Dopamine-dependent responses to morphine depend on glucocorticoid receptors

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ABSTRACT Previous work has shown that glucocorticoid hormones facilitate the behavioral and dopaminergic effects of morphine. In this study we examined the possible role in these effects of the two central corticosteroid receptor types: mineralocorticoid receptor (MR), and glucocorticoid receptor (GR). To accomplish this, specific antagonists of these receptors were infused intracerebroventricularly and 2 hr later we measured: (i) locomotor activity induced by a systemic injection of morphine (2 mg/kg); (ii) locomotor activity induced by an infusion of morphine (1 μg per side) into the ventral tegmental area, which is a dopamine-dependent behavioral response to morphine; (iii) morphine-induced dopamine release in the nucleus accumbens, a dopaminergic projection site mediating the locomotor and reinforcing effects of drugs of abuse. Blockade of MRs by spironolactone had no significant effects on locomotion induced by systemic morphine. In contrast, blockade of GRs by either RU38486 or RU39305, which is devoid of antiprogestosterone effects, reduced the locomotor response to morphine, and this effect was dose dependent. GR antagonists also reduced the locomotor response to intraventral tegmental area morphine as well as the basal and morphine-induced increase in accumbens dopamine, as measured by microdialysis in freely moving rats. In contrast, spironolactone did not modify dopamine release. In conclusion, glucocorticoids, via GRs, facilitate the dopamine-dependent behavioral effects of morphine, probably by facilitating dopamine release. The possibility of decreasing the behavioral and dopaminergic effects of opioids by an acute administration of GR antagonists may open new therapeutic strategies for treatment of drug addiction.

Glucocorticoid hormones are important factors in determining the behavioral effects of drugs of abuse (1). Plasma concentrations of corticosterone, the major glucocorticoid in the rat, are positively correlated to the propensity of an individual to develop psychostimulant self-administration (2). In addition, administration of corticosterone before a self-administration session increases the reinforcing effects of amphetamine (2). Finally, suppression of corticosterone secretion by adrenalectomy decreases the psychomotor and reinforcing effects of psychostimulant or opiate drugs (3–6) and prevents the stress-induced sensitization of these effects (7, 8).

Glucocorticoids have been hypothesized to facilitate behavioral effects of drugs of abuse by acting on mesolimbic dopaminergic neurons (1), one of the principal neurobiological substrates for the psychomotor and reinforcing effects of drugs of abuse (9–11). Thus, blockade of corticosterone secretion also reduces the locomotor activity induced by an infusion of morphine in the ventral tegmental area (VTA) (4, 12) and of cocaine in the nucleus accumbens (4, 12), which are dopamine-dependent behavioral responses (13, 14). Furthermore, administration of corticosterone increases dopamine release in the nucleus accumbens (15) whereas depletion of corticosterone by adrenalectomy (16) or by the synthesis inhibitor metyrapone (17) has opposite effects.

The aim of this report was to further investigate the mechanisms by which glucocorticoids facilitate the behavioral and dopaminergic effects of drugs of abuse with a focus on the role of the different corticosteroid receptors. Glucocorticoids exert their action on the brain through two types of central corticosteroid receptors. The mineralocorticoid receptor (MR or type I), is mainly located in limbic structures and shows high affinity for corticosterone (18). The glucocorticoid receptor (or type II) has a more widespread distribution in the brain and has a lower affinity for corticosterone (18). MRs are believed to mediate tonic basal actions of glucocorticoids, whereas GRs appear to mediate phasic responses such as those to stress (19, 20).

Three specific questions were addressed in this report. (i) What is the influence of each corticosteroid receptor type on the behavioral effects of morphine? (ii) Does this influence involve dopamine-dependent behavioral responses? (iii) Is this influence mediated by changes in dopamine release? To answer these questions, we studied the effects of corticosteroid receptor blockade on: the locomotor response to a systemic injection of morphine; locomotion induced by an infusion of morphine into the ventral tegmental area (VTA); morphine-induced dopamine release in the nucleus accumbens, a dopaminergic projection site mediating locomotor and reinforcing effects of drugs of abuse (9–11).

Dopamine release was estimated by monitoring extracellular dopamine in freely moving rats by means of the microdialysis technique. Corticosteroid receptors were blocked by intracerebro-ventricular (i.c.v.) infusion of MR or GR antagonists. The most commonly employed corticosteroid antagonists, spironolactone and RU38486, were used to block the MR and GR, respectively. However, as RU38486 also blocks progestosterone receptors (21, 22), we compared some of its effects to those of a more selective GR antagonist, RU39305, which is devoid of anti progesterone effects (22).

METHODS AND MATERIALS

Animals. Male Sprague–Dawley rats (Iffa Credo) weighing 300–320 g were used. Animals were housed four per cage upon arrival, and then individually after surgery. They had ad libitum access to food and water. The light-dark cycle (lights on from 6 a.m. to 8 p.m.), temperature (22°C), and humidity (60%) were kept constant in the animal room. Animals were allowed at least 1 week to acclimatize to the animal room before starting any manipulation.

Abbreviations: MR, mineralocorticoid receptor; GR, glucocorticoid receptor; i.c.v., intracerebroventricular; VTA, ventral tegmental area.

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**Locomotor Activity.** Locomotor activity was measured in circular corridors (10 cm wide and 70 cm in diameter) by four photoelectric cells placed at the perpendicular axis of the apparatus. Because it has been shown that the locomotor response to novelty is correlated with the sensitivity to the psychomotor and dopaminergic response to drugs of abuse (2, 23–25), we ensured a homogenous distribution of this factor throughout the experimental groups.

**Stereotaxic Implantation.** Rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and placed in a stereotaxic apparatus (Kopf Instruments, Tujunga, CA) with incisor bar 5 mm above the interaural line. Chronic guide cannulae (23-gauge stainless steel for cerebral infusions and CMA/11 Carnegie Medicin, Stockholm, for microdialysis) were implanted according to the atlas of Pellegrino et al. (26). Guide cannulae were secured in place with the use of stainless steel screws and dental cement, and sealed with a 30-gauge stainless steel stylet. Rats were left to recover 10–15 days before the start of the experiments.

Guide cannulae for i.c.v. infusions were aimed at the lateral ventricle and implanted unilaterally 1.5 mm above the final injection site. The stereotaxic coordinates were: anteroposterior (AP) +0.3, lateral (L) +1.5, vertical (V) −2.3.

Guide cannulae for intra-VTA infusions were implanted bilaterally 2.5 mm above the final injection site at AP −3.4, L ±0.5, V −6.9.

Guide cannulae for microdialysis were implanted unilaterally over the nucleus accumbens shell, 2 mm above the location of the probe tip and at a lateral angle of +6° by using the following coordinates: AP +3.7, L +1.7, V −6.4.

At the end of the experiments, cannula placements were verified histologically on 100 μm thionin-stained coronal sections. Only animals with correctly placed probes were included in the statistical analyses.

**Drugs and drug administration.** The MR antagonist spironolactone (Sigma) and the GR antagonists RU38486 and RU39305 (kindly provided by Roussel-UCLAF) were initially dissolved in absolute ethanol and then diluted in a vehicle solution reproducing the electrolytic content of cerebrospinal fluid containing 145 mM NaCl, 1.2 mM CaCl₂, 2.7 mM KCl, 1.0 mM MgCl₂, and buffered with 0.2 mM Na₂HPO₄/NaH₂PO₄ at pH 7.4. Antagonists, or vehicle, were administered i.c.v. in a volume of 6 μl, the final solution containing 1.5% ethanol. Corticosteroid receptor antagonists were administered at the dose of 100 ng for either spironolactone or RU38486, and of 200 ng for RU39305. These doses were chosen because they ensure a similar saturation of corticosteroid receptors (22, 27, 28). For the dose-response studies, spironolactone was administered at the doses of 0, 30, 100, 300, and 1,000 ng/6 μl and RU39305 at the doses of 0, 50, 100, and 200 ng/6 μl. The i.c.v. infusions were performed by gravity over a period of 20–30 sec in rats loosely restrained by hand. The injection cannula (30-gauge stainless steel) was connected to a 30-cm long Pe10 tubing and descended 1.5 mm below the guide cannula. It was left in place for 30 sec after the administration period.

Morphine sulfate (Francopina, Gentilly, France) was dissolved in sterile 0.9% NaCl solution for subcutaneous injections (2 mg/kg per ml), and in artificial cerebrospinal fluid for intra-VTA infusions (1 μg/μl per side over a 90 sec period). The injection cannulae (30-gauge stainless steel, connected to a pump-driven syringe via Pe 10 tubing) descended 2.5 mm below the guide cannula and were left in place for 60 sec both before and after the administration period.

**Microdialysis.** Two days before the microdialysis experiment, a dialysis probe (CMA/11, 2 mm cuprophane membrane length, Carnegie Medicin) was inserted through the guide cannula, and animals returned to their home cage. The in vitro recovery of each probe had been determined before the implantation so as to distribute this factor throughout the groups. On the day of the testing, each animal was transferred to the dialysis cage (23 × 33 × 21 cm), the probe was connected to a syringe pump (Harvard Apparatus 22) via peek tubing connected to a two-channel swivel, and the perfusion (2 μl/min) started immediately. The perfusion fluid was a modified artificial cerebrospinal fluid as that mentioned above. Dialysate samples were collected in 40-μl sample loops and injected with a fully automated on-line system. HPLC coupled to a coulometric detector (Coulomb Vet II, ESA, Bedford, MA) was used to detect dopamine (0.3 pg/40 μl detection limit).

**Statistical Analysis.** ANOVA for repeated measures was used to analyze the activity scores and extracellular dopamine. The different treatments were used as between factor, and time as within factor. Newman–Keuls tests were used for post hoc analysis.

**PROCEDURES**

**Effect of Corticosteroid Receptor Antagonists on the Locomotor Response to a Systemic Injection of Morphine.** Three groups of animals (n = 8 each) received an i.c.v. infusion of either vehicle, the MR antagonist spironolactone or the GR antagonist RU38486. Animals received these i.c.v. infusions between 10 and 11 a.m. and were then immediately placed in the circular corridors. After 2 hr of habituation to the apparatus, they received a subcutaneous injection of 0.9% NaCl saline solution and their locomotor activity recorded for 3 hr over 30-min intervals. Four days later, the same procedure was repeated, but instead of receiving saline, the animals were administered morphine subcutaneously. This experiment was replicated a second time in independent groups of animals (n = 5–6 for each group).

To test the GR-specificity of the effects of RU38486, in a subsequent experiment, we compared the effects of this compound to those of RU39305, a more selective antiGR, which is devoid of antiprogesterone effects (22). Three different groups of animals were used: vehicle, RU38486, and RU39305 (n = 8–9 per group). The same exact procedure as the one just described was utilized to study the locomotor response to a subcutaneous injection of saline and morphine.

To perform the dose-response study, after i.c.v. infusion of the selective MR antagonist spironolactone (0, 30, 100, 300, and 1,000 ng/6 μl), or of GR antagonist RU39305 (0, 50, 100, or 200 ng/6 μl), the animals (n = 6 to 10 per group) were tested for the locomotor response to morphine. Each animal was tested with one dose of the antagonist only. The dose-response effects for spironolactone and for RU39305 were studied in independent experiments.

A 2-hr interval was used between the infusion of the corticosteroid antagonists and the injection of morphine to allow enough time for the appearance of possible genomic effects of these compounds.

**Effect of GR Antagonists on the Locomotor Response to an Intra-VTA Infusion of Morphine.** Animals received an i.c.v. infusion of either vehicle or the GR antagonist RU38486 (n = 6 each) between 10 and 11 a.m. Only the GR antagonist was tested because the results of the previous experiment revealed that only the blockade of GRs reduced the locomotor effects of morphine. Immediately after the i.c.v. infusion, rats were placed in the circular corridors. After a habituation period of 2 hr, they received an intra-VTA infusion of vehicle. Four days later, the same procedure was repeated, but instead of receiving an intra-VTA vehicle, the rats received an intra-VTA infusion of morphine. Locomotor activity was recorded for 3 hr over 30 min intervals.

**Effect of MR or GR Antagonists on the Morphine-Induced Increase in Extracellular Dopamine in the Nucleus Accumbens.** Two separate experiments were performed; the first tested the effects of the MR antagonist spironolactone, whereas the second tested the effects of both GR antagonists.
Results

Effect of Corticosteroid Receptor Antagonists on the Locomotor Response to a Systemic Injection of Morphine. Corticosteroid receptor antagonists did not modify the locomotor response to a subcutaneous injection of saline [treatment effect, 8.41 (2, 21), P < 0.05; RU38486 vs. vehicle: not significant]. Both antagonists vs. vehicle: not significant]. Males yielded comparable results [treatment effect, 8.41 (2, 21), P < 0.05; RU38486 vs. vehicle: not significant]. The i.c.v. administration of the GR antagonist RU38486 (100 ng/kg) reduced the locomotor response to morphine, whereas the MR antagonist spironolactone (100 ng/kg) did not have any significant effects. Each point represents the mean ± SEM of activity scores cumulated over 30 min. Columns show total activity scores over 3 hr. Antagonists were administered 2 hr before the injection of morphine.

Fig. 1. Effect of corticosteroid receptor antagonists on the locomotor response to a subcutaneous injection of saline and morphine (2 mg/kg). The locomotor response to saline was not modified by these treatments. The i.c.v. administration of the GR antagonist RU38486 (100 ng/kg) reduced the locomotor response to morphine, whereas the MR antagonist spironolactone (100 ng/kg) did not have any significant effects. Each point represents the mean ± SEM of activity scores cumulated over 30 min. Columns show total activity scores over 3 hr. Antagonists were administered 2 hr before the injection of morphine.

Fig. 2. Effect of i.c.v. administration of the GR antagonists RU38486 (100 ng/6 μl) or RU39305 (200 ng/6 μl) on the locomotor response to a subcutaneous injection of saline and morphine (2 mg/kg). Groups did not differ in their response to saline, however both GR antagonists similarly reduced (50% each) the locomotor response to morphine. Each point represents the mean ± SEM of activity scores cumulated over 30 min. Columns show total activity scores over 3 hr. The antagonists were administered 2 hr before the injection of morphine.
represents the mean response to intra-VTA morphine of about 50%. Each point represents the mean ± SEM of activity scores cumulated over 3 hr. Antagonist were administered 2 hr before the injection of morphine.

(Fig. 6b) shows that the increase in extracellular dopamine induced by morphine was lower in animals treated with GR antagonists than in animals receiving vehicle [treatment effect, \( F(2, 14) = 5.13, P < 0.025 \)], an effect that was time-dependent [Treatment × Time interaction, \( F(16, 112) = 2.88, P < 0.001 \)]. Again, the groups treated with the two antagonists did not differ and both had lower dopamine levels than vehicle-treated rats [Newman–Keuls, \( P < 0.03 \)]. In contrast, no significant differences between the three groups were found when results were expressed as percentage increase from baseline [treatment effect, \( F(2, 14) = 1.52, P > 0.25 \)] (Fig. 6c).

DISCUSSION

Three principal findings highlight the results of the present experiments. (i) The behavioral response to morphine is modulated by GRs. Thus, GR antagonists dose-dependently decreased the locomotor response to a systemic injection of the drug, whereas the MR antagonist had no significant effects. Such an effect does not seem to depend on a general impairment of motor behavior because GR antagonists did not reduce the motor activation induced by a saline injection. (ii) The blockade of GRs decreased the behavioral effect of morphine by a dopamine-dependent mechanism. GR antagonists reduced in a comparable manner the locomotor response to morphine injected systemically or intra-VTA (50% in both cases). This points to an involvement of dopamine because the locomotor activity induced by intra-VTA opiates depends on the activation of the mesencephalic dopaminergic transmission (13, 29, 30). (iii) GRs control dopamine-dependent behavioral effects of morphine probably by modulating the release of dopamine in the nucleus accumbens, which is one of the major drug-induced neurochemical changes mediating the psychomotor stimulant effects of morphine (31). Thus, GR antagonists reduced dopamine in the nucleus accumbens in basal conditions and in response to the injection of morphine whereas the MR antagonist had no significant effect. These results confirm previous work suggesting that glucocorticoids facilitate the behavioral and dopaminergic (4, 16) response to morphine and extend them by showing that these effects of glucocorticoids are mediated by GR corticosteroid receptors.

The GR antagonists did not significantly reduce the dopaminergic response to morphine when the microdialysis results were expressed as percentage increase from baseline. However, the lack of such an effect does not exclude an action of GR antagonists on dopamine release. It has been previously shown that it is the absolute outflow of dopamine, the one GR antagonists profoundly reduce, and not the percentage increase, that correlates with the behavioral response to dopaminestimulating drugs (32). Furthermore, the lack of effect of GR antagonists on the morphine-induced percentage increase in dopamine gives some insights on the possible mechanisms of action of these compounds. These results suggest that blockade of GRs probably has a tonic inhibitory effect, as shown by the decrease in the basal release of dopamine, only on a subpopulation of dopaminergic neurons. The response of these neurons to subsequent stimuli will be consequently reduced, as shown by the decrease in the net outflow of dopamine after morphine in animals treated with GR antagonists. However, the responsiveness to morphine of the dopa-
expressed as: dopamine concentrations in pg/ml sample and the mean value of the baseline.

The mean dose of spironolactone used here (100 ng) has been shown to occupy MRs to the same extent the dose of RU38486 occupies GRs (27, 28). Furthermore, the range of doses of spironolactone that have been tested largely covers the ones that are active on other biological and behavioral parameters, such as corticosterone secretion (34, 35) and performance in the Morris water maze (36). It is likely, therefore, that MRs do not mediate the effects of glucocorticoids on the locomotor response to morphine or on dopaminergic transmission. Consistent with this hypothesis, a dose of corticosterone that almost saturates MRs, but that only scarcely occupies GR receptors, did not reinstate the decrease in the locomotor response to morphine induced by adrenalectomy (4).

The presence of GRs on the cell bodies of mesencephalic dopaminergic neurons (33) suggests a direct action of glucocorticoids on mesolimbic dopaminergic neurons. This hypothesis is also supported by the recent finding in our laboratory (F. Rougé-Pont, D. N. Abrours, M.L.M., P.V.P., unpublished observations), that on cultures of dopaminergic mesencephalic neurons, GR antagonists have identical effects as those observed in vivo, i.e., they decrease basal and depolarization-induced dopamine release. It is noteworthy that the MR antagonist had no effect on dopamine release in these cultures.

The mechanisms by which glucocorticoids, through GRs, may modulate the functional status of dopaminergic neurons are currently unknown. However several hypotheses could be put forward. (i) Glucocorticoids could modify the firing of dopaminergic cells as shown in a recent study by Overton et al. (37). (ii) These hormones could stimulate dopamine synthesis. In fact, the injection of corticosterone has been shown to increase the activity of tyrosine hydroxylase in the brain (38) whereas depletion of glucocorticoids by adrenalectomy reduces the activity of this enzyme in the hypothalamus (39) and VTA (40). However, it is unknown if this is a GR-mediated effect.

Glucocorticoids could also act through the opioid, glutamatergic, GABAergic, or serotonergic systems (41, 42) that regulate dopamine neurons in the VTA (43). However, GR-mediated effects of glucocorticoids on these neuronal systems are unlikely to modulate the changes in dopaminergic activity observed here.

Glutamatergic and enkephalineric inputs to the VTA facilitate dopaminergic activity (43). Even though glucocorticoids can potentiate the functional activity of opioid (44, 45) and glutamatergic transmission (46, 47) this seems mainly a MR receptor-mediated effect (41). In contrast, via GRs, glucocorticoids reduce excitatory amino acid (a-amino-3-
hydroxy-5-methyl-4-isoxazolepropionic acid and N-methyl-D-aspartate) and opioid (\(\mu\) and \(\delta\)) receptor-mediated responses (41, 48). Consequently, GR antagonists acting on opioid and glutamate transmissions would be hypothesized to increase and not decrease dopaminergic activity.

GABAergic and serotonergic inputs to the VTA also exert a tonic inhibitory control over the activity of dopaminergic neurons (43). GABA-mediated responses, in particular inhibitory postsynaptic potentials, are potentiated by glucocorticoids through GRs, consequently, if glucocorticoids have the same effects on the raphe-VTA serotonergic projection, GR antagonists should increase and not decrease dopaminergic activity.

In conclusion, our results indicate that glucocorticoids, via GRs, facilitate dopamine-dependent behavioral effects of morphine, probably by modifying dopamine release. These findings are consistent with the hypothesis (1) that an interaction between glucocorticoids and mesolimbic dopaminergic neurons could constitute a pathophysiological mechanism underlying vulnerability to develop drug addiction. Furthermore, the possibility of modifying behavioral and dopaminergic effects of opiates by an acute administration of GR antagonists, opens new insights for the development of therapeutic strategies for treatment of drug abuse.

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