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Dexamethasone reduces morphine-induced straub reaction in mice

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Abstract

This study examined the effect of dexamethasone on morphine-induced straub reaction in mice. When morphine was administered in doses of 7.5, 15 and 30 mg kg^{-1} intraperitoneally, a dose-dependent straub reaction was produced. Dexamethasone per-se (0.1–10 mg kg^{-1} i.p.) did not modify the tail of control mice.

Pre-treatment with dexamethasone 120 min before morphine injection caused a dose-dependent reduction of straub reaction. Cycloheximide (15 mg kg^{-1} i.p.) administered 2 h before morphine did not change morphine-induced straub reaction, but was able to prevent the effects of dexamethasone on morphine-induced straub reaction. The glucocorticoid receptor antagonist RU-38486 (15 mg kg^{-1} i.p.) did not affect morphine-induced straub reaction, whereas it was able to block the effects of dexamethasone on morphine-induced straub reaction. Results of this study indicate that dexamethasone reduced morphine-mediated straub reaction in mice, indicating a further important functional interaction between dexamethasone and the opioid system. The ability of cycloheximide and RU-38486 to block dexamethasone's effects indicates that the steroid's interference with morphine-mediated straub reaction involves a protein-synthesis-dependent mechanism via glucocorticoid receptors.

Introduction

Morphine causes a rigid elevation of the tail in rats and mice. This response is called Straub reaction, since it was first described by Straub in 1911 (Gupta et al 1988), and there have been several studies of the neurotransmitter mechanisms involved in this phenomenon. Besides the central dopaminergic system (Jaju & Srivastava 1984), a variety of other neurotransmitters including acetylcholine (Racagni et al 1977), histamine (Gupta et al 1979), serotonin (Beaumont & Hughes 1979), gamma-aminobutyric acid (GABA) (Jaffe & Martin 1985) and noradrenaline (norepinephrine) (Cools 1977) have been implicated in the mechanism of the central actions of morphine.

Recently, we found that dexamethasone exerts consistent inhibition against the effects induced by opioids. In fact, dexamethasone is able to modify the effects of opioids in analgesia, constipation, hypermotility, epilepsy, dependence and hypotension (Capasso et al 1991, 1992a, b, 1996; Pieretti et al 1991, 1992, 1994; Sorrentino et al 2001), indicating the existence of an important interaction between glucocorticoids and the opioid system.

Although the dexamethasone–opioid interaction has been widely studied by the authors, the effects of dexamethasone on the straub reaction induced by morphine is unknown. Therefore, this study examines whether dexamethasone is able to modify morphine-induced straub reaction in mice.

Furthermore, we investigated the influence of the glucocorticoid receptor antagonist RU-38486 (Proulx-Terland et al 1982) on the effects induced by morphine in mice pre-treated with dexamethasone. Finally, since it has been reported that cycloheximide prevented some effects of dexamethasone (Holaday et al 1978; Pieretti et al 1992; Capasso et al 1996; Sorrentino et al 2001), we investigated whether dexamethasone's

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effects may be prevented by cycloheximide as previously reported (Pieretti et al 1992; Capasso et al 1996; Sorrentino et al).

Materials and Methods

Drugs

Dexamethasone-21-phosphate and cycloheximide were obtained from Sigma (St Louis, MO). Morphine hydrochloride was from Carlo Erba (Milano, Italia). RU-38486 was from Roussell-Uclaf (Paris, France).

Animals

Male Swiss mice, 20–25 g, were supplied by Charles River (Italy). The mice were housed in colony cages (10 mice each) under standard light (light on: 0700–1900 h), temperature (22±1°C) and room-humidity (60±10%) conditions for at least 1 week before the experimental sessions. Food and water were freely available. In all experiments attention was paid to the ethical guidelines for experiments in conscious animals (Zimmerman 1983).

Straub tail measurement

Mice were placed individually in a glass cylinder (30 cm wide, 50 cm long) and allowed to habituate for 30 min before drug administration. Immediately after drug injection, each mouse was replaced into the cylinder and the response was recorded by direct observation. The duration of straub tail was scored every 2 min over 1 h as follows: 0, absent; 1, the tail forms an angle of more than 30° to the bottom of the cage. A score of 0 indicated no straub tail. A score of 30 indicated maximum straub tail. Each mouse was tested for straub reaction only once.

Experimental procedure

The effect of dexamethasone on morphine-induced straub reaction was studied according to the following experimental schedule: dexamethasone (0.1, 1.0, 10 mg kg^{-1}) or saline was injected intraperitoneally 120 min before intraperitoneal injection of morphine (7.5, 15 or 30 mg kg^{-1}) or saline; RU-38486 and cycloheximide were injected intraperitoneally at a dose of 15 mg kg^{-1} 2 h before dexamethasone administration.

Data analysis

Due to the presence of cut-off values in this study, we preferred to express all the data as medians and to process them through non-parametric tests. Statistical analysis was performed by analysis of variance for comparison between baseline latency in all groups and by the Mann–Whitney *U*-test for between-group differences. The former statistical analysis did not reveal significant differences among groups and a common baseline value was therefore reported (= 0). A probability of less than 0.05 (two tailed) was considered statistically significant.

Results and Discussion

Straub reaction induced by morphine in mice

Morphine (7.5, 15 and 30 mg kg^{-1} i.p.) produced a dose-dependent straub reaction ranging from 12 to 30 (Figure 1). The effect was detectable within 10 min of morphine administration and the peak effect was reached within 15 min. The effect lasted for 3 h.

Effect of dexamethasone on morphine-induced straub reaction in mice

Dexamethasone (0.1, 1.0, 10 mg kg^{-1} i.p.), RU-38486 (15 mg kg^{-1} i.p.) and cycloheximide (15 mg kg^{-1} i.p.) did not significantly modify the tail of mice: a score of 0 was observed, indicating no straub tail.

However, when dexamethasone was administered 120 min before morphine injection, significant effects on straub reaction were observed. Dexamethasone, at all doses used (0.1, 1.0, 10 mg kg^{-1} i.p.), was able to reduce morphine-induced straub reaction in a dose-dependent manner; this reduction lasted for the whole recording period (Figure 2).

The effect induced by RU-38486 in mice pre-treated with dexamethasone is shown in Figure 3. RU-38486 administration (15 mg kg^{-1} i.p.) was able to prevent dexamethasone reduction of straub reaction induced by morphine. Similar results were found in mice treated with the protein synthesis inhibitor, cycloheximide. The administration of cycloheximide (15 mg kg^{-1} i.p.) by itself did not modify the morphine-induced straub reaction, whereas

Figure 1 Effect of morphine on straub reaction in mice. The bars represent the median of one determination 120 min after morphine treatment (n = 12).

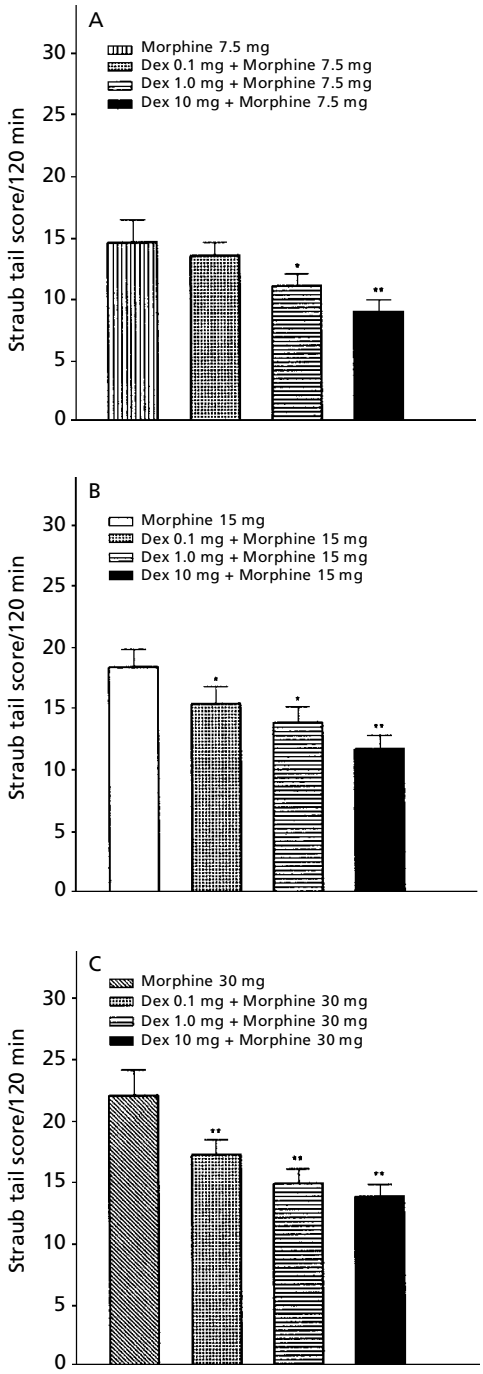

Figure 2 Effect of dexamethasone (Dex; 0.1, 1.0, 10 mg kg^{-1} i.p.) on straub reaction induced by intraperitoneal injection of morphine 7.5 mg kg^{-1} (A), 15 mg kg^{-1} (B) or 30 mg kg^{-1} (C) in mice. The bars represent the median of one determination 120 min after morphine treatment (n = 12). $*P < 0.05$; $**P < 0.01$, vs morphine alone.

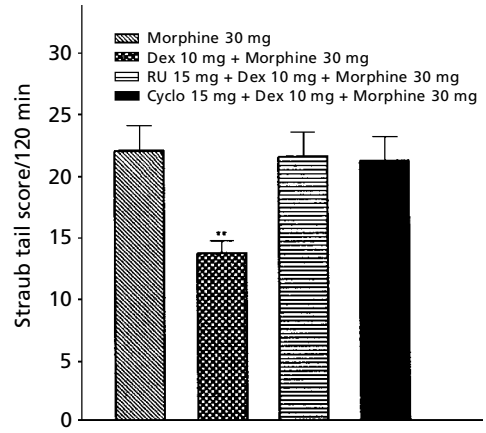

Figure 3 Effect of RU-38486 (RU) and cycloheximide (Cyclo; 15 mg kg^{-1} i.p.) on dexamethasone (Dex; 10 mg kg^{-1} i.p.) reduction of straub reaction induced by morphine (30 mg kg^{-1} i.p.) in mice. The bars represent the median of one determination 120 min after morphine treatment (n = 12). $*P < 0.05$; $**P < 0.01$, vs morphine alone.

when administered 1 h before dexamethasone, it prevented the reduction of the morphine straub reaction induced by dexamethasone (Figure 3).

This study indicates that dexamethasone reduces morphine-induced straub reaction and the steroid was effective in preventing hypotension when it was administered 120 min before opioid agonists.

Since corticosteroids mediate their effects through specific glucocorticoid receptors and protein synthesis, we also investigated the effects of the glucocorticoid receptor antagonist RU-38486 (Moguilewski & Philbert 1984; Gaillard et al 1984, 1985; Peers et al 1988; Ratka et al 1988a, b) and the protein synthesis inhibitor cycloheximide (Blackwell et al 1980) on the effects induced by dexamethasone on the morphine-induced straub reaction. Both RU-38486 and cycloheximide were capable of blocking dexamethasone, preventing its effect on morphine straub reaction. These results confirm and extend our previous findings (Pieretti et al 1991, 1992, 1994; Capasso et al 1992, 1996; Sorrentino et al 2001), indicating a further important functional interaction between dexamethasone and the opioid system.

Corticosteroids exert many of their effects on target cells through intracellular receptor mechanisms (Thompson & Lippman 1974). Two types of receptors for corticosteroids are known – type 1 and type 2 (De Kloet & Reul 1987). Type 2 receptors display a higher affinity for synthetic glucocorticoids, such as dexamethasone, than corticosterone (Reul & De Kloet 1985), and RU-38486 antagonizes dexamethasone's effects at the type-2 receptor binding sites (Gaillard et al 1984, 1985; Moguilewski & Philbert 1984; Peers et al 1988). In this study, the possible involvement of glucocorticoid receptors was confirmed by virtue of RU-38486 blocking dexamethasone's reduction of the morphine-induced straub reaction. Dexamethasone also displays a time-related inhibitory effect on opioid hypotension. Various reports in the literature support the view that effects mediated by corticosteroids are time related and involve intracellular mechanisms or protein synthesis (Thompson & Lippman 1974). Our results indicate that the inhibitory effects of dexamethasone on opioid

hypotension are prevented by cycloheximide, a protein synthesis inhibitor, which suggests that dexamethasone may exert its inhibitory effects on morphine straub reaction through a protein-synthesis-dependent mechanism. The dexamethasone lag time of 120 min, required for the appearance of corticosteroid action, further enforces this hypothesis. Therefore, considering that steroid action depends on receptor occupation and protein synthesis (Blackwell et al 1980), the results obtained with RU-38486 and cycloheximide suggest that in our experiments dexamethasone reduces morphine straub reaction through a synthesis-dependent mechanism via glucocorticoid receptors.

The discussion on the possible mechanism by which dexamethasone causes a reducing effect on opioid hypotension is still open. Some authors suggest that glucocorticoid-induced reduction of opioid effects might be mediated through an alteration in drug metabolism by the liver, presumably through an enhancement of drug metabolism, leading to lower drug concentrations. This would finally result in a decreased effect of the drug. Holaday et al (1978) reported a greater concentration of morphine in the blood and brain of adrenalectomized versus sham control mice, and showed that dexamethasone treatment in adrenalectomized mice restored morphine levels to those observed in sham control mice. Furthermore, Holaday et al (1978) reported that dexamethasone-induced effects were reduced by cycloheximide pre-treatment and that dexamethasone also reduced morphine levels in the blood and brain of sham control mice, indicating that glucocorticoids might induce an overall opioid-metabolizing activity. In our study we could not exclude the possibility that the reduction of morphine straub reaction in mice pre-treated with dexamethasone might occur via an alteration of morphine metabolism as previously reported (Holaday et al 1978).

On the other hand, we cannot exclude another possibility, that dexamethasone may exert its action on morphine straub reaction by a mechanism wherein the sensitivity to opioids may be altered. This hypothesis is supported by several studies showing a link between glucocorticoids and the opioid system, most prominently regarding the glucocorticoid-induced changes of opioid receptors. In this regard, it has been suggested that glucocorticoids may act, directly or indirectly, upon the same neuronal pathways as do recognized opioids (La Bella et al 1978). This supports the possibility that glucocorticoids may interact with receptors having lesser affinity for recognized opioids but coupled to the activation of the same neuronal pathway. Also, glucocorticoids may produce their effects by controlling the rate of production, release or degradation of endogenous opioid ligands (La Bella et al 1978). Furthermore, the steroid-dependent changes in opioid receptors and related to altered opioid-induced antinociception has been suggested as a possible mechanism underlying this interaction (Ratka et al 1988b). This hypothesis was further supported by data showing that the number of opioid receptors and some morphine effects are reduced in stressed animals in which high corticosteroid levels are detected (Seeger et al 1984).

In conclusion, whatever may be the mechanism underlying the action of dexamethasone, these results further indicate an important interaction between corticosteroids and the opioid system.

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