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

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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

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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

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.

2.7. Corticosterone assay

On experimental days, rats were killed with 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.

2.8. Statistical analysis

The results are expressed as mean±SEM. The difference in MPE% (antinociception) and corticosterone levels between groups over the time course of study was determined by two- or one-way analysis of variance (ANOVA), respectively followed by the Newman–Keuls test with 5% level of significance ($p<0.05$).

3. Results

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

As shown in the Table 1, plasma corticosterone concentrations were significantly reduced (to undetectable levels) in ADX 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 sham operated animals (218.8±25.3 ng/ml) ($p>0.05$). In this group (ADX+CORT) plasma corticosterone concentrations were similar during different days of testing (data is not shown).

Table 1
Effect of adrenalectomy and corticosterone replacement via drinking water on plasma corticosterone level in rats

	Plasma corticosterone concentration (ng/ml)
SHAM	245.6±28.8
ADX	undetectable
ADX+CORT	218.8±25.3

Values represent mean±SEM ($n=8$).

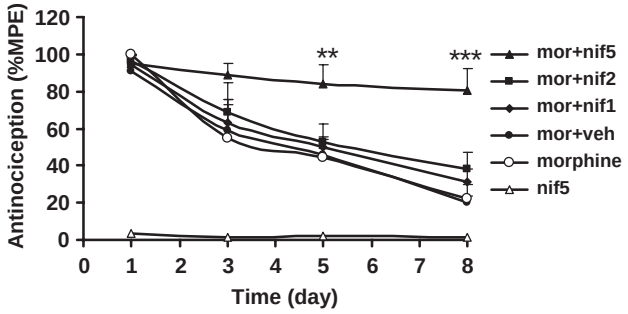

Fig. 1. The effect of nifedipine on the development of tolerance to analgesic effect of morphine in sham operated animals. Values represent mean±SEM ($n=8$). **$p<0.01$ ***$p<0.001$ as compared with the group that received morphine.

3.2. The effect of nifedipine on the development of tolerance to analgesic effect of morphine in the presence or absence of adrenal glands

As it is shown in Fig. 1, in sham operated animals, after chronic administration of morphine (15 mg/kg) for 8 days, significant tolerance occurred to its analgesic effect. In rats that received morphine together with nifedipine (5 mg/kg), no tolerance developed during the experiment. In contrast, nifedipine (1 and 2 mg/kg) did not prevent the development of tolerance to analgesic effect of morphine. However, concomitant treatment of saline with nifedipine (5 mg/kg) had no antinociceptive effect.

Adrenalectomized rats receiving chronic morphine also displayed tolerance to antinociceptive effect of morphine (Fig. 2). Concomitant treatment of morphine with nifedipine in doses 2 and 5 mg/kg, but not in 1 mg/kg prevented the development of tolerance to analgesic effect of morphine. Similar to sham operated rats, adrenalectomized rats chronically treated with saline followed by nifedipine (5 mg/kg) on days 1–8, did not show any antinociceptive effect (Fig. 2).

In ADX animals that were replaced with corticosterone, repeated injection of morphine induced significant decrease in its analgesic effect. Nifedipine (2 mg/kg) could not prevent this analgesic tolerance. This effect was similar to sham operated rats (Fig. 3).

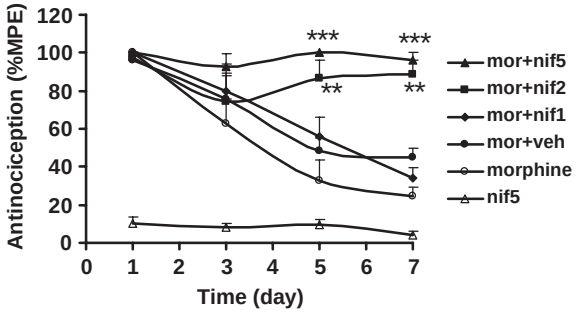

Fig. 2. The effect of nifedipine on the development of tolerance to analgesic effect of morphine in adrenalectomized rats. Values represent mean±SEM ($n=8$). **$p<0.01$ ***$p<0.001$ as compared with the group that received morphine.

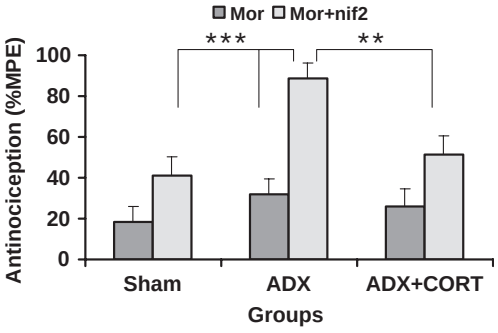

Fig. 3. The analgesic effect of morphine on day 8 in groups that received morphine (15 mg/kg) and morphine concurrently with nifedipine (15 mg/kg+2 mg/kg) for 7 days. Each bar represents mean±SEM ($n=6-8$ rats per group). $^{**}p<0.01$ $^{***}p<0.001$.

3.3. The effect of nifedipine on the development of tolerance to neurosecretory effect of morphine on HPA axis

In this part of study, we investigated the changes in HPA activity upon acute and chronic exposure to morphine, as well as the contribution of nifedipine on this effect. As shown in Fig. 4, the acute administration of morphine (15 mg/kg) produced a significant increase in plasma level of corticosterone, 30 min after injection as compared with the saline injected group ($p<0.001$). Administration of a low dose of nifedipine (2 mg/kg) with morphine, did not prevent the effect of morphine on corticosterone secretion, but in a higher dose (5 mg/kg) it significantly attenuated this effect ($p<0.05$).

Fig. 5 depicts plasma corticosterone concentration 30 min after saline or morphine (15 mg/kg i.p.) injection on day 8, in rats that received morphine alone, and morphine together with nifedipine for 7 days.

Our data shows that in groups which received chronic morphine for 7 days, on day 8, morphine could not increase the corticosterone level compared to chronic saline treated group. It means that a tolerance was developed to this neurosecretory effect (Fig. 5). However,

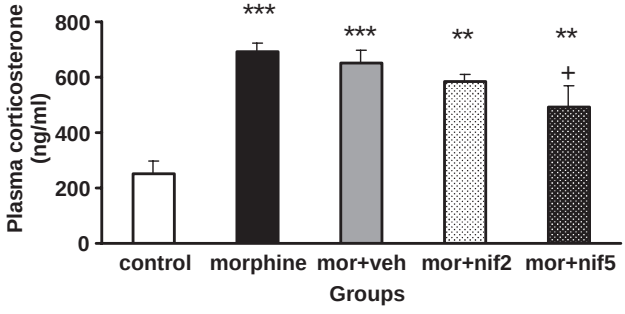

Fig. 4. Plasma corticosterone concentration in rats after injection of saline (control), morphine and morphine concomitant with nifedipine. Animals were decapitated 30 min after the injection. Values are means±SEM. $^{**}p<0.01$, $^{***}p<0.001$ significantly different versus control (saline) group. $^{+}p<0.05$ versus the group which received morphine alone.

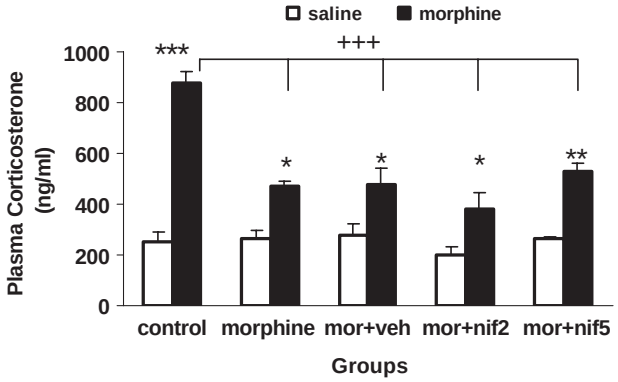

Fig. 5. Plasma corticosterone concentration 30 min after injecting either saline or morphine on day 8 in rats that received saline as control group, and morphine concurrently with or without nifedipine (2 and 5 mg/kg) for 7 days. Each bar represents mean±SEM. Asterisks indicate significant differences from corresponding saline injected group. $^{*}p<0.05$ $^{**}p<0.01$ $^{***}p<0.001$. Cross indicates significant differences between the effect of morphine on experimental and control groups $^{+++}p<0.001$ ($n=6-8$ rats per group).

concomitant treatment of morphine with nifedipine in both doses could not prevent the development of this neurosecretory tolerance.

4. Discussion

Although it has been shown that administration of calcium channel blockers can prevent morphine tolerance but the role of adrenal glands and their corticosteroids in this effect has not been identified yet. Our results showed that the development of tolerance to analgesic effect of morphine appears in adrenalectomized rats similar to the sham operated animals. In the absence of adrenal glands, nifedipine could prevent morphine tolerance development in a lower dose, i.e. 2 mg/kg than in the presence of adrenal glands.

Several lines of evidence indicate that glucocorticoids potentiate calcium influx and accelerate the release of Ca^{2+} from intracellular stores (Zhou et al., 2000; Karast et al., 2002; Takahashi et al., 2002; Machida et al., 2003). This action is opposite to the effect of nifedipine in blockage of Ca^{2+} channels and decreasing Ca^{2+} influx. Therefore, it seems logic that with adrenalectomy, nifedipine is more effective in preventing Ca^{2+} influx into the structures involved in morphine tolerance and as a result, attenuates the development of tolerance to morphine analgesia.

In addition, it has been reported that glucocorticoids can induce mRNA expression of calcium channel subunits (Nair et al., 1998). Moreover, one of the mechanisms underlying the development of morphine tolerance is up regulation of calcium channels (Ramkumar and El-Flakani, 1984, 1988; Diaz et al., 1995). Therefore, it seems that by removal of glucocorticoids, this process has been attenuated and thereby nifedipine has become more effective. However, this possible mechanism needs further investigation.

Our results show that Ca^{2+} influx is necessary for acute opioid action on HPA axis, because nifedipine (5 mg/kg) can attenuate the effect of morphine on corticosterone. Other groups have found similar results using other Ca^{2+} channel blockers e.g. nimodipine or verapamil (Martinez-Pinero et al., 1993; Vargas et al., 1997). The action of opioids on the HPA axis is thought to be mediated, directly, by the release of corticotropin releasing factor (CRF) (Pechnick, 1993; Foote and Maurer, 1982; Buckingham and Cooper, 1986) or indirectly, by releasing noradrenalin (NA) which in turn increases CRF release (Martinez-Pinero et al., 1993, 1994; Milanes et al., 1993; Fuertes et al., 2000). It has been reported that CRF stimulates Ca^{2+} entry through L-type calcium channels in rat coticotrope cells and that voltage sensitive calcium channels have an important role in releasing ACTH (Stojilkovic et al., 1988; Kuryshev et al., 1996). Patch clamp studies show that ACTH can stimulate L-type calcium channels in human adrenal cells (Gallo-Payet et al., 1996). Based on these reports, nifedipine may also affect morphine induced corticosterone secretion in pituitary or adrenal gland and attenuates this effect. Other possible site for the effect of nifedipine is prevention of NA release induced by opioids. Other investigators have shown that acute treatment with nimodipine or verapamil attenuate morphine induced HPA axis activation by inhibiting NA release (Vargas et al., 1997). Previous studies have shown the involvement of α-adrenoceptors of paraventricular nucleus in stimulation of the hypothalamic-pituitary-adrenocortical axis (Itoi et al., 1994) and nifedipine can inhibit the responses to α-adrenoceptor stimulation (Forster et al., 1993). Therefore, nifedipine possibly exerts its effect through antagonism of NA effect and consequently, attenuation of HPA function, in other words Ca^{2+} influx especially from L-type Ca^{2+} channels is important for the expression of acute opioid action on HPA axis.

Since the mechanisms responsible for opioid-induced tolerance on HPA axis and the effect of nifedipine on this aspect of tolerance has not been clarified, in this part of our study, we tested the chronic effect of morphine on HPA activity in the presence of nifedipine, in order to elucidate the probable mechanism responsible for opioid-induced tolerance on HPA axis. Our results show that nifedipine has no effect on the development of neurosecretory tolerance.

There are some reports showing that the effect of Ca^{2+} channel blocker on prevention of the development of tolerance to analgesic effect of morphine is mediated through the inhibition of Ca^{2+} channel up regulation and alteration of Ca^{2+} homeostasis (Ramkumar and El-Flakani, 1988; Contreras et al., 1988; Dierssen et al., 1990; Diaz et al., 1995). Since in our experiment nifedipine could not prevent the development of neurosecretory tolerance, it seems that Ca^{2+} channel up regulation is not responsible for this phenomenon. Further studies are necessary to elucidate this matter.

In summary, our results show that concomitant administration of morphine and nifedipine prevent the development of tolerance to analgesic effect of morphine and following the exclusion of adrenal glands, this drug can act more effectively even in a lower dose. But nifedipine cannot prevent the chronic effect of morphine on HPA function, indicating its negligible effect on this phenomenon.

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