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Involvement of hypothalamic pituitary adrenal axis on the nifedipine-induced antinociception and tolerance in rats

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Abstract

Nifedipine, a calcium channel blocker, can modulate the nociceptive threshold. However, the underlying mechanism, especially the role of HPA axis, on this effect has still not been elucidated. In the present study we investigated the analgesic effect of nifedipine in intact and adrenalectomized (ADX) male rats and we also measured the effect of nifedipine on HPA function. The Tail-Flick test was used to assess the nociceptive threshold before and 15, 30, 60, 90, and 120 min after drug administration. Corticosterone level was measured by radioimmunoassay as a marker of HPA function. Our results showed that in intact and sham operated animals, administration of 10 mg/kg nifedipine induces an antinociceptive effect. But at the dosage of 2 and 5 mg/kg animals do not exhibit this effect. With repeated injections, its analgesic effect was decreased, a phenomenon prevented by adrenalectomy. Acute administration of nifedipine produced significant decrease in plasma corticosterone level. In ADX animals, had a potent antinociceptive effect nifedipine at high dosage (10 mg/kg) as well as at lower dosage (5 mg/kg) that reversed with corticosterone replacement. In conclusion, the results of our study show that the elimination of HPA function through adrenalectomy potentiates the antinociceptive effect of nifedipine and attenuates its analgesic tolerance. Both effects are reversed by corticosterone replacement.
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Keywords: Nifedipine; Antinociception; HPA axis; Corticosterone; Rats

1. Introduction

Several investigators have reported that calcium ion has a physiological role in the regulation of pain sensitivity, and inhibition of calcium movement contributes to antinociception (Schmidt and Way, 1980; Venegas and Schaible, 2000; Heinke et al., 2004; Galeotti et al., 2004; Chen et al., 2005; Weiss and De Waard, 2006). L-type Ca^{2+} channel antagonists produce analgesia after peripheral and ICV administration (Del Pozo et al., 1987, 1990; Miranda et al., 1993; Weissman et al., 1999; Todorovic et al., 2004). In addition, nifedipine, as a calcium channel blocker can elicit analgesic response (Wong et al., 1993; 1998). Wong et al. (1996) reported that after chronic administration of nifedipine a tolerance-like phenomenon occurred in its antinociceptive effect.

Ca^{2+} and related channels, particularly the L-type, participates in the control of HPA axis (Stojilkovic et al., 1988; Guerineau et al., 1991; Kuryshev et al., 1996; Robidoux et al., 2000).

Many *in vitro* studies have demonstrated that glucocorticoids can potentiate Ca^{2+} influx through high voltage activated calcium channel (L-type) and accelerate the release of Ca^{2+} from intracellular stores. (Nair et al., 1998; Zhou et al., 2000; Karast et al., 2002; Takahashi et al., 2002; Machida et al., 2003; Sun et al., 2004).

Since the interaction between corticosterone and calcium channels has not been clarified *in vivo*, and the role of HPA axis in analgesic effects of calcium channel blockers has not been fully elucidated, the present study was designed to analyze the contribution of HPA axis and its glucocorticoids to the effect of

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nifedipine, as a calcium channel blocker, on pain threshold by using Tail-Flick test in rats.

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 followed the guidelines on ethical standard for investigation of experimental pain in animals (Zimmermann, 1983).

2.2. Drugs

Nifedipine (Sigma, USA) was dissolved in dimethyl sulfoxide (DMSO) and saline. This drug was given in a volume of 1 ml/kg, i.p. Corticosterone (Sigma, USA) was dissolved in absolute ethanol then combined with 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 the 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 nifedipine (2, 5 and 10 mg/kg), and the reaction latency was determined 15, 30, 60, 90 and 120 min after the injection. The Tail-Flick latencies were converted to the percentage of antinociception according to the following formula:

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

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 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.

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 surgery). With this manner plasma corticosterone level was close to the sham operated animals (Table 1).

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 the 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 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 to sham operated animals (237.6±35.4 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 (211.2±42.3 ng/ml) ($p>0.05$).

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

Plasma corticosterone concentration (ng/ml)	
SHAM	237.6±35.4
ADX	undetectable
ADX+CORT	211.2±42.3

Values represent mean±SEM ($n=8$).

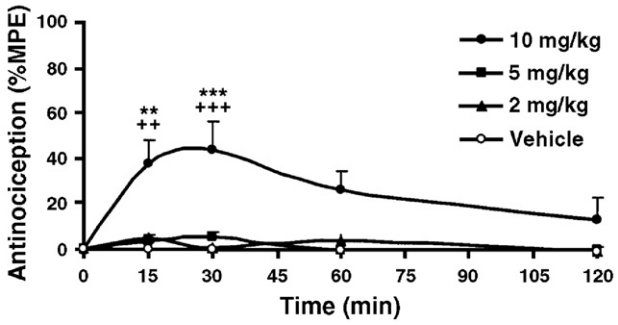

Fig. 1. Antinociceptive effect of nifedipine on Tail-Flick test in sham operated animals. Values represent mean±SEM (n=8).++p<0.01 +++p<0.001 significantly different versus before drug administration. ⋆⋆p<0.01 ⋆⋆⋆p<0.001 versus other groups in the same time.

3.2. The analgesic effect of nifedipine in the presence or absence of adrenal glands

In sham operated animals the baseline Tail-Flick latency was 2.76±0.144 s (n=8). Nifedipine only in high dose (10 mg/kg) induced significant antinociceptive effect at 15 and 30 min after the injection. Maximal antinociceptive effect of nifedipine was observed 30 min after injection. Administration of nifedipine (2 and 5 mg/kg) and vehicle had no effect on nociceptive threshold (Fig. 1).

There was no significant difference in baseline Tail-Flick latency between sham operated and adrenalectomized animals (3.01±0.19 s, n=8). In ADX rats, nifedipine in doses 5 and 10 mg/kg, but not in 2 mg/kg exerted an antinociceptive activity that peaked at 30 min and returned to baseline by 120 min after injection. Similar to sham operated rats, ADX rats that received vehicle did not show any antinociceptive effect (Fig. 2).

Nifedipine in 2 mg/kg had no antinociceptive effect in sham operated, ADX and ADX animals that replaced with corticosterone (ADX+CORT) 30 min after injection as shown in Fig. 3. Although nifedipine (5 mg/kg) failed to modulate nociceptive threshold in sham operated and ADX rats replaced with corticosterone, it did significantly induce antinociception in ADX animals 30 min after injection. Administration of 10 mg/kg nifedipine in sham operated group showed an

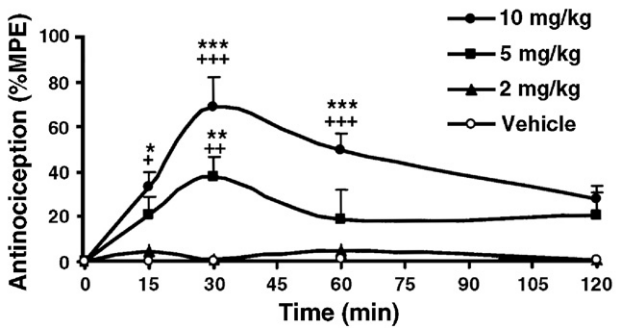

Fig. 2. Antinociceptive effect of nifedipine on Tail-Flick test in ADX animals. Values represent mean±SEM (n=8). +p<0.05 ++p<0.01 +++p<0.001 significantly different versus before drug administration. ⋆p<0.05 ⋆⋆p<0.01 ⋆⋆⋆p<0.001 as compared with the vehicle group.

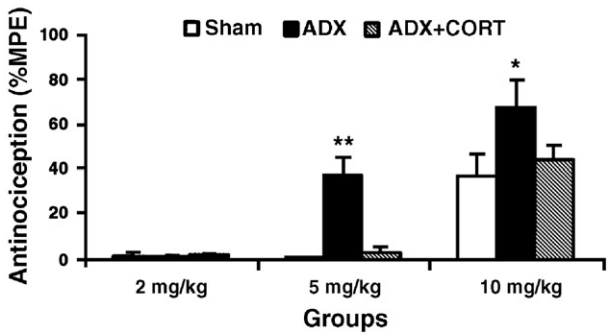

Fig. 3. The analgesic effect of nifedipine 30 min after injection in sham operated (Sham), adrenalectomized (ADX) and adrenalectomized rats that received corticosterone in drinking solution (ADX+CORT). Values represent mean±SEM (n=6–8 rats per group). ⋆p<0.05 ⋆⋆p<0.01 as compared with Sham and ADX+CORT groups in same dose.

antinociceptive response that was potentiated by adrenalectomy. Similar to sham operated group, ADX rats that received corticosterone (ADX+CORT) showed a mild antinociceptive effect (Fig. 3). In other words, adrenalectomy increases the analgesic effect of nifedipine, and corticosterone replacement reverses this response.

3.3. Tolerance-like phenomenon to the analgesic effect of nifedipine in the presence or absence of adrenal glands

In this part of the study, we investigated the antinociceptive activity of 10 mg/kg nifedipine, 30 min after injection, on the rats which were treated chronically with either vehicle or nifedipine (10 mg/kg, once daily for 7 days). As shown in Fig. 4, in sham operated animals, antinociceptive activity of nifedipine was significantly decreased (p<0.01) due to repeated administration of this drug. In other words, a tolerance-like phenomenon had occurred. Whereas, injection of nifedipine to both ADX rats that received repeated nifedipine or vehicle induced a potent antinociceptive effect. No difference between the level of nifedipine-induced antinociception in vehicle and nifedipine treated animals was observed. These effects were reversed by corticosterone replacement (Fig. 4).

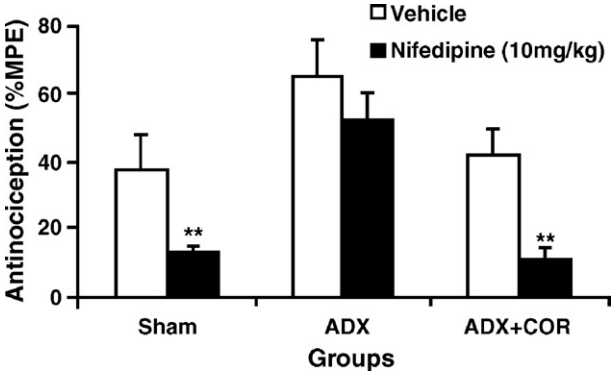

Fig. 4. The analgesic effect of 10 mg/kg nifedipine, 30 min after injection, in Sham, ADX and ADX+CORT animals that received vehicle or 10mg/kg nifedipine once daily for 7 days. Values represent mean±SEM (n=6–8 rats per group). ⋆⋆p<0.01 significantly different versus vehicle treated group.

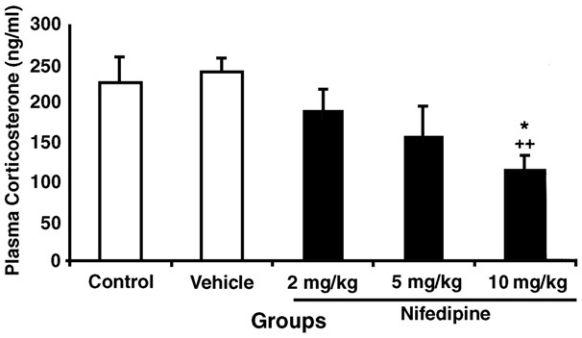

Fig. 5. Plasma corticosterone concentration in rats after injection of vehicle or nifedipine. Animals were decapitated 30 min after the injection. Data are the mean ± SEM. $\star p < 0.05$ significantly different versus control animals. $++p < 0.01$ versus the group that received vehicle.

3.4. The effect of nifedipine on HPA axis

In this section of the study, we assessed the changes in HPA activity upon exposure to nifedipine. As shown in Fig. 5, acute administration of nifedipine (10 mg/kg) produced a significant decrease in plasma level of corticosterone, 30 min after injection as compared to the control ($p < 0.05$) and vehicle injected groups ($p < 0.01$). However, lower doses of nifedipine (2 and 5 mg/kg) had no significant effect on corticosterone level.

4. Discussion

Although it has been shown that administration of calcium channel blockers can modulate nociceptive threshold and induce an analgesic effect, the role of adrenal glands and their glucocorticoids in this effect has not been identified yet. This article is the first *in vivo* study that shows the interaction between glucocorticoids and L-type calcium channels. Our results showed that in the absence of adrenal glands, nifedipine-induced an antinociceptive effect with lower dose of nifedipine i.e. 5 mg/kg than in the presence of adrenal glands, and analgesic effect of higher dose (10 mg/kg) was potentiated. This drug in dose that had significant inhibitory effect on corticosterone secretion also had prominent antinociceptive effect.

Several lines of evidence indicate that nociception is related to the intraneuronal Ca^{2+} level. The lowering of the neuronal Ca^{2+} induces analgesia. Not surprisingly, drugs such as nifedipine, which reduce Ca^{2+} availability, could exert analgesic effect.

The data showed that in the absence of the adrenal glands, nifedipine could exert analgesia in sub-effective dose and induce potent antinociceptive activity in effective dose than in presence of adrenal glands (Fig. 3). Therefore, it seems that the effect of nifedipine is not as simple as mentioned above and modulation of nociceptive threshold by this drug is not accounted for only by direct suppression of Ca^{2+} influx and diminished calcium dependent neurotransmitter release.

There are reports indicating 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; Sun et al., 2004). This action is opposite to the effect of nifedipine in blockage of Ca^{2+} channels and decrease Ca^{2+} influx. Therefore, it is apparent logic that with adrenalectomy, nifedipine is more effective in preventing Ca^{2+} influx into the structure involved in pain processing and as a result, modulates nociceptive threshold.

It is well known that pro-opiomelanocortin, ACTH and beta-endorphin levels increase in ADX rats (Bogdanov and Yarushkina, 2004; Vissers et al., 2004). Beta-endorphin can modulate calcium channel activity and inhibit Ca^{2+} influx (Mazorow et al., 1994). The antinociceptive effect of endomorphin-1 (a novel endogenous mu-opioid ligand) microinjected into the ventrolateral periaqueductal gray is potentiated by concomitant administration of nifedipine (Hao et al., 2003). Therefore, the potentiation of nifedipine-induced analgesia following adrenalectomy could also be due, at least in part, to altered beta-endorphin levels.

In sham operated animals the effective dose of nifedipine on corticosterone secretion could elicit prominent analgesic effect. Therefore, its influence on corticosterone level could be helpful on induction of its antinociceptive effect. It has been shown that Ca^{2+} influx especially from L-type Ca^{2+} channels is important for normal function of HPA axis. Stojilkovic et al. (1988) and Kuryshev et al. (1996) found that CRF stimulates Ca^{2+} entry through L-type calcium channels in rat corticotrop cells and these channels have an important role in releasing ACTH. Patch clamp studies show that ACTH can stimulate L-type calcium channel in adrenal cells (Gallo-Payet et al., 1996). Previous studies have shown the inhibitory effect of nifedipine (Esmaeili Mahani et al., 2005a) and other Ca^{2+} channel blockers e.g. nimodipine or verapamil (Martinez-Pinero et al., 1993) on morphine-induced corticosterone secretion.

Our results show that repeated injections of nifedipine produced a tolerance-like phenomenon in its analgesic activity. Other investigators also have found similar results but with different dosage and regimen (Wong et al., 1996). One possible reason of this tolerance is changes in dihydropyridine receptors density.

There is evidence that shows glucocorticoids can induce mRNA expression of calcium channel subunits (Nair et al., 1998; Gu et al., 2001; Karast et al., 2002; Qin et al., 2004), and significantly increase the number of dihydropyridine-binding sites in nervous and non-nervous tissues (Fomina et al., 1996; Takimoto et al., 1997). Other groups demonstrated that chronic administration of nifedipine also induces up-regulation of calcium channels (Chiappe De Cingolani et al., 1994; Morgan et al., 1999; Verde et al., 2002). Therefore, the effects of glucocorticoids and nifedipine on dihydropyridine Ca^{2+} channel density seem to be synergistic. With elimination of corticosterone through adrenalectomy, one of the factors that affect calcium channel density is removed and, thereby tolerance is not exhibited. However, this possible mechanism needs to be clarified by further investigation.

We have reported that hypothalamic pituitary adrenal axis has an important role in the effect of nifedipine on morphine

analgesia and tolerance and also demonstrated an *in vivo* interaction between glucocorticoids and calcium channels (Esmaeili Mahani et al., 2005a,b). According to the present data HPA axis is also involved in the antinociceptive effect of nifedipine. Therefore, it seems that there is a general interaction between HPA axis factors especially its glucocorticoids and nifedipine which could affect other therapeutic effects of nifedipine. However, this phenomenon needs to be clarified by further investigations.

In summary, our results show that nifedipine, as a Ca^{2+} channel blocker, could exert an antinociceptive activity in rats and with repeated injection a tolerance-like phenomenon is induced. Following the exclusion of adrenal glands, nifedipine is more effective on induction of analgesia even in sub-effective doses and tolerance does not occur. Thus, HPA function has an important role on the analgesic effect of nifedipine.

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