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Involvement of hypothalamic pituitary adrenal axis on the analgesic cross-tolerance between morphine and nifedipine

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Abstract

Bidirectional cross-tolerance develops between opioids and Ca^{2+} channel blockers relating to their antinociceptive effects; however, the role of hypothalamic pituitary adrenal (HPA) axis on this action has not been elucidated yet. We examined the analgesic cross-tolerance between morphine and nifedipine, a dihydropyridine calcium channel blocker, in intact and adrenalectomized (ADX) rats and also evaluated modification of HPA activity during this phenomenon. The tail-flick test was used to assess the nociceptive threshold. The plasma level of corticosterone, as a marker of HPA function, was measured by radioimmunoassay. Our results showed that, in sham operated rats which were chronically treated with morphine, nifedipine failed to affect nociceptive threshold but it could induce significant antinociceptive effect in ADX morphine treated animals. This effect was reversed by corticosterone replacement. Furthermore, morphine could not induce analgesic effect either in sham operated or in ADX animals that received chronic nifedipine. Chronic morphine inhibited the effect of nifedipine on corticosterone secretion but nifedipine treatment had no effect on morphine-induced corticosterone secretion. Based on these results, we can conclude that HPA axis is involved in the induction of cross-tolerance between morphine and nifedipine due to chronic morphine and not nifedipine treatment.

Keywords: Morphine; Nifedipine; Analgesia; Cross-tolerance; HPA axis; Corticosterone

1. Introduction

For centuries, opioids have been used for the treatment of pain. However, their prolonged administration for chronic pain produces tolerance to the analgesic effects requiring escalating doses that are associated with side effects such as respiratory depression, and thus limiting their therapeutic potential. A lot of work has been dedicated to identify molecular mechanisms of tolerance and now it is well admitted that alteration in the Ca^{2+} homeostasis, that is defined as an increase of dihydropyridine Ca^{2+} channel density and also basal free intracellular Ca^{2+} concentration after sustained agonist activation, is closely connected to this phenomenon (Ramkumar and El-Flakani, 1984, 1988; Welch and Olson, 1991; Diaz et al., 1995, 2000).

Calcium channel antagonists prevent the development of opioid tolerance and also attenuate the signs of physical dependence (Contreras et al., 1988; Antkiewicz-Michaluk et al., 1993; Michaluk et al., 1998; Smith et al., 1999; Dogrul et al., 2005). Furthermore, calcium channel blockers have been found to potentiate the analgesic effect of morphine (Assi, 2001; Dogrul et al., 2001; Shimizu et al., 2004; Esmaeili Mahani et al., 2005b). Despite these beneficial interactions, chronic nifedipine treatment and subsequent withdrawal, decreases the analgesic effect of morphine (Piepponen et al., 1999). Previous study has also demonstrated that the analgesic effect of nifedipine, a dihydropyridine calcium channel blocker, is reduced in morphine pretreated animals (Ohnishi et al., 1988). Taken together, these data suggest that bidirectional cross-tolerance develops

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between opioids and Ca^{2+} channel blockers in their analgesic effect.

Not only opioids (Buckingham and Cooper, 1986; Gonzalvez et al., 1991; Little and Kuhn, 1995; Coventry et al., 2001; Nock et al., 1998, 2005) but also Ca^{2+} and related channels, particularly dihydropyridine channels, are important regulatory factors participating in the control of HPA axis function (Matsuki et al., 1996; Mamczarz et al., 1999; Robidoux et al., 2000). Furthermore, *in vitro* studies show that there is an interaction between corticosterone and dihydropyridine calcium channels. Glucocorticoids can potentiate Ca^{2+} influx and accelerate the release of Ca^{2+} from intracellular stores, and also corticosterone can increase Ca^{2+} entry through the high voltage activated (L-type) calcium channels (Nair et al., 1998; Zhou et al., 2000; Karast et al., 2002; Machida et al., 2003; Sun et al., 2004; Chameau et al., 2007). We previously reported that hypothalamic pituitary adrenal axis and its glucocorticoids have an important role in the effect of nifedipine on nociception, on morphine analgesia and tolerance (Esmaeili Mahani et al., 2005a,b, 2006). Since both chronic morphine and chronic nifedipine could affect HPA activity the present study was designed to analyze the contribution of hypothalamic pituitary adrenal axis and its glucocorticoids to the analgesic cross-tolerance between nifedipine and morphine and also to evaluate modification in the activity of HPA axis during treatment with these drugs.

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 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 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 initially dissolved in dimethyl sulfoxide (DMSO) and diluted with saline. The percentage of DMSO and saline in the final volume were 60% and 40% respectively. These drugs were given a volume of 1 ml/kg, i.p. Corticosterone (Sigma, USA) was dissolved in absolute ethanol then combined with saline, yielding a 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 the mean of these trials 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 and 120 min after injection. The tail-flick latencies were converted to the percentage of antinociception according to the following formula:

$$\%\ \text{Maximal Possible analgesic effect (\%MPE)} = (\text{Reaction time of test} - \text{Basal reaction time}) \div (\text{Cut off time} - \text{Basal reaction time}).$$

2.4. 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 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 a 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 percentage of ethanol in each drinking solution was 0.2%. The amount of drinking solution consumed by each rat was analyzed to determine whether there were any group differences. This procedure resulted in a plasma corticosterone level in adrenalectomized rats which was close to the sham operated animals (Esmaeili Mahani et al., 2005b).

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 1000 ×*g* for 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. Experimental design

To induce analgesic cross-tolerance between morphine and nifedipine, two sets of experiments were performed. First,

morphine was given chronically (7 mg/kg i.p. once daily for 10 days) and on the 11th day the analgesic effect of 10 mg/kg nifedipine was evaluated in saline or morphine treated groups. In the second set, nifedipine (10 mg/kg i.p.) was administrated once daily for 10 days. The rats were given morphine (3 mg/kg), 24 h after the last vehicle or nifedipine administration, and nociceptive threshold was measured. The experiments were performed in both sham operated and ADX animals. To evaluate modification in the activity of HPA axis, drugs were given according to the same schedule as mentioned above in the sham operated rats. Animals were randomly sacrificed in the last day of experiment for measurement of plasma corticosterone concentration.

2.8. Statistical analysis

The results are expressed as mean±S.E.M. 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–Keul's test. $P<0.05$ was considered significant.

3. Results

3.1. The basal tail-flick latency in experimental groups

Table 1, shows the basal tail-flick latency in sham operated and adrenalectomized animals that received chronic morphine, nifedipine and their vehicles. Baseline tail-flick latency in sham operated animals (control animals) was 2.76±0.14 s. This value did not differ significantly from groups which were chronically treated with saline, morphine, nifedipine and vehicle [$F_{(4,35)}=0.449$, $P=0.7724$].

In adrenalectomized animals the baseline tail-flick latency was 3.01±0.19 s which was not significantly different from the adrenalectomized animals that received chronic drug or vehicles [$F_{(4,35)}=1.381$, $P=0.2606$].

3.2. The antinociceptive effect of nifedipine after chronic morphine treatment in the presence or absence of adrenal glands

Nifedipine (10 mg/kg) induced an antinociceptive effect in sham operated animals that received saline for 10 days [$F_{(4,30)}=5.600$, $P=0.0017$]. However, in the group that was chronically treated with 7 mg/kg morphine, it failed to affect nociceptive threshold [$F_{(4,28)}=1.599$, $P=0.2022$] (Fig. 1). In ADX animals, nifedipine could induce significant antinociceptive effect in both saline and morphine treated groups [$F_{(4,25)}=16.068$, $P=0.0001$] (Fig. 1).

Table 1
The basal tail-flick latency in intact and groups that received chronic morphine, nifedipine and their vehicles

	The basal tail-flick latency (s)	
	Sham operated	Adrenalectomized
Intact	2.76±0.14	3.01±0.19
Saline treated	2.70±0.12	3.09±0.17
Morphine treated	2.69±0.14	2.55±0.17
Vehicle treated	2.65±0.11	3.17±0.28
Nifedipine treated	2.88±0.16	3.15±0.25

Values represent mean±SEM ($n=8$).

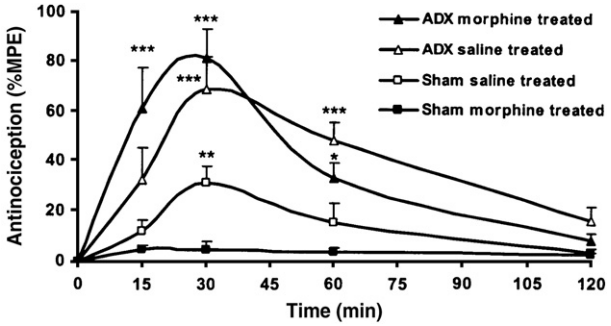

Fig. 1. The antinociceptive effect of 10 mg/kg nifedipine on day 11 in adrenalectomized (ADX) and sham operated (Sham) animals that received morphine (7 mg/kg) and saline for 10 days. Values are mean±S.E.M. *$P<0.05$ **$P<0.01$ ***$P<0.001$ significantly different versus before drug administration ($n=6$–8 rats per group).

As shown in Fig. 2, the analgesic effect of 10 mg/kg nifedipine was at a reduced level in the sham morphine treated group relative to sham saline treated rats 30 min after injection ($P<0.05$). In adrenalectomized rats, antinociceptive response of 10 mg/kg nifedipine was potentiated significantly 30 min after injection and there was no significant difference between saline and morphine pretreated animals. In adrenalectomized rats that had corticosterone in drinking solution (ADX+CORT) the antinociceptive effect of nifedipine was reduced due to the previous morphine treatment ($P<0.05$). This outcome was similar to the sham operated group (Fig. 2).

Not only in sham operated but also in adrenalectomized animals, the analgesic effect of morphine was significantly decreased due to chronic administration of morphine (7 mg/kg i.p. once daily for 10 days). The development of tolerance to anal1-gesic effect of morphine appears in adrenalectomized rats similar to the sham operated animals (data not shown).

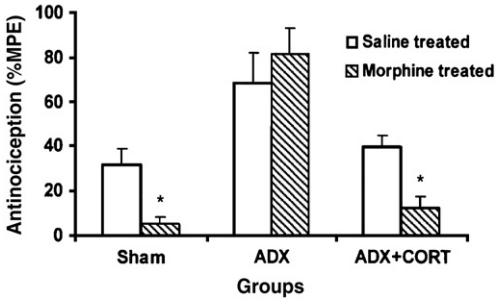

Fig. 2. The analgesic effect of nifedipine 30 min after injection in sham operated (Sham), adrenalectomized (ADX) and adrenalectomized rats that received corticosterone in drinking solution (ADX+CORT). Values represent mean±S.E.M ($n=6$–8 rats per group). *$P<0.05$ as compared with saline treated animals.

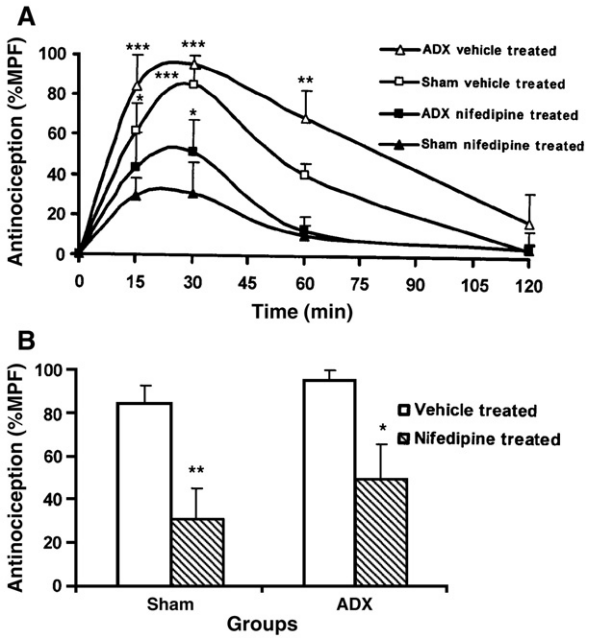

Fig. 3. (A) The antinociceptive effect of 3 mg/kg morphine on day 11 in adrenalectomized (ADX) and sham operated (Sham) animals that received nifedipine (10 mg/kg) and vehicle for 10 days. (B) Comparison of the analgesic effect of 3 mg/kg morphine, 30 min after injection, in groups that received nifedipine (10 mg/kg) or vehicle for 10 days. Values are mean±S.E.M. *$P<0.05$, **$P<0.01$ and ***$P<0.001$ significantly different versus before drug administration (A) *$P<0.05$ and **$P<0.01$ as compared with vehicle treated animals (B) ($n=6$–8 rats per group).

3.3. The antinociceptive effect of morphine after chronic nifedipine pretreatment in the presence or absence of adrenal glands

As it is shown in Fig. 3A, morphine (3 mg/kg) produced an analgesic response that reached a peak 30 min and lasted 120 min after injection in vehicle treated sham operated animals [$F_{(4,25)}=12.791$, $P=0.0001$]. However, morphine had no such antinociceptive effect in nifedipine pretreated animals [$F_{(4,25)}=2.675$, $P=0.0552$].

In adrenalectomized rats, after chronic treatment with vehicle, morphine (3 mg/kg) could induce a significant analgesic response peaked 30 min after injection [$F_{(4,30)}=17.557$, $P=0.0001$] but it showed a moderate but significant antinociception only 30 min after injection in rats which received chronic nifedipine [$F_{(4,29)}=4.062$, $P=0.0098$] (Fig. 3A).

As shown in Fig. 3B, the analgesic effect of morphine, 30 min after injection, in both sham and ADX animals was significantly decreased due to previous nifedipine treatment [($P<0.01$) and ($P<0.05$) respectively].

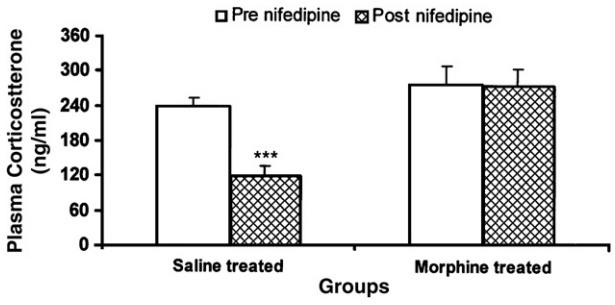

Fig. 4. Plasma corticosterone concentration before and 30 min after injecting nifedipine on day 11 in rats that received saline or morphine for 10 days. Each bar represents mean±S.E.M. ($n=8$) ***$P<0.001$.

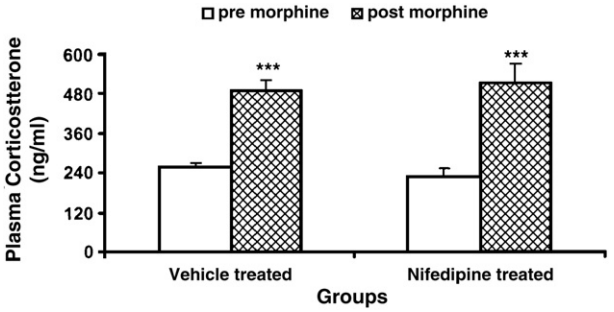

Fig. 5. Plasma corticosterone concentration before and 30 min after injecting morphine on day 11 in rats that received vehicle as control group, and nifedipine for 10 days. Each bar represents mean±S.E.M. Asterisks indicates significant difference with corresponding predrug situation. ***$P<0.001$ ($n=8$).

3.4. The effect of nifedipine and morphine on HPA function

In this part of study, the changes in HPA activity upon interaction with either morphine or nifedipine treatment was investigated. Our results show that corticosterone level in chronic saline or morphine treated groups was not statistically different (238.1±15.5 versus 278.08±31.6 respectively). Also, these levels were not significantly different from the control untreated group (245.6±28.8). As shown in Fig. 4, 30 min after nifedipine injection, a significant decrease in corticosterone concentration was found in chronic saline treated group as compared with before nifedipine injection ($P<0.001$) but in group which received chronic morphine, nifedipine could not change the corticosterone level.

Fig. 5, depicts plasma corticosterone concentration before and 30 min after morphine injection on day 11, in rats that received nifedipine or vehicle for 10 days. Our data show that chronic treatment with vehicle or nifedipine had no effect on corticosterone level compared to control animals ($P>0.05$).

Administration of morphine in both vehicle ($P<0.001$) and nifedipine treated ($P<0.001$) groups produced a significant increase in plasma level of corticosterone, 30 min after injection.

4. Discussion

Our results showed that the antinociceptive response of nifedipine or morphine is reduced due to chronic treatment of morphine and nifedipine respectively. In other words, a bidirectional analgesic cross-tolerance was developed between morphine and nifedipine. In adrenalectomized rats, chronic nifedipine attenuated the antinociceptive effect of acute morphine while chronic morphine did not affect nifedipine analgesia indicating that cross-tolerance was not developed in ADX

animals due to chronic administration of morphine, while it was induced after chronic nifedipine administration.

Several studies indicate that dihydropyridine Ca^{2+} channels are increased in animals which were injected chronically with morphine (Ramkumar and El-Flakani, 1984, 1988; Diaz et al., 1995, 2000). In addition, 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).

Inhibitory interaction between opioid receptors and voltage-dependent calcium channels have been demonstrated by electrophysiological and biochemical methods (Attali et al., 1989; Wilding et al., 1995). It is well known that opioids modulate not only N, P/Q calcium channels (Wu et al., 2004) but also L-type channels (Piros et al., 1995, 1996; Przewlocki et al., 1999; Xiao et al., 2005). Therefore, it seems that there is a common site of interaction between dihydropyridine calcium channel blockers and morphine for regulation of pain sensitivity which would appear, in turn, to induce an analgesic cross-tolerance.

Evidence exists to show that 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). Therefore, the effect of glucocorticoids, morphine and nifedipine on dihydropyridine calcium channel density appears to be synergistic. It is well known that morphine stimulates the hypothalamic pituitary adrenal axis in rodents and increases plasma corticosterone levels (Gonzalvez et al., 1991; Little and Kuhn, 1995; Coventry et al., 2001; Nock et al., 2005). Since, nifedipine had an antinociceptive property in morphine adrenalectomized rats and did not have this property in the morphine treated sham animals, it seems that one of the routes for Ca^{2+} channel up-regulation following chronic morphine is through morphine effects on HPA activity. Our data indicate that the mechanism underlying the modulation of dihydropyridine Ca^{2+} channels by morphine involves mediation, at least in part, by the effect of morphine on HPA function.

Since the rate of morphine tolerance in sham operated and ADX animals can influence the responsiveness to nifedipine therefore, it is important to know if tolerance to morphine itself developed at the same rate in ADX and sham operated rats. Our results not only in this work but also in our previous research (Esmaeili mahani et al., 2005a) showed that the development of tolerance to analgesic effect of morphine appears in adrenalectomized rats similar to the sham operated animals.

Our results show that nifedipine has a suppressive effect on corticosterone level but in morphine treated rats nifedipine failed to affect corticosterone secretion (Fig. 4). Hence it would appear that a neurosecretory cross-tolerance involving nifedipine and morphine had developed.

It is documented that corticosterone can increase Ca^{2+} entry through the high voltage activated (L-type) calcium channels which is opposite in effect compared to nifedipine-induced Ca^{2+} channel blockage (Zhou et al., 2000; Karast et al., 2002; Machida et al., 2003; Sun et al., 2004). We previously showed that the effect of nifedipine on corticosterone level could be helpful on induction of its antinociceptive effect (Esmaeili Mahani et al., 2006). Therefore, the elimination of nifedipine effect on the HPA axis following chronic usage of morphine (neurosecretory cross-tolerance) could be one possible reason in reduction in nifedipine-induced antinociception (analgesic cross-tolerance). This potential mechanism does, however, require further study.

In 1999 Piepponen et al., showed that cross-tolerance develops between opioids and Ca^{2+} channel antagonists selectively to their nociceptive effects (Piepponen et al., 1999). However, our data demonstrate that cross-tolerance could also develop between morphine and nifedipine in their neurosecretory effect on HPA function.

This study shows that the analgesic effect of morphine, in both sham and ADX animals declined due to repeated administration of nifedipine (Fig. 3A). Thus, the HPA axis has an important role in the reduction of analgesic effect of nifedipine due to chronic usage of morphine. However, under conditions of chronic nifedipine administration, the HPA axis was not found to be involved in morphine analgesic reduction.

According to our results morphine had a stimulatory effect on HPA function in both vehicle and nifedipine treated animals (Fig. 5). Since, chronic administration of nifedipine had no effect on morphine-induced corticosterone secretion, it can therefore be inferred that changes in dihydropyridine Ca^{2+} channel density could not influence the effect of morphine on HPA function. This finding would suggest that dihydropyridine Ca^{2+} channel up-regulation is not responsible for the neurosecretory cross-tolerance due to chronic nifedipine. This phenomenon is similar to morphine neurosecretory tolerance which has been reported previously (Esmaeili Mahani et al., 2005a).

The present data demonstrated bi-directional cross-tolerance to antinociceptive effect of morphine and nifedipine but mono-directional cross-tolerance to neurosecretory effects of these drugs on HPA function. It is well known that morphine and nifedipine have similar effects on nociception and both of them can induce analgesia. Pharmacological studies have demonstrated that modulation of Ca^{2+} influx through L-type calcium channels can potentiate the analgesic effect of morphine (Dogrul et al., 2001; Shimizu et al., 2004). In contrast, morphine and nifedipine have apposite effects (stimulatory and suppressive, respectively) on corticosterone secretion. Several reports indicate that acute treatment with L-type calcium channels attenuates morphine-induced corticosterone secretion (Martinez-Pinero et al., 1993; Esmaeili Mahani, 2005b). Taken together, these findings may indicate a common pathway or similar calcium-dependent mechanisms involved in the antinociceptive effects of morphine and nifedipine as well as in the development of analgesic cross-tolerance between these drugs but a different pathway in the induction of neurosecretory cross-tolerance. Further studies are necessary to elucidate this matter.

In summary, our results show that a bidirectional analgesic cross-tolerance occurs between morphine and nifedipine in their antinociceptive effects, and hypothalamus pituitary adrenal axis is involved in the induction of analgesic cross-tolerance between these drugs due to chronic morphine and not nifedipine

treatment. In addition, a neurosecretory cross-tolerance was developed to inhibitory effect of nifedipine on HPA axis due to chronic administration of morphine.

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