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Research report

Increased calcium/calmodulin-dependent protein kinase II activity by morphine-sensitization in rat hippocampus

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HIGHLIGHTS

- Acute morphine did not alter mRNA expression and activity of CaMKII in hippocampus.
- Morphine sensitization increased mRNA expression of CaMKII in the hippocampus.
- Morphine sensitization increased CaMKII activity in the rat hippocampus.
- Naloxone decreased mRNA expression of CaMKII in morphine-sensitized rats.
- Naloxone decreased CaMKII activity in morphine-sensitized rats.

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ABSTRACT

Repeated exposure to drugs of abuse, such as morphine, elicits a progressive enhancement of drug-induced behavioral responses, a phenomenon termed behavioral sensitization. These changes in behavior may reflect long-lasting changes in some of the important molecules involved in memory processing such as calcium/calmodulin-dependent protein kinase II (CaMKII). In the present study, we investigated the effect of morphine sensitization on mRNA expression of α and β isoforms and activity of CaMKII in the hippocampus of male rats. Animals were treated for 3 days with saline or morphine (20 mg/kg) and following a washout period of 5 days, a challenge dose of morphine (5 mg/kg) were administered. The results indicate that morphine administration in pre-treated animals produces behavioral sensitization, as determined by significant increase in locomotion and oral stereotypy behavior. In addition, repeated morphine treatment increased mRNA expression of both α and β isoforms of CaMKII in the hippocampus. The present study also showed that induction of morphine sensitization significantly increased both Ca2+/calmodulin-independent and Ca2+/calmodulin-dependent activities of CaMK II in the rat hippocampus. However, acute administration of morphine (5 mg/kg) did not alter either α and β CaMKII mRNA expression or CaMKII activity in the hippocampus. The stimulation effects of morphine sensitization on mRNA expression and activity of CaMKII were completely abolished by administration of naloxone, 30 min prior to s.c. injections of morphine (20 mg/kg/day × 3 days). Our data demonstrated that induction of morphine sensitization could effectively modulate the activity and the mRNA expression of CaMKII in the hippocampus and this effect of morphine was exerted by the activation of opioid receptors.

1. Introduction

Calcium/calmodulin-dependent protein kinase II (CaMKII), a multiple functional enzyme, is one of the most abundant kinases in the brain, comprising 2% of total hippocampal proteins [16]. CaMKII is a major calcium-regulated signal transducer that

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regulates many neuronal systems including receptor-gated ion channels, calcium-dependent ion currents, and the synthesis and release of neurotransmitters [47]. Persistent activation of CaMKII occurs as a result of autophosphorylation which is dependent on Ca2+/calmodulin [15]. Autophosphorylation of CaMKII in the presence of Ca2+ and calmodulin produces substantial Ca2+/calmodulin-independent activity and prolongs the duration of its effect [13]. At least four isoforms of CaMKII (α, β, γ, and δ) are found to express in the rat brain and to function in the form of homomultimers or heteromultimers [16]. Among them, the α and β isoforms are restricted in nervous tissues, especially in the hippocampus, whereas γ and δ are found in most tissues besides brain [47]. It has been demonstrated that the activation of CaMKII in the hippocampus has a central role in synapse formation, receptor and ion channel function, gene expression, memory processing and neuroplasticity [1,5,15]. The role of CaMKII in learning and memory has been studied intensively in different experimental models of memory formation [14,15,19]. Studies revealed that formation of some types of learning and memory such as hippocampal-dependent spatial learning is strongly dependent on the activity of this enzyme [29,50]. Pharmacological inhibition [56], inactivation of this kinase [36] or prevention of CaMKII autophosphorylation in mutant mice [15] impairs spatial learning and memory tasks in rodents. However, overexpression of CaMKII in transgenic mice resulted in the enhancement of spatial memory acquisition [37].

It has been shown that repeated administration of morphine followed by a period of drug-free treatment can induce sensitization and can result in long-lasting augmentation of morphine behavioral effects [21,45]. The circuitry involved in sensitization is complex [56], because sensitization represents a cascade of events involving different neurotransmitter systems and different brain regions such as nucleus accumbens, ventral tegmental area and the hippocampus [56]. Behavioral sensitization reflects drug-induced neuroadaptive long-lasting changes in reward-related circuits in the brain [26]. This form of long-term plasticity in the brain requires relative stable changes in gene expression, which may alter neurotransmission and the structure of target neurons [43,56]. Because learning and memory are suggested to be essentially involved in opiate addiction [23], it is intriguing to hypothesize that selective modulation of those genes that play key roles in learning and memory processes could affect the development of opiate tolerance, dependence and behavioral sensitization.

Although several studies have shown that development of morphine dependence and tolerance are related on the activity of CaMKII [3,8,32], there are few studies dealing with the relation of CaMKII activity and behavioral sensitization. Licata et al. reported that administration of a CaMKII inhibitor into the ventral tegmental area can impair the development of behavioral sensitization [28].

Our previous studies showed that morphine-induced impairment of acquisition and retrieval of spatial learning reversed by pre-exposure to morphine for 3 days [9,11].

Considering the common neuronal pathways in the induction of behavioral sensitization and memory processing [56], we hypothesized that the improvement of spatial memory by morphine sensitization may be due to changes in the expression and activity of CaMKII in the hippocampus.

In the present study, to confirm the induction of locomotor and behavioral sensitization by our used method, we first investigated the effects of repeated pre-exposure to morphine on locomotor activity and stereotypy behavior and then the effects of acute and repeated pre-exposure to morphine on mRNA expression and activity of CaMKII in the hippocampus were examined.

2. Materials and methods

2.1. Animals and drugs

Male albino Wistar rats, weighing 180–220 g, were housed under controlled environmental conditions at a temperature of 23 ± 1 °C with free access to food and water. In the behavioral experiment, each rat was used only once and was killed immediately after the experiment. For real time PCR and activity assay experiments, rats were decapitated and their brains removed immediately. Tissues were kept at –80 °C until further analysis. All experimental procedures were in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Morphine sulfate (Temad, Tehran, Iran) and naloxone hydrochloride (Tolid-Daru, Tehran, Iran) were dissolved in sterile 0.9% saline just before the experiment and were injected subcutaneously (s.c.).

2.2. Measurement of locomotor activity

Rats were placed individually in plastic cages (20 × 20 × 45 cm) for 15 min to habituate them to the environment. Immediately after administration of either morphine or saline, locomotion was measured for 60 min with an activity meter (Animex, Type S; LKB, Farrad Electronics, Hagerstein, Sweden), as described by Heidari et al. [18]. Oral stereotypy behavior was also measured during the same observation period.

2.3. Oral stereotypy measurement

Stereotypy was scored from 0 to 6 according to a rating scale described by Creese and Iversen [4] as follows: 0, asleep or motionless; 1, active; 2, active with intermittent bursts of oral stereotypy; 3, discontinuous sniffing, licking and biting; 4, frequent sniffing, licking and biting; 5, continuous sniffing, licking and biting; 6, continuous, intense oral stereotypy that disrupt gross motility. All behavioral sessions were digitally video-recorded for 60 min immediately following the injections and a stereotypy score was assigned to each rat.

2.4. Total RNA extraction

Rats were killed by decapitation and their brains removed quickly. The hippocampus was dissected on ice-chilled plates. Samples were lysed immediately in guanidinium isothiocyanate (GITC), homogenized using a Silent Crusher S homogenizer (Heidolph, Schwabach, Germany) and subjected to total RNA extraction using a High Pure Tissue RNA extraction kit (Roche Applied Science, Indianapolis, IN, USA) according to the manufacturer's instructions. The quality of the RNA extracted was checked on denatured agarose gels and quantified by Biophotometer (Eppendorf Biophotometer, Cambridgeshire, UK).

2.5. Reverse transcription

Reverse transcription (RT) was performed using 1 μL total RNA as a template, 1 μL expand reverse transcriptase, 4 μL buffer, 1 μL dNTPs (10 mmol/L), 1 μL dithiothreitol and 1 μL oligo(dT)15 (20 pmol) in a total volume of 20 μL at 42 °C for 60 min.

2.6. Real-time polymerase chain reaction

The polymerase chain reaction was used to amplify cDNA for β-actin (as an internal standard), α-CaMKII and β-CaMKII using the following primers: β-actin forward primer, 5′-TGAAGTACCCCATTGAACATG-3′; β-actin reverse primer,