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ScienceDirect
Neuroscience Letters 430 (2008) 213–217
Neuroscience Letters
www.elsevier.com/locate/neulet

Epinephrine inhibits analgesic tolerance to intrathecal administrated morphine and increases the expression of calcium–calmodulin-dependent protein kinase IIα

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Received 17 July 2007; received in revised form 13 October 2007; accepted 29 October 2007

Abstract

Activation of hypothalamic–pituitary–adrenal (HPA) axis inhibits development of morphine tolerance. Also, the expression of CaMKIIα is increased following chronic administration of morphine. In the current study, we tried to examine the effect of epinephrine, on the development of morphine tolerance; and also evaluate the expression of CaMKIIα as a molecular index for tolerance development. Analgesic tolerance was induced by intrathecal (i.t.) injection of morphine 15 μg/rat, twice a day for 5 days. To study the effect of epinephrine on development or reversal of morphine tolerance, epinephrine was administrated 20 min before morphine injections. Analgesia was assessed using tail flick test. Gene expression assays were done using RT-PCR. Following 5 days of combined administration of morphine and epinephrine (2, 5 or 10 μg/rat), in day 6, morphine produced potent analgesia. Administration of saline and morphine during days 1–5, caused reduced analgesic effect of morphine on day 6. After tolerance induction during 5 days, co-administration of epinephrine and morphine for another 5 days, significantly reversed the tolerance. Both morphine and epinephrine increased the expression of CaMKIIα. The expression of CaMKIIα was highly increased following combined administration of epinephrine and morphine. Our results showed the inhibition and reversal of analgesic tolerance to local administrated morphine by epinephrine. We observed the increased expression of CaMKIIα without development of morphine tolerance in animals treated with combined epinephrine and morphine.
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Keywords: Tolerance; Morphine; Opioids; Epinephrine; Calcium/calmodulin-dependent protein kinase IIα; Rats

Morphine and related opioids produce analgesia by activating specific receptors expressed in brain, spinal cord and peripheral tissues. Chronic administration of opioids, however, leads to development of both tolerance and dependence (reviewed in [32]). Analgesic tolerance is decreased sensitivity to pain relieving effect of drug. Mechanisms underlying the development of tolerance are complex and poorly understood. Different mechanisms including activation of NMDA and metabotropic glutamate receptors [21,22,25,28,32,33], translocation and activation of protein kinases [21,23,24,32], increase in nitric oxide production [21,22,25], uncoupling of opioid receptors with adenylate cyclases and G proteins [14,23,22,32], adenylate cyclases supersensitivity [8,21,23,25,32] and opioid receptors down-regulation and internalization [21,25,32], have been suggested to be involved in the development of opioid tolerance. It has been reported that analgesic tolerance to morphine dose not develop in stressful situations such as chronic pain induced by subcutaneous injection of formalin [6,14,29]. The inhibitory effect of pain on analgesic tolerance development, have been attributed to the stress aspect of pain and activation of hypothalamic–pituitary–adrenal (HPA) axis. Either adrenalectomy or hypophysectomy have shown to potentiate opiate tolerance [13]. The effect of hypophysectomy can be reversed by adrenocorticotropic hormone (ACTH) [13]. Also, stress blocks the development of morphine tolerance in intact, but not in adrenalectomized mice [30]. ACTH has been found to prevent the development of tolerance to analgesic effect of morphine [30].

Stress activates HPA axis and results in increased release of corticotropin-releasing hormone, ACTH, glucocorticoides and epinephrine [3]. Previous studies have reported the effect of corticostrone and other glucocorticoides (e.g. dexamethasone) on

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0304-3940/$ – see front matter © 2007 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.neulet.2007.10.038

development of morphine tolerance [15,30]. These studies have focused on the role of glucocorticoids in the inhibitory effect of pain on morphine tolerance development and no possible role is addressed for adrenal medulla and epinephrine. In stressful situation, ACTH directly acts on the adrenal medulla and increases the level of dopamine beta-hydroxylase and tyrosine hydroxylase activity. Moreover, epinephrine and norepinephrine result in the release of ACTH, from anterior lobe of pituitary gland [3]. In this study, we have tried to find out if co-administration of epinephrine as the product of adrenal medulla is effective on development of analgesic tolerance to morphine.

Calcium/calmodulin-dependent protein kinase IIα (CaMKIIα) is a ubiquitous multifunctional Ser/Thr kinase. It is of particular importance in the central nervous system [5,12,37]. This kinase is concentrated at synapses and is the main protein of postsynaptic density, where it plays a key role in synaptic signaling [17,36]. It is colocalized with mu-opioid receptors in distinct pain-processing brain regions [7]. Brief Ca^{++} signals activate CaMKIIα, and this enzyme has the ability to autophosphorylate. Thus, the kinase can maintain its active state after the Ca^{++} removal. These distinctive features allow CaMKIIα to regulate important neuronal functions including synaptic plasticity, learning and memory [4,27]. Its activity and expression is increased following chronic administration of morphine and its inhibition using KN-62 or others inhibitors, inhibits development and expression of morphine analgesic tolerance [11,18,20,31]. In the current study, as a molecular index for morphine tolerance, we tried to investigate the expression level of CaMKIIα following chronic morphine, chronic epinephrine and chronic combined administration of epinephrine and morphine.

Male Wistar rats (Pasture institute, Iran) (weighing 200 ± 20 g) were housed four per cage and maintained on a 12 h light/12 h dark cycle with food and water ad libitum. All animal protocols used have been approved by animal ethics committee of our university. Catheterization into the lumbar spinal cord were done according to Yaksh and Ruddy [35] method. Briefly, 2 days before the experiments, the rat was anesthetized by i.p. injection of ketamin (50 mg/kg) and xylazin (5 mg/kg). After making an incision on the occipital area of animal head, muscles were removed and small hole was made in the membrane. Prepared PE-10 tube was gently pushed down to the lumbar subaraqnoid space to provide our access to the lumbar spinal cord for intrathecal injections.

Morphine sulfate (Temad, Iran) was dissolved in physiological saline and administrated intrathecally. To induce morphine tolerance, morphine (15 μg/rat) was injected twice a day (08:00 and 17:00) for 5 days.

Fourteen to sixteen hours after the last injection (i.e. day 6), all animals received a dose of morphine (15 μg/rat, i.t.) and analgesia was evaluated using tail-flick test (Harvard Apparatus) [10]. Three tail-flick latencies were recorded and averaged as baseline latency. Five minutes after i.t. administration of morphine, another set of tail-flick latencies were measured and averaged as test latency. An 8 s cut-off time period was imposed to avoid damage to tail tissues. The percent of analgesia from maximum possible effect (%MPE) was calculated as follow:

$$\%\,\mathrm{MPE} = \left[\frac{\text{Test latency} - \text{basal latency}}{\text{Cut-off time} - \text{basal latency}}\right] \times 100$$

To evaluate the effect of epinephrine on morphine tolerance development, three groups of rats received 2, 5 or 10 μg/rat of epinephrine 20 min prior to all i.t. injections of morphine for 5 days. Two control groups received saline + saline and/or epinephrine (10 μg/rat) + saline injections, respectively, twice a day for 5 days. Tail flick assays were done on day 6 as described above.

To evaluate the possible reversal of tolerance by epinephrine, three additional groups of animals were considered. Group 1 received i.t. saline for 10 days, twice a day. Group 2 received 15 μg/rat morphine twice a day for 10 days. Group 3 received 15 μg/rat morphine twice a day in days 1–5 and epinephrine (10 μg/rat) combined with morphine (15 μg/rat) in days 6–10, twice a day. The analgesic effect of a single dose of morphine (15 μg/rat) was assessed at morning sessions on days 1, 6 and 11 using tail flick test.

For gene expression assays, four groups of rats were used. Group 1 contained the animals which received saline–saline (10 μl, i.t.) for 5 days. Using a same paradigm, groups 2–4 received saline–morphine, epinephrine–saline or epinephrine–morphine, respectively, for 5 days. Fourteen to sixteen hours after the last injection (day 6), animals were decapitated. After spinal cord extraction, the dorsal part of lumbar spinal cord was immediately separated and preserved in liquid nitrogen. As described elsewhere [14], gene expression assay was done using semi-quantitative RT-PCR.

A nested-PCR method was used. Briefly, total cellular RNAs were isolated using a modification of guanidine isothiocyonate–phenol–chloroform method [2], using RNX$^+$ reagent (CinnaGen, Iran). The reverse transcription was performed using oligo-dT primer and M-MuLV reverse transcriptase (Fermentas) based on the manufacture's protocol. PCR reactions were carried out using selective forward and reverse primers for β-actin (internal standard) or CaMKIIα. The sequences of primers are presented in Table 1. Taq DNA polymerase (CinnaGen, Iran) was used for DNA amplification. The reactions were set up based on the manufacture's protocol. To amplify the cDNA of CaMKIIα, first PCR reaction included incubation at 95 °C for 5 min, followed by 10 cycles of ther-

Table 1
The sequences of primers used for PCR amplification of calcium-dependent-protein kinase IIα (CaMKIIα) and β-actin mRNAs

Gene name	Primer	Sequence
CaMKIIα	F_{out}	5′-AGGGAGAGCAGCAGGCAT-3′
CaMKIIα	R_{out}	5′-GTGGTGTTGGTGCTCTCAG-3′
CaMKIIα	F_{in}	5′-CTCTTCCCTCCGGAGAAGTT-3′
CaMKIIα	R_{in}	5′-CAGGGACACCTGGATACCTC-3′
β-Actin	F	5′-CCCAGAGCAAGAGAGGCATC-3′
β-Actin	R	5′-CTCAGGAGGAGCAATGATCT-3′

CaMKIIα, calcium-dependent-protein kinase IIα; F, forward; R, reverse; out, outer primer; in, inner primer.

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