THE INTRACEREBROVENTRICULAR (ICV) ADMINISTRATION OF W-7, A CALMODULIN INHIBITOR, ATTENUATES THE DEVELOPMENT OF MORPHINE TOLERANCE IN RATS

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ABSTRACT

The present study was performed to determine the effect of intracerebroventricular (ICV) administration of W-7, a specific calmodulin inhibitor, on the development of tolerance to antinociceptive effect morphine administration. This study was carried out on male wistar rats, weighing 200-250 g. Morphine was administered daily (15 mg/kg for 8 days). The threshold to thermal nociceptive stimuli was measured by tail-flick test. W-7 (0.25, 0.5 and 1 µmol/rat) was injected through ICV. Maximal possible effect percentage (MPE %) was considered as analgesia index.

Our result showed that chronic morphine exposure induced tolerance to its antinociceptive effect and administration of W-7 (0.5 and 1 µmol/rat) decreased the development of tolerance to it.

In conclusion these data showed that chronic injection of W-7 inhibited the development of morphine tolerance which indicates that calmodulin and its dependent pathways may play a role in the morphine tolerance processes.

Keywords: Calmodulin inhibitor (W-7), morphine tolerance, rat.

INTRODUCTION

Tolerance is indicated by a decreased efficacy of the drug after chronic use, leading to the requirement for a higher dose to get the desired effect (Hamdy *et al*., 2004). Accumulating evidences have revealed that prolong exposure to Opiates such as morphine and heroin can significantly alter brain function, leading to the development of tolerance to opiates (Eisch *et al*., 2000, Nestler and Aghajanian, 1997, Kelley *et al*., 2000). However, the underlying central mechanisms for opiate tolerance are not entirely understood.

Calcium ions are thought to play an important role in many cellular processes. Interaction of calcium with several calcium-binding proteins, the main one of which, Calmodulin (Gnegy, 1993), is a critical step for activating or deactivating of different cellular pathways such as enzyme activation, plasma $Ca2^{+}$ pump regulation and protein phosphorylation & dephosphorylation cascades (Dinsmore and Sloboda, 1988, Cheung, 1982, Ye *et al*., 2004). A number of studies indicate that opioid tolerance is associated with alteration in the calcium homeostasis and free intracellular Ca2+ concentration is higher in the brain (Welch and Olson, 1991, Diaz *et al*., 1995). Increase in cytosolic calcium concentration activate several intracellular enzymes including protein kinases (Wroblewski and Danysz, 1989). Protein kinases such as Ca2+/calmodulin-dependent protein kinases (CaMK) have been reported to phosphorylate opioid receptor, leading to receptor desensitization (Koch *et al*., 1997, Mestek *et al*., 1995); a phenomenon which plays a critical role in opioid tolerance (Yabaluri and Medzihradsky, 1997, Breivogel *et al*., 1997). If this were to be the case, an essential process for opioid receptor phosphorylation and desensitization could be the activation of the Ca2+/calmodulin complex. A previous report which described the inhibition of morphine tolerance by intraventricular application of CaMK II inhibitor favors this possibility (Tang *et al*., 2006). However, the effect of specific calmodulin inhibitors such as W-7 on tolerance to morphine is not determined yet. So the major aim of the present work was to study the effect of supraspinal inhibition of calmodulin by its specific inhibitor, W-7, on development of tolerance to morphine in male rats.

MATERIALS AND METHODS

Animals

Male Wistar rats, weighing 200-250 g were used in this study. Subjects were housed four per cage in a temperature-controlled room at 25 ± 1°C on 12:12-hour light-dark cycle with lights on at 07:00 am. The experiments were carried out during the light phase of the cycle. The animals had free access to commercial food for rodents (Teklad Rodent Diet, Iran) and drinking water.

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Rats were divided randomly into several experimental groups, each comprising 7-9 animals. All of the procedures were in accordance with guidelines for caring and using of laboratory animals in Neuroscience Research Center of Kerman University of Medical Sciences and the European Communities Council Directive of 24 November 1986 (86/609/EEC).

Drugs

Morphine hydrochloride (Temad Co, Iran) was dissolved in saline. W-7 (N-(6-aminohexyl)-5-chloro-1-naphtalenesulfonamide) was purchased from Alexis Company; USA and was dissolved in 100% DMSO as vehicle (Dimethyl Sulfoxide, Merk Company, Germany).

Surgical procedure

Each rat was anaesthetized by an intraperitoneal injection (i.p) of 30 mg/kg ketamine and 5 mg/kg xylazine. They were then placed in the stereotaxic instrument (Stoelting Company, USA). A single incision was made on the midline of the scalp. Once the area had been prepped, a stainless steel 21 gauge cannula was placed in the lateral ventricle according to the Paxinos and Watson atlas (Paxinos, 1986). The coordinates were 1.0 mm posterior to the bregma and 1.5 mm lateral of the midline and 3.0 mm inferior of the duramater. The cannula was kept in place on the skull by dental cement and bone screws. The rats were allowed a 7-day recovery period after the surgery for implantation of the cannula. The injections were done, using a 23 gauge stainless steel cannula attached to polyethylene tubing and 10 µL Hamilton syringe (Hamilton Inc., Reno, NV).

After the completion of experiments, the dye (Evans Blue 20mg/kg) was injected through the cannula to mark the ventricular space. Then animals were killed by an overdosage of ketamine and xylazine combination. The brain sections were visually examined to verify that the tip of the cannula was located in the lateral cerebral ventricle.

Tail-flick test

Antinociception was assessed by tail-flick test. Radiant heat was applied at 5-8 cm from the tip of the tail using a tail-flick apparatus (PANLAB 7160, Spain). Tail flick latency (TFL) was measured as the time of the beam exposure to the withdrawal time of the tail. The mean of three consecutive TFL was measured at 1-min intervals before drug or solvent administration (basal latency) and then similar TFL was measured at specific times after drug or solvent administration, (experimental latencies) (Esmaeili-Mahani *et al*., 2005). The intensity of radiant heat was adjusted to establish the basal latency of 3-5 second. To avoid tissue damage, a cut-off time of 15 second was set. Trials were automatically terminated if a response did not occur within 15 second (Doi *et al*., 1988). Maximal possible effect percentage (MPE %) was considered as analgesia index which was calculated by the following formula:

$$\text{MPE\%} = \frac{\text{Experimental latency-Basal latency}}{\text{15- Basal latency}} * 100$$

Morphine tolerance

All rats underwent surgical procedure and were tested 7 days after cannulation. Tolerance to morphine was induced by daily morphine injections (15 mg/kg/i.p) for 8 days. Tail-flick latency (TFL) was measured both before and 30 min after morphine administration in days 1, 3, 5 and 8 (Esmaeili-Mahani *et al*., 2005). W-7 (0.25, 0.5 and 1 µmol/rat) was administered intracerebroventricularly (ICV) 10 minutes before morphine administration but in days that tail-flick test was performed (1, 3, 5 and 8), W-7 was injected after doing the tail-flick test. All of ICV injections were performed with a micro injector in an amount of 10 µL at a constant rate within 1 min.

Experimental group

1. Rats that received morphine (15 mg/kg/i.p) for 8 days
2. Rats that received saline i.p for 8 days
3. Rats that received saline i.p and DMSO intracerebroventricularly (ICV) for 8 days
4. Rats that received morphine (15 mg/kg/ i.p) and DMSO ICV for 8 days
5. Rats that received saline i.p and maximum dose of W-7 (1 µmol/rat /ICV) for 8 days

6-8: Experimental treated rats that received ICV injection of W-7 (0.25, 0.5 and 1 µmol/rat) and morphine (15 mg/kg/ i.p) for 8 days.

Statistical Analysis

The results are expressed as mean ± SEM. The difference in MPE% (antinociception) between groups was determined by analysis of variance (ANOVA), followed by the Tukey post hoc test with 5% level of significance ($p < 0.05$). For assessing tail flick latencies or %MPE in one group for several days we used repeated measure ANOVA followed by paired t-test for post hoc comparisons.

RESULTS

Effect of ICV injection of W-7 on the development of tolerance to analgesic effect of morphine

As shown in fig. 1, chronic administration of morphine alone for 8 days induced tolerance to its antinociceptive effect; the %MPE on day 5(45%) and day 8 (30%) was significantly reduced compared with MPE% on the first day (80%) in this group (RMA followed by paired t-test, all $P<0.05$). The tolerance to the analgesic effect of morphine treatment alone was not significantly different from that of DMSO + morphine-treated group. ICV injection of W-7 (1 µmol/rat) with i.p administration of saline did not affect the Maximal possible effect

percentage (MPE %) compared with saline- treated group (ANOVA, P>0.05) (fig. 1).

The %MPE on day 8 in morphine + W-7 (0.25 micromol/rat) group was about 35% which slightly increased in comparison with morphine + W-7 Vehicle-treated rats. However, this increase was not statistically significant (ANOVA, P>0.05). %MPE on day 5 and 8 in rats receiving morphine + W-7 (0.5 micromol/rat) was about 90% and 62% respectively which showed a significant increase compared with that of morphine + DMSO-treated rats (ANOVA, both P<0.05). %MPE on 5^{th} and 8^{th} day in group treated with morphine + W-7 (1µmol/rat) was 85% and 65% which significantly increased in comparison with rats treated with morphine + DMSO (ANOVA, P<0.05). It means that chronic ICV administration of W-7 in dose 0.5 and 1 µmol/rat could effectively inhibited the development of morphine tolerance (fig. 2).

DISCUSSION

The present study was designed to evaluate the effect of calmodulin inhibition by microinjection of its specific inhibitor "W-7" into the lateral ventricle of the rat's brain on the development of morphine tolerance. We found that chronic ICV administration of W-7 strongly attenuated the development of tolerance to chronic morphine exposure, as evidenced by a significant increased in the Maximal possible effect (MPE %) on day 5 and 8 in comparison with morphine + W-7 Vehicle- treated group.

It has been shown that calmodulin content and location changed during chronic morphine administration. Chronic

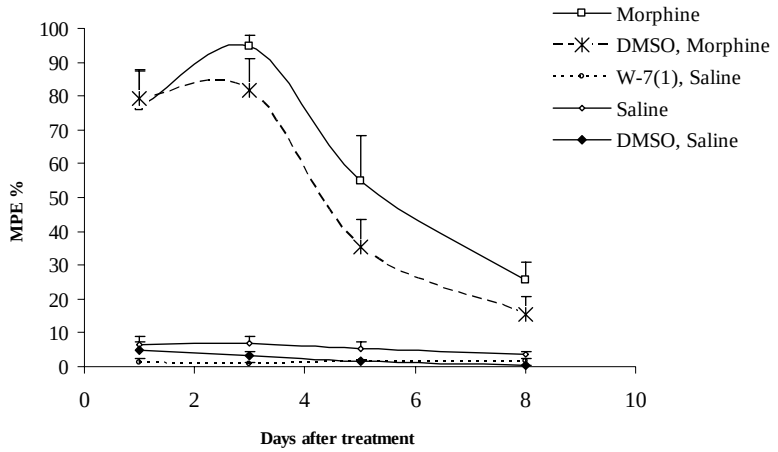

Fig. 1: Tolerance to analgesic effects of morphine induced by chronic morphine administration.
*means significant difference in MPE% at day 1 with days 5 and 8 in morphine, DMSO group (RMA followed by paired t-test, all P<0.001)

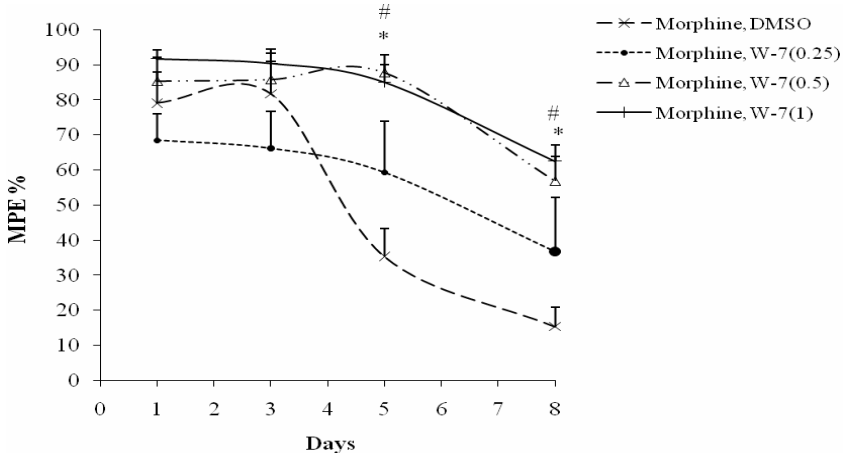

Fig. 2: The effect of ICV administration of W-7 (0.25, 0.5 and 1 µmol/rat) on the development of tolerance to analgesic effect of morphine (15 mg/kg/8d/ i.p) in rats. Other notations are the same as in fig. 2.
*means significant difference in MPE% between morphine, DMSO and morphine, W7 (0.5) in days 5 and 8 (ANOVA, all P<0.05)
#means significant difference in MPE% between morphine, DMSO and morphine, W7 (1) in days 5 and 8 (ANOVA, all P<0.05)

morphine treatment increased calmodulin contents in the rat's brain (Nehmad *et al*., 1982). Moreover, the intracellular calcium elevation by opioid receptor stimulation (Welch and Olson, 1991, Diaz *et al*., 1995) lead to calmodulin dissociation from cell membrane (Wang *et al*., 1999). The increase in cytosolic calmodulin prompts the translocation of calmodulin into the nucleus (Wang *et al*., 2000) which regulates the gene expression by morphine (Deisseroth *et al*., 1998, Niu *et al*., 2000). However, it is unclear whether these finding may play a role in the development of morphine tolerance or not.

In addition, several lines of evidence indicated that calmodulin dependent pathways such as Ca2+/calmodulin kinase II (CaMKII) activation may involve in the morphine tolerance (Liang *et al*., 2004). It has been reported that intraventricular application of CaMK II inhibitor attenuated morphine tolerance (Tang *et al*., 2006). However, an important characteristic of CaMK II is its autophosphorylation which enables this kinase to phosporylate substrates in a Ca2+/calmodulin-independent manner and thus prolongs the duration of its effect (Lou *et al*., 1999).

In summary, our results indicated that chronic inhibition of calmodulin activity by ICV injection of its specific inhibitor, W-7, attenuated development of morphine tolerance in rat.

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