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Nifedipine potentiates antinociceptive effects of morphine in rats by decreasing hypothalamic pituitary adrenal axis activity

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Abstract

It has been shown that nifedipine, as a calcium channel blocker can potentiate the antinociceptive effect of morphine; however, the role of Hypothalamic–Pituitary–Adrenal (HPA) axis on this action has not been elucidated. We examined the effect of nifedipine on morphine-induced analgesia in intact and adrenalectomized (ADX) rats and on HPA activity induced by morphine. To determine the effect of nifedipine on morphine analgesia, nifedipine (2 mg/kg i.p.) that had no antinociceptive effect, was injected concomitant with sub-effective dose of morphine (1 and 2 mg/kg). The tail-flick test was used to assess the nociceptive threshold, before and 15, 30, 60, 90, 120 and 180 min after drug administration. Our results showed that, nifedipine could potentiate the antinociceptive effect of morphine and this effect of nifedipine in ADX was greater than sham operated rats which, was reversed by corticosterone replacement. Nifedipine has an inhibitory effect on morphine -induced corticosterone secretion. Thus, the data indicate that the mechanism underlying the potentiation of morphine analgesia by nifedipine involves mediation, at least in part, by attenuating the effect of morphine on HPA axis.

Keywords: Morphine; Analgesia; Nifedipine; HPA axis; Corticosterone

1. Introduction

Opioids have been used for treating moderate to severe pain. Activation of opioid receptor inhibits adenylyl cyclase activity via inhibitory G-proteins, inhibits voltage activated calcium channels, reducing the Ca^{++} influx, thus inhibits neurotransmitter release and attenuates pain sensation (Childers, 1991). Due to the fact that calcium influx is essential for normal sensory processing, inhibition of Ca^{++} movement would contribute to antinociception (Schmidt et al., 1980; Venegas and Schaible, 2000; Todorovic et al., 2002; Heinke et al., 2004; Galeotti et al., 2004). Not surprisingly, Ca^{++} channel antagonists have been shown to induce antinociceptive effect (Del Pozo et al., 1990; Miranda et al., 1993; Weizman et al., 1999; Todorovic et al., 2004; Chen et al., 2005). Many investigators reported that calcium channel blockers potentiate the analgesic effect of morphine (Hoffmeister and Tettenborn, 1986; Contreras et al., 1988; Antkiewicz-Michaluk et al., 1993; Santilan et al., 1994; Michaluk et al., 1998; Assi, 2001; Dogrul et al., 2001; Maeda et al., 2002; Fukuizumi et al., 2003; Yokoyama et al., 2004; Shimizu et al., 2004a,b). In many in vitro studies, it has been demonstrated that glucocorticoids can potentiate Ca^{++} influx and accelerate the release of Ca^{++} from intracellular stores, and corticosterone can increase Ca^{++} entry through the high voltage activated (L-type) calcium channel (Nair et al., 1998; Zhou et al., 2000; Kole et al., 2001; Karast et al., 2002;

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Takahashi et al., 2002; Machida et al., 2003; Sun et al., 2004).

Not only opioids (Buckingham and Cooper, 1986; Gonzalvez et al., 1991; Pechnick, 1993; Little and Kuhn, 1995; Nock et al., 1998; Cerezo et al., 2002) but also Ca^{++} channel blockers (Guerineau et al., 1991; Kuryshev et al., 1995, 1996; Robidoux et al., 2000) could affect HPA function. It has been reported that hypothalamic pituitary adrenal axis (HPA) and its glucocorticoids have an important role on the effect of nifedipine in the development of morphine tolerance (Esmaeili Mahani et al., 2005). Since the interaction between corticosterone and calcium channels has not been clarified in vivo and the role of HPA axis on the effects of calcium channel blockers in morphine analgesia, has not been elucidated, the present study was designed to: first, analyze the contribution of HPA axis and its glucocorticoids to the analgesic effect of morphine that potentiate with nifedipine by using intact and adrenalectomized (ADX) rats. Second, evaluate modifications in the activity of the HPA axis during treatments with morphine in the presence of nifedipine.

2. Materials and methods

2.1. Animals

All experiments were carried out on male Wistar rats, weighing 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 follow 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 and mean was designated as baseline latency before drug 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 drugs, and the reaction latency was determined 15, 30, 60, 90, 120 and 180 min after injection. The Tail-Flick latencies were converted to the percentage of antinociception according to the following formula:%Antinociception (%MPE)=(Reaction time of test−basal reaction time)/(cut off time−basal reaction time).

2.4. 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.

2.5. Corticosterone replacement

For corticosterone replacement in adrenalectomized rats, corticosterone was dissolved in 2 ml of ethyl alcohol then combined with 0.9% NaCl, yielding final concentration of 100 μg/ml of drinking solution (continuously from the time of ADX). The amount of drinking solution consumed by each rat was analyzed to determine whether there were any group differences. With this manner plasma corticosterone level was close to the sham operated animals.

2.6. Corticosterone assay

On experimental days, rats were killed with decapitation between 9:00–10:00 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.

2.7. Statistical analysis

The results are expressed as mean±SEM. The difference in MPE% (antinociception) and corticosterone levels