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Clonidine-induced antinociception and locomotor hypoactivity are reduced by dexamethasone in mice

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Abstract

The effects of dexamethasone pretreatment on clonidine-induced antinociception and locomotor hypoactivity were investigated in mice. In the hot-plate and the tail-flick tests, dexamethasone administered intraperitoneally at a dose of 1 mg kg^{-1}, 30 or 60 min before clonidine, reduced clonidine antinociception in both tests and reduced clonidine-induced locomotor hypoactivity in the activity cage. When administered 15 min before clonidine, dexamethasone had no effect on clonidine antinociception. A higher dexamethasone dose (10 mg kg^{-1}) induced the same effects observed at a dose of 1 mg kg^{-1} in the hot-plate and the tail-flick tests, but the former dose had a stronger effect on locomotor hypoactivity. Dexamethasone (10 ng/mouse) administered intracerebroventricularly 30 min before clonidine was also able to reduce both clonidine-induced antinociception and locomotor hypoactivity. The protein synthesis inhibitor, cycloheximide, administered intraperitoneally at the dose of 10 mg kg^{-1}, 2 h before clonidine, was able to prevent dexamethasone effects on clonidine-induced antinociception. The glucocorticoid receptor antagonist RU-38486, administered intracerebroventricularly at the dose of 1 ng/mouse, was also able to block dexamethasone effects on clonidine-induced antinociception and locomotor hypoactivity, whereas both cycloheximide and RU-38486 per se did not influence pain sensitivity or locomotor activity. These results suggest that the dexamethasone effects on clonidine-induced antinociception and locomotor hypoactivity depend on the stimulating effects that dexamethasone exert, on the protein synthesis via the glucocorticoid receptor in the brain.

Introduction

Clonidine is mainly considered as an α_2-adrenergic agonist that, besides having hypotensive action, induces antinociception and hypoactivity. Clonidine reduces the response to pain induced by a wide variety of noxious stimuli including chemicals (Skingle et al 1982; Luttinger et al 1984), heat (Lin et al 1980), pressure (Fielding et al 1978) and electrical stimuli (Paalzow & Paalzow 1976). The site of clonidine action is the central nervous system (Drew 1976; Drew et al 1979; Skingle et al 1982), where clonidine antinociception and locomotor hypoactivity are mediated by α_2-adrenergic receptors.

Several investigations showed that glucocorticoids interfere with some effects induced by opiates. Glucocorticoid pretreatment reduces morphine-induced antinociception in rats and mice (Chatterjee et al 1982; Pieretti et al 1991; Capasso et al 1992), reduces or enhances morphine-induced hypermotility in a dose-dependent manner in mice (Capasso et al 1991), and blocks morphine-induced hippocampal seizures in rats and rabbits (Pieretti et al 1992).

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In the present study we examined the possibility that peripheral or central dexamethasone administration could influence antinociception and locomotor hypoactivity induced by clonidine in mice. This investigation followed the working hypothesis that glucocorticoids could exert strong interference not only on antinociception (and cerebral excitability) induced by drugs active on the opioid system, but also on antinociception (and locomotor hypoactivity) exerted by clonidine. Clonidine produces analgesia on its own, and potentiates the analgesia produced by opiates (Furst 1999). Although antinociception and locomotor hypoactivity induced by a relatively high dose of clonidine are not antagonized by opioid antagonists (Fielding et al 1978), a certain degree of cross-tolerance was found between them (Flacke & Flacke 1993).

Since in previous studies it has been demonstrated that dexamethasone exerts its effects with a certain time lag (Tsurufuji et al 1979), and that dexamethasone may exert different effects when administered at low and high doses (Capasso et al 1991), we administered dexamethasone at various doses and at various time intervals before clonidine administration. Furthermore, we investigated the influence of the glucocorticoid receptor antagonist RU-38486 (Proulx-Terland et al 1982) on the effects induced by clonidine in mice pretreated with dexamethasone. Finally, since it has been reported that cycloheximide prevented some dexamethasone effects (Holaday et al 1978; Pieretti et al 1992), we performed some experiments to investigate whether dexamethasone effects may be prevented by cycloheximide pretreatment.

Materials and Methods

Animals

Male CD-1 mice (Charles River, Italy), 25–30 g, were used in the experiments. The mice were housed in colony cages with free access to food and water before the experiments. They were maintained in a climate- and light-controlled room (22 ± 1°C, 12-h dark–light cycle with lights on at 0700 h) for at least 7 days before the experiments. All experiments were performed from 0800 to 1400 h and each mouse was used in only one experimental session.

Drugs

Clonidine hydrochloride, yohimbine hydrochloride, dexamethasone-21 phosphate, and cycloheximide (Sigma Chemical Co., St Louis, MO) were dissolved in either 0.9% NaCl solution for intraperitoneal administration, or in distilled water for intracerebroventricular administration. RU-38486 (Roussel-Uclaf, France) solutions were prepared by dissolving the steroid in absolute ethanol; fractions of this solution were used for subsequent dilution in distilled water for intracerebroventricular treatment, and were administered after sonication. Drugs were injected in a volume of 5 mL kg^{-1} for intraperitoneal administration or in a volume of 5 μL/mouse for intracerebroventricular administration. Intracerebroventricular injection was performed according to the method described by Haley & McCormick (1957). At the end of the experimental session, the injection site was verified by using 1% methylene blue and examining the distribution of the dye in the cerebrum.

Nociceptive assays

Changes in nociceptive threshold were measured using the hot-plate and the tail-flick tests (Pieretti et al 1991; Capasso et al 1992). The hot-plate test was conducted using an analgesimeter (Socrel Model DS-37; Ugo Basile, Italy) consisting of a metal plate (25 × 25 cm^2) heated at constant temperature (55 ± 0.5°C) on which a plastic cylinder (20 cm diam. × 16 cm high) was placed. Latency was measured as the time that elapsed between the moment the mouse was placed on the hot plate and when it licked its paw. The recording was terminated if the latency exceeded the cut-off time (30 s). In untreated mice, the basal latency was 8–10 s. Tail-flick latency was obtained using a device (Socrel Model DS-20; Ugo Basile, Italy) consisting of an infrared source, radiant light (100 W bulb) with adjustable intensity which was focussed by an aluminized parabolic mirror on a photocell. Radiant heat was focussed on a blackened spot 1–2 cm from the tip of the tail, and the latency time until the mouse flicked its tail was recorded. The measurement was terminated if the latency exceeded the cut-off time (10 s). Beam intensity was adjusted to produce a tail-flick latency of 2–3 s in untreated mice. The mice were gently restrained during trials by using a glove. Each mouse was tested twice, 60 and 30 min before the first injection in the baseline determination. The tests were performed five more times, at 15, 30, 60, 90 and 120 min after clonidine administration. In all experiments attention was paid to the ethical guidelines for investigations of experimental pain in conscious animals (Zimmerman 1983).

In these experiments, yohimbine, dexamethasone, RU-38486 or cycloheximide were injected before intra-

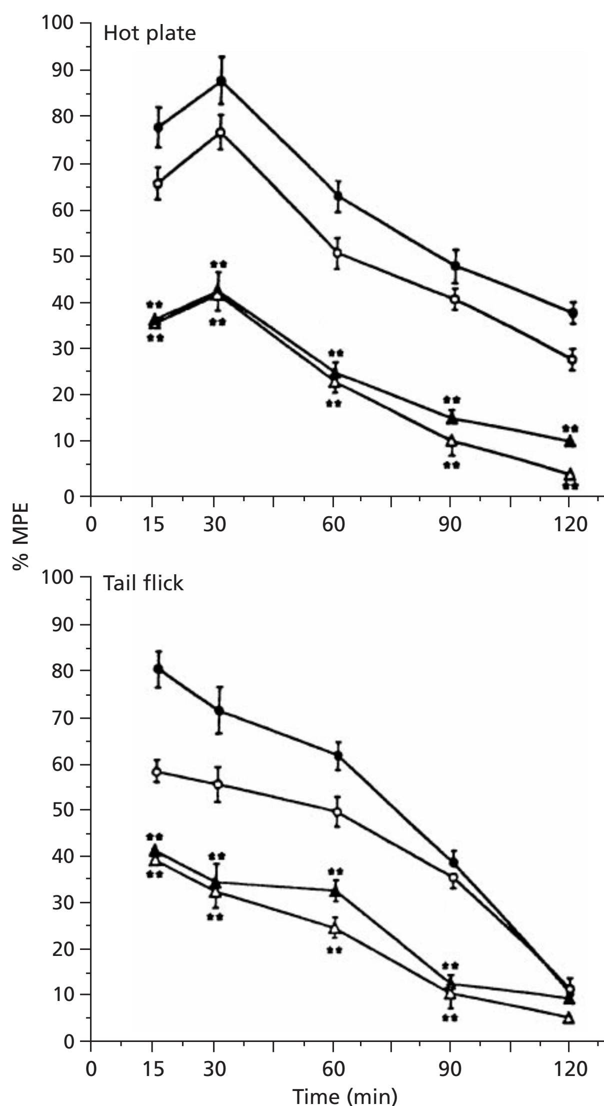

Figure 1 Effects induced by dexamethasone administered intraperitoneally at a dose of 1 mg kg^{-1} 15 min (○), 30 min (▲) or 1 h (△) before clonidine administered intraperitoneally at a dose of 1 mg kg^{-1}, in the hot-plate and tail-flick tests (●, 1 mg kg^{-1} clonidine alone). Values are the mean ± s.e. (n = 15) of the percentage maximum possible effect (% MPE). $^{**}P < 0.01$ vs clonidine.

peritoneal administration of clonidine (1 mg kg^{-1}), according to the following experimental schedule: yohimbine (5 mg kg^{-1}, i.p.) 20 min before clonidine; dexamethasone (1 mg kg^{-1}, i.p.) 15 min, 30 min or 1 h before clonidine; dexamethasone (0.1 and 10 mg kg^{-1}, i.p.) 30 min before clonidine; dexamethasone (10 ng/mouse, i.c.v.) 30 min before clonidine (1 or 2 mg kg^{-1}); cycloheximide (10 mg kg^{-1}, i.p.) 1.5 h before dexamethasone (1 mg kg^{-1}, i.p.) and 2 h before clonidine (1 mg kg^{-1}); RU-38486 (1 ng/mouse, i.c.v.) 15 min before clonidine (1 mg kg^{-1}) in mice treated with dexamethasone (1 mg kg^{-1}, i.p.) 30 min before clonidine. Results were expressed as a percentage of the maximum possible effect (% MPE = (test latency − basal latency/cut-off latency − basal latency) × 100). Statistical significance was determined using analysis of variance followed by Scheffe's test.

Locomotor activity

Locomotor activity was measured as previously reported (Capasso et al 1991) with some modifications. Mice were randomly assigned to an experimental group, and placed for 2 h in the activity cage (Ugo Basile, Italy) in groups of four, each day for four days before the experimental session. On the day of the experiment the mice were placed in the activity cage for at least 30 min before drug administration. Uniform temperature, sound and light conditions were maintained during the course of the experiments. Measurements were carried out at 20-min intervals and cumulative counts were recorded after 2 h. In these experiments yohimbine, dexamethasone or RU-38486 were injected before clonidine (0.05 mg kg^{-1}, i.p.) according to the following experimental schedule: yohimbine (0.5 mg kg^{-1}, i.p.) 20 min before clonidine; dexamethasone (1 mg kg^{-1}, i.p.) 15 min, 30 min or 1 h before clonidine; dexamethasone (0.1 and 10 mg kg^{-1}, i.p.) 30 min before clonidine; dexamethasone (10 ng/mouse, i.c.v.) 30 min before clonidine; RU-38486 (1 ng/mouse, i.c.v.) 15 min before clonidine in mice treated with dexamethasone (1 mg kg^{-1}, i.p.) 30 min before clonidine. Data were statistically analysed by one-way analysis of variance followed by multiple post-hoc Newman-Keuls test.

Results

Effects of dexamethasone on clonidine antinociception

Clonidine (0.1–2 mg kg^{-1}) induced a dose-dependent antinociceptive effect in both the hot-plate and the tail-flick tests, and this effect reached a peak 30 and 15 min after administration in the hot-plate and tail-flick tests, respectively. ED50 (probit method) was similar for the two tests (0.72 and 0.71 mg kg^{-1}, respectively); the duration of effect was 30–60 min for lower doses, and 120–180 min for higher doses (data not shown). In the first series of experiments we investigated time–effect and dose–effect induced by dexamethasone on clonidine

Table 1 Effects induced by yohimbine (5 mg kg^{-1}, i.p.) and dexamethasone (0.1, 1 and 10 mg kg^{-1}, i.p.) in mice treated with clonidine (1 mg kg^{-1}, i.p.) in the hot-plate and tail-flick tests.

Test	Time elapsed after clonidine administration (min)				
	15	30	60	90	120
Hot-plate					
Clonidine	77 ± 3.3	91 ± 1.6	68 ± 3.2	57 ± 2.1	33 ± 2.6
Yohimbine + clonidine	28 ± 1.3**	26 ± 1.9**	17 ± 1.1**	10 ± 0.7**	9 ± 0.8**
Dexamethasone (0.1 mg kg^{-1}) + clonidine	80 ± 2.8	91 ± 2.0	74 ± 2.0	55 ± 1.7	40 ± 2.2
Dexamethasone (1 mg kg^{-1}) + clonidine	42 ± 1.7**	53 ± 2.7**	32 ± 1.8**	27 ± 1.4**	18 ± 1.2**
Dexamethasone (10 mg kg^{-1}) + clonidine	45 ± 1.4**	56 ± 2.8**	36 ± 1.8**	23 ± 1.0**	18 ± 1.1**
Tail-flick					
Clonidine	69 ± 2.6	55 ± 2.3	46 ± 1.3	26 ± 1.5	19 ± 1.3
Yohimbine + clonidine	36 ± 1.2**	28 ± 1.7**	26 ± 1.3**	17 ± 1.5**	11 ± 1.6**
Dexamethasone (0.1 mg kg^{-1}) + clonidine	80 ± 1.7	73 ± 2.6	65 ± 2.2	27 ± 1.6	18 ± 0.1
Dexamethasone (1 mg kg^{-1}) + clonidine	39 ± 1.4**	30 ± 1.6**	28 ± 1.4**	21 ± 1.3**	9 ± 0.6**
Dexamethasone (10 mg kg^{-1}) + clonidine	40 ± 2.1**	36 ± 1.5**	29 ± 1.8**	14 ± 1.1**	12 ± 0.8**

Yohimbine was administered 20 min before clonidine; dexamethasone was administered 30 min before clonidine. Values are mean ± s.e. (n = 15) of the percentage maximum possible effect. **$P < 0.01$ vs clonidine.

antinociception. Dexamethasone administered intraperitoneally at the dose of 1 mg kg^{-1}, 30 min or 1 h before clonidine, produced a significant reduction in the antinociceptive effects induced by clonidine both in the hot-plate and tail-flick tests (Figure 1), whereas dexamethasone administered 15 min before clonidine did not induce consistent changes. Dexamethasone at the dose of 10 mg kg^{-1}, 30 min before clonidine administration, exerted the same effects as 1 mg kg^{-1}, and analogous effects to those obtained with the antagonist yohimbine (Table 1), whereas the dose of 0.1 mg kg^{-1} did not effect clonidine-induced antinociception. Dexamethasone administered intracerebroventricularly at the dose of 10 ng/mouse, 30 min before clonidine (1 or 2 mg kg^{-1}), induced a strong reduction in clonidine-induced antinociception in the hot-plate and tail-flick tests (Figure 2).

Inhibitory activity of cycloheximide and RU-38486 on dexamethasone effects

Since these experiments showed that dexamethasone, administered 30 or 60 min before clonidine, was able to reduce clonidine-induced antinociception, we decided to investigate whether these effects could be reversed by the protein synthesis inhibitor cycloheximide, or by the glucocorticoid receptor antagonist RU-38486. The results of these experiments are shown in Table 2. In mice treated with cycloheximide (10 mg kg^{-1}, i.p.) 1.5 h before dexamethasone (1 mg kg^{-1}) and 30 min before clonidine (1 mg kg^{-1}), cycloheximide completely prevented dexamethasone effects on clonidine-induced antinociception. The same results were found in mice treated with the RU-38486 (1 ng/mouse, i.c.v.) administered 15 min before clonidine. Dexamethasone administered intraperitoneally or intracerebroventricularly, cycloheximide administered intraperitoneally and RU-38486 administered intracerebroventricularly by themselves were not able to change the nociceptive threshold (data not shown). Cycloheximide injected 2 h before clonidine and RU-38486, injected intracerebroventricularly 15 min before clonidine, did not prevent clonidine-induced effects.

Locomotor activity

Clonidine administered at doses of 0.05, 0.1 and 0.5 mg kg^{-1} produced a marked reduction in locomotor activity, which was prevented by yohimbine (Table 3). Dexamethasone, administered intraperitoneally 30 min or 1 h before clonidine at the dose of 1 mg kg^{-1}, was able to significantly reduce the hypoactivity induced by clonidine; the dose of 10 mg kg^{-1} induced a stronger inhibitory effect. Dexamethasone administered intracerebroventricularly at a dose of 10 ng/mouse, 30 min before clonidine, was also able to reduce clonidine-induced locomotor hypoactivity, whereas a lower dose (0.1 mg kg^{-1}) or a dose of 1 mg kg^{-1} administered 15 min before clonidine were ineffective. Also RU-38486 was able to prevent dexamethasone effects on the reduction of locomotor activity induced by clonidine. RU-38486

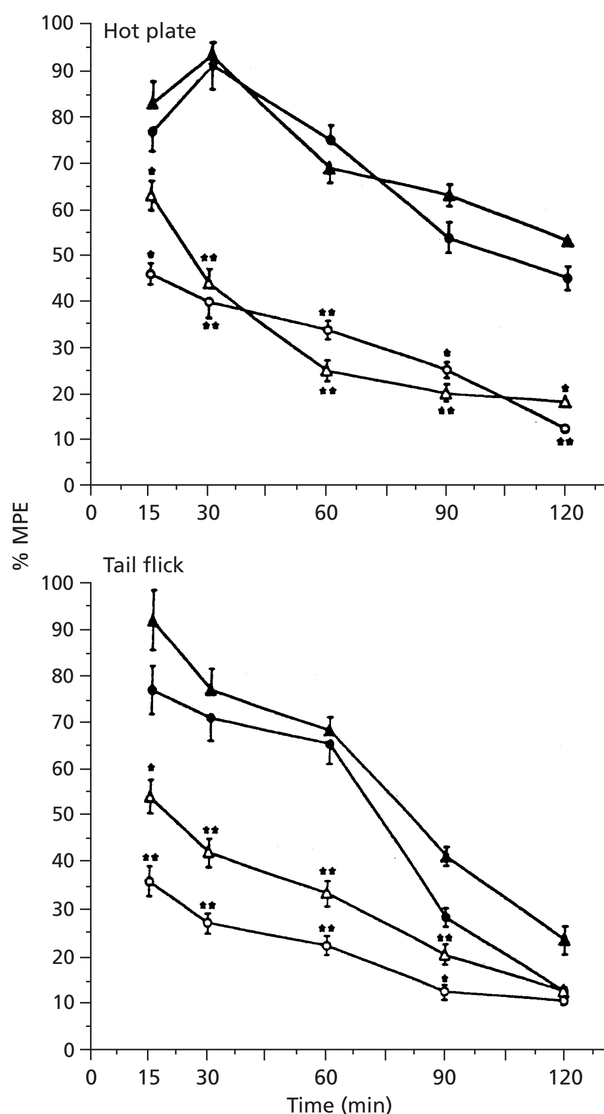

Figure 2 Effects induced by dexamethasone administered intracerebroventricularly at the dose of 10 ng/mouse, 30 min before clonidine administered intraperitoneally at a dose of 1 or 2 mg kg^{-1}, in the hot-plate and tail-flick tests (●, 1 mg kg^{-1} clonidine; ▲, 2 mg kg^{-1} clonidine; ○, dexamethasone + 1 mg kg^{-1} clonidine; △, dexamethasone + 2 mg kg^{-1} clonidine). Values are the mean ± s.e. (n = 15) of the percentage maximum possible effect (% MPE). $^{**}P < 0.01$ vs clonidine.

administered alone did not change clonidine-induced locomotor hypoactivity (Table 4).

Discussion

The data obtained in our experiments indicated that dexamethasone strongly prevented both the antinociception and the locomotor hypoactivity induced by clonidine in mice. Moreover, dexamethasone exerted maximum effects 30–60 min after administration. These results appear to be very similar to results we obtained in previous studies on the antagonistic effect of dexamethasone and opioid drugs, that is, dexamethasone pretreatment prevented antinociception induced by μ- and δ-opioid receptor agonist drugs; this effect was exerted with at least 30 min delay, and was prevented by cycloheximide or by RU-38486 (Capasso et al 1992; Pieretti et al 1992). Glucocorticoids are known to exert many of their effects with a certain time-lag, through stimulation of protein synthesis via an intracellular receptor mechanism (Thompson & Lippman 1974). Therefore dexamethasone was injected after administration of the protein synthesis inhibitor, cycloheximide, to test if protein synthesis was involved in dexamethasone effects. As expected, cycloheximide blocked the capacity of dexamethasone to reduce clonidine-induced effects. It has been suggested that glucocorticoid-induced reduction of the opioid effects might be mediated through an alteration in drug metabolism by the liver, presumably through an enhancement of drug metabolism and therefore producing lower drug concentrations, and this would finally result in a decreased effect of the drug (Holaday et al 1978). In our study we cannot exclude that the reduction of clonidine effects in mice pretreated with dexamethasone might occur also via an alteration of clonidine metabolism. However, the results of our study suggest another hypothesis. Dexamethasone was able to reduce sensitivity to clonidine even when dexamethasone was injected intracerebroventricularly at a low dose. Moreover, in the nociceptive assays, the effects induced by dexamethasone administered peripherally were similar to those observed after central administration, thus suggesting a central site in the action of dexamethasone. Furthermore, in our experiments dexamethasone effects were prevented by central RU-38486 administration. Type 2 receptors for corticosteroids are present both in neurons and glial cells, and show widespread localization in the brain (Reul & De Kloet 1986). These receptors display higher affinity for synthetic glucocorticoids such as dexamethasone than does corticosterone (Reul & De Kloet 1986), and mediate the increase in synthesis of some proteins that occur in the brain after glucocorticoid treatment (Schlatter & Dokas 1988). RU-38486 antagonizes dexamethasone at type 2 receptor binding sites (Moguilewski & Philbert 1984), blocks the inhibitory action exerted by dexamethasone on the pituitary–adrenal axis (Gaillard et al 1984), inhibits bethamethasone-induced vasocostriction (Gail-

Table 2 Effects induced by dexamethasone (1 mg kg^{-1}, i.p.), cycloheximide (10 mg kg^{-1}, i.p.) and RU-38486 (1 ng/mouse, i.c.v.) in mice treated with clonidine (1 mg kg^{-1}, i.p.) in the hot-plate and tail-flick tests.

Test	Time elapsed after clonidine administration (min)				
	15	30	60	90	120
Hot-plate					
Clonidine	75±3.9	89±2.7	65±3.1	45±2.0	35±3.0
Dexamethasone+clonidine	39±2.3**	45±3.9**	27±2.3**	17±1.2**	12±1.8**
Cycloheximide+clonidine	81±5.4	90±3.6	60±3.0	43±2.3	32±21
Cycloheximide+dexamethasone+clonidine	66±3.9††	83±4.7††	58±2.1	36±2.4††	30±3.2††
RU-38486+clonidine	82±4.3	93±4.5	75±2.5	65±3.1	44±2.9
Dexamethasone+RU-38486+clonidine	80±3.2††	90±5.0††	65±3.4††	50±3.7††	40±2.3††
Tail-flick					
Clonidine	74±2.9	67±3.7	60±3.3	30±1.0	20±1.0
Dexamethasone+clonidine	41±1.4**	34±2.9**	32±1.5**	12±1.3**	10±0.8**
Cycloheximide+clonidine	72±4.0	66±2.6	57±2.3	27±2.1	17±1.9
Cycloheximide+dexamethasone+clonidine	73±4.9††	69±3.9††	52±3.1††	35±1.7††	29±2.2††
RU-38486+clonidine	82±3.3	76±5.3	62±3.4	41±3.0	34±3.0
Dexamethasone+RU-38486+clonidine	70±3.9††	65±3.1††	57±4.0††	34±2.2††	23±2.3††

Dexamethasone was administered 30 min before clonidine; RU-38486 was administered 15 min before clonidine; cycloheximide was administered 2 h before clonidine. In the experiments performed with cycloheximide+dexamethasone, cycloheximide was administered 1.5 h before dexamethasone and 2 h before clonidine. In the experiments performed with RU-38486+dexamethasone, RU-38486 was administered 15 min after dexamethasone and 15 min before clonidine. Values are mean±s.e. (n = 15) of the percentage maximum possible effect. $^{**}P < 0.01$ vs clonidine; $^{††}P < 0.01$ vs dexamethasone+clonidine.

Table 3 Effects induced by yohimbine (0.5 mg kg^{-1}, i.p.) and dexamethasone (0.1, 1 and 10 mg kg^{-1}, i.p., and 10 ng/mouse, i.c.v.) on the reduction of the locomotor activity induced by clonidine (0.05 mg kg^{-1}, i.p.).

Treatment	Cumulative counts after clonidine administration (min)					
	0–20	20–40	40–60	60–80	80–100	100–120
Saline	439±23	828±39	1303±41	1504±61	1804±80	1668±112
Clonidine	55±5	75±8	92±10	128±13	260±30	395±40
Yohimbine+clonidine	476±1**	945±27**	1412±54**	1616±73**	1712±81**	1786±123**
Dexamethasone (0.1 mg kg^{-1})+clonidine	54±6	71±8	100±14	124±18	280±34	386±41
Dexamethasone (1 mg kg^{-1})+clonidine	328±17**	463±42**	547±26**	687±53**	728±55**	934±62**
Dexamethasone (10 mg kg^{-1})+clonidine	346±25**	728±40**	1343±47**	1564±53**	1644±87**	1688±132**
Dexamethasone (10 ng/mouse)+clonidine	356±23**	488±45**	523±37**	665±84**	767±79**	904±66**

Yohimbine was administered 20 min before clonidine; dexamethasone was administered 30 min before clonidine. Values are the mean±s.d. (n = 6) of the cumulative counts recorded every 20 min for 2 h after clonidine administration. $^{**}P < 0.01$ vs clonidine.

lard et al 1985), and reverses the anti-inflammatory effects of dexamethasone (Peers et al 1988).

All these findings suggest that, in our experiments, dexamethasone reduces clonidine-induced effects through protein synthesis stimulation via type 2 glucocorticoid receptors in the brain.

Corticosteroids may modulate peripheral as well as central binding to α_2-adrenoreceptors, but this is still a matter for debate. Some reports have indicated that chronic corticosteroid treatment reduces the binding of clonidine in the brain cortex of rats (Szentendrei & Fekete 1990), and that some hypothalamic nuclei exhibit a diurnal variation of [^{3}H]para-aminoclonidine binding, with a significant decline in binding when plasmatic

Table 4 Effects induced by dexamethasone (1 mg kg^{-1}, i.p.) or RU-38486 (1 ng/mouse, i.c.v.) on the reduction of the locomotor activity induced by clonidine (0.05 mg kg^{-1}, i.p.).

Treatment	Cumulative counts after clonidine administration (min)					
	0–20	20–40	40–60	60–80	80–100	160–120
Clonidine	65 ± 7	87 ± 10	102 ± 13	135 ± 15	289 ± 33	401 ± 42
Dexamethasone + clonidine	341 ± 32**	445 ± 28**	574 ± 35**	685 ± 33**	789 ± 56**	912 ± 68**
RU-38486 + clonidine	57 ± 8	72 ± 4	83 ± 7	124 ± 11	244 ± 35	388 ± 32
Dexamethasone + RU-38486 + clonidine	106 ± 13††	128 ± 15††	173 ± 17††	275 ± 24††	367 ± 29††	434 ± 36††

Dexamethasone was administered 30 min before clonidine; RU-38486 was administered 15 min before clonidine. In the experiments performed with RU-38486 + dexamethasone, RU-38486 was administered 15 min after dexamethasone and 15 min before clonidine. Values are mean ± s.d. (n = 6) of the cumulative counts recorded every 20 min for 2 h after clonidine administration. **$P < 0.01$ vs clonidine; ††$P < 0.01$ vs dexamethasone + clonidine.

corticosterone levels are high (Jhanwar-Uniyal et al 1986). Maeda et al (1983) studied the changes in [^{3}H]clonidine binding in the vas deferens of reserpinized rats and found that the decrease in [^{3}H]clonidine binding was prevented by hydrocortisone or dexamethasone addition to the culture medium. Furthermore, Maeda et al (1983) reported that the effect of glucocorticoid was blocked by the inhibitors of protein synthesis, cycloheximide and puromycin.

Adrenalectomy specifically reduced the binding of [^{3}H]para-aminoclonidine to the α_2 receptors in the hypothalamic paraventricular nucleus, and this down-regulation was reversed by corticosterone replacement (Jhanwar-Uniyal et al 1985). These findings may suggest the possibility that dexamethasone effects on clonidine-induced antinociception and locomotor hypoactivity depend on the possible influence that dexamethasone exerts on α_2 receptor binding properties.

Other hypotheses cannot be excluded. Dexamethasone may exert its antagonistic effects also by interacting with other neuronal systems involved in clonidine-induced effects. Indeed, many findings point towards the potential involvement of serotonergic and cholinergic systems. Paalzow & Paalzow (1976) demonstrated that the depletion of serotonin by parachlorophenyl-alanine increased the effect of clonidine on the threshold for vocalization during stimulation and after withdrawal of stimulus. Duan & Sawynok (1987) reported that serotonin depletion induced by 5,7-dihydroxytryptamine potentiated the antinociceptive effect of systemically administered clonidine both in the hot-plate and tail-flick tests. It was also reported that the serotonin agonist RU-24969, the serotonin antagonists ketanserin and ritanserin, and the serotonin depletor 5,7-dihydroxytryptamine, enhance clonidine-induced hypoactivity (Heal & Philpot 1987). Furthermore, it has been reported that atropine antagonizes the prolongation of chloral hydrate sleep induced by clonidine, and that atropine reduces clonidine-induced hypothermia (Delbarre & Schmitt 1971; Tsoucaris-Kupfer & Schmitt 1972). Maj et al (1975) reported that the hypoactivity induced by clonidine in mice was antagonized by atropine and scopolamine, and that clonidine increased the acetylcholine content in rat striatum. The treatment with glucocorticoids increased brain serotonin levels (Neckers & Sze 1975; Ulrich et al 1975) and chronic as well as acute corticosteroid treatments increase high-affinity choline uptake as a result of an enhancement in the maximum choline transport velocity in brain limbic structures (Riker et al 1979). These findings suggest that the dexamethasone-induced reduction of clonidine-induced antinociception and locomotor hypoactivity may depend in part on the effect that dexamethasone exerts on the serotonergic and cholinergic systems.

Recent evidence also suggested that α_2-adrenergic agonists and corticosteroids differentially influence G protein subunits. G proteins modulate different effector units, such as adenylate cyclase, cyclic GMP phosphodiesterase, phospholipase C and ion channels (Gilman 1987). Experiments performed in-vivo showed that treatment with pertussis toxin reduced clonidine-induced antinociception and locomotor hypoactivity, thus suggesting that the G proteins functionally coupled to α_2-receptors are involved in clonidine-induced effects (Nomura et al 1987; Sanchez-Blazquez & Garzon 1991). In-vitro studies showed that α_2-adrenergic receptor stimulation induces a variety of neuronal activity changes via G proteins, such as inhibition of adenylate cyclase in rat brain membranes (Kitamura et al 1985) and in neuroblastoma x glioma hybrid cells (Kurose et

al 1983). Pertussis toxin also blocked the inhibition of adenylate cyclase mediated by α_2-receptor activation (Kurose et al 1983). Some reports showed that G protein subunits, namely Gsα, are under control of glucocorticoids (Saito et al 1989), and that dexamethasone enhances adenylate cyclase activity in rat pituitary cell line GH3 mediated, at least in part, by an increase in the cellular content of the α subunit of Gs protein (Chang & Bourne 1987). In this respect, the literature suggests that the opposite influence exerted by dexamethasone and clonidine on G proteins may explain the inhibitory effects induced by dexamethasone on clonidine-induced antinociception and locomotor hypoactivity.

Recent studies suggest another possible explanation: the ATP-sensitive K^+ channels play an important role not only in the antinociception induced by clonidine (Galeotti et al 1999a), but also in the antinociceptive effects induced by morphine (Vergoni et al 1992; Lohmann & Welch 1999; Rodrigues & Duarte 2000), H_1 antihistamines (Galeotti et al 1999b) and by adenosine H_1-receptor agonists (Ocana & Baeyens 1994). We may speculate that K^+-channel function is bound to a common mechanism of antinociception, which is influenced by dexamethasone. Although experimental evidence connecting ATP-gated K^+ channels and glucocorticoids is limited, we know that dexamethasone upregulates some voltage-gated K^+ gene transcription and expression in isolated cells (Takimoto et al 1993), and, in this context, we know that blockade of a voltage-gated K^+channel can prevent clonidine antinociception (Galeotti et al 1999a) and, finally, that μ-opioid receptor activation in the brain is bound to the activation of a voltage-dependent K^+ channel (Christie et al 2000).

Whatever the mechanism, our study provides evidence that dexamethasone reduces some of the pharmacological effects induced by an α_2-agonist, and that the interference of dexamethasone with clonidine-induced antinociception and locomotor hypoactivity are apparently mediated by protein synthesis activation via type 2 glucocorticoid receptors in the brain.

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