subcutaneously with two morphine or placebo pellets (75 mg morphine base/pellet; placebo contains no morphine; NIDA, Rockville, MD, USA). The antinociception produced by morphine (10 μg, i.th.) in the tail-flick test before the implantation of pellets was 91 ± 5%MPE (maximal possible effect). On day 6, the same dose i.th. morphine produced significantly reduced antinociceptive response in morphine-treated rats (Fig. 1), indicative of the presence of antinociceptive tolerance. In comparison, the same dose morphine still produced significant antinociception in the placebo pellet-treated rats ($p>0.05$ vs. pre-drug antinociception). Spinal CaMKII activity, determined by an antibody recognizing the activated form of CaMKII

* Corresponding author. Tel.: +1-312-996-0888; fax: +1-312-996-0098.
E-mail address: zjwang@uic.edu (Z.J. Wang).

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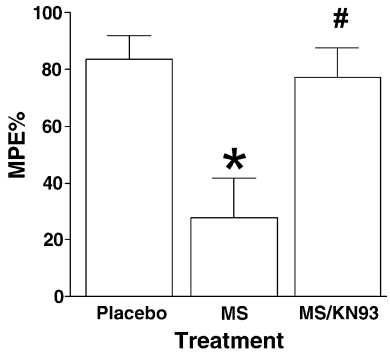

Fig. 1. Effect of KN93 on morphine antinociceptive tolerance. Male Sprague–Dawley rats received subcutaneous implantation of placebo ("Placebo" group) or morphine pellets ("MS", "MS/KN93" groups) (two 75-mg pellets or 150 mg/animal). Latency of a rapid tail flick to 52 °C warm water was determined before and 30 min after i.th. morphine (10 μg) injection. Data, expressed in mean ± standard error ($n = 6–10$/group), are converted to %MPE (maximal possible effect), defined as 100 × (test control)/(cutoff control), where control is the pre-drug observation and test is the post-drug observation. A cutoff of 10 s was applied to prevent tissue damage. The exposure to subcutaneous morphine pellets for 5 days resulted in antinociceptive tolerance to the drug indicated by a significant reduction ($^{*}p < 0.01$ compared to "Placebo" group; Student's *t*-test) of antinociceptive response to acute morphine (10 μg, i.th.). KN93 (15 nmol, i.th.), given 15 min before the acute i.th. morphine ("MS/KN93" group), was able to restore morphine antinociceptive effect ($^{\#}p < 0.05$ compared to "MS" group; $p > 0.05$ compared to the "Placebo"; Student's *t*-test).

(Promega, Madison, WI), was increased in the morphine-tolerant rats as compared with that in placebo rats (data not shown). Increase in CaMKII activity is in agreement with previous findings that chronic opioids can increase calmodulin level and activity and cytosolic-free Ca^{2+} (e.g., Lou et al., 1999; Quillan et al., 2002), although it remains to be tested whether these events are directly or indirectly connected.

When the selective CaMKII inhibitor 2-[*N*-(2-hydroxyethyl)]-*N*-(4-methoxybenzenesulfonyl)]amino-*N*-(4-chlorocinnamyl)-*N*-methylbenzylamine) (KN93) (15 nmol, i.th., Calbiochem, San Diego, CA) was given 15 min before i.th. morphine, KN93 was able to reverse morphine antinociceptive tolerance (Fig. 1). In contrast, KN92, an inactive structural analog of KN93, was ineffective in modulating morphine antinociceptive tolerance (data not shown). Neither KN93 nor KN92 affected acute antinociception of morphine (data not shown). These data suggest that inhibition of spinal CaMKII can effectively disrupt opioid antinociceptive tolerance.

In cellular tolerance models, mu opioid receptor (μOR) desensitization was enhanced when CaMKII was overexpressed (Koch et al., 1997; Mestek et al., 1995). This study provides evidence for direct involvement of CaMKII in opioid antinociceptive tolerance. Applying directly to the lumbar spinal region for a short duration, KN93 is believed to have minimal effect on hippocampal CaMKII; nevertheless, it can effectively reverse morphine antinociceptive tolerance. The quick onset is conceivable since CaMKII and μOR are co-localized in the superficial layers of spinal cord dorsal horn (Bruggemann et al., 2000).

Although interaction of CaMKII and NMDA receptor serves as a most probable underlying mechanism for the role of CaMKII in opioid tolerance, it is almost certain that enhanced CaMKII activity can phosphorylate and modulate functions of other proteins. For example, CaMKII can phosphorylate and activate the cAMP response element binding protein (CREB), another key element in opioid tolerance (Yokota et al., 2001). The cloned human μOR contains several consensus sites for phosphorylation by CaMKII (Mestek et al., 1995). Therefore, CaMKII may play a direct and central role in promoting opioid tolerance.

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