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Nifedipine potentiates antinociceptive effects of morphine in rats by decreasing hypothalamic pituitary adrenal axis activity

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Abstract

It has been shown that nifedipine, as a calcium channel blocker can potentiate the antinociceptive effect of morphine; however, the role of Hypothalamic–Pituitary–Adrenal (HPA) axis on this action has not been elucidated. We examined the effect of nifedipine on morphine-induced analgesia in intact and adrenalectomized (ADX) rats and on HPA activity induced by morphine. To determine the effect of nifedipine on morphine analgesia, nifedipine (2 mg/kg i.p.) that had no antinociceptive effect, was injected concomitant with sub-effective dose of morphine (1 and 2 mg/kg). The tail-flick test was used to assess the nociceptive threshold, before and 15, 30, 60, 90, 120 and 180 min after drug administration. Our results showed that, nifedipine could potentiate the antinociceptive effect of morphine and this effect of nifedipine in ADX was greater than sham operated rats which, was reversed by corticosterone replacement. Nifedipine has an inhibitory effect on morphine -induced corticosterone secretion. Thus, the data indicate that the mechanism underlying the potentiation of morphine analgesia by nifedipine involves mediation, at least in part, by attenuating the effect of morphine on HPA axis.

Keywords: Morphine; Analgesia; Nifedipine; HPA axis; Corticosterone

1. Introduction

Opioids have been used for treating moderate to severe pain. Activation of opioid receptor inhibits adenylyl cyclase activity via inhibitory G-proteins, inhibits voltage activated calcium channels, reducing the Ca^{++} influx, thus inhibits neurotransmitter release and attenuates pain sensation (Childers, 1991). Due to the fact that calcium influx is essential for normal sensory processing, inhibition of Ca^{++} movement would contribute to antinociception (Schmidt et al., 1980; Venegas and Schaible, 2000; Todorovic et al., 2002; Heinke et al., 2004; Galeotti et al., 2004). Not surprisingly, Ca^{++} channel antagonists have been shown to induce antinociceptive effect (Del Pozo et al., 1990; Miranda et al., 1993; Weizman et al., 1999; Todorovic et al., 2004; Chen et al., 2005). Many investigators reported that calcium channel blockers potentiate the analgesic effect of morphine (Hoffmeister and Tettenborn, 1986; Contreras et al., 1988; Antkiewicz-Michaluk et al., 1993; Santilan et al., 1994; Michaluk et al., 1998; Assi, 2001; Dogrul et al., 2001; Maeda et al., 2002; Fukuizumi et al., 2003; Yokoyama et al., 2004; Shimizu et al., 2004a,b). In many in vitro studies, it has been demonstrated that glucocorticoids can potentiate Ca^{++} influx and accelerate the release of Ca^{++} from intracellular stores, and corticosterone can increase Ca^{++} entry through the high voltage activated (L-type) calcium channel (Nair et al., 1998; Zhou et al., 2000; Kole et al., 2001; Karast et al., 2002;

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Takahashi et al., 2002; Machida et al., 2003; Sun et al., 2004).

Not only opioids (Buckingham and Cooper, 1986; Gonzalvez et al., 1991; Pechnick, 1993; Little and Kuhn, 1995; Nock et al., 1998; Cerezo et al., 2002) but also Ca^{++} channel blockers (Guerineau et al., 1991; Kuryshev et al., 1995, 1996; Robidoux et al., 2000) could affect HPA function. It has been reported that hypothalamic pituitary adrenal axis (HPA) and its glucocorticoids have an important role on the effect of nifedipine in the development of morphine tolerance (Esmaeili Mahani et al., 2005). Since the interaction between corticosterone and calcium channels has not been clarified in vivo and the role of HPA axis on the effects of calcium channel blockers in morphine analgesia, has not been elucidated, the present study was designed to: first, analyze the contribution of HPA axis and its glucocorticoids to the analgesic effect of morphine that potentiate with nifedipine by using intact and adrenalectomized (ADX) rats. Second, evaluate modifications in the activity of the HPA axis during treatments with 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 (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 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 dissolved in dimethyl sulfoxide (DMSO) plus saline. These drugs were given in the volume of 1 ml/kg, i.p. Corticosterone (Sigma, USA) was dissolved in absolute ethanol then combined with 0.9% NaCl water, yielding final concentration of 100 μg/ml of drinking solution.

2.3. Antinociceptive test

Antinociception was assessed by Tail-Flick test (D'Amour and Smith, 1941). The Tail-Flick latency for each rat was determined three times and 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 drugs, and the reaction latency was determined 15, 30, 60, 90, 120 and 180 min after injection. The Tail-Flick latencies were converted to the percentage of antinociception according to the following formula:%Antinociception (%MPE)=(Reaction time of test−basal reaction time)/(cut off time−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 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 ADX). The amount of drinking solution consumed by each rat was analyzed to determine whether there were any group differences. With this manner plasma corticosterone level was close to the sham operated animals.

2.6. 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 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

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

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

Values represent mean±SEM ($n=8$).

between groups over the time course of study was determined by two or one-way analysis of variance (ANOVA), respectively followed by the Newman–Keuls test with 5% level of significance ($p<0.05$).

3. Results

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

As shown in the Table 1, plasma corticosterone concentrations were significantly reduced (to undetectable levels) in ADX compared with sham operated animals (245.6±28.8 ng/ml). In ADX animals that had corticosterone replaced in their drinking water (ADX+CORT), the plasma corticosterone concentration was not different than sham operated animals (218.8±25.3 ng/ml) ($p>0.05$).

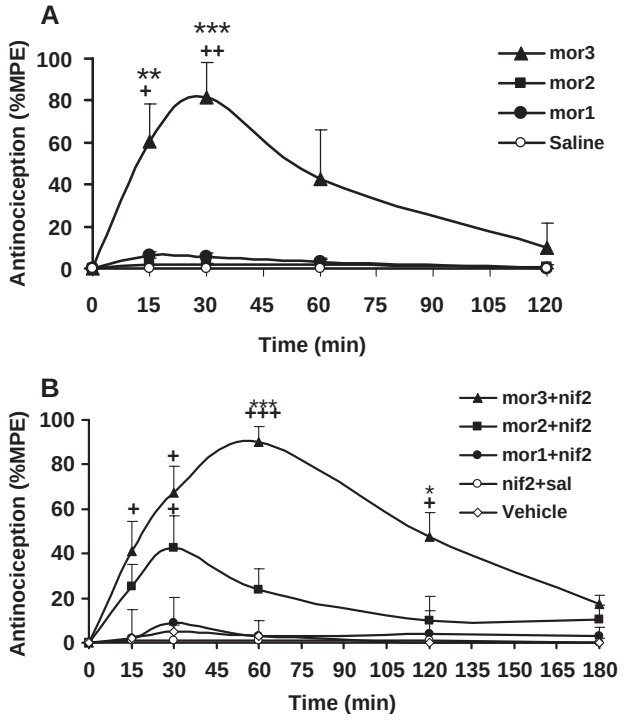

Fig. 1. The effect of morphine (A) and morphine concomitant with 2 mg/kg nifedipine (B) on nociceptive threshold in sham operated animals. Values represent mean±SEM ($n=8$). $+p<0.05$ $++p<0.01$ $+++p<0.001$ significantly different versus before drug administration. $*p<0.05$ $**p<0.01$ $***p<0.001$ versus the other groups in the same time.

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

As it is shown in Fig. 1, morphine (3 mg/kg) produced an analgesic response in sham operated animals that reached a peak 30 min after injection. Morphine (1 and 2 mg/kg) had no antinociceptive activity (Fig. 1A). Nifedipine (2 mg/kg) had not any antinociceptive effect but, concomitant administration of nifedipine with a sub-effective dose of morphine (2 mg/kg) produced significantly antinociceptive effect 30 min following administration (Fig. 1B). In addition, nifedipine significantly enhanced the antinociception elicited by injection of 3 mg/kg morphine that reached a peak 60 min after injection and lasted for about 120 min. Co-administration of nifedipine and morphine (1 mg/kg) could not induce any effect (Fig. 1B).

In adrenalectomized rats, morphine not only in 3 mg/kg but also in the sub-effective dose (2 mg/kg) could affect nociceptive threshold and induce analgesic response peaked 30 min after injection (Fig. 2A). Morphine at the dosage of 1 mg/kg failed to show antinociceptive effect. In the presence of nifedipine, all doses of morphine had a potent and prolong antinociceptive effect that lasted more than 120 min in groups that received 2 or 3 mg/kg morphine (Fig. 2B).

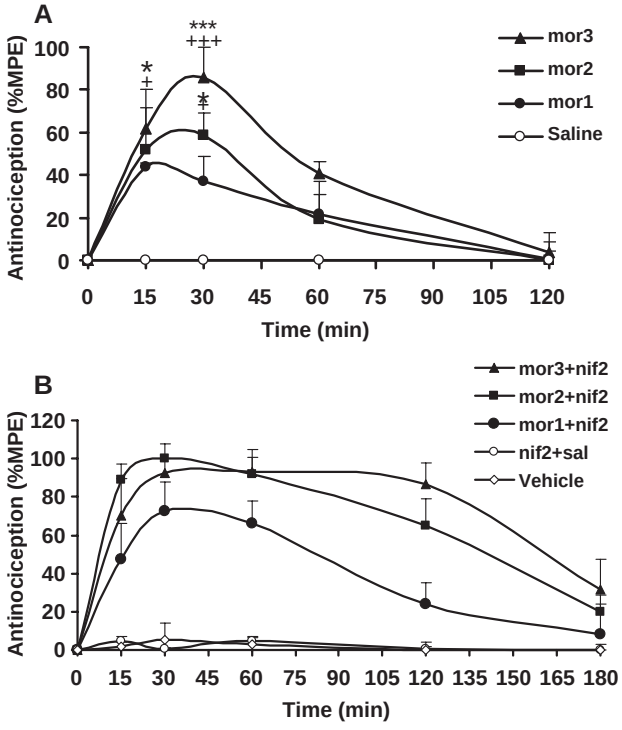

Fig. 2. The effect of morphine (A) and morphine concomitant with 2 mg/kg nifedipine (B) on nociceptive threshold in ADX animals. Values represent mean±SEM ($n=8$). In part A, $+p<0.05$ $+++p<0.001$ significantly different versus before drug administration. $*p<0.05$ $***p<0.001$ as compared with saline treated group. In part B, all of values except mor1+nif2 in 120 min and all doses in 180 min, have significant difference versus before drug administration, vehicle and nifedipine treated groups.

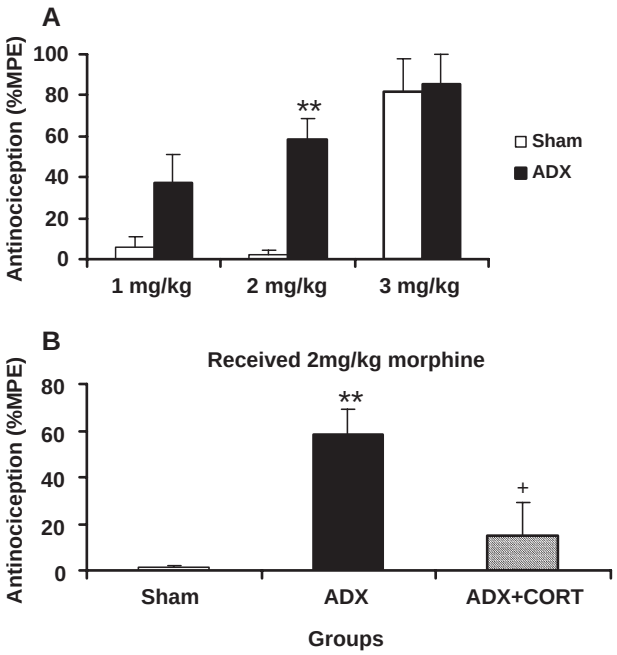

Fig. 3. The analgesic effect of morphine (1, 2 and 3 mg/kg) in sham operated (Sham) and adrenalectomized (ADX) rats 30 min after injection (A). Corticosterone replacement (ADX+CORT) significantly reversed the analgesic effect of 2 mg/kg morphine. Values represent mean ± SEM ($n = 6–8$ rats per group). $^{**}p < 0.01$ as compared with sham operated group in same dose. $+p < 0.05$ as compared with ADX.

With adrenalectomy, morphine in sub-effective dose became effective. As shown in Fig. 3A, the analgesic effect of 2 mg/kg morphine in ADX rats was greater than sham operated animals 30 min after injection ($p < 0.01$). This effect was reversed with corticosterone replacement (Fig. 3B).

The antinociceptive effect of different doses of morphine accompanied with nifedipine 30 min after injection showed

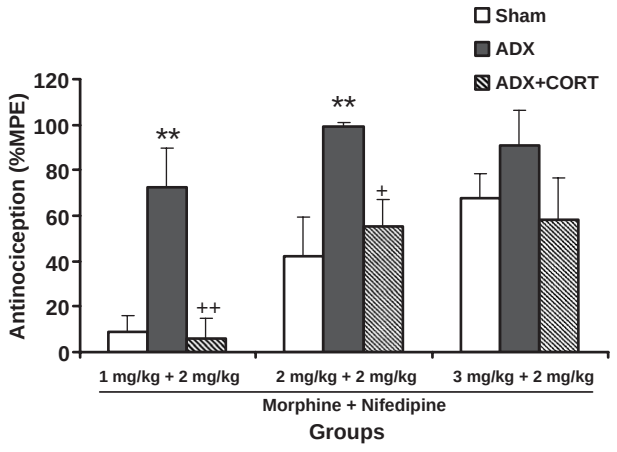

Fig. 4. The analgesic effect of different doses of morphine concomitant with 2 mg/kg nifedipine 30 min after injection in sham operated (Sham), adrenalectomized (ADX) and adrenalectomized rats that received corticosterone in drinking solution (ADX+CORT). Values represent mean ± SEM ($n = 6–8$ rats per group). $^{**}p < 0.01$ as compared with Sham and $+p < 0.05$ $++p < 0.01$ as compared with ADX group.

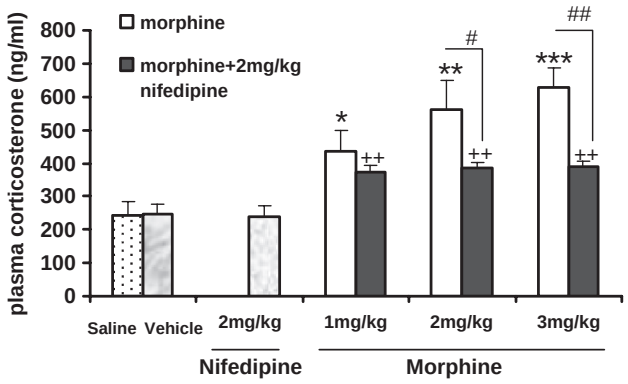

Fig. 5. Plasma corticosterone concentration 30 min after injecting either morphine or morphine concurrently with nifedipine (2 mg/kg). Each bar represents mean ± SEM ($n = 6–8$ rats per group). $\#p < 0.05$ $\#\#p < 0.01$. Asterisks indicate significant differences from saline injected group. $^{*}p < 0.05$ $^{**}p < 0.01$ $^{***}p < 0.001$. Crosses indicate significant differences from vehicle and nifedipine treated group. $++p < 0.01$.

that nifedipine not only in sham operated but also in ADX rats, could potentiate the antinociceptive effect of morphine. The effect of nifedipine on morphine-induced analgesia in ADX was significantly greater than sham operated rats, especially in groups that received the sub-effective doses of morphine (1 and 2 mg/kg) ($p < 0.01$). However, corticosterone replacement significantly reversed this analgesic effect to the level that was similar to the sham operated group (Fig. 4).

3.3. The effect of nifedipine on the neurosecretory effect of morphine in HPA axis

In this part of study, we investigated the changes in HPA activity upon acute exposure to morphine, as well as the contribution of nifedipine on this effect. As shown in Fig. 5, the acute administration of morphine produced significant increase in plasma level of corticosterone, 30 min after injection as compared to the saline treated group. Administration of nifedipine (2 mg/kg) with morphine, attenuated the effect of morphine on corticosterone secretion, especially in dosage 2 ($p < 0.05$) and 3 mg/kg ($p < 0.01$) of morphine. Injection of nifedipine (2 mg/kg) had no any significant effect on plasma corticosterone concentration ($p > 0.05$).

4. Discussion

Although it has been shown that co-administration of calcium channel blockers (CCB_S) with morphine potentiates the analgesic effect of morphine but the role of adrenal glands and their corticosteroids in this effect has not been identified yet. Our results showed that nifedipine in both sham operated and ADX animals could potentiates the antinociceptive property the sub-effective doses of morphine but this phenomenon in ADX rats was potent than

sham operated animals and with corticosterone replacement returned to the value similar to sham group.

Inhibitory interaction between opioid receptors and voltage-dependent calcium channels have been demonstrated by electrophysiological and biochemical methods (Attali et al., 1989). Therefore, there is a common site of interaction between calcium channel blockers and morphine for regulation of pain sensitivity. Not surprisingly, a synergic effect can be inducing when nifedipine and morphine use concomitantly. Several line of evidence indicate that the interaction between opioids and CCB_S is not as simple as mentioned above and the regulation of morphine analgesia is not accounted for only by suppression of Ca^{++} influx and diminish calcium dependent neurotransmitter release. Other aspects of the interaction between these drugs are related to pharmacokinetics. Maeda et al. (2002) reported that diltiazem augmented the magnitude of morphine-induced analgesia in part via increase in morphine level in serum. Moreover, some results showed that the elevation of serum morphine in presence of CCB_S (verapamil and nimodipine) is due to inhibition of morphine metabolism. L-type CCB_S have been reported to be competitive inhibitors of morphine metabolizing enzymes (Murray and Butler, 1996; Thummel and Wilkinson, 1998; Liu et al., 2000). Some of L-type CCB_S and morphine are both substrates for P-glycoprotein (P-gp), a drug efflux pump in blood brain barrier (Callaghan and Riordan, 1993; Barancik et al., 1994; Dagenais et al., 2004). Other groups find that administration of verapamil and diltiazem increase the level of morphine in serum and also in the brain (Shimizu et al., 2004b). In addition, other investigators demonstrated that the brain-to-serum ratio of morphine was increased by treatment with verapamil (Zong and Pollack, 2000) and it may be a mechanism involving potentiation of morphine analgesia by CCB_S. Our results indicate that in the absence of adrenal glands, nifedipine could potentiate morphine analgesia in sub-effective doses of morphine, i.e. 1 and 2 mg/kg than in presence of adrenal glands (Fig. 4).

Several lines of evidence indicate that glucocorticoids potentiate calcium influx and accelerate the release of Ca^{++} 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^{++} channels and decreasing Ca^{++} influx. Therefore, it seems logical that with adrenalectomy, nifedipine is more effective in preventing Ca^{++} influx into the structures involved in morphine analgesia and as a result, potentiates better its analgesic property. The elimination of corticosterone with the same manner could affect even morphine analgesia. It has been reported that ADX significantly potentiate morphine analgesia in low and high doses of morphine (Miyamoto et al., 1988, 1989; Candido et al., 1992; Suzuki et al., 1995). In low doses as same as doses used in this study, ADX increased sensitivity to morphine (Miyamoto et al., 1990). Since both morphine and corticosterone affect calcium channel activity in opposite directions, therefore this potentiation, at least in part, may be due to a lack of corticosterone effect on Ca^{++} channels.

In addition, it has been reported that glucocorticoids can induce mRNA expression of calcium channel subunits (Nair et al., 1998). An in vitro study indicates that 3 or 7 days after adrenalectomy calcium current amplitude was decreased in dentate granule cells (Karast and Joels, 2001). Moreover, ablations of calcium channel by knocking out the gene encoding the subunit of these channels caused antinociception and reduce nociceptive behaviour in persistent pain (Saegusa et al., 2000, 2001; Kim et al., 2001). So, it seems that by removal of glucocorticoids, the expression of calcium channels and their number is decreased. Therefore, nifedipine has become more effective in the potentiation of morphine antinociception. However, this possible mechanism needs to be clarified by further investigations.

Our results show that nifedipine has an inhibitory effect on morphine-induced corticosterone secretion (Fig. 5). When this drug had significant inhibitory effect on morphine-induced corticosterone, secretion also had a prominent effect on antinociceptive response elicited by morphine.

It seems that one of the routes for nifedipine-induced potentiation of morphine analgesia is through its effect on corticosterone secretion.

In summary, our results show that nifedipine could potentiate the analgesic effect of morphine and following the exclusion of adrenal glands, this drug can enhance morphine induced-antinociception more effectively even in sub-effective doses. Nifedipine can attenuate the effect of morphine on HPA function, indicating other pharmacokinetic interaction between morphine and L-type calcium channel blockers. Thus, the data indicate that the mechanism underlying the potentiation of morphine analgesia by nifedipine involves mediation, at least in part, by attenuating the effect of morphine on HPA axis.

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References

Antkiewicz-Michaluk L, Michaluk J, Romanska I, Vetulani J. Reduction in morphine dependence and potentiation of the analgesia by chronic co-administration of nifedipine. Psychopharmacology (Berlin) 1993;111:457–64.

Assi AA. The influence of divalent cations on the analgesic effect of opioid and non-opioid drugs. Pharmacol Res 2001;43:521–9.

Attali B, Saya D, Nah SY, Vogel Z. Opiate agonists inhibit Ca^{++} influx in rat spinal cord–dorsal root ganglion co-cultures: involvement of a GTP-binding protein. J Biol Chem 1989;264:347–53.

Barancik M, Polekova L, Mrazova T, Breier A, Stankovicova T, Slezak J. Reversal effects of several Ca(2+)-entry blockers, neuroleptics and local

anaesthetics on P-glycoprotein-mediated vincristine resistance of L1210/VCR mouse leukaemic cell line. Drugs Exp Clin Res 1994;20: 13–8.

Buckingham JC, Cooper TA. Pharmacological characterization of opioid receptor influencing the secretion of corticotropin releasing factor in the rat. Neuroendocrinology 1986;44:36–40.

Callaghan R, Riordan JR. Synthetic and natural opiates interact with P-glycoprotein in multidrug-resistant cells. J Biol Chem 1993;268: 16059–64.

Candido J, Lutfy K, Billings B, Sierra V, Duttaroy A, Inturrisi CE, et al. Effect of adrenal and sex hormones on opioid analgesia and opioid receptor regulation. Pharmacol Biochem Behav 1992;42:685–92.

Cerezo MM, Laorden ML, Milanes V. Inhibition of protein kinase C but not protein kinase A attenuate morphine withdrawal excitation of rat hypothalamus–pituitary–adrenal axis. Eur J Pharmacol 2002;452: 57–66.

Chen JQ, Zhang YQ, Dai J, Luo ZM, Liang SP. Antinociceptive effects of intrathecally administered huwentoxin-I, a selective N-type calcium channel blocker, in the formalin test in conscious rats. Toxicon 2005;45: 15–20.

Childers SR. Opioid receptor coupled second messengers. Life Sci 1991;48: 1991–2003.

Contreras E, Tamayo I, Amigo M. Ca^{++} channel antagonists increase morphine induced analgesia and antagonize morphine tolerance. Eur J Pharmacol 1988;148:463–6.

Dagenais C, Graff CL, Pollack GM. Variable modulation of opioid brain uptake by P-glycoprotein in mice. Eur J Pharmacol 2004;483:249–58.

D'Amour FE, Smith DL. A method of determining loss of pain sensation. J Pharmacol Exp Ther 1941;27:74–9.

Del Pozo E, Rouiz Garcia C, Bayens JM. Analgesic effect of diltiazem and verapamil, after central and peripheral administration in the hot plate test. Gen Pharmacol 1990;21:681–5.

Dogrul A, Yesilyurt O, Isimer A, Erdal Guzeldemir M. L-type and T-type calcium channel blockade potentiate the analgesic effects of morphine and selective μ opioid agonist, but not to selective δ and κ opioid agonist at the level of spinal cord in mice. Pain 2001;93:61–8.

Esmaeili Mahani S, Motamedi F, Javan M, Ahmadiani A. Involvement of hypothalamic pituitary adrenal axis on the effect of nifedipine in the development of morphine tolerance in rats. Pharmacol Biochem Behav 2005;81:152–7.

Fukuizumi T, Ohkubo T, Kitamura K. Spinally delivered N-, P/Q- and L-type Ca^{2+}-channel blockers potentiate morphine analgesia in mice. Life Sci 2003;73:2873–81.

Galeotti N, Bartolini A, Ghelardini C. Role of intracellular calcium in acute thermal pain perception. Neuropharmacology 2004;47:935–44.

Gonzalvez ML, Milanes MV, Vargas ML. Effect of acute and chronic administration of μ and δ opioid agonists on the hypothalamic–pituitary–adrenocortical (HPA) axis in the rat. Eur J Pharmacol 1991; 200:155–8.

Guerineau N, Corcuff JB, Tabarin A, Molard P. Spontaneous and corticotropin releasing factor-induced cytosolic calcium transients in corticotrops. Endocrinology 1991;129:409–20.

Heinke B, Balzer E, Sandkuhler J. Pre-and postsynaptic contributions of voltage-dependent Ca^{2+} channels to nociceptive transmission in rat spinal lamina I neurons. Eur J Neurosci 2004;19:103–11.

Hoffmeister F, Tettenborn D. Calcium agonists and antagonists of the dihydropyridine type: antinociceptive effects, interference with opiate-mu-receptor agonists and neuropharmacological actions in rodents. Psychopharmacology (Berl) 1986;90:299–307.

Karast H, Joels M. Calcium currents in rat granule cells are altered after adrenalectomy. Eur J Neurosci 2001;14:503–12.

Karast H, Nair S, Velzing E, Rumpuff-van Essen L, Slagter E, Shinnick-Collagher P, et al. Glucocorticoids alter calcium conductance and calcium channel subunit expression in basolateral amygdala neuron. Eur J Neurosci 2002;16:1083–9.

Kim C, Jun K, Lee T, Kim SS, McEnery MW, Chin H, et al. Altered nociceptive response in mice deficient in the alpha(1B) subunit of the voltage-dependent calcium channel. Mol Cell Neurosci 2001;18: 235–45.

Kole MH, Koolhaas JM, Luiten PG, Fuchs E. High-voltage-activated Ca^{2+} currents and the excitability of pyramidal neurons in the hippocampal CA3 subfield in rats depend on corticosterone and time of day. Neurosci Lett 2001;307:53–6.

Kuryshev YA, Childs GV, Ritchie AK. Corticotropin-releasing hormone stimulate calcium entry is particularly dependent on protein kinase A. Endocrinology 1995;136:3925–33.

Kuryshev YA, Childs GV, Ritchie AK. Corticotropin-releasing hormone stimulates calcium entry through L and P-type channels in rat corticotrops. Endocrinology 1996;137:2269–77.

Little PJ, Kuhn MC. Ontogenetic studies of tolerance development: effects of chronic morphine on the hypothalamic–pituitary–adrenal axis. Psychopharmacology 1995;122:78–84.

Liu XQ, Ren YL, Qian ZY, Wang GJ. Enzyme kinetics and inhibition of nimodipine metabolism in human liver microsomes. Acta Pharmacol Sin 2000;21:690–4.

Machida K, Ishibashi R, Hara T, Ohtsuka A. Effect of corticosterone on Ca^{++} uptake and myofibrilar disassembly in primary muscle cell culture. Biosci Biotechnol Biochem 2003;67:244–9.

Maeda T, Kishioka S, Fan X, Fukazawa Y, Shimizu N, Ozaki M, et al. Effect of diltiazem and MK-801 on morphine analgesia and pharmacokinetics in mice. Neurosci Lett 2002;326:216–8.

Michaluk J, Karokewicz B, Antkiewicz-Michaluk L, Vetulani J. Effect of various Ca^{++} channel antagonists on morphine analgesia Tolerance and dependence, and on blood pressure in the rat. Eur J Pharmacol 1998; 352:189–97.

Miranda HF, Pelissier T, Sierralta F. Analgesic effect of intracerebroventricular administration of calcium channel blockers in mice. Gen Pharmacol 1993;24:201–4.

Miyamoto Y, Ozaki M, Yamamoto H. Effects of adrenalectomy on pharmacokinetics and antinociceptive activity of morphine in rats. Jpn J Pharmacol 1988;46:379–86.

Miyamoto Y, Ozaki M, Yamamoto H. Effect of adrenalectomy on correlation of analgesia with tissue content of morphine. Eur J Pharmacol 1989;167:11–20.

Miyamoto Y, Ozaki M, Kishioka S, Yamanishi T, Kitabata Y, Morita N, et al. Adrenalectomy-induced potentiation of morphine analgesia: reversal by prednisolone. Pharmacol Biochem Behav 1990;37:703–6.

Murray M, Butler AM. Enhanced inhibition of microsomal cytochrome P450 3A2 in rat liver during diltiazem biotransformation. J Pharmacol Exp Ther 1996;279:1447–52.

Nair SM, Workman TR, Craig J, Finnell R, Joels M, Eberwine JH. Corticosteroid regulation of ion channel conductance and mRNA level in individual hippocampal CA1 neurons. J Neurosci 1998;18: 2685–95.

Nock B, Cicero TJ, Wich M. Chronic exposure to morphine decreases physiologically active corticosterone in both male and female rats but by different mechanisms. J Pharmacol Exp Ther 1998;286:875–82.

Pechnick RN. Effect of opioids on the hypothalamo-pituitary-adrenal axis. Ann Rev Pharmacol Toxicol 1993;32:353–8.

Robidoux J, Simoneau L, Masse A, Lafond J. Activation of L-type calcium channels induces corticotropin-releasing factor secretion from human placental trophoblasts. J Clin Endocrinol Metab 2000;85:3356–64.

Saegusa H, Kurihara T, Zong S, Minowa O, Kazuno A, Han W, et al. Altered pain responses in mice lacking alpha 1E subunit of the voltage-dependent Ca^{2+} channel. Proc Natl Acad Sci U S A 2000;97:6132–7.

Saegusa H, Kurihara T, Zong S, Kazuno A, Matsuda Y, Nonaka T, et al. Suppression of inflammatory and neuropathic pain symptoms in mice lacking the N-type Ca^{2+} channel. EMBO J 2001;20:2349–56.

Santilan R, Maestre JM, Hurle MA, Florez J. Enhancement of opiate analgesia by nimodipine in cancer patients chronically treated with morphine: a preliminary report. Pain 1994;58:129–32.

Schmidt R, Joo F, Zimmermann H. Preferential calcium staining of mitochondria in stimulated cholinergic nerve endings. Exp Brain Res 1980;38:419–24.

Shimizu N, Kishioka S, Maeda T, Fukazawa Y, Dake Y, Yamamoto C, et al. Involvement of peripheral mechanism in the verapamil-induced potentiation of morphine analgesia in mice. J Pharmacol Sci 2004a; 95:425–57.

Shimizu N, Kishioka S, Maeda T, Fukazawa Y, Yamamoto C, Ozaki M, et al. Role of pharmacokinetic effects of potentiation of morphine analgesia by L-type calcium channel blockers in mice. J Pharmacol Sci 2004b;94:240–5.

Sun C, Liu N, Li H, Zhang M, Liu S, Liu X, et al. Experimental study of effect of corticosterone on primary cultured hippocampal neurons and their Ca^{2+}/CaMKII expression. J Huazhong Univ Sci Technolog Med Sci 2004;24:543–6.

Suzuki T, Sugano Y, Funada M, Misawa M. Adrenalectomy potentiates the morphine – but not cocaine – induced place preference in rats. Life Sci 1995;56:339–44.

Takahashi T, Kimoto T, Tanabe N, Hahori TA, Yasumatso N, Kawato S. Corticosterone acutely prolong N-methyle-D-aspartate receptor-mediated Ca^{++} elevation in culture rat hippocampal neuron. J Neurochem 2002;83:1441–551.

Thummel KE, Wilkinson GR. In vitro and in vivo drug interactions involving human CYP3A. Annu Rev Pharmacol Toxicol 1998;38: 389–430.

Todorovic SM, Meyenburg A, Jevtovic-Todorovic V. Mechanical and thermal antinociception in rats following systemic administration of mibefradil, a T-type calcium channel blocker. Brain Res 2002;951: 336–40.

Todorovic SM, Pathirathna S, Meyenburg A, Jevtovic-Todorovic V. Mechanical and thermal antinociception in rats after systemic administration of verapamil. Neurosci Lett 2004;360:57–60.

Venegas H, Schaible HG. Effects of antagonists to high threshold calcium channels upon spinal mechanisms of pain. Pain 2000;85:9–18.

Weizman R, Getslev V, Pankov IA, Schrieber S, Pick CG. Pharmacological interaction of the calcium channel blockers verapamil and flunarizine with the opioid system. Brain Res 1999;818:187–95.

Yokoyama K, Kurihara T, Saegusa H, Zong S, Makita K, Tanabe T. Blocking the R-type (Cav23) Ca^{2+} channel enhanced morphine analgesia and reduced morphine tolerance. Eur J Neurosci 2004;20: 3516–9.

Zhou JZ, Zheng JQ, Zhang YX, Zhou JH. Corticosterone impairs cultured hippocampal neuron and facilitates calcium influx through voltage-dependent Ca^{++} channel. Acta Pharmacol Sin 2000;21:156–60.

Zimmermann M. Ethical guidelines for investigations of experimental pain in conscious animals. Pain 1983;16:109–10.

Zong J, Pollack GM. Morphine antinociception is enhanced in mdr1a gene-deficient mice. Pharm Res 2000;17:749–53.