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# Nifedipine suppresses morphine-induced thermal hyperalgesia: Evidence for the role of corticosterone

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**Abstract**

It has been shown that systemic administration of morphine induced a hyperalgesic response at an extremely low dose. We have examined the effect of nifedipine, as a calcium channel blocker, on morphine-induced hyperalgesia in intact and adrenalectomized rats and on hypothalamic–pituitary–adrenal axis activity induced by ultra-low dose of morphine. To determine the effect of nifedipine on hyperalgesic effect of morphine, nifedipine (2 mg/kg i.p. and 10 μg i.t.) that had no nociceptive effect, was injected concomitant with morphine (1 μg/kg i.p. and 0.01 μg i.t. respectively). The tail-flick test was used to assess the nociceptive threshold, before and 30, 60, 120, 180, 240 and 300 min after drug administration. The data showed that low dose morphine systemic administration could produce hyperalgesic effect in adrenalectomized rats equivalent to sham-operated animals while intrathecal injection of morphine only elicited hyperalgesia in sham-operated animals. Nifedipine could block morphine-induced hyperalgesia in sham and adrenalectomized rats and even a mild analgesic effect was observed in the adrenalectomized group which was reversed by corticosterone replacement. Systemic administration of low dose morphine produced significant increase in plasma level of corticosterone. Nifedipine has an inhibitory effect on morphine-induced corticosterone secretion. Thus, the data indicate that dihydropyridine calcium channels are involved in ultra-low dose morphine-induced hyperalgesia and that both the pattern of morphine hyperalgesia and the blockage of it by nifedipine are modulated by manipulation of the hypothalamic pituitary adrenal axis.


*Keywords:* Morphine; Hyperalgesia; L-type calcium channel; Nifedipine; Corticosterone

## 1. Introduction

Opioids are the drugs of choice for the treatment of moderate to severe acute and chronic pain. Besides their well-known analgesic activity, numerous clinical and laboratory reports exist demonstrating that opioids can enhance sensitivity to noxious stimuli (Laulin et al., 1998, 2002; Guignard et al., 2000; Celerier et al., 2000; Crain and Shen, 2001; Angst et al., 2003; Compton et al., 2003; Van Elstraete et al., 2005).

Morphine, at higher doses, induces analgesia via Pertussis toxin-sensitive inhibitory G protein ($G_{\alpha i}$), inhibits cAMP formation and $Ca^{2+}$ conductance, and activates $K^+$ conductance, leading to hyperpolarization of cells and exerting an inhibitory effect (Nestler, 2004). In extremely low doses, morphine could elicit acute hyperalgesia in animal models of pain. The exact mechanism(s) underlying opioid-induced hyperalgesia has not been clarified. Since cholera toxin blocked the opioid-induced hyperalgesia, it was suggested that the excitatory effect may be due to coupling of opioid receptors to stimulatory $G_{\alpha s}$ proteins (Crain and Shen, 2001). Other investigators have reported that

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acute opioid hyperalgesia is mediated by the phosphoinositide-signaling pathway (Galeotti et al., 2006).

It is well known that opioids can affect the hypothalamic–pituitary–adrenal axis, exerting a stimulatory effect in rodents, which has been repeatedly demonstrated by elevation of corticosterone levels (Buckingham and Cooper, 1984; Milanes et al., 1993; Pirnik et al., 2001; Esmaeili Mahani et al., 2005a). In addition, corticosterone and other glucocorticoids suppress the analgesic effect of morphine (Capasso et al., 1994; Takenaka et al., 2003). It has been reported that adrenalectomy significantly potentiates morphine-induced analgesia (Miyamoto et al., 1989, 1990; Candido et al., 1992; Esmaeili Mahani et al., 2005b).

Furthermore, calcium channel blockers potentiate the analgesic effect of morphine (Assi, 2001; Dogrul et al., 2001; Shimizu et al., 2004; Casey et al., 2006). We previously indicated that the mechanism underlying the potentiation of morphine analgesia by nifedipine involves mediation, at least in part, by attenuating the effect of morphine on corticosterone levels (Esmaeili Mahani et al., 2005b). However, the role of the L-type calcium channels in low-dose morphine-induced thermal hyperalgesia has not been elucidated.

In the present report, we determined the effect of nifedipine, as a calcium channel blocker, on morphine-induced hyperalgesia in sham-operated and adrenalectomized rats and also evaluated modifications in the plasma concentration of corticosterone during treatments with ultra-low dose of morphine in the presence of nifedipine.

## 2. Materials and methods

### 2.1. Animals

All experiments were carried out on male Wistar rats, weighing 200–250 g, that were housed four per cage under a 12 h light/dark cycle in a room with controlled temperature (22 ± 1 °C). Food and water were available *ad libitum* except in adrenalectomized rats (the adrenalectomized rats were maintained on 0.9% NaCl drinking solution). 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 nonspecific stress responses. Rats were divided randomly into several experimental groups, each comprised of 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 initially dissolved in dimethyl sulfoxide (DMSO) and diluted with saline. The percentages of DMSO and saline in the final volume were 60% and 40% respectively. These drugs were given in a volume of 1 ml/kg (i.p.) and in a total volume of 10 μl (i.t.). Corticosterone (Sigma, USA) was dissolved in absolute ethanol and 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 intensity of the beam was adjusted to produce a mean control reaction time between 4 and 6 s. The cut-off time was fixed at 15 s in order to avoid any damage to the tail. In this manner, we were able to reveal potential, subtle alternations that may occur in basal thermal nociception. The tail-flick latency for each rat was determined three times and mean was designated as baseline latency before drug injection. After determination of baseline latencies, rats received drugs, and the reaction latencies were determined 30, 60, 120, 180, 240 and 300 min after injection. Experimentally-induced increases in control tail-flick latency provide a measure of antinociceptive or analgesic effect, whereas decreases in tail-flick latency provide an indication of hyperalgesic effect.

### 2.4. Intrathecal catheter implantation and drug delivery

Animals were anesthetized with ketamine (50 mg/kg) and xylazine (5 mg/kg) i.p. An intrathecal catheter (PE-10) was implanted in each rat according to a previously published method (Yaksh and Rudy, 1976). Animals that exhibited neurological deficits (e.g. paralysis) after the catheter implantation or during drug delivery were excluded from the experiments.

### 2.5. Adrenalectomy

Adrenalectomy was performed under the same surgical conditions mentioned for catheterization and 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. In some animals the catheterization was performed at the same time. 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 a final concentration of

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

| Groups | Plasma corticosterone concentration (ng/ml) |
|---|---|
| Sham | 245.6 ± 28.8 |
| ADX | Undetectable |
| ADX + CORT | 224.3 ± 27.4 |

Values represent mean ± SEM ($n = 8$).

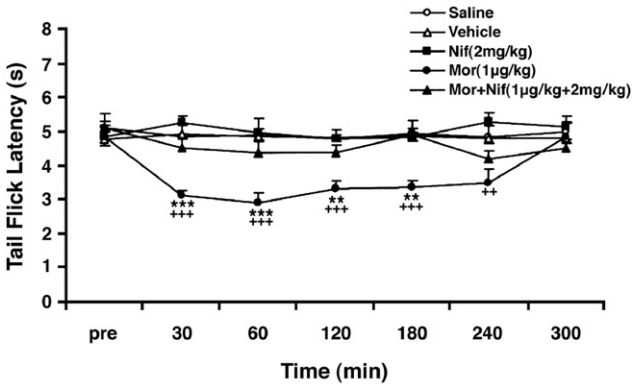


Fig. 1. The effect of 1 μg/kg (i.p.) morphine and morphine concomitant with nifedipine (2 mg/kg i.p.) on nociceptive threshold in sham-operated animals. Values represent mean±S.E.M ($n$=8). $^{++}P<0.01$, $^{+++}P<0.001$ significantly different versus before drug administration. $^{**}P<0.01$, $^{***}P<0.001$ versus the other groups at the same time.

100 μg/ml of drinking solution (continuously from the time of adrenalectomy). The percentage of ethanol in each drinking solution was 0.2%. With this treatment, plasma corticosterone levels were close to the sham-operated animals.

### 2.7. Corticosterone assay

On experimental days, rats were killed, 30 min after drug treatment, by 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 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.

### 2.8. Statistical analysis

The results are expressed as mean±S.E.M. The difference in mean tail-flick latency 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 ($P<0.05$).

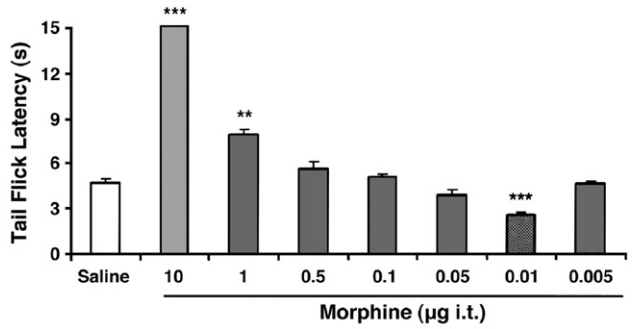


Fig. 2. Effect of decreasing morphine doses on the tail-flick latency, 30 min after intrathecal injection, in sham-operated animals. Vertical bars represent mean±S.E.M ($n$=8). $^{**}P<0.01$, $^{***}P<0.001$ in comparison with saline treated group.

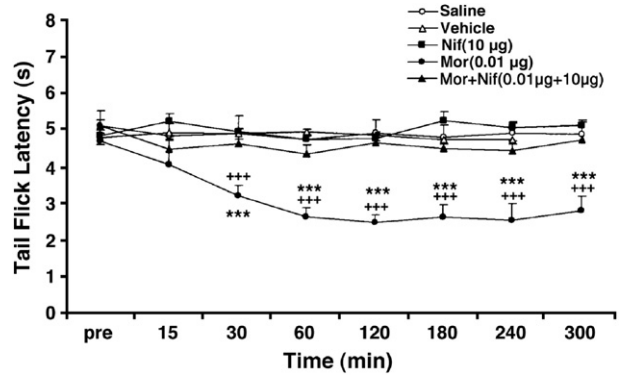


Fig. 3. The effect of 0.01 μg (i.t.) morphine and morphine concomitant with nifedipine (10 μg i.t.) on nociceptive threshold in sham-operated animals. Values represent mean±S.E.M ($n$=8). $^{+++}P<0.001$ significantly different versus before drug administration. $^{***}P<0.001$ versus the other groups at the same time.

## 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 adrenalectomized animals compared with sham-operated animals (245.6±28.8 ng/ml). In adrenalectomized rats that had corticosterone replaced in their drinking water, the plasma corticosterone concentration was not different from sham-operated animals (224.3±27.4 ng/ml) ($P>0.05$).

### 3.2. The effect of nifedipine on the hyperalgesic effect of morphine in the presence or absence of adrenal glands

As is shown in Fig. 1, morphine (1 μg/kg i.p.) produced a hyperalgesic response in sham-operated animals. The maximum hyperalgesic effect was reached at 30 min after injection and

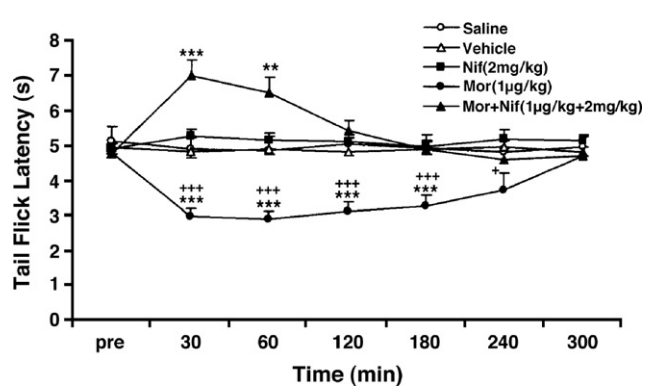


Fig. 4. The effect of intraperitoneal injection of morphine and morphine concomitant with nifedipine on nociceptive threshold in adrenalectomized animals. Values represent mean±S.E.M ($n$=8). $^{+}P<0.05$, $^{++}P<0.01$, $^{+++}P<0.001$ significantly different versus before drug administration. $^{***}P<0.001$ as compared with other groups at the same time.

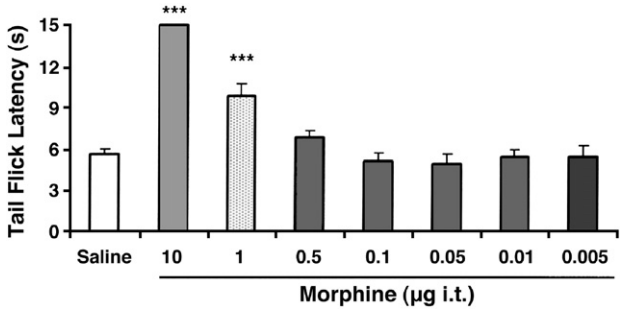


Fig. 5. Effect of decreasing morphine doses on tail-flick latency, 30 min after intrathecal injection, in adrenalectomized rats. Vertical bars represent mean±S.E.M ($n$=8). ***$P$<0.001 significantly different versus saline treated group.

persisted almost unchanged up to 180 min and then diminished. Administration of saline, Vehicle, and nifedipine (2 mg/kg i.p.) did not show a nociceptive response. Nifedipine (2 mg/kg) co-administration completely blocked the hyperalgesic effect of 1 µg/kg morphine (Fig. 1).

Fig. 2 shows the effect of intrathecal morphine at different doses on tail-flick latency, 30 min after injection, in sham-operated animals. Administration of 10 µg morphine elicited a potent analgesic effect. Morphine (1 µg i.t.) induced a moderate analgesia, whereas, doses ranging between 0.5 and 0.05 did not show any effect on the nociceptive threshold. A hyperalgesic effect was observed in animals that received 0.01 µg morphine. Dose 0.005 µg could not elicit any nociceptive response. As is shown in Fig. 3, morphine (0.01 µg i.t.) produced a hyperalgesic response which appeared 30 min after injection, reaching a peak 60 min after injection and persisting almost unchanged up to 300 min (hyperalgesia lasted for approximately 36 h after morphine injection). Nifedipine (10 µg i.t.) administration completely blocked the hyperalgesic effect of 0.01 µg morphine. Administration of saline, Vehicle, and nifedipine (10 µg i.t.) did not show any nociceptive response.

In adrenalectomized rats, administration of morphine (1 µg/kg i.p.) produced a hyperalgesic effect equivalent to sham-operated animals that reached a peak 30 min after injection and lasted for

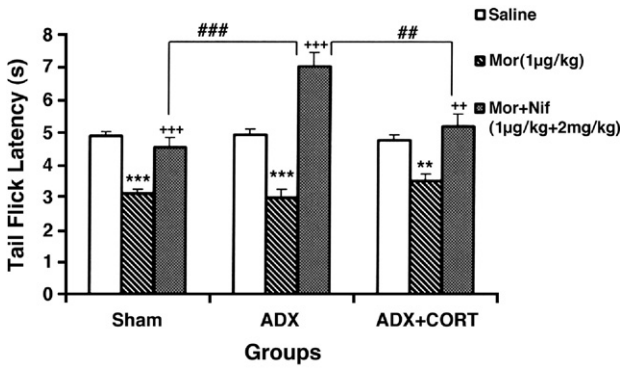


Fig. 6. The effect of saline, morphine and morphine concomitant with nifedipine on nociceptive threshold, 30 min after intraperitoneal injection, in sham-operated (Sham), adrenalectomized (ADX) and adrenalectomized rats that received corticosterone in drinking solution (ADX+CORT). Values represent mean±S.E.M ($n$=6–8 rats per group). **$P$<0.01, ***$P$<0.001 as compared with saline in the same group. $^{++}P$<0.01, $^{+++}P$<0.001 as compared with morphine in the same group. $^{\#\#}P$<0.01, $^{\#\#\#}P$<0.001.

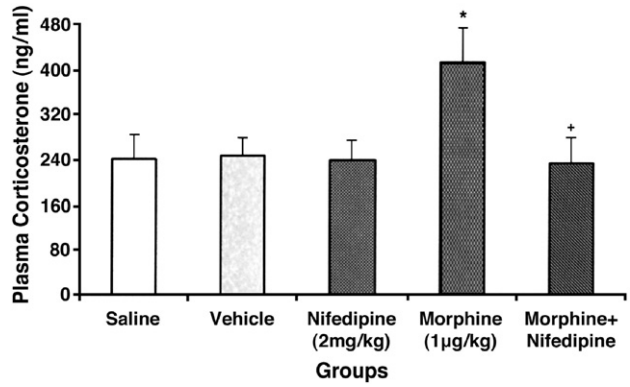


Fig. 7. Plasma corticosterone concentration 30 min after injecting either morphine (1 µg/kg i.p.) or morphine concurrently with nifedipine (1 µg/kg+2 mg/kg i.p.). Each bar represents mean±S.E.M ($n$=7–8 rats per group). Asterisk indicates significant differences from saline, vehicle and nifedipine treated groups, *$P$<0.05. Cross indicates significant differences from morphine treated group, $^{+}P$<0.05.

about 240 min. In the presence of nifedipine, the hyperalgesic effect of morphine was reversed and a small antinociceptive response was observed (Fig. 4). Adrenalectomized rats that received saline, vehicle, and nifedipine did not show any antinociceptive or hyperalgesic response. Intrathecal administration of morphine at different doses did not show a hyperalgesic effect (Fig. 5). Injection of morphine (0.01 µg i.t.) elicited a hyperalgesic property in adrenalectomized animals that had corticosterone replacement in their drinking water. The hyperalgesic effect of morphine in both i.t. and i.p. groups was also completely blocked by naloxone (data not shown).

The effect of morphine and morphine accompanied with nifedipine on nociceptive threshold, 30 min after injection, in sham-operated and adrenalectomized animals showed that the hyperalgesic effect of 1 µg/kg morphine in adrenalectomized rats is the same as sham-operated rats. Nifedipine completely abolished morphine-induced hyperalgesia in sham-operated animals and returned the nociceptive threshold to a value similar to control and vehicle-treated rats. In adrenalectomized group systemic morphine-induced hyperalgesia was reversed by nifedipine and surprisingly a mild antinociceptive effect was induced. However, corticosterone replacement significantly

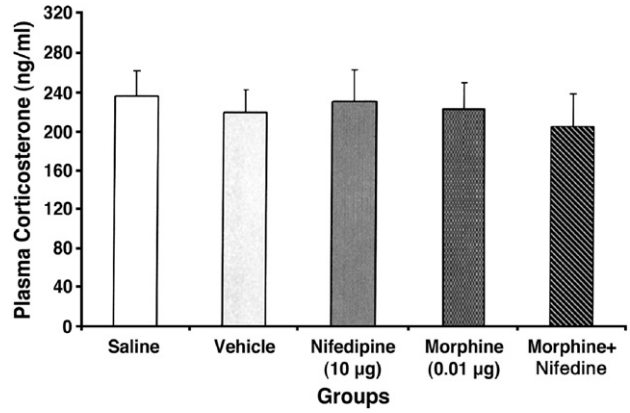


Fig. 8. Plasma corticosterone concentration 30 min after intrathecal injection of either morphine (0.01 µg) or morphine concurrently with nifedipine (0.01 µg+10 µg). Each bar represents mean±S.E.M ($n$=7–8 rats per group).

reversed these effects to a level similar to that of the sham-operated group (Fig. 6).

### 3.3. The effect of nifedepine on corticosterone responses to morphine

In this part of study, we investigated changes in corticosterone concentration upon acute exposure to ultra-low dose of morphine, as well as the contribution of nifedipine on this effect. As shown in Fig. 7, acute administration of 1 μg/kg i.p. morphine produced a significant increase in plasma level of corticosterone (412.3 ± 60.2) 30 min after injection as compared to the saline treated group (241.7 ± 42.5). Administration of nifedipine (2 mg/kg i.p.) with morphine completely blocked morphine-induced corticosterone secretion. Injection of nifedipine or vehicle had no significant effect on plasma corticosterone concentration ($P > 0.05$).

Fig. 8 depicts plasma corticosterone concentration 30 min after intrathecal injection of morphine alone, and morphine together with nifedipine or vehicle in sham-operated animals. Our data shows that plasma corticosterone levels were not affected by these treatments.

## 4. Discussion

Although it has been shown that co-administration of calcium channel blockers with morphine potentiates the analgesic effect of morphine and this phenomenon involves mediation, at least in part by attenuating the effect of morphine on hypothalamic pituitary adrenal axis but the effect of dihydropyridine calcium channel blockers on morphine-induced hyperalgesia and the role of adrenal glands and their corticosteroids in these effects have not yet been identified.

Our results showed that low dose morphine systemic administration could elicit hyperalgesic property in both sham-operated and adrenalectomized animals, while intrathecal injection of morphine only elicited hyperalgesia in sham-operated rats. Nifedipine completely reversed the hyperalgesic effect of morphine.

The interactions between opioid receptors and voltage-dependent calcium channels have been demonstrated by electrophysiological and biochemical methods (Attali et al., 1989). The effects of opioids on calcium channels have been controversial. Both inhibition (Johnson et al., 2006) and activation (Lorentz et al., 1988; Chen et al., 2000; Xiao et al., 2005) have been reported.

It is documented that mu-opioid receptors couple to L-type $Ca^{2+}$ channels (Piros et al., 1995, 1996). Morphine could prolong action potential duration by increasing L-type $Ca^{2+}$ current (Xiao et al., 2005). In addition, lower concentrations (1–10 nM) of mu-opioid agonists elicit excitatory effects, i.e. prolongation of the calcium component of the action potential in the sensory neurons (Shen and Crain, 1989).

As mentioned above, opioids in ordinary (analgesic) doses have an inhibitory effect on calcium channels, but could activate these channels in very low doses which elicit a hyperalgesic property. Therefore, it seems logical that nifedipine could block morphine-induced hyperalgesia and also potentiate morphine analgesia.

Galeotti et al. (2006) found that activation of *N*-methyl-d-aspartate receptors involves in the hyperalgesic effect of low dose morphine. In addition it is documented that nifedipine can eliminate the effect of *N*-methyl-d-aspartate on L-type voltage-dependent calcium channels in the neuronal cell culture (Chaban et al., 2004). Therefore, it appears that nifidipine can inhibit morphine hyperalgesia by closing calcium channels and indirectly by elimination of NMDA receptor function. However, this possible mechanism needs to be clarified by further investigations.

Dogrul et al. (2005) also found that blockade of L-type calcium channels can prevent the tactile hypersensitivity and thermal hyperalgesia produced following chronic morphine.

The present study shows that adrenal glands are not involved in inducing hyperalgesia by low-dose morphine systemic administration, while hyperalgesia appeared to be dependent on adrenal glands when morphine is intrathecally administrated. It seems that intact hypothalamic pituitary adrenal axis activity and basal levels of corticosterone are necessary for the induction of intrathecal morphine-induced hyperalgesia.

Our results show that low-dose morphine systemic administration induced a significant increase in plasma levels of corticosterone (Fig. 4). This finding is similar to the effect of ordinary doses (Buckingham and Cooper, 1984; Milanes et al., 1993; Pirnik et al., 2001) and indicates that morphine dose not have a dual inhibitory/excitatory effect on hypothalamic pituitary adrenal axis function. Furthermore, it appears that calcium influx is necessary for the action of ultra-low doses of morphine on hypothalamic pituitary adrenal axis because the effect of morphine on plasma corticosterone level was blocked in the presence of nifedipine. Other groups have found that $Ca^{2+}$ influx also has an important role on the effect of ordinary doses of morphine on hypothalamic pituitary adrenal axis function (Martinez-Pinero et al., 1993; Vargas et al., 1997). Further investigations are needed to clarify the mechanism(s) underlying changes in hypothalamic pituitary adrenal axis activity induced by ultra-low doses of morphine.

In adrenalectomized rats, nifedipine not only prevent morphine hyperalgesia but also elicit a mild analgesic effect. There are several lines of evidence for involvement of calcium in pronociception (Prado, 2001). Several reports indicate that glucocorticoids potentiate calcium influx and accelerate the release of $Ca^{2+}$ from intracellular stores (Zhou et al., 2000; Karst et al., 2002; Machida et al., 2003). The corticosterone effect is in opposition to the effect of nifedipine on the blockage of $Ca^{2+}$ channels and decreasing $Ca^{2+}$ influx (Machida et al., 2003). In addition it is well known that beta-endorphin plasma levels increase in adrenalectomized rats (Bogdanov and Yarushkina, 2004; Vissers et al., 2004) and also beta-endorphin could modulate calcium channel activity and inhibit $Ca^{2+}$ influx (Mazorow et al., 1994). Therefore, we speculate that the observed increased tail-flick latency in adrenalectomized rats after concomitant systemic injection of low dose morphine and nifedipine is the result of an increased concentration of beta-endorphin and absence of corticosterone.

In summary, our results show that nifedipine could prevent the hyperalgesic effect of morphine, and that following the exclusion of adrenal glands, coadministration of morphine and nifedipine elicits a mild analgesia. Nifedipine can attenuate the effect of morphine on hypothalamic pituitary adrenal function. Thus, the data indicate that L-type calcium channels have an important role in morphine-induced hyperalgesia and the mechanism underlying the suppression of morphine-induced hyperalgesia by nifedipine involves mediation, at least in part, by attenuating the effect of morphine on hypothalamic pituitary adrenal axis.

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