Glucocorticoids and Behavioral Effects of Psychostimulants. II: Cocaine Intravenous Self-administration and Reinstatement Depend on Glucocorticoid Levels

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ABSTRACT
Observations suggest that corticosterone, the principal glucocorticoid hormone in the rat, can modulate the behavioral effects of drugs of abuse. In this report, the influence of corticosterone on intravenous self-administration of cocaine was studied. In the first experiment, cocaine intravenous self-administration in adrenalectomized rats and in adrenalectomized rats receiving corticosterone replacement treatments was studied as a function of corticosterone concentrations and as a function of cocaine doses (0.025, 0.05, 0.1, 0.2, 0.4, 0.8 mg/kg/infusion). In a second experiment, we tested, in intact rats, the effect of different doses of corticosterone (0.09, 0.18, 0.37, 0.58, 0.75 mg/kg) on the reinstatement of an extinguished cocaine self-administration behavior. It is reported that adrenalectomy markedly shifts the cocaine self-administration dose-effect curve downward. This effect was dose-dependently reversed by corticosterone; a complete restoration being obtained for corticosterone levels in the range of those induced by stress. Corticosterone administration also precipitated dose-dependently the reinstatement of cocaine self-administration. The maximal effect was obtained for a dose of corticosterone producing an increase in plasma levels similar to the increase produced by an intense stress. In conclusion, our results show that glucocorticoids facilitate the reinforcing effects of cocaine and support the hypothesis that glucocorticoids are one of the biological factors determining vulnerability to substance abuse.
corticosterone and the reinforcing effects of the drug, the influence of corticosterone on the reinstatement of cocaine SA was also investigated. Reinstatement of drug SA is believed to reflect craving for the drug (de Wit and Stewart, 1981, 1983).

In the first experiment, we studied intravenous SA of cocaine (0.8 mg/kg/infusion) in rats in which endogenous corticosterone had been removed by ADX and in adrenalectomized rats receiving substitutive treatments reproducing different plasma corticosterone levels. Once the corticosterone-substitutive treatment reversing the effects of ADX was identified, a full dose-response curve for cocaine SA was performed. In the second experiment, we tested the effects of the administration of different doses of corticosterone (0.09, 0.18, 0.37, 0.58, 0.75 mg/kg i.v.) on the reinstatement of cocaine SA in intact rats which were submitted to extinction after the stabilization of cocaine SA (0.25 mg/kg/infusion). Finally, in the third experiment, we measured the effect, on plasma corticosterone levels, of the two doses of corticosterone (0.37 and 0.58 mg/kg i.v.) which had a significant effect on reinstatement of cocaine SA.

Methods

General Methods

Subjects. Male Sprague-Dawley rats (Iffa Credo, Lyon, France) weighing 280 to 300 g were used. Animals were individually housed with ad libitum access to food and water for 10 days before beginning the experiments. A constant dark-light cycle (on 12:00 A.M., off 12:00 P.M.) was maintained in the animal house. Temperature (22°C) and humidity (60%) were also controlled.

Drugs. Cocaine HCl (Coopération Pharmaceutique Française, Bordeaux, France) was dissolved in saline (0.9%). Corticosterone 21-hemisuccinate (Agrar, Italy) was used for corticosterone replace-ment treatments. The concentrations of the hormone are expressed as base.

Constitution of experimental groups. Because it has been shown previously that locomotor activity in a novel environment is positively correlated with the sensitivity to the psychomotor and reinforcing effects of psychomotor stimulants (Piazza et al., 1989; Hooks et al., 1991a, b; Deroche et al., 1993b), we ensured a homog-enous distribution of this factor throughout the different experimental groups. For this purpose, after a period of 10 days of habituation to the housing conditions and before any other manipulation, animals were exposed to a novel environment and were then evenly distributed in the different experimental groups according to their activity score accumulated over the 2 h of testing from 6:00 to 8:00 A.M. The novel environment was a circular corridor (10 cm wide and 70 cm in diameter). Four photoelectric cells placed at the perpendicular axis of the apparatus automatically recorded locomotion.

Catheter implantation. The Silastic catheter (12 μl dead volume) used was similar to that described by Caine et al. (1993). Under ether anesthesia, the catheter was inserted in the right auricle through the external jugular vein. The catheter was then passed under the skin and fixed in the mid scapular region. For the first 3 days after surgery, the catheters were daily flushed with 0.1 ml of saline mixed with heparin (100 U/ml) and gentamicin (1 mg/kg). Thereafter, the catheters were flushed with saline-heparin mixture after each SA session.

Adrenalectomy and corticosterone replacement treatments. ADX was performed, via the dorsal route, under ether anesthesia, between 1:30 and 3:30 A.M., i.e., at the beginning of the light period. Sham-operated animals underwent the same surgical procedure except that the adrenals were not removed. Following surgery, NaCl (0.9%) was added to the drinking water of adrenalectomized rats.

Some of the adrenalectomized rats received a corticosterone replacement treatment. These animals were implanted, at the time of ADX, with a subcutaneous pellet which releases a stable amount of corticosterone in the range of the diurnal basal levels. Such pellets contained 50 mg of corticosterone adjusted to 100 mg with cholesterol (Meyer et al., 1979). To test the influence of plasma corticosterone levels on cocaine SA, corticosterone was also added to the drinking water. Three concentrations of the hormone were tested successively: 25, 50 and 100 μg/ml. The 50 and 100 μg/ml concentrations had been previously shown to reproduce mild (around 12 μg/100 ml; Marinelli et al., 1994) to severe (around 40 μg/100 ml; Deroche et al., 1995) stress levels of corticosterone, respectively.

Corticosterone assay. Blood for corticosterone assay was collected on ethylenediaminetetraacetic acid. Plasma, obtained after centrifugation, was stored at −20°C until assay. Plasma corticosterone was measured by radioimmunoassay (RIA Kit, ICN Biomedica, Inc., Costa Mesa, CA) with a highly specific corticosterone antiserum with a detection threshold of 0.1 μg/100 ml.

Intravenous self-administration. Each SA chamber (35 × 33 cm floor area, 50 cm high) was provided with two holes in the middle of each of the larger sides at 5 cm from the floor of the cage. A syringe pump was activated to deliver, over 2 sec, 20 μl of a cocaine solution from a 5-ml syringe. The 5-ml syringe was connected via a tygon tube to a swivel mounted on a balance arm above the SA chamber. The tygon tube attached to the exit of the swivel entered the chamber from above and was connected to the external cannula of the i.v. catheter in the rat. By introducing their nose (nose-poke) into one of the holes, defined as active, rats triggered a photocell, activated the pump and initiated an infusion of 20 μl of cocaine solution over 2 sec. Each infusion was followed by a 20-sec time-out period during which further nose-pokes were recorded but did not result in additional intravenous infusions. A nose-poke in the other hole, defined as inactive, was without followed sequences at any time. The number of responses at both holes and the number of reinforcers earned were recorded for the entire session.

SA sessions (one per day, 1 hour each) were conducted between 1:00 and 6:00 P.M. The criterion of acquisition ("base-line" criterion) of cocaine SA was defined as at least three consecutive SA sessions in which the total number of reinforcers remained constant within 10% deviation from the mean of three consecutive sessions.

Procedures

Experiment 1: Influence of corticosterone on cocaine intravenous self-administration. Six days after catheter implantation, 14 rats were adrenalectomized (ADX) and 7 rats were sham-operated (Sham). Six of the ADX rats were submitted to the corticosterone replacement treatment (ADX+Cort25). Six days later, animals were tested for acquisition of cocaine SA at the dose of 0.8 mg/kg/infusion. After base-line criterion was reached (10 days), the substitutive corticosterone treatment was increased progressively (ADX+Cort50 and ADX+Cort100). Each dose (n = 6/dose) was tested during at least three consecutive sessions. The criterion of stability of cocaine SA was less than 10% variation of responding over two consecutive sessions. The mean of these last 2 days was used for computation. A cocaine dose-response study was then performed comparing the Sham, ADX and ADX+Cort100 groups. Five supplementary doses of cocaine were tested in this order: 0.4, 0.2, 0.1, 0.05, 0.025 mg/kg/infusion. Each dose was tested during at least three consecutive sessions and the criterion of stability of cocaine SA was the same as the one described above. This criterion was reached during the last 2 days of testing for each dose of cocaine. The mean of these 2 days was used for computation.

Experiment 2: Effect of corticosterone on the reinstate-ment of cocaine self-administration. Six days after catheter implantation, 15 rats were tested for acquisition of cocaine SA at the dose of 0.25 mg/kg/infusion; a dose inducing a high rate of responding as observed in experiment 1. Once base-line criterion was met (10 days), an extinction procedure was performed, i.e., the same protocol
was applied but nose-pokes in the active hole were without scheduled consequences. This extinction procedure was maintained until active and inactive nose-pokes did not differ anymore significantly for three consecutive sessions. This criterion was reached after 20 days of extinction. To habituate the animals to the infusion, rats were first tested with the vehicle for four consecutive sessions. At this point (24 days after the last cocaine SA session), the effect of i.v. corticosterone infusion on the reinstatement of SA behavior was studied. Then, five doses of corticosterone (0.09, 0.18, 0.37, 0.58, 0.75 mg/kg) were tested with a Latin square design. Each dose was tested once. Corticosterone and vehicle were infused immediately before the session.

**Experiment 3: Effect of intravenous corticosterone administration on plasma corticosterone levels.** Ten animals were implanted with two catheters, one in each jugular vein. One catheter was used to administer the corticosterone solutions (0.37 or 0.58 mg/kg), the other one to collect blood. The manipulation which the rat underwent was identical with that used for SA. The experiment was performed after 7 days of recovery and conducted over 2 days starting every day at 1 P.M. Using a Latio square design, each rat was tested for the two corticosterone doses, one each day. Blood (300 μl) was collected immediately before the corticosterone administration (basal level) and 5 min after; a time point chosen to estimate the peak in corticosterone increase. The blood withdrawn was immediately replaced with the same volume of saline.

**Statistical Analyses**

For all the experiments, analysis of variance for repeated measures was used. For the analysis of the SA data, the treatment (Sham, ADX, two levels; or Sham, ADX, ADX+Cort(100), three levels) was used as a between-subjects factor and, depending on the experiment, the hole (active vs. inactive, two levels), the dose of cocaine (six levels) or the concentration of corticosterone (ADX+Cort 25, ADX+Cort50, ADX+Cort100, three levels) were used as within subject factors. For the experiment on corticosterone-induced reinstatement, the hole (active vs. inactive, two levels) and the dose of corticosterone (0, 0.09, 0.18, 0.37, 0.58, 0.75 mg/kg) were used as within subjects factors. Plasma concentrations of corticosterone were analyzed using the dose of corticosterone (0.37 mg/kg and 0.58 mg/kg, two levels) and time of sampling (0 and 5 minutes) as within subjects factors.

Newman-Keuls and simple main effects analyses were used to determine the locus of significant main effects and interactions. A significance level of P < .05 was used for all statistical analyses.

**Results**

**Experiment 1: Influence of corticosterone on cocaine intravenous self-administration.** Suppression of corticosterone secretion by ADX reduced cocaine (0.8 mg/kg/infusion) SA (fig. 1). ADX had a different effect on the number of nose-pokes in the active and inactive holes [Treatment × hole interaction, F(1,13) = 4.22, P < .05]. Thus, ADX animals exhibited a lower number of nose-pokes in the active hole than controls [Treatment effect, F(1,25) = 7.32, P < .01], while the two groups did not differ for the number of inactive responses [Treatment effect, F(1,25) = 0.15, P > .7]. Furthermore, in ADX rats, the number of nose-pokes in the two holes did not differ [Hole effect, F(1,13) = 0.048, P = .83]. In contrast, in Sham rats, the number of active responses was significantly higher than the number of inactive ones [Hole effect, F(1,13) = 6.82, P < .05].

Administration of corticosterone dose-dependently reversed the effects of ADX (fig. 1). Corticosterone administration differentially modified the responses in the active and inactive holes [Dose × hole interaction, F(2,15) = 4.64, P < .05], increasing dose-dependently the number of nose-pokes in the active hole [Dose effect, F(2,27) = 4.16, P < .05], without modifying responding in the inactive hole [Dose effect, F(2,27) = 0.242, P > .70]. The effects of ADX were fully compensated with the 100 μg/ml dose of corticosterone. At this dose, animals exhibited a higher number of active than inactive responses [Hole effect, F(1,12) = 5.68, P < .05] and did not differ from controls for the number of nose-pokes in the active hole [Treatment effect, F(1,23) = 0.192, P > .60].

Suppression of corticosterone by ADX also modified the dose-response function for cocaine SA (fig. 2). The effects of ADX on the number of nose-pokes in the active and inactive holes were different [Treatment × hole × dose interaction, F(5,65) = 2.207, P < .05]. Thus, as compared with Sham rats, adrenalectomized animals showed a lower number of active nose-pokes [Treatment × dose interaction, F(5,65) = 1.963, P < .05], but a similar number of inactive responses [Treatment effect, F(1,13) = 0.289, P > .6]. Corticosterone administration reversed the effect of ADX. ADX+Cort(100) rats exhibited a higher number of active nose-pokes than ADX animals [Treatment × dose interaction, F(5,60) = 2.71, P < .05], but did not differ from Sham rats [Treatment × dose interaction, F(5,55) = 1.03, P > .40]. It is noteworthy that in the three conditions studied a classical inverted-U-shaped dose-response function was found for the number of nose-pokes in the active hole; the dose-effect was significant for the three experimental groups [ADX: F(5,90) = 2.53, P < .05; Sham: F(5,90) = 10.07, P < .0001; ADX+Cort100: F(5,90) = 3.74, P < .005]. In contrast, the dose of cocaine per infusion did not modify the number of responses in the inactive hole [Dose effect, F(5,90) = 0.96, P > .44 for Sham; F(5,90) = 1.096, P > .37 for ADX; F(5,90) = 0.299, P > .91 for ADX+Cort100].

ADX also reduced the intake of cocaine [Treatment effect, F(1,13) = 11.18, P < .01] and this effect depended on the dose [Treatment × dose interaction, F(5,65) = 7.37, P < .0001] (fig. 3). Thus ADX rats did not differ from Sham animals for the intake of cocaine at low doses (0.025 and 0.05 mg/kg/infusion.), whereas they exhibited a lower intake of cocaine for the highest doses of the drug (0.1, 0.2, 0.4 and 0.8 mg/kg/infusion). Also, this effect of ADX was reversed by the ad
administration of corticosterone. ADX+Cort100 rats had a higher intake of cocaine than ADX animals [Treatment ×
dose interaction, F(5,60) = 2.81, P < .05], but did not differ
from the Sham group [Treatment × dose interaction, F(5,55) =
1.23, P > .3].

Experiment 2: Effects of corticosterone on reinstatement of cocaine self-administration. Intravenous administration of corticosterone differentially modified the number
of nose-pokes in the two holes, previously defined as active
and inactive [Dose × hole interaction, F(5,70) = 5.64, P <
.0005] (fig. 4A). Thus, corticosterone dose-dependently in-
creased the number of active nose-pokes. The increase was
significantly higher with 0.37 mg/kg than with the 0.58
mg/kg dose (P < .05).

Experiment 3: Effects of intravenous corticosterone administration on plasma corticosterone levels. Intravenous administration of corticosterone produced a signifi-
cant increase in plasma corticosterone levels [Time effect,
F(1,18) = 307.12, P < .0001] as measured 5 min after the
injection (fig. 4B). The effect of corticosterone intravenous
administration on plasma corticosterone levels depended
upon the dose [Time × dose interaction, F(1,18) = 13.06, P <
.005]. The dose of 0.37 mg/kg, which was the most powerful in
inducing reinstatement, enhanced corticosterone plasma lev-
els (P < .0001) in the range of the ones produced by an
electric foot-shock (Bassett et al., 1973).

Discussion

The results of the present experiments suggest that corti-
costerone increases the capacity of cocaine to act as a positive
reinforcer. Suppression of endogenous glucocorticoids by
ADX blunted the dose-response function for cocaine SA,
which suggests that the positive reinforcing effects of cocaine
are lower in adrenalectomized rats. In parallel, an adminis-
tration of corticosterone, at doses raising its plasma levels in
the range of stress-induced levels, was able to precipitate the
reinstatement of cocaine SA, which suggested that the hor-
mone increases the craving for this drug.

These results confirm and extend previous observations
which suggest that corticosterone increases the capacity of
psychostimulants to act as positive reinforcers. Administra-
tion of corticosterone induces acquisition of amphetamine SA
in animals that do not develop this behavior spontaneously
(Piazza et al., 1991). In parallel, animals that spontaneously
acquire SA have a longer stress-induced corticosterone secre-
tion (Piazza et al., 1991) and a higher sensitivity to the
behavioral and dopaminergic effect of this hormone (Piazza
et al., 1993). Finally, a chronic treatment with the corticoste-
rone synthesis inhibitor metyrapone reduces cocaine SA in a
test for relapse (Piazza et al., 1994). Consequently, cortico-
sterone appears as a biological factor which is able to influ-
ence all the different phases of drug intake, acquisition, retention and relapse.

Results obtained by studying SA seem in contrast with the ones recently published by Suzuki et al. (1995) showing that ADX does not modify cocaine-induced place preference. This is not really surprising because it has been previously shown that place-preference induced by intraperitoneal injections of cocaine, as in Suzuki et al. (1995), does not depend on the same neurobiological mechanisms than the one induced by intravenous injections (the route of administration used in our experiments). In particular, the dopaminergic system, which is considered as one of the principal substrates of intravenous cocaine-induced reinforcement, both in SA and place preference, does not seem to mediate the place preference induced by intraperitoneal injections of cocaine (Spyraki et al., 1982).

Observations suggest that glucocorticoids affect cocaine-directed behaviors by modulating specifically the drug effects. First, manipulations of glucocorticoids levels do not seem to profoundly alter motor behaviors. Thus, in the present work, we showed that the number of nose-pokes in the inactive hole was not modified by ADX or by corticosterone administration. Furthermore, a chronic metyrapone treatment, which decreases cocaine intake, does not modify the spontaneous locomotor or hole exploratory activities exhibited by naive rats in the SA cages (Piazza et al., 1994). In this last experiment (Piazza et al., 1994), the rate of nose-poking (around 50 responses per hole in 1 h) shown by metyrapone-treated animals was similar to the rate of active nose-poking demonstrated by sham rats at 0.2 mg/kg infusion of cocaine in the present work. Second, manipulation of glucocorticoids levels does not seem to produce general alterations of motivation. ADX does not alter the acquisition of an operant responding reinforced by food (Micco and McEwen, 1980; Micco et al., 1979), and a chronic metyrapone treatment does not modify the motivation for food as measured in the straight alley test (Piazza et al., 1994). However, it has to be mentioned that the effects of the manipulations of glucocorticoid levels on behaviors reinforced by cocaine and by food have not been tested by the same schedules of reinforcement.

Suppression of glucocorticoid by ADX seems to modify, in a similar way, the dose-response curve for cocaine-induced locomotion and SA. In both cases, a vertical shift in the dose-response function was found, which suggests that ADX reduces the efficacy of the drug. This idea is supported, in the first place, by the fact that vertical shifts in dose-response functions are generally a result of changes in efficacy. Furthermore, some of the biological effects of ADX on the dopaminergic system, that mediate both locomotor and reinforcing effects of cocaine, are among the ones that could mediate changes in efficacy. Indeed, ADX decreases the basal dopaminergic activity, as measured by in vivo microdialysis (Piazza et al., 1996) and the number of dopaminergic receptors (Biron et al., 1992), in a glucocorticoid-dependent manner.

Although cocaine-induced locomotion and SA were similarly modified by ADX, the effects of glucocorticoids on the two behaviors do not seem to be identical. Thus, although in adrenalectomized rats, the locomotor response to cocaine was fully restored by low basal diurnal levels of the hormone (around 5 μg/100 ml) (Marinelli et al., 1994), much higher levels of corticosterone (around 40 μg/100 ml) (Deroche et al., 1995), in the range of those observed after cocaine injection (Marinelli et al., 1997, companion paper), were necessary to recover cocaine-induced SA. It is then probable that cocaine-induced corticosterone secretion, which does not influence the locomotor effects of cocaine (Marinelli et al., 1997, companion paper), is required for the expression of SA. This hypothesis is supported by a previous report with use of the corticosterone synthesis inhibitor metyrapone. Metyrapone, at doses which do not reduce basal corticosterone plasma levels but block stress- and cocaine-induced corticosterone secretions (Marinelli et al., 1996), decreases cocaine SA (Piazza et al., 1994).

The differences in the concentration of corticosterone required to restore locomotion and SA suggest that glucocorticoids may influence the two behaviors by different mechanisms. In particular, type I corticosteroid receptors might be implicated in mediating locomotion, whereas the occupation of the type II receptors may be required for SA. Type I receptors (or mineralocorticoid receptors) are almost completely saturated at basal corticosterone levels, the ones al-

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**Fig. 4.** Effects of intravenous corticosterone administration on: (a) the reinstatement of cocaine SA and (b) on plasma corticosterone levels. (a) Corticosterone dose-dependently increased the number of nose-pokes in the hole previously associated to cocaine (Active hole) (*P < .05). (b) Corticosterone induced a significant and dose-dependent increase in plasma corticosterone levels measured 5 min after injection (*n = 10*). The 0.37 mg/kg dose of corticosterone enhanced corticosterone levels in the range of the ones induced by an electric foot-shock stress (shaded area, recalculated from Bassett et al., 1973). (*P < .05*).
lowing cocaine-induced locomotion to recover. In contrast, a full occupation of type II receptors (or glucocorticoid receptors) is observed only for higher corticosterone concentrations, the ones required to compensate for the effects of ADX on SA. These differences in occupancy of the two corticosteroid receptors by corticosterone are explained by the higher affinity for corticosterone of the type I receptor (McEwen et al., 1986).

Our results also suggest that corticosterone could play a role in stress-induced relapse to drug-taking (Carroll, 1985; Krueger, 1981; Shaham et al., 1996; Shaham and Stewart, 1995, 1996). Thus, corticosterone administration increased cocaine-reinforced responding for doses producing plasma levels of the hormone similar to those reached after an electric foot-shock (Bassett et al., 1973), a stress that has been shown to precipitate the reinstatement of responding for heroin (Shaham et al., 1996; Shaham and Stewart, 1995, 1996). It is noteworthy that the dose-response function for corticosterone-induced reinstatement is an inverted-U-shaped curve; the medium dose of corticosterone being the most effective. The basis for this bell-shaped effect is unknown, but such a pattern of responses could reflect, as proposed by Münck et al. (1984), a dissociation between physiological and pharmacological actions of glucocorticoids. Now, the maximal effect on reinstatement is obtained for a dose of hormone producing plasma levels in the range of the highest levels physiologically observed, i.e. the stress-induced levels (Bassett et al., 1973).

The possible involvement of glucocorticoids in stress-induced reinstatement enlarges our knowledge on the role of glucocorticoids in the mediation of the interaction between stress and sensitivity to drugs of abuse. That is, stress-induced corticosterone secretion has been previously shown to mediate stress-induced sensitization of the behavioral and dopaminergic effects of drugs of abuse (Deroche et al., 1992b, 1993a, 1994, 1995). Thus, the stress-induced increase in the psychomotor effects of amphetamine and morphine (Deroche et al., 1992b, 1993a, 1994, 1995) as well as in cocaine-induced dopamine release (Barrot et al., 1994) is suppressed in rats in which corticosterone secretion has been blocked. In parallel, repeated administration of corticosterone, in a similar manner as repeated exposures to stress, increases the psychomotor effects of amphetamine (Deroche et al., 1992a).

In conclusion, our results show that glucocorticoids facilitate the reinforcing effects of cocaine, probably by increasing the efficacy of this drug. These data support the hypothesis that glucocorticoids are one of the biological factors determining vulnerability to substance abuse and might contribute to open new therapeutic strategies of addiction.

References


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