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Adrenalectomy potentiates the antinociceptive effects of calcium channel blockers

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ABSTRACT

Calcium channel blockers can modulate the nociceptive threshold. However, the underlying mechanism(s), especially the role of hypothalamic–pituitary–adrenal (HPA) axis, on this effect has not yet been clarified. In the present study we investigated the analgesic effect of verapamil, diltiazem and nimodipine in intact and adrenalectomized (ADX) male rats and also measured the effect of these drugs on HPA function. The tail-flick and hot-plate tests were used to assess the nociceptive threshold before and 15, 30, 60 and 120 min after drug administration. Corticosterone level was measured by radioimmunoassay as a marker of HPA function. Our results showed that these drugs could elicit antinociceptive effects which were more prominent in the hot-plate than in the tail-flick tests. Following the exclusion of adrenal glands these drugs showed stronger analgesic effects. Acute administration of verapamil, diltiazem and nimodipine produced significant decrease in plasma corticosterone level that was more prominent by nimodipine. In conclusion, the results of our study show that the HPA function has an important role in the antinociceptive effect of calcium channel blockers.

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 central administration (Del Pozo et al., 1990; Miranda et al., 1993; Wong et al., 1998; Todorovic et al., 2004; Esmaeili-Mahani et al., 2006). Moreover, the effects of L-type Ca^{2+} channel antagonists on nociception differ depending on the drug, dosage, and route of administration and the algesimetric test used (Prado, 2001).

It is documented that Ca^{2+} and related channels, particularly the L-type, participate in the control of hypothalamic–pituitary–adrenal (HPA) axis (Stojilkovic et al., 1988; Kuryshev et al., 1996; Robidoux et al., 2000). Furthermore, many *in vitro* studies have demonstrated that glucocorticoids can potentiate Ca^{2+} influx through high 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 analgesic effect of nifedipine (Esmaeili-Mahani et al., 2006).

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 three different kinds of calcium channel blockers i.e. verapamil, diltiazem and nimodipine, on pain threshold using tail-flick (TF) and hot-plate (HP) tests 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/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 the adrenalectomized (ADX) rats. Animals were handled daily (between 9:00 and 10:00 AM) 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).

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2.2. Drugs

Verapamil and diltiazem (Sigma, USA) were dissolved in physiological saline and nimodipine (Sigma, USA) was dissolved in dimethyl sulfoxide (DMSO) plus saline. The percentages of DMSO and saline in the final volume were 60% and 40% respectively. Verapamil, diltiazem and nimodipine were given in the volume of 1 ml/kg, i.p. Corticosterone (Sigma, USA) was dissolved in absolute ethanol, and then combined with drinking water.

2.3. Evaluation of nociceptive response

2.3.1. Tail-flick test

Standard tail-flick test (D'Amour and Smith, 1941) was used. The tail-flick latency for each rat was determined three times at 3 min intervals and mean was designated as baseline latency before drug injection. Radiant heat was focused on 4–7 cm from the tail distal end. 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 verapamil (5, 10, 15, 30 and 50 mg/kg), diltiazem (5, 10, 15, 30 and 50 mg/kg) and nimodipine (2, 5 and 10 mg/kg) and the reaction latency was determined 15, 30, 60 and 120 min after the injection. The tail-flick latencies were converted to the percentage of antinociception according to the following formula:

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

2.3.2. Hot-plate test

Rats were individually placed on a hot-plate maintained at 55±0.2 °C and the time of licking of the hind paws or attempt to jump out of the beaker was recorded as the latency period. The cut-off time was 60 s to avoid tissue damage. Before drug administration, baseline latency was examined. The paw withdrawal latency was tested after drug administration. The maximum possible effect (MPE) was calculated as:

$$\text{MPE}\% = (\text{latency after drug administration} - \text{baseline latency}) / (60 - \text{baseline latency}) \times 100.$$

2.4. Measurement of locomotor activity in open field

The locomotor activity in open field was carried out in an activity monitoring apparatus. The activity chambers (27×30×26 cm, width×length×height) were placed in soundproof boxes with dim illumination and a fan. Horizontal locomotor activity was recorded by the breaking of infrared beams. After 5 min acclimatization, the locomotor activity was recorded for 30 min.

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 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.

2.6. 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 surgery). With this manner plasma corticosterone level was close to the sham-operated animals (Esmaeili Mahani et al., 2007).

2.7. Corticosterone assay

On experimental days, rats were killed with decapitation between 9:00 and 10:00 AM and trunk blood was collected into tubes containing 5% EDTA. Plasma was obtained by centrifugation of blood at 2500 rpm (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

Plasma corticosterone concentrations were significantly reduced (2.03±0.56 ng/ml) in ADX animals compared with sham-operated animals (201.12±21.06 ng/ml $p<0.001$). In ADX animals that had corticosterone replaced in their drinking water (ADX+CORT), the plasma corticosterone concentration was close to that of sham-operated animals (175.4±9.7 ng/ml) ($P>0.05$).

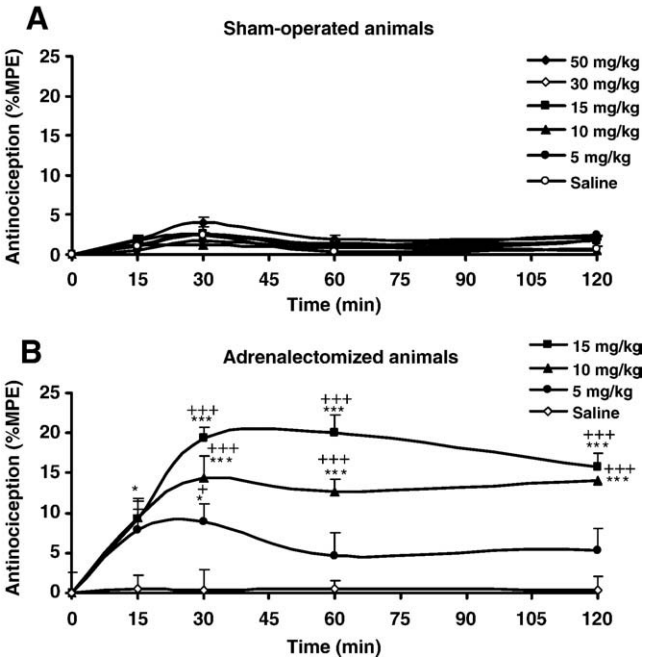

Fig. 1. Antinociceptive effect of verapamil on tail-flick test in the sham-operated (A) and adrenalectomized animals (B). Values represent mean±SEM (n=6–8). *p<0.05, ***$p<0.001$ significantly different versus before drug administration. $^{+}p<0.05$, $^{+++}p<0.001$ versus saline treated group at the same time.

3.2. The analgesic effect of verapamil 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). Administration of verapamil (5, 10, 15, 30 and 50 mg/kg i.p.) and saline had no significant effect on nociceptive threshold (Fig. 1A). Baseline tail-flick latency in adrenalectomized animals was 3.03 ± 0.22 s. This value did not differ significantly from sham-operated rats (P>0.05).

In ADX rats, verapamil in doses of 10 and 15 mg/kg, but not in 5 mg/kg exerted an antinociceptive activity at 30, 60 and 120 min after the injection on tail-flick test (Fig. 1B). Verapamil in doses above 15 mg/kg had toxic and lethal effects in ADX animals.

Administration of 50 mg/kg verapamil in sham-operated group showed an antinociceptive response on hot-plate test. In contrast, lower doses of verapamil had no antinociceptive property in this test (Fig. 2A). Following adrenalectomy verapamil could elicit analgesic effects in doses of 10 and 15 mg/kg in all the time points of the test. However, administration of 5 mg/kg of verepamil could affect nociceptive threshold and induce moderate analgesic property at 15 and 30 min after injection (Fig. 2B).

3.3. The analgesic effect of diltiazem in the presence or absence of adrenal glands

As is shown in Fig. 3A, diltiazem (50 mg/kg i.p.) produced an analgesic response in sham-operated animals on tail-flick test. The maximum analgesic effect was reached at 30 min after injection and persisted up to 60 min. Administration of saline or diltiazem (5, 10, 15, 30 mg/kg i.p.) did not show any nociceptive response. Fig. 3B shows the effect of diltiazem at different doses on tail-flick test in adrenalectomized animals. Administration of 15 mg/kg diltiazem elicited a significant analgesic effect which picked at 30 and 60 min after the injection and persisting significant up to 120 min. In addition, diltiazem (10 mg/kg i.p.) induced a moderate analgesia at 15, 30 and 60 min after administration, whereas, 5 mg/kg did not show any effect on the nociceptive threshold. Diltiazem at doses more than 15 mg/kg had toxic effect in ADX animals.

Hot-plate data from sham-operated animals showed that diltiazem (50 mg/kg i.p.) could elicit an antinociceptive effect which appeared 15 min after the injection, reaching a peak at 30 min and persisting almost unchanged up to 120 min. In addition, 30 mg/kg diltiazem induced a moderate analgesia 30 min after the injection. Administration

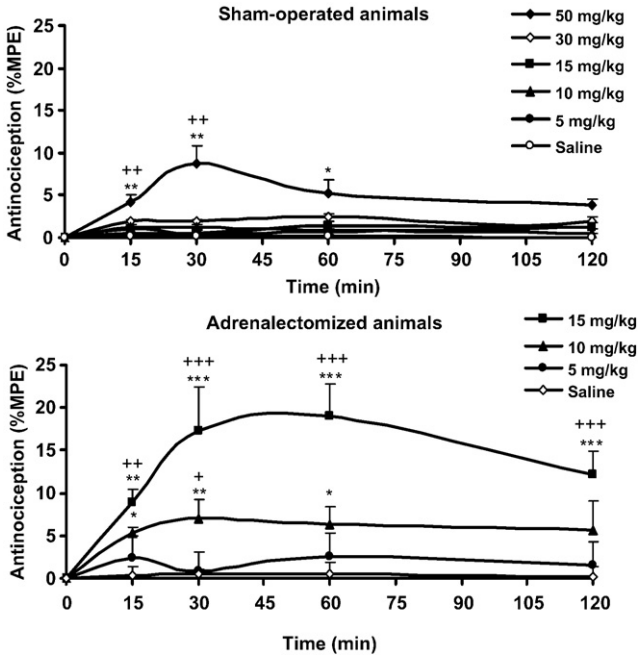

Fig. 3. Antinociceptive effect of diltiazem on tail-flick test in the sham-operated (A) and adrenalectomized animals (B). Values represent mean ± SEM (n=6–8). *p<0.05, **p<0.01, ***p<0.001 significantly different versus before drug administration. ^{+}p<0.05, ^{++}p<0.01, ^{+++}p<0.001 versus saline treated group at the same time.

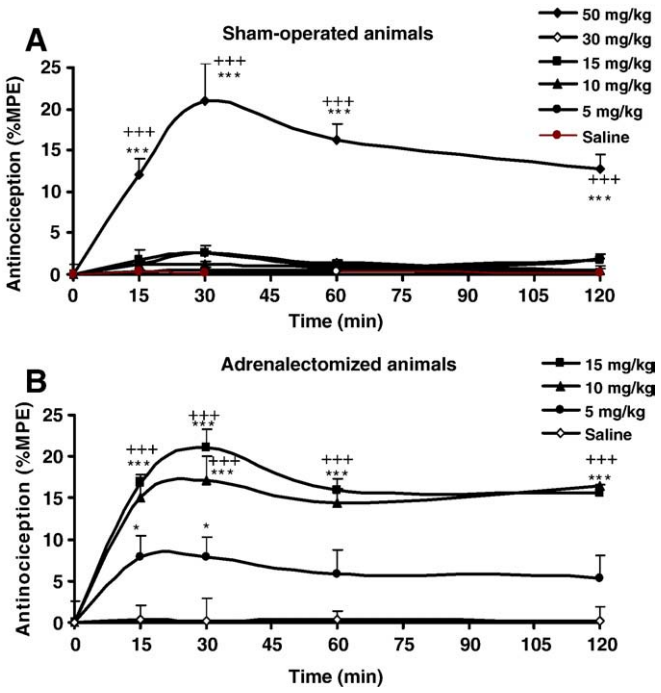

Fig. 2. Antinociceptive effect of verapamil on hot-plate test in the sham-operated (A) and adrenalectomized animals (B). Values represent mean ± SEM (n=6–8). *p<0.05, ***p<0.001 significantly different versus before drug administration. ^{+}p<0.05, ^{+++}p<0.001 as compare with saline treated group at the same time.

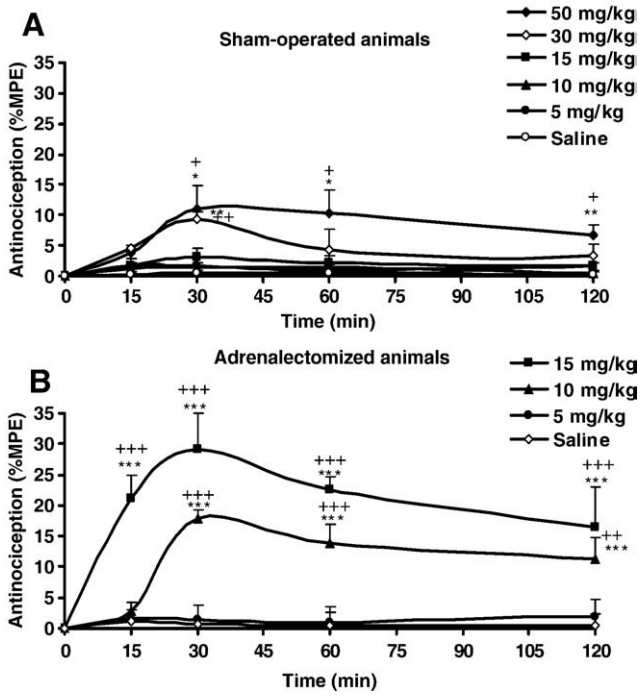

Fig. 4. Antinociceptive effect of diltiazem on hot-plate test in the sham-operated (A) and adrenalectomized animals (B). Values represent mean ± SEM (n=6–8). *p<0.05, **p<0.01, ***p<0.001 significantly different versus before drug administration. ^{+}p<0.05, ^{++}p<0.01, ^{+++}p<0.001 versus saline treated group at the same time.

of saline, 5, 10 and 15 mg/kg diltiazem did not show any nociceptive response (Fig. 4A).

Following adrenalectomy, diltiazem produced analgesic effect in lower doses so that, administration of 15 mg/kg diltiazem elicited a potent analgesic effect. In addition, diltiazem (10 mg/kg i.p.) induced analgesia, whereas 5 mg/kg did not show any effect on nociceptive threshold (Fig. 4B). Furthermore, the antinociceptive effect of diltiazem was significantly greater in hot-plate than tail-flick test.

3.4. The analgesic effect of nimodipine in the presence or absence of adrenal glands

In sham-operated animals nimodipine in high dose (10 mg/kg) induced significant antinociceptive effect on tail-flick test. Maximal antinociceptive effect of nimodipine was observed 15 min after injection. Administration of 5 mg/kg nimodipine showed analgesic effect only at 60 min after injection. However, nimodipine in dose of 2 mg/kg and vehicle did not show any effect on nociceptive threshold (Fig. 5A).

In ADX rats, 10 mg/kg nimodipine exerted a potent antinociceptive activity that peaked at 30 min after the injection. In addition, a significant analgesic effect was observed in ADX animals that received 5 mg/kg nimodipine. A moderate but significant antinociceptive effect was elicited at 60 min after nimodipine (2 mg/kg) injection. Similar to sham-operated rats, ADX rats that received vehicle did not show any antinociceptive effect (Fig. 5B).

Fig. 6A and B depict the effects of various doses of nimodipine on the hot-plate test in sham-operated and adrenalectomized animals, respectively. In the sham-operated rats nimodipine at 10 mg/kg produced a significant antinociception. The maximum analgesic effect was reached at 15 min after the injection and persisted up to 120 min. Administration of 5 mg/kg nimodipine showed analgesic effect only at 30 and 60 min after the injection. Injection of nimodipine (2 mg/kg i.p.) and vehicle had no antinociceptive property. Following adrenalectomy, all doses of nimodipine could induce the analgesic effect (Fig. 6B).

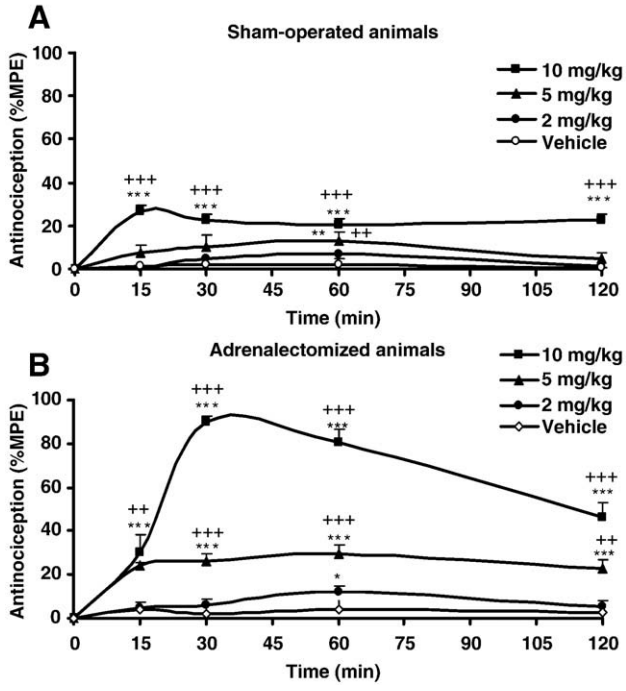

Fig. 5. Antinociceptive effect of nimodipine on tail-flick test in the sham-operated (A) and adrenalectomized animals (B). Values represent mean±SEM (n=6–8). *p<0.05, **p<0.01, ***p<0.001 significantly different versus before drug administration. ^{++}p<0.01, ^{+++}p<0.001 significantly different versus vehicle treated group at the same time.

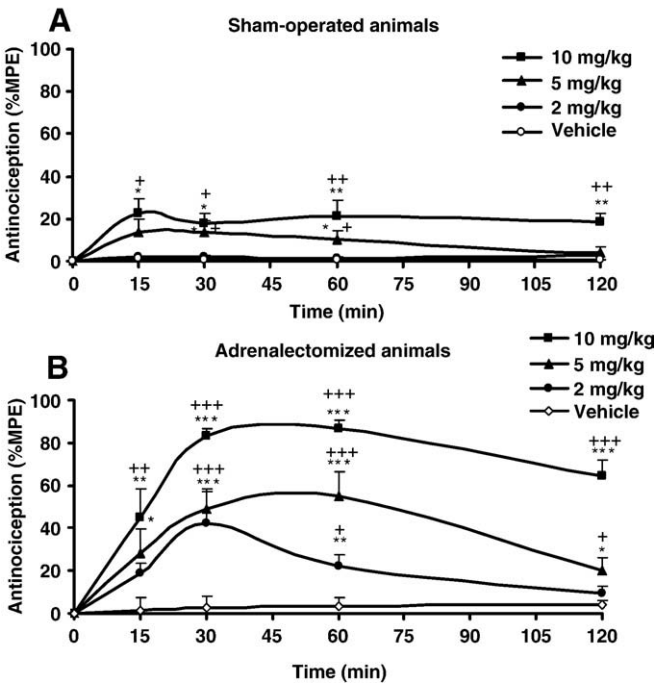

Fig. 6. Antinociceptive effect of nimodipine on hot-plate test in the sham-operated (A) and adrenalectomized animals (B). Values represent mean±SEM (n=6–8). *p<0.05, **p<0.01, ***p<0.001 significantly different versus before drug administration. ^{+}p<0.05, ^{++}p<0.01, ^{+++}p<0.001 significantly different versus vehicle treated group at the same time.

3.5. The effect of corticosterone replacement on the analgesic effects of verapamil, diltiazem and nimodipine

As shown in Fig. 7A, verapamil (10 mg/kg) did not produce analgesic effect in the sham-operated rats while, it produced analgesic effects in ADX animals, 30 min after the injection, in both tail-flick and hot-plate tests. Verapamil had no antinociceptive property in the ADX rats that received corticosterone in their drinking water. In other words, adrenalectomy potentiates the analgesic effect of verapamil which is reversed by corticosterone replacement.

In adrenalectomized animals that had corticosterone in their drinking water the antinociceptive effect of 10 mg/kg diltiazem, 30 min after injection, was similar to those in the sham-operated rats (Fig. 7B). In the ADX animals the analgesic effect of 10 mg/kg diltiazem on the hot-plate test was significantly greater than the tail-flick test (P<0.001).

Surprisingly, nimodipine in dose of 10 mg/kg had a full analgesic property in ADX animals which was also attenuated by corticosterone replacement (Fig. 7C).

3.6. The effect of verapamil, diltiazem and nimodipine on HPA axis

In this section of study, we assessed the changes in HPA activity upon exposure to calcium channel blockers. As shown in Fig. 8A, acute administration of verapamil (50 mg/kg) produced a significant decrease in plasma level of corticosterone, 30 min after injection as compared to the control and saline injected groups (p<0.001). However, lower doses of verapamil (5, 10, 15 and 30 mg/kg) had no significant effect on the corticosterone level. Fig. 8B shows that acute administration of 50 mg/kg i.p. diltiazem had a significant decreasing effect on plasma level of corticosterone 30 min after injection as compared to the saline treated and control groups (p<0.001). In contrast, administration of diltiazem in doses of 5, 10, 15 and 30 mg/kg had no significant effect on plasma corticosterone concentration (P>0.05).

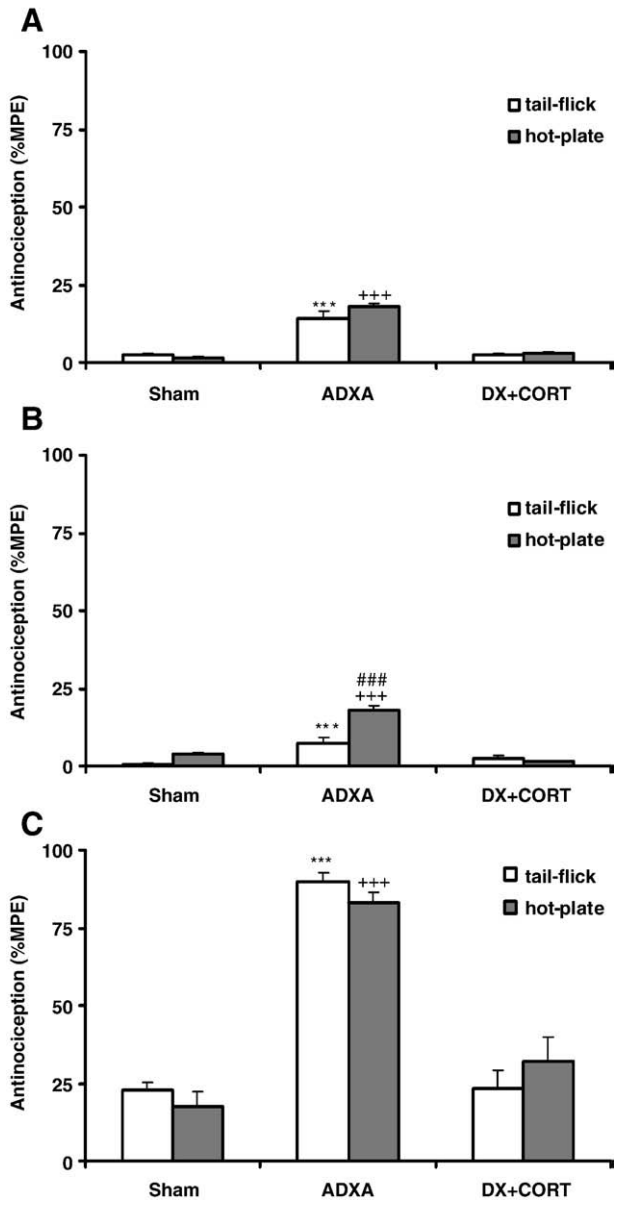

Fig. 7. The analgesic effect of 10 mg/kg verapamil (A), diltiazem (B) and nimodipine, 30 min after injection, in the sham-operated (Sham), adrenalectomized (ADX) and adrenalectomized rats that received corticosterone in drinking solution (ADX+CORT). Values represent mean±SEM (n=6–8 rats/group). $^{***}p<0.001$ significantly different versus sham and ADX+CORT groups tested with tail-flick test. $^{+++}p<0.001$ significantly different versus sham and ADX+CORT groups tested with hot-plate test. $^{###}p<0.001$ significantly different versus tail-flick antinociception score in ADX animals.

Fig. 8C, depicts plasma corticosterone concentration 30 min after intraperitoneal injection of different doses of nimodipine or vehicle in sham-operated animals. Our data shows that plasma corticosterone level was not affected by 2 mg/kg nimodipine while, it was decreased following 5 and 10 mg/kg nimodipine ($p<0.05$ and $p<0.001$ respectively).

4. The effect of verapamil, diltiazem and nimodipine on locomotor activity

In order to determine whether the thermal antinociception following the systemic injection of verapamil, diltiazem and nimodipine could be attributed to a diminished sensory and/or motor performance in the tested animals (due to cardiovascular side effects, e.g. hypotension), we performed sensorimotor testing at 30 min post-treatment (at the peak of the antinociceptive effect) using the highest dose of verapamil, diltiazem (50 mg/kg in the sham-operated and 15 mg/kg in the ADX animals) and nimodipine (10 mg/kg in both sham-operated and ADX animals). Drug treatment did not significantly affect walk performance and distance traveling compared to control animals suggesting that their sensory and motor capabilities remained intact (Fig. 9).

5. 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 fully identified. Our results showed that in the sham-operated animals different L-type calcium channel blockers induced an antinociceptive effect and following adrenalectomy, their analgesic effect was significantly potentiated. In the sham-operated animals, a prominent antinociceptive effect was observed in doses that had significant inhibitory effect on corticosterone secretion.

Several lines of evidence indicate that nociception is related to the intraneuronal Ca^{2+} level. The lowering of the neuronal Ca^{2+} induces analgesia (Schmidt and Way, 1980; Venegas and Schaible, 2000; Heinke et al., 2004; Galeotti et al., 2004; Chen et al., 2005; Weiss and De Waard,

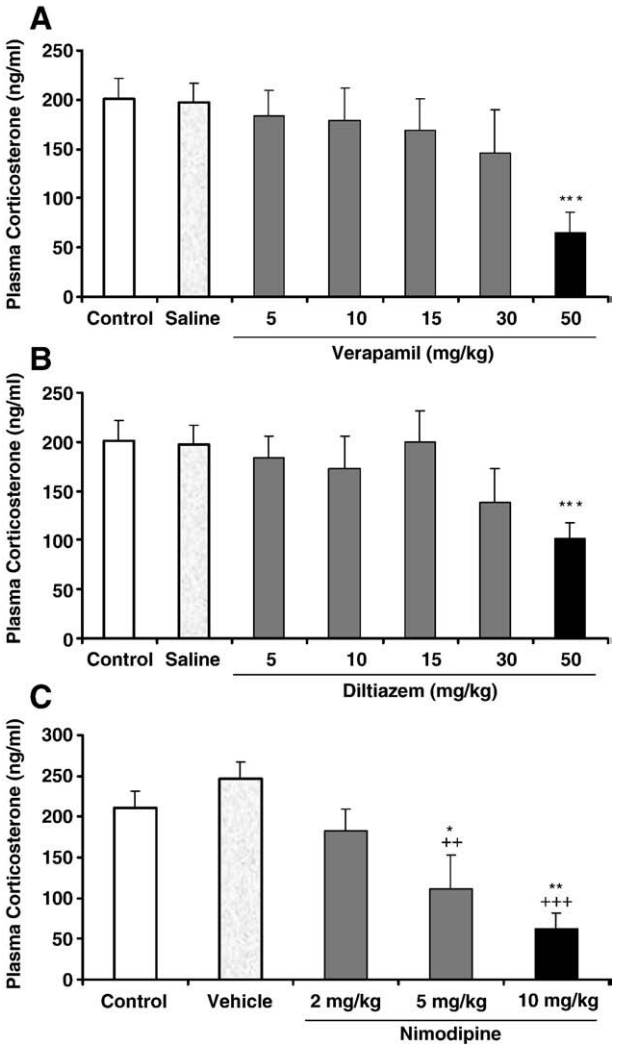

Fig. 8. Plasma corticosterone concentration 30 min after injecting different doses of verapamil, diltiazem and nifedipine. Each bar represents mean±SEM (n=6–8). $^{*}p<0.05$, $^{**}p<0.01$ $^{***}p<0.001$ significantly different versus control animals. $^{++}p<0.01$, $^{+++}p<0.001$ significantly different versus vehicle treated rats.

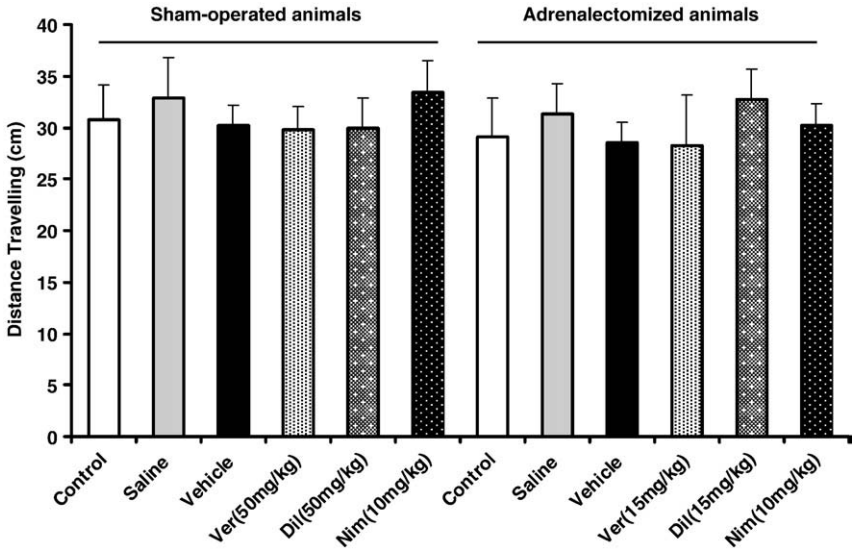

Fig. 9. Locomotor activity in the sham-operated and adrenalectomized animals in response to saline, vehicle and high doses of verapamil, diltiazem and nimodipine. The data are expressed as mean±SEM of distance traveled during 30 min test session (n=7–8).

2006). Not surprisingly, drugs such as verapamil, diltiazem and nimodipine, which reduce Ca^{2+} availability, could exert analgesic effect.

It seems that the effect of calcium channel blockers is not as simple as mentioned above and modulation of nociceptive threshold by these drugs 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 calcium channel blockers in blockage of Ca^{2+} channels and decrease of Ca^{2+} influx. Therefore, it is logical that with adrenalectomy, these drugs are more effective in preventing Ca^{2+} influx into the structures involved in pain processing and as a result, modulating 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(an 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 calcium channel blockers-induced analgesia following adrenalectomy could also be due, at least in part, to the altered beta-endorphin levels which need further investigation.

In sham-operated animals the effective doses of verapamil, diltiazem and nimodipine that could reduce the corticosterone secretion elicited a prominent analgesic effect. It seems that there is a negative correlation between the plasma corticosterone levels and the analgesic effect of these calcium channel blockers. Therefore, their influence on corticosterone level could be helpful on induction of its antinociceptive effect. However, 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 corticotroph 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 reported the inhibitory effect of nifedipine (Esmaeili Mahani et al., 2005) and other Ca^{2+} channel blockers e.g. nimodipine or verapamil (Martinez-Pinero et al., 1993) on morphine-induced corticosterone secretion.

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; Chameau et al., 2007), and significantly increase the number of dihydropyridine-binding sites in nervous and non-nervous tissues (Fomina et al., 1996; Takimoto et al., 1997). With elimination of corticosterone through adrenalectomy, one of the factors that affect calcium channel density is removed. However, this possible mechanism needs to be clarified by further investigation.

Our result also suggests the antinociceptive effect of calcium channel blockers verapamil, diltiazem and nimodipine at the spinal and supraspinal levels using tail-flick and hot-plate tests. Wong et al. (1994) reported that another L-type calcium channel blocker, nifedipine, significantly increased HP and TF latencies when administered epidurally to rats. However, the pattern of antinociceptive effects on hot-plate and tail-flick tests was different so that the stronger effect was shown in hot-plate test.

Nociceptive information is processed and integrated peripherally as well as at spinal and supraspinal levels within the central nervous system. In the present study, diltiazem and verepamil had little or no effect in the tail-flick test, a spinally integrated nociceptive reflex, and had modest effects in the hot plate test, a complex response that is supraspinally integrated. Thus, The observed differences indicate that the supraspinal mechanisms are more significant in antinociceptive effects of L-type calcium channel blockers than the spinal mechanisms.

Descending pain inhibitory circuits contribute to the supraspinal control of spinal transmission of nociceptive information. Descending inhibitory circuits include the neuronal connections between the ventrolateral periaqueductal gray and rostral ventral medulla that in turn project to the spinal cord dorsal horn lamina. The electrical stimulation or microinjection of morphine into these regions produces antinociception by inhibiting the responses of dorsal horn neurons to the peripheral noxious stimuli (Fields et al., 1991). The synergic effect of calcium channel blockers and opioid system can be considered as a mechanism for better action of these drugs at the supraspinal level in modulatinon of pain. Activation of opioid receptors inhibits adenylyl cyclase activity via activation of inhibitory G proteins, thus reducing the Ca^{2+} influx, inhibits N-type and L-type calcium channels (Nestler, 2004). In addition, calcium channel blockers potentiate the analgesic effect of opioids (Prado, 2001). Weissman and colleagues (1999) have demonstrated that verapamil evokes antinociception in the mouse hot-plate test and showed that these effects might be due to the agonistic activity of verapamil at μ-, δ- and κ-receptor subtypes.

According to our results nimodipine had a more prominent antinociceptive effect than verapamil and diltiazem. Since, the calcium

channel blockers are classified on the basis of their activation and inactivation kinetics, conductance, ion specificity, and sensitivity to drugs and toxins, drugs in the L-type group may have heterogeneous characteristics (Hara et al., 1998). In fact, each drug has a different effect or potency on the inhibition of aspartate release and neuronal calcium influx (Mangano et al., 1991). These differences support the possibility of different potencies in the antinociceptive effects of L-type calcium channel blockers.

The data indicate that nimodipine was more effective than the remaining blockers in the sham-operated and especially in the ADX rats. It is documented that nimodipine is more lipophilic than other L-type calcium channel blockers thus capable of crossing the blood brain barrier (Ray and Mehra, 2008). Nimodipine have a cerebroselective effect so that it dilates cerebral blood vessels at much lower doses than that require for peripheral vasodilatation (Kazda, 1985). In addition, nimodipine is more effective than many other Ca^{2+} channel blockers in inhibiting $^{45}Ca^{2+}$ uptake by neuroblastoma cells (Park and Azmitia, 1991). Moreover, nimodipine could decrease the release of substance P from neurons of the dorsal root ganglia and also inhibit the release of glutamate from synaptosomes prepared from the cerebral cortex (Ray and Mehra, 2008).

Our results showed that verapamil and diltiazem at doses more than 15 mg/kg had toxic effects in ADX rats. Potential toxic effects include severe hypotension and death. Diltiazem and especially verapamil tend to produce the most hypotension, bradycardia and deaths of the Ca^{+2} channel blockers. Nimodipine and nifedipine are generally less lethal. We found that mortality rate was low or absent in intact and sham-operated rats, whereas it was dramatically increased by adrenalectomy. However, replacement treatments with corticosterone to ADX rats could attenuate Ca^{+2} channel blockers toxicity (our unpublished data). We conclude that the integrity of the hypothalamic–pituitary–adrenal axis is needed to tolerate the toxicity of various L-type calcium channel blockers. Complex mechanisms (Pharmacokinetics or pharmacodynamics or other reason?) appear to underlie such toxicity and need to be clarified by further studies. The opposite action of corticosterone and Ca^{+2} blockers on Ca^{+2} channels can be considered as one of the reasons for such toxicity so that in the absence of corticosterone, channel blockage is more potent especially in the structures that are involved in cardiovascular performance. Interruption of calcium fluxes leads to decreased intracellular calcium producing cardiovascular dysfunction that, in the most severe situations, results in cardiovascular collapse (DeWitt and Waksman, 2004).

Previously 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., 2005, 2006, 2007, 2008). According to present data HPA axis also involves in the antinociceptive effect of verapamil, diltiazem and nimodipine. Therefore, it seems that there is a general interaction between HPA axis factors especially its glucocorticoids and calcium channels which could affect other therapeutic effects of calcium channel blockers. However, this phenomenon needs to be clarified by further investigations.

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