

Interaction between glucocorticoids and opioids in nociception in young and adult rats

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Background: The aim of this study was to examine the relationship between the glucocorticoid and opioid systems in the modulation of nociception in young and adult rats.

Materials and methods: The experiments were done in young and adult Wistar rats, using morphine 5 mg/kg, and naloxone 2 mg/kg as a μ -opioid receptor agonist. Dexamethasone 1 mg/kg and mifepristone (RU486) 20 mg/kg were used as a glucocorticoid receptor agonist and antagonist, respectively. Hind paw licking latency was measured by hot plate after intraperitoneal administration of drugs.

Results: The results showed that morphine and dexamethasone had significant analgesic effects ($P < 0.001$, $P < 0.01$, respectively) in both age groups. Coadministration of morphine and dexamethasone did not induce a greater analgesic effect in comparison with morphine alone in either age group. Mifepristone prevented the analgesic effect of morphine in the adult animals ($P < 0.001$), but had no effect in young animals. The analgesic effect of dexamethasone was inhibited by naloxone in both groups ($P < 0.01$).

Conclusion: These results suggest that glucocorticoids regulate opioid-induced analgesia from the age of puberty, but opioids regulate glucocorticoid-induced analgesia in prepubescent animals. Thus, there is a clear overlapping effect between the two pain modulation systems.

Keywords: opioid, glucocorticoid, naloxone, mifepristone, hot plate

Introduction

Pain as a stressful stimulus activates the hypothalamic–pituitary–adrenal axis.¹ Glucocorticoids that are the final products of hypothalamic–pituitary–adrenal axis² play important roles in responses to stressful stimuli, including pain.³ For several years glucocorticoids have been used to reduce postoperative pain,⁴ suggesting that they can interact with and modify the effects of opioids and analgesia.² Opioids are the therapeutic mainstay in clinical pain management,^{5,6} but they induce a considerable number of adverse side effects, including respiratory depression, constipation, tolerance, and addiction,⁷ which has led to an active search for novel opioid compounds exhibiting a favorable dissociation between analgesic activity and the development of dependence/tolerance and other side effects.⁸ Recent reports show that chronic morphine upregulates expression and activation of glucocorticoid receptors.^{9,10} High levels of glucocorticoids associated with stress are known to activate glucocorticoid receptors, which modify the pain threshold and the antinociceptive effects of morphine.^{11,12}

Some nonopioid analgesics with widespread uses, when administered at clinically relevant concentrations, cause antinociception by activating endogenous opioidergic circuits in the descending pain control system.¹³ Some experiments have indicated an

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important functional interaction between glucocorticoids and the opioid system, eg, their interactions at the κ -opioid receptor in hypotension induced by opioids or the inhibitory effect of dexamethasone on the development of morphine tolerance.¹⁴ Dexamethasone may exert its action on opiate-induced hypotension by a mechanism that alters the sensitivity to opioids. This hypothesis is supported by the results of several studies that show a link between glucocorticoids and the opiate system.⁸

The effect of age on pain sensitivity is still unclear. This could be due to the fact that aging and pain are multidimensional phenomena.¹⁵ The effect of age on pain reactivity in animals has been investigated by several groups.⁶ Conflicting results have been reported showing either a decrease¹⁶ in pain reactivity, an increase,¹⁷ or no effect.¹⁸ There are a number of developmental processes that would suggest the peripheral analgesic action of opiates differ from those of the adult animal.¹⁹

The purpose of this study was to examine the interaction of glucocorticoid and opioid systems in modulating nociception in young (1 month old) and adult (3 months old) male rats. We investigated whether the antinociceptive effect of morphine acts via the glucocorticoid system, and whether morphine's side effects may be reduced by dexamethasone while still retaining its analgesic effects. Another aim of this study was to compare the pain sensitivity and antinociceptive effect of the drugs in the two different rodent age groups.

Our results clearly indicate, for the first time, a difference in the interactions of the glucocorticoid and opioid systems in modulating nociception in young and adult rats.

Material and methods

Animals

Our experiments were performed using 98 male Wistar rats, comprising 49 young rats (1 month old and weighing 40–45 g) and 49 adult rats (3 months old and weighing 170–180 g). The animals were kept at a constant temperature ($22 \pm 2^\circ\text{C}$) on a 12-hour light/dark cycle (lights on from 07:00 hours to 19:00 hours) with free access to food and water. They were last handled 4–5 days before the test. All experiments were carried out between 09:00 and 11:00 hours. Both age groups of animals were divided into six groups, and received saline, morphine 5 mg/kg, dexamethasone 1 mg/kg, morphine 5 mg/kg + dexamethasone 1 mg/kg, mifepristone 20 mg/kg + morphine 5 mg/kg, or naloxone 2 mg/kg + dexamethasone 1 mg/kg.

Drugs

Morphine (Temad Co, Tehran, Iran) and dexamethasone (Iran Hormone Co, Tehran, Iran) were dissolved in saline.

Naloxone (Tolidaru Co, Tehran, Iran) and mifepristone (Sigma Co, St Louis, MO) were dissolved in sterile distilled water. All injections were given intraperitoneally in a volume of 5 mL of saline or sterile water.

Hot plate test

The hot plate test was performed using a method²⁰ adapted for rats. The evaluated parameters were the latency time for paw licking responses on exposure to the hot plate surface. For the hot plate test, the rats were placed on a metal plate heated to a mean temperature of $52 \pm 0.2^\circ\text{C}$ and the reaction time was determined. The apparatus consisted of a 25×25 cm Plexiglas cage fitted over the hot plate and a foot-switch timer. Pain thresholds were measured by latency time (analgesia time) to nociceptive responses (paw lick) with a maximum exposure time of 120 seconds. Latency time was recorded and the results are expressed as hot plate analgesic index.

All animals were tested on the hot plate. Each rat was placed in the center of the hot plate and animals were tested individually. After each trial the hot plate was cleaned to minimize lingering olfactory cues. Feces or urine were first removed with paper towels and the central platform was cleaned with 95% ethanol. After towel drying the solution, the apparatus was further allowed to air dry for about two minutes before another animal was tested. Before the hot plate test, the animal was allowed to acclimatize and familiarize to the environment of the apparatus one day before experiment for only two minutes.

Statistical analysis

All results are presented as mean \pm standard error of the mean for seven animals per group. A two-tailed independent samples *t*-test was used to compare the mean frequency of the behaviors between groups as showed in the results. Some data were assessed by analysis of variance. Following a significant *F* value, post hoc analyses (least significant difference test) were performed for assessing specific group comparisons, where $P < 0.05$ was considered statistically significant. Calculations were performed using the SPSS (version 11; SPSS Inc., Chicago, IL) statistical package.

Results

Treatment of young and adult rats with morphine + dexamethasone

In the young rats, morphine 5 mg/kg and dexamethasone 1 mg/kg administration induced a significant increase in latency time (analgesic effect) for the hot plate test (Figure 1) and there was a similar effect in adult rats (Figure 2).

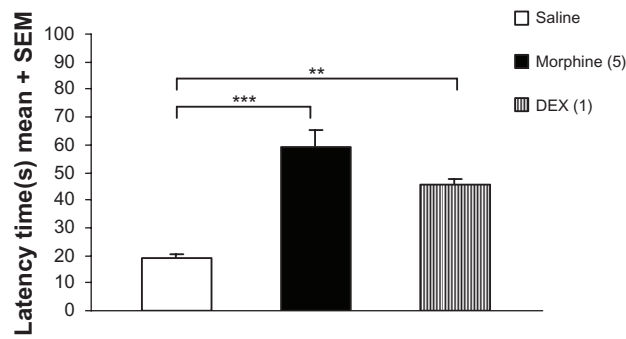


Figure 1 Latency time (mean ± standard error of the mean) 30 minutes after receiving saline, morphine 5 mg/kg, and dexamethasone 1 mg/kg intraperitoneally in male young rats ($n = 7$ per group) for the hot plate test.

Notes: *** $P < 0.01$, ** $P < 0.001$ compared with animals receiving saline.

Abbreviation: DEX, dexamethasone.

Analgesic effect of morphine ± mifepristone in young and adult rats

Here we used mifepristone 20 mg/kg as a glucocorticoid receptor antagonist five minutes before administration of morphine 5 mg/kg. Figure 3 shows that there was no significant difference between groups receiving morphine and morphine + mifepristone. Thus, mifepristone did not change the latency time (analgesia time) of morphine.

In adult animals, a significant difference was observed between mifepristone p+ morphine and morphine alone, indicating that mifepristone can inhibit the analgesic effect of morphine (Figure 4).

Analgesic effect of dexamethasone ± naloxone in young and adult rats

Here naloxone 2 mg/kg was used as an opioid receptor (μ) antagonist five minutes before administration of dexamethasone 1 mg/kg. As shown in Figure 5, there was a significant difference between dexamethasone 1 mg/kg and dexamethasone 1 mg/kg ± naloxone 2 mg/kg in latency

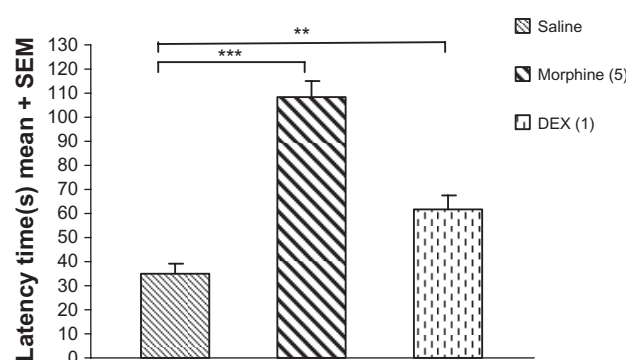


Figure 2 Latency time (mean ± standard error of the mean) 30 minutes after receiving saline, morphine, and dexamethasone intraperitoneally in male adult rats ($n = 7$ per group) in the hot plate test.

Notes: *** $P < 0.01$, ** $P < 0.001$ compared with animals receiving saline.

Abbreviation: DEX, dexamethasone.

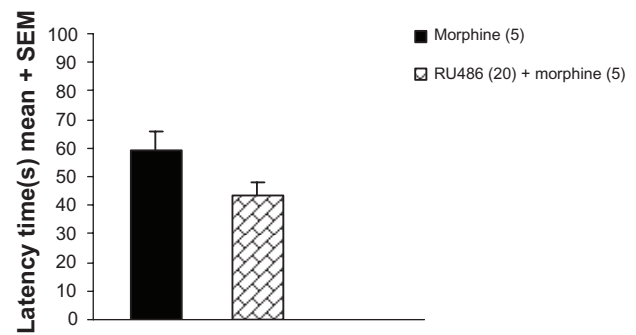


Figure 3 Comparison between latency time (mean ± standard error of the mean) between groups that received morphine 5 mg/kg alone and morphine 5 mg/kg + mifepristone (RU486) 20 mg/kg in male young rats ($n = 7$ per group) for the hot plate test.

Note: There was no significant difference between the two groups.

time in the young rats. Thus, the analgesic effect of DEX was inhibited by the opioid receptor antagonist, naloxone. A similar effect was also noted in adult rats (Figure 6).

Effect of coadministration of morphine + dexamethasone on latency time

Coadministration of morphine 5 mg/kg and dexamethasone 1 mg/kg in the young and adult groups showed no significant differences with morphine 5 mg/kg (Figure 7) in latency time. Thus, morphine and dexamethasone have overlapping analgesic effects.

Discussion

The results of these experiments clearly demonstrate that systemic injection of morphine 5 mg/kg and dexamethasone 1 mg/kg provide effective analgesia in both young and adult groups, as shown in many previous studies,^{13,21–25} Our results are in agreement with a previous study showing no difference in the analgesic effect of morphine between young and adult rats using the latency of the tail-flick response.²⁶ It has also

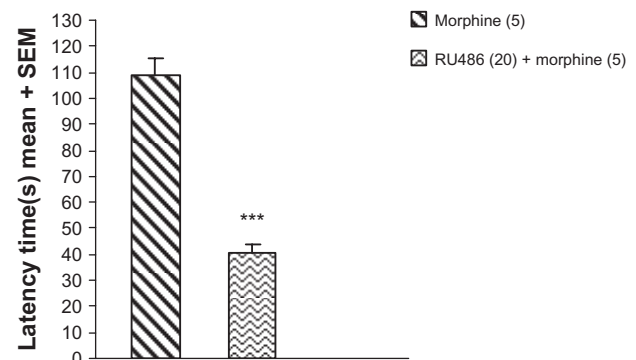


Figure 4 Comparison of latency time (mean ± standard error of the mean) between the groups that received morphine 5 mg/kg alone and morphine 5 mg/kg + mifepristone (RU486) 20 mg/kg in male adult rats ($n = 7$ per group) for the hot plate test.

Note: *** $P < 0.001$ compared with animals receiving morphine alone.

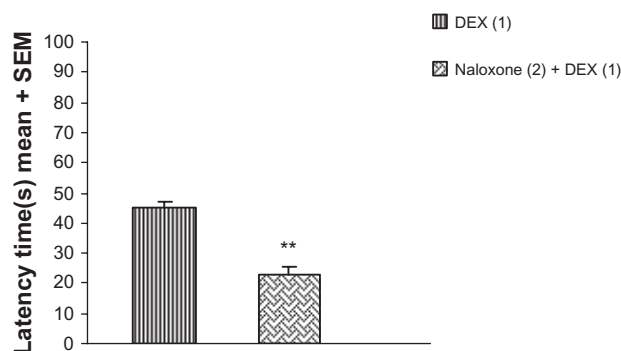


Figure 5 Comparison between latency time (mean \pm standard error of the mean) between groups that received dexamethasone 1 mg/kg alone and naloxone 2 mg/kg + dexamethasone 1 mg/kg in male young rats ($n = 7$ per group) for the hot plate test. **Note:** ** $P < 0.01$ compared with animals receiving dexamethasone alone.

Abbreviation: DEX, dexamethasone.

been shown that intraarticular morphine produces analgesia of a magnitude similar to that of dexamethasone.²⁷

There is evidence indicating that the opioid system at supraspinal sites modulates noxious peripheral input by activating the descending inhibitory pathways and significantly changing pain behaviors.²³ Modulation of adenylyl cyclase activity by opioids has an important role in the mechanisms of analgesia, and opioid tolerance occurs through intracellular adenylyl cyclase, cAMP, and cAMP-dependent protein kinase.²⁸ With respect to the analgesic effects of dexamethasone, our results are consistent with those of a previous experimental study¹³ and clinical trials^{27,29} that demonstrated that dexamethasone can alleviate pain effectively. It was shown that systemic administration of glucocorticoids to rats led to increases in the latency period of the tail-flick reaction.²¹ Furthermore, dexamethasone is used in the treatment of inflammatory disease and pain^{27,29} but experimental results, using the hot-water immersion test

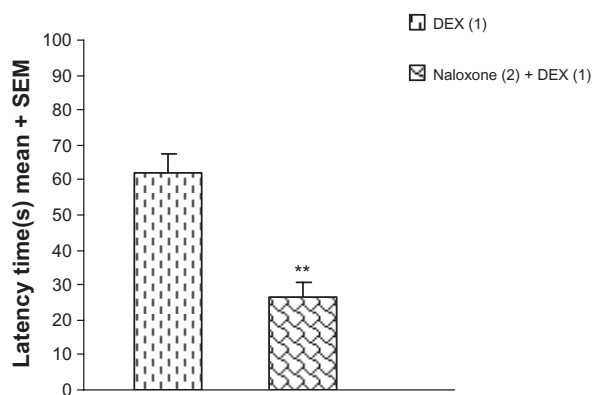


Figure 6 Comparison between latency time (mean \pm standard error of the mean) between groups that received dexamethasone 1 mg/kg alone and naloxone 2 mg/kg + dexamethasone 1 mg/kg in male adult rats ($n = 7$ per group) for the hot plate test. **Note:** ** $P < 0.001$ compared with animals receiving dexamethasone alone.

Abbreviation: DEX, dexamethasone.

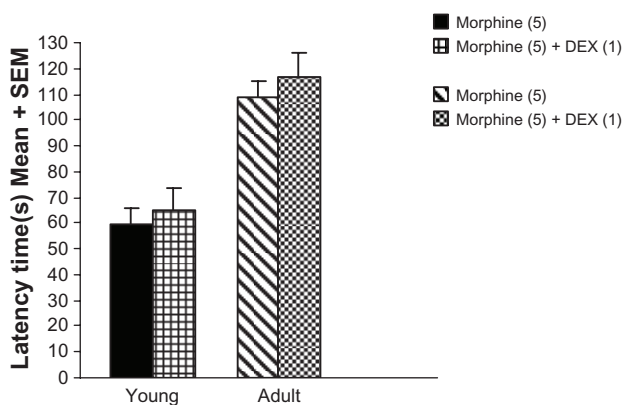


Figure 7 Comparison of latency time (mean \pm standard error of the mean) between the groups that received morphine 5 mg/kg + dexamethasone 1 mg/kg and morphine 5 mg/kg alone in male young and adult rats ($n = 7$ per group) for the hot plate test.

Note: There was no significant difference between two groups.

Abbreviation: DEX, dexamethasone.

in rats, showed that acute injection of dexamethasone did not produce antinociception.¹³ This difference in pain reactivity following dexamethasone administration could be related to the method used to evaluate pain perception, or various pain parameters and use of different age groups.¹⁵

Coinfusion of morphine 5 mg/kg with dexamethasone 1 mg/kg showed similar antinociceptive effects to those of morphine 5 mg/kg in young and adult rats. This result was supported by a previous study showing that a single dexamethasone injection did not enhance morphine's antinociceptive effect in adult rats but attenuated morphine's antinociceptive tolerance.¹³

Some experiments indicated an important functional interaction between glucocorticoids and the opioid system in analgesia, constipation, hypermotility, epilepsy, and dependence.^{11,12,30-32} However, concerning the similarity between the coinfusion of morphine + dexamethasone and administration of morphine alone, it seems that there is either a similar pathway or overlapping pathways for the pain-modulating effects of morphine and dexamethasone.

The present study showed that the analgesic effect of morphine could be prevented by the glucocorticoid receptor antagonist, mifepristone, in adult but not young rats. This inhibitory effect of mifepristone is in agreement with the findings of Maggi et al³³ who demonstrated that mifepristone inhibits the binding of labeled dihydromorphine to μ -opioid receptors present on membrane preparations derived from rat and mouse brain, as well as in human neuroblastoma cells. The inhibitory effect of mifepristone was dose-dependent and linked to a decrease in the affinity of labeled dihydromorphine to μ -opioid receptors. Kinetic experiments have shown that mifepristone induces a decrease of the association rate

constant of dihydromorphine. Mifepristone also demonstrated an ability to dissociate the dihydromorphine- μ opioid receptor complex.³³

In another study, it was shown that mifepristone did not block the immunosuppressive effects of morphine, suggesting that such effects are independent of activation of the hypothalamic–pituitary–adrenal axis.³⁴ There are studies that demonstrated differential effects of prenatal opiate exposure on the tail flick latency response to opiates in adults.³⁵ With these different data, it could be suggested that the inhibitory effect of mifepristone on opioid receptors is related to an overlapping phenomenon between these systems in the pain modulation pathway, because we have also shown that the analgesic effect of dexamethasone is inhibited for animals in which opioid receptors are blocked by naloxone. This result is supported by a study showing that adrenocorticotrophic hormone-induced analgesia in conscious rats is mediated by opioid receptors.²¹ Interactions between opioids and glucocorticoids have been shown in the central nervous system, where it has been reported that opioid blockade with naloxone could prevent negative feedback at the level of the hypothalamic–pituitary–adrenal axis.³⁶

These results suggest that there is a clear overlapping effect in pain modulation between the opioid and glucocorticoid systems. Opioids regulate glucocorticoid-induced analgesia and, after puberty, glucocorticoids regulate opioid-induced analgesia. These novel findings may help us to prevent the side effects of drugs like morphine, perhaps including morphine addiction.

Disclosure

The authors report no conflicts of interest in this work.

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