Available online at www.sciencedirect.com

SCIENCE DIRECT

Pharmacology, Biochemistry and Behavior 81 (2005) 152 – 157

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

www.elsevier.com/locate/pharmbiochembeh

Involvement of hypothalamic pituitary adrenal axis on the effects of nifedipine in the development of morphine tolerance in rats

Saeed Esmaeili Mahani[a,b], Fereshteh Motamedi[a], Mohammad Javan[c], Abolhasan Ahmadiani[a,*]

[a]*Department of Physiology and Pharmacology, Neuroscience Research Center, Shahid Beheshti University of Medical Sciences, P.O. Box 19835-355, Tehran, Iran*

[b]*Department of Biology, Faculty of Science, Shahid Bahonar University, Kerman, Iran*

[c]*Department of Physiology, Faculty of Medical Science, Tarbiat Modares University, Tehran, Iran*

Received 9 August 2004; received in revised form 12 February 2005; accepted 12 March 2005
Available online 21 April 2005

Abstract

It has been shown that nifedipine, as a calcium channel blocker, can attenuate the development of tolerance to the antinociceptive effect of morphine; however, the role of HPA axis on this action has not been elucidated. We examined the effect of nifedipine on morphine analgesic tolerance in intact and adrenalectomized (ADX) rats and on HPA activity induced by morphine. Adult male rats were rendered tolerant to morphine by daily injection of morphine (15 mg/kg i.p.) for 8 days. To determine the effect of nifedipine on the development of morphine tolerance, nifedipine (1, 2 and 5 mg/kg i.p.) was injected concomitant with morphine. The tail-flick test was used to assess the nociceptive threshold, before and 30 min after morphine administration in days 1, 3, 5 and 8. Our results showed that despite the demonstration of tolerance in both ADX and sham operated rats, nifedipine in ADX rats prevented morphine tolerance development at a lower dose (2 mg/kg) than in sham operated rats, however corticosterone replacement prevented nifedipine effect in ADX rats. Acute administration of morphine produced significant increase in plasma corticosterone level, and with repeated injection, a tolerance to this neurosecretory effect was developed. Nifedipine (5 mg/kg) attenuated the acute effect of morphine, but could not block its neurosecretory tolerance.

Keywords: Morphine tolerance; Nifedipine; HPA axis; Corticosterone

1. Introduction

Opioids have been used for treating moderate to severe pain but chronic treatment with these drugs lead to the development of tolerance and dependence. Numerous reports indicate that opioid tolerance is associated with alteration in the Ca^{2+} homeostasis and basal free intracellular Ca^{2+} concentration is higher in the brain and spinal cord (Yamamoto et al., 1981; Ramkumar and El-Flakani, 1984; Welch and Olson, 1991; Diaz et al., 1995). In morphine tolerance, dihydropiridine Ca^{2+} channel density is increased (Ramkumar and El-Flakani, 1984, 1988; Diaz et al., 1995). Not surprisingly, Ca^{2+} channel antagonists have been shown to prevent the development of opioid tolerance, reverse tolerance (Contreras et al., 1988; Dierssen et al., 1990; Antkiewicz-Michaluk et al., 1993; Michaluk et al., 1998; Smith et al., 1999) and also attenuate the signs of physical dependence in animals (Antkiewicz-Michaluk et al., 1993; Baeyens et al., 1987). In 1994, Santilan et al., reported that tolerance was reversed in humans by using these drugs.

It is also well known that opioids are important regulators of the hypothalamic pituitary adrenal axis (HPA) in rodents. Morphine administration influences HPA axis, exerting a stimulatory effect through releasing CRF from hypothalamus in rats (Bukingham and Cooper, 1984). Tolerance to HPA stimulation by morphine has been demonstrated in adult rats (Kokka et al., 1973; Ignar and Kuh, 1990; Gonzalvez et al., 1991; Pechnick, 1993; Little et al., 1995; Nock et al., 1998; Cerezo et al., 2002). Another regulatory

* Corresponding author. Fax: +98 21 2403154.
E-mail address: aahmadiani@yahoo.com (A. Ahmadiani).

0091-3057/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2005.03.004

factor participating in the control of HPA axis is Ca^{2+} and related channels, particularly L-type Ca^{2+} channels (Guerineau et al., 1991; Kuryshev et al., 1995, 1996; Robidoux et al., 2000).

In many in vitro studies, it has been demonstrated that glucocorticoids can potentiate Ca^{2+} influx and accelerate the release of Ca^{2+} from intracellular stores, and corticosterone can increase Ca^{2+} entry through the high voltage activated (L-type) calcium channel (Nair et al., 1998; Zhou et al., 2000; Karast et al., 2002; Takahashi et al., 2002; Machida et al., 2003).

Therefore, since the interaction between corticosterone and calcium channels has not been clarified in vivo and the role of HPA axis in the effects of calcium channel blockers on tolerance to analgesic effect of morphine, has not been elucidated, the present study was designed to: first, analyze the contribution of HPA axis and its glucocorticoids to the effect of nifedipine, as a calcium channel blocker, on tolerance to analgesic effect of morphine by using intact and adrenalectomized (ADX) rats. Second, evaluate modifications in the activity of the HPA axis during acute and chronic treatments with morphine in the presence of nifedipine.

2. Materials and methods

2.1. Animals

All experiments were carried out on male wistar rats, weight 200–250 g, that were housed four per cage under a 12 h light/dark cycle in a room with controlled temperature (22±1 °C). Food and water were available ad libitum except in adrenalectomized (ADX) rats. Animals were handled daily (between 9:00 and 10:00 A.M.) for 5 days before the experiment day in order to adapt them to manipulation and minimize nonspecific stress responses. Rats were divided randomly into several experimental groups, each comprising 6–8 animals. All experiments followed the guidelines on ethical standard for investigation of experimental pain in animals (Zimmermann, 1983).

2.2. Drugs

Morphine hydrochloride was dissolved in physiological saline and nifedipine (Sigma, USA) was dissolved in dimethyl sulfoxide (DMSO) plus saline. These drugs were given in the volume of 1 ml/kg, i.p. Corticosterone (Sigma, USA) was dissolved in absolute ethanol then combined with 0.9% NaCl water, yielding final concentration of 100 μg/ml of drinking solution.

2.3. Antinociceptive test

Antinociception was assessed by Tail-Flick test (D'Amour and Smith, 1941). The Tail-Flick latency for each rat was determined three times at 3 min intervals and mean was designated as baseline latency before morphine injection. The intensity of the beam was adjusted to produce mean control reaction time between 2 and 4 s. The cut-off time was fixed at 10 s in order to avoid any damage to the tail. After determination of baseline latencies, rats received intraperitoneal injection of morphine (15 mg/kg), and the reaction latency was determined 30 min after injection. The Tail-Flick latencies were converted to the percentage of antinociception according to the following formula:

$$\%\ \text{Antinociception}(\%\text{MPE}) = (\text{Reaction time of test} - \text{basal reaction time}) / (\text{cut off time} - \text{basal reaction time})$$

2.4. Morphine tolerance

To induce tolerance to analgesic effect, morphine was given chronically in a daily dose of 15 mg/kg from days 1 to 8. Nifedipine or saline was given according to the same schedule as control groups. Nociceptive testing was performed both before and 30 min after drug administration in days 1, 3, 5 and 8. To determine the effect of nifedipine on the development of morphine tolerance, nifedipine (1, 2 and 5 mg/kg) was given concomitant with morphine but in days that nociceptive testing was measured, morphine was injected first and antinociception was measured 30 min after drug administration and then, nifedipine was injected.

To induce tolerance to neurosecretory effect of morphine on HPA axis, morphine was given chronically the same as the above-mentioned procedure. On days 1–7 rats were injected i.p. with morphine (15 mg/kg). Control animals received saline using the same time course. To test the role of nifedipine, as a calcium channel blocker, in the development of tolerance to neurosecretory effect of morphine, nifedipine (2 and 5 mg/kg) was injected concomitant with morphine. On the 8th day rats were divided in two groups, that each received either saline or morphine (15 mg/kg, i.p.) and sacrificed 30 min later for measurement of plasma corticosterone concentration.

2.5. Adrenalectomy

Animals were anesthetized with ketamin (50 mg/kg) and xylazine (5 mg/kg) i.p. Both adrenal glands were removed through two dorsal incisions. The sham operation consisted of bilateral dorsal incision, plus locating and exposing the adrenals. All adrenalectomized rats were maintained on 0.9% NaCl drinking solution, whereas the sham operated rats were kept on tap water. The animals were tested 5 days after the adrenalectomy or sham procedure.

2.6. Corticosterone replacement

For corticosterone replacement in adrenalectomized rats, corticosterone was dissolved in 2 ml of ethyl alcohol then