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L-type calcium channel blockade attenuates morphine withdrawal: In vivo interaction between L-type calcium channels and corticosterone

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Abstract

Both opioids and calcium channel blockers could affect hypothalamic–pituitary–adrenal (HPA) axis function. Nifedipine, as a calcium channel blocker, can attenuate the development of morphine dependence; however, the role of the HPA axis in this effect has not been elucidated. We examined the effect of nifedipine on the induction of morphine dependency in intact and adrenalectomized (ADX) male rats, as assessed by the naloxone precipitation test. We also evaluated the effect of this drug on HPA activity induced by naloxone. Our results showed that despite the demonstration of dependence in both groups of rats, nifedipine is more effective in preventing of withdrawal signs in ADX rats than in sham-operated rats. In groups that received morphine and nifedipine concomitantly, naloxone-induced corticosterone secretion was attenuated.

Thus, we have shown the involvement of the HPA axis in the effect of nifedipine on the development of morphine dependency and additionally demonstrated an *in vivo* interaction between the L-type Ca^{2+} channels and corticosterone.

Keywords: Morphine dependence; L-type Ca^{2+} channel; HPA axis; Corticosterone

Introduction

Opioids have been used for treating moderate to severe pain but chronic treatment with these drugs leads to the development of tolerance and dependence. Numerous reports indicate that opioid tolerance and dependence are associated with alteration in Ca^{2+} homeostasis so that basal-free intracellular Ca^{2+} concentration is increased in the brain and spinal cord (Yamamoto et al., 1981; Ramkumar and El-Flakani, 1984; Welch and Olson, 1991; Diaz et al., 1995). Furthermore, chronic morphine treatment increases dihydropyridine Ca^{2+} channel density (Ramkumar and El-Flakani, 1984, 1988; Antkiewicz-Michaluk et al., 1990; Bernstein and Welch, 1995; Diaz et al., 1995, 2000). Not surprisingly, Ca^{2+} channel antagonists have been shown to attenuate the signs of physical dependence in animals (Antkiewicz-Michaluk et al., 1993; Kishioka et al., 1994; Michaluk et al., 1998; Vitcheva and Mitcheva, 2004).

It is also well known that opioids are important regulatory factors participating in the control of hypothalamic–pituitary–adrenal (HPA) axis function in rodents. Morphine administration influences the HPA axis, exerting a stimulatory effect through the release of CRF from the hypothalamus in rats (Buckingham and Cooper, 1986). In addition, the HPA axis also plays an important role in morphine withdrawal syndrome (Kishioka et al., 1994; Brugger et al., 1998; Milanes et al., 1998; Laorden et al., 2000, 2002; McNally and Akil, 2002).

Other regulatory factors participating in the control of the HPA axis are Ca^{2+} and related channels, particularly L-type Ca^{2+} channels (Stojilkovic et al., 1988; Guerineau et al., 1991; Kuryshev et al., 1996; Mamczarz et al., 1999; Robidoux et al., 2000). Furthermore, many *in vitro* studies have demonstrated that glucocorticoids can potentiate Ca^{2+} influx through high

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voltage activated (L-type) calcium channels and accelerate the release of Ca^{2+} from intracellular stores (Nair et al., 1998; Karast et al., 2002; Machida et al., 2003; Chameau et al., 2007). We previously reported that the HPA axis and its glucocorticoids have an important role in the effect of nifedipine on morphine-induced analgesia, hyperalgesia and also on morphine analgesic tolerance (Esmaeili Mahani et al., 2005a,b; 2007a,b).

Because both morphine and nifedipine could affect HPA activity and the HPA axis plays an important role in the morphine withdrawal syndrome, the present study was designed with two goals in mind: First, to analyze the contribution of the HPA axis and its glucocorticoids to the effect of nifedipine, as a calcium channel blocker, on morphine dependence by using intact and adrenalectomized (ADX) rats; and second, to evaluate modifications in the activity of the HPA axis during naloxone-precipitated withdrawal.

Materials and methods

Animals

All experiments were carried out on male Wistar rats, weighing 200–250 g, 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 procedure in order to adapt them to manipulation and minimize non-specific stress responses. Rats were divided randomly into several experimental groups, each comprising 6–8 animals. All experiments followed the guidelines on ethical standards for investigation of experimental pain in animals (Zimmermann, 1983).

Drugs

Morphine hydrochloride and naloxone hydrochloride were dissolved in physiological saline and nifedipine (Sigma, USA) was dissolved in dimethyl sulfoxide (DMSO) plus saline. The percentage of DMSO and saline in the final volume was 60% and 40%, respectively. 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 a drinking solution with a final concentration of 100 μg/ml.

Induction of morphine dependence

To develop morphine dependence, we injected rats intraperitoneally with morphine twice daily for 7 days. The dose of morphine on days 1 and 2 was 2.5 mg/kg; this dose was doubled every day thereafter to reach a total dose of 40 mg/kg on day 6. On day 7, the animals received the last injection of morphine, 50 mg/kg.

Induction of withdrawal syndrome

Animals received naloxone (3 mg/kg i.p.) 5 h after the last injection of morphine on the seventh day. Immediately after naloxone injection, each animal was placed in a transparent acrylic cylinder to observe the frequency of withdrawal manifestations. Two classes of signs were distinguished: graded signs (weight loss, abdominal contraction), which were quantified numerically, and checked signs (diarrhea, teeth chattering, ptosis) in which only presence or absence were evaluated.

Experimental design

To induce morphine dependency, we gave morphine, as mentioned above, from days 1–7. To determine the effect of nifedipine on the development of morphine dependence, we gave nifedipine (1, 2 and 5 mg/kg i.p.) concomitant with morphine. Nifedipine or saline was given according to the same schedule as control groups.

On the 7th day (5 h after the last injection of morphine), rats were randomly divided in two groups. In one group, naloxone-induced withdrawal signs were assessed and in the other animals were sacrificed 30 min after receiving naloxone to evaluate plasma corticosterone concentration.

Adrenalectomy

Animals were anesthetized with ketamine (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 adrenalectomized animals were monitored throughout the study to insure that they were healthy, active, showed no noticeable weight loss and had clean fur. All animals were retained in the study and appeared active and healthy .The animals were tested 5 days after the adrenalectomy or sham procedure. Plasma level of corticosterone and also postmortem examination of the ADX animals confirmed that the adrenal glands had been completely removed.

Corticosterone replacement

For corticosterone replacement in adrenalectomized rats, corticosterone was dissolved in 2 ml of ethyl alcohol and then combined with 0.9% NaCl, yielding final concentration of 100 μg/ml of drinking solution (continuously from the time of adrenalectomy). In this manner, plasma corticosterone level was maintained at a level close to that of the sham-operated animals.

Corticosterone assay

On experimental days, rats were killed by decapitation between 9:00 and 10:00 A.M. and trunk blood was collected into tubes containing 5% EDTA. Plasma was obtained by centrifugation of blood at 2500 r.p.m (10 min). Samples were frozen immediately and stored until the time of corticosterone assay at −20 °C. Plasma level of corticosterone was measured by radioimmunoassay using a commercial kit for rats ([^{125}I] corticosterone, DRG International, Inc., USA). The sensitivity of assay was 0.25 ng/ml and the antibody cross-reacted 100% with corticosterone, 0.34% with desoxycorticosterone and less than 0.10% with other steroids.

Statistical analysis

The results are expressed as mean±SEM. The difference in graded signs and corticosterone levels between groups over the course of study was determined by one-way analysis of variance (ANOVA), respectively, followed by the Newman–Keuls test. Checked sign behaviors were quantified as the number of animals exhibiting the sign/total number of animals observed, and data obtained were analyzed non-parametrically with the Fisher exact test. The level of significance in all tests was set at $P<0.05$.

Results

The effect of adrenalectomy and corticosterone replacement on the levels of plasma corticosterone

Plasma corticosterone concentrations were significantly reduced (to undetectable levels) in ADX animals compared with sham-operated animals (245.6±28.8 ng/ml). In ADX animals that had corticosterone replaced in their drinking water (ADX+CORT), the plasma corticosterone concentration was similar to that of sham-operated animals (218.8±25.3 ng/ml) ($P>0.05$).