

Glucocorticoids and Behavioral Effects of Psychostimulants. I: Locomotor Response to Cocaine Depends on Basal Levels of Glucocorticoids¹

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ABSTRACT

In this study, we explored the influence of corticosterone, the major glucocorticoid in the rat, on the locomotor response to cocaine. In particular, in a first series of experiments, we determined the effects of suppressing endogenous glucocorticoids by adrenalectomy on a full dose-response curve of cocaine-induced locomotion and the influence, on this behavioral response, of different corticosterone concentrations, by implanting different corticosterone pellets in adrenalectomized rats. Adrenalectomy decreased the locomotor response to cocaine, inducing a vertical shift in the dose-response curve, and corticosterone dose-dependently reversed the decrease induced by adrenalectomy. The effects of adrenalectomy were fully replicated by the acute central infusion of corticosteroid receptor antagonists, and the action of glucocorticoids did not seem to depend on nonspecific effects such as a general alteration of motor responses or drug metabolism. Thus, nei-

ther adrenalectomy, corticosterone receptor antagonists nor corticosterone replacement modified saline-induced locomotion and the administration of corticosterone did not increase locomotion. Furthermore, adrenalectomy slightly increased brain concentrations of cocaine, an effect that cannot account for the decrease in drug-induced locomotion it induced. In a second series of experiments, we tested whether corticosterone levels at the time of adrenalectomy could influence the outcome of this surgical procedure on the locomotor response to cocaine. We thus adrenalectomized rats under different conditions resulting in different levels of the hormone. Corticosterone levels at the moment of adrenalectomy had dose-dependent long-term facilitatory effects on the response to the drug. These findings underline a facilitatory role of glucocorticoids in the behavioral effects of psychostimulant drugs.

Glucocorticoid hormones are the final step of the activation of the hypothalamo-pituitary-adrenal axis. These hormones play an important role in adaptation; they contribute to the regulation of circadian activities and constitute one of the principal biological responses to stress (for review, see Selye, 1973; Krieger and Aschoff, 1979; Münck *et al.*, 1984; McEwen *et al.*, 1986).

Several recent experiments suggested that an enhanced glucocorticoid secretion, spontaneously present in certain subjects or induced by repeated stress in others, may determine a higher predisposition to develop drug intake. In particular, the behavioral effects of psychostimulant drugs seem to be facilitated by glucocorticoids (Piazza *et al.*, 1991a, 1996b; Piazza and Le Moal, 1996). First, plasma levels of corticosterone, the main glucocorticoid in the rat, are positively correlated with the propensity to acquire psychostimu-

lant self-administration (Piazza *et al.*, 1991b, Goeders and Guerin, 1994). Second, acute or repeated administration of corticosterone increases the reinforcing (Piazza *et al.*, 1991b) and the locomotor (Piazza *et al.*, 1991b; Deroche *et al.*, 1992; Cador *et al.*, 1993) effects of psychostimulant drugs. Third, a total or partial suppression of endogenous glucocorticoids decreases the psychomotor and reinforcing properties of psychostimulants (Marinelli *et al.*, 1994, 1997; Piazza *et al.*, 1994) and prevents the stress-induced sensitization of these effects (Deroche *et al.*, 1993, 1994, 1995; Rougé-Pont *et al.*, 1995).

Although it seems clear that glucocorticoids influence the behavioral response to psychostimulants, the pharmacological features of this interaction are largely unknown. Indeed, until now single-dose investigations have been reported almost exclusively. Without complete dose-response information, it is difficult to understand the precise effects of these hormones on the response to psychostimulants. For this reason, in this report, we performed a large pharmacological

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ABBREVIATIONS: i.c.v., intracerebroventricular; i.v., intravenous; s.c., subcutaneous; i.p., intraperitoneally; ADX, adrenalectomized.

characterization of the influence of glucocorticoids on the locomotor response to the psychostimulant cocaine.

In a first series of experiments we investigated the effects of corticosterone on the locomotor response to cocaine as a function of the dose of cocaine and the concentration of plasma corticosterone. Dose-response functions of cocaine-induced locomotion were compared in animals with an intact glucocorticoid secretion and in animals in which the endogenous source of corticosterone was removed by adrenalectomy. Subsequently, cocaine-induced locomotion was studied in adrenalectomized animals that received corticosterone replacement treatments which fixed plasma levels of corticosterone at different concentrations. To further characterize the effects of glucocorticoids we also studied: 1) the effects of adrenalectomy on brain levels of cocaine; 2) the effects of corticosterone itself on locomotor activity; 3) the effects, as compared with those of adrenalectomy, of the central (i.c.v.) injection of corticosteroid receptor antagonists. The effects of cocaine on corticosterone secretion were also determined.

In a second series of experiments, we investigated the influence of different experimental manipulations at the time of adrenalectomy on the outcome of this surgical procedure on the locomotor response to cocaine. Indeed, it was reported by Ratka and co-workers (1988) that if adrenalectomy is performed in conditions in which glucocorticoid levels are high, as for example at the beginning of the dark period, the effects of this manipulation on the analgesic effects of morphine are largely decreased. This report is most peculiar because a single rise in corticosterone levels before adrenalectomy is shown to suppress the outcome of this manipulation up to 2 weeks later (Ratka *et al.*, 1988). Consequently, this phenomenon, if replicated and extended to other drugs, not only has important methodological implications, but could also highlight one of the most impressive relationships between glucocorticoids and brain plasticity. For this reason, we studied the effect of four different experimental conditions that induced different rises in plasma corticosterone levels on the outcome of adrenalectomy on the locomotor response to cocaine.

Methods

Subjects

Male Sprague-Dawley rats (Iffa Credo; Lyon, France) weighing 260 to 280 g upon arrival were used. Animals were individually housed with *ad libitum* access to food and water. A 14:10 light/dark cycle (lights on from 6:00 A.M. to 8:00 P.M.) was maintained in the animal room, and temperature (22°C) and humidity (66%) were kept constant. Animals were allowed at least 1 week to acclimatize to the animal room before beginning any experiment.

General Methods

Locomotor activity and constitution of experimental groups. Locomotor activity was measured in a circular corridor (10 cm wide and 70 cm in diameter). Four photoelectric cells placed at the perpendicular axis of this apparatus automatically recorded locomotion. It has been shown previously that the locomotor response to novelty is correlated with sensitivity to the locomotor effects of psychostimulant drugs (Piazza *et al.*, 1989, 1991b; Hooks *et al.*, 1991). Therefore, we ensured a homogeneous distribution of this factor throughout the different experimental groups. Animals were prescreened for their locomotor response to novelty over 2 h and

evenly distributed among the different experimental groups according to their activity score.

For the study of the effects of corticosterone itself on locomotor activity, different activity cages (35 × 33 cm floor area, 50 cm high) were used. This apparatus was used because it was adapted to perform intravenous infusions (see "Procedures"). The cage was equipped with two photocell beams placed 15 cm from each other and located parallel to the shorter sides of the cage. Total beam breaks were recorded (total counts) as well as the number of consecutive breaks of both beams (crossings).

Adrenalectomy. Unless specified otherwise, adrenalectomy was performed between 7:00 and 9:00 A.M. under ether anesthesia. Each rat was brought into the surgery room in its home cage and quickly placed in a jar containing ether. Adrenals were removed *via* the dorsal approach within 2.5 to 3.5 min from when the cage was taken from the rack in the animal room. After surgery, NaCl (0.9%) was added to the drinking water of ADX rats. Sham animals underwent the same procedure, except that the adrenals were not removed.

Intravenous catheter implantation. Rats were anesthetized under ether, and a silastic catheter was inserted in the right auricle through the external jugular vein, passed under the skin and fixed in the mid scapular region. Catheters were flushed daily with a heparin/saline solution (10 IU in 100 μ l volume).

Stereotaxic implantation. Rats were anesthetized with sodium pentobarbital (60 mg/kg injected i.p.) and placed in a stereotaxic apparatus (Kopf Instruments, Dusseldorf, Germany) with incisor bar 5 mm above the interaural line. A chronic guide cannula (23-gauge stainless steel) was implanted according to the atlas of Pellegrino *et al.* (1979), taking the bregma and surface of skull as reference. This guide cannula for i.c.v. infusions was aimed at the lateral ventricle and implanted unilaterally 1.5 mm above the final injection site. The stereotaxic coordinates were: AP +0.3, L +1.5, V -2.3. Each cannula was secured in place with the use of stainless steel screws and dental cement, and a removable 30-gauge stainless steel stylet was inserted to prevent clogging. Within 4 to 7 days after the end of the experiment, all rats were anesthetized with sodium pentobarbital, and 10 μ l of ink was infused by gravity in the lateral ventricle. The rats were sacrificed thereupon, and ink diffusion verified on a sagittal section of the brain. Only animals showing a diffusion of the ink in all the ventricles were included in the statistical analyses.

Drugs. Corticosterone 21-hemisuccinate (Agrar, Italy) was used for subcutaneous pellets (see "Procedures") and i.v. injections. For i.v. injections, corticosterone was dissolved in 0.9% NaCl solution (saline); in all cases, concentrations are expressed as corticosterone base. Corticosterone (Sigma, Saint Quentin Fallavier, France), suspended in sesame oil, was used for s.c. injections of the hormone. Corticosteroid receptor antagonists (spironolactone, SIGMA, and RU 38486, kindly donated by Roussel UCLAF, Romanville, France) for i.c.v. infusions were initially dissolved in absolute ethanol and then diluted (final concentration of ethanol: 1.5%) in a vehicle solution reproducing the electrolytic content of cerebrospinal fluid and containing 145 mM NaCl, 1.2 mM CaCl₂, 2.7 mM KCl, 1.0 mM MgCl₂, and buffered with 0.2 mM of Na₂HPO₄/NaH₂PO₄ at pH 7.4. Cocaine-HCl (Coopérative Pharmaceutique Française, Bordeaux, France) was dissolved in 0.9% saline and injected i.p.

Corticosterone assay. Blood for corticosterone assay was collected in heparinized tubes. Plasma, obtained after centrifugation, was stored at -20°C until assay. Plasma corticosterone was measured by radioimmunoassay (RIA Kit, ICN Biomedicals, Inc., Costa Mesa, CA) with a highly specific corticosterone antiserum with a detection threshold of 0.1 μ g/100 ml.

Cocaine assay. To determine cocaine concentrations, we employed a modified version from Benuck *et al.* (1987). Briefly, each brain was separated from its cerebellum and cut into 2 hemispheres according to the medial axis. The left hemisphere was sonicated in acetonitrile, and supernatant collected after centrifugation. Cocaine content was measured in the supernatant by high performance liquid chromatography coupled with U.V. detection. The chromato-

graphic system consisted of a Milton Roy Constametric pump, a refrigerated automatic injector (CMA200 Carnegie Medicine, Stockholm Sweden), a precolumn and a C18 Kromasil column. Detection of cocaine was made by a UV detector (Shimadzu-SPD-A) at $\lambda = 235$ nm. Results are expressed as micrograms per gram of brain.

Procedures

Experiment 1: Influence of adrenalectomy on the dose-response curve of cocaine-induced locomotion. To determine the effects of adrenalectomy on a full dose-response curve of cocaine-induced locomotion, 1 week after surgery, independent groups of *Sham* and *ADX* animals were tested for their locomotor response to 0 (saline), 3.25, 7.5, 15, 30 or 60 mg/kg of cocaine ($n = 6-9$ per group). Rats were placed in the circular corridor at 8:00 A.M. After 3 h of habituation to the apparatus, they received an i.p. injection of saline. Two hours later, they received an injection of saline or the assigned dose of cocaine and their locomotor response was recorded for 2 h over 10-min intervals.

To test the effects of adrenalectomy on brain levels of cocaine and the effect of this drug on corticosterone secretion, animals from the previous experiment were used. Twenty-four hours after the locomotor activity test, the rats received the same treatment as the previous day (*i.e.*, saline or the assigned dose of cocaine) in their home cage. Twenty minutes later they were sacrificed by decapitation. Trunk blood was collected for corticosterone assay, brains were removed, rapidly frozen and stored at -80°C until assayed for cocaine levels. Samples were obtained 20 min after drug injection because this corresponded to the time of maximal locomotor activation.

Experiment 2: Influence of corticosteroid receptor antagonists on cocaine-induced locomotion. To verify that the effects of adrenalectomy were specific to a suppression of glucocorticoid action, we determined the effects of corticosteroid receptor antagonists on the locomotor response to cocaine. Two weeks after stereotaxic surgery, animals received an i.c.v. infusion of either vehicle ($n = 6$), or the selective corticosteroid receptor antagonists spironolactone + RU 38486 (100 ng each, in a 3 μl volume) ($n = 7$). The infusion was performed by gravity over a period of 10 to 15 sec in rats loosely restrained by hand. The injection cannula (30 gauge, stainless steel) was connected to a 20-cm-long polyethylene tube and descended 1.5 mm below the guide cannula. It was left in place for 15 sec after the administration period. Animals received these infusions between 9:00 and 10:00 A.M. and were then immediately placed in the circular corridors. After a habituation period of 2 h, the animals received an i.p. injection of saline and locomotor activity was recorded for 2 h over 10-min intervals. Four days later the same procedure was repeated but instead of receiving saline, the animals received cocaine (15 mg/kg). Locomotor activity was again monitored over a 2-h period.

Experiment 3: Influence of plasma concentrations of corticosterone on cocaine-induced locomotion. To determine whether there was a dose-response relationship between basal levels of corticosterone and the locomotor response to cocaine, different groups of *ADX* animals received, upon removal of the adrenal glands, subcutaneous pellets containing different concentrations of corticosterone. These pellets contained either 0, 3.125, 12.5 or 50 mg of corticosterone adjusted to 120 mg with cholesterol; such pellets release a constant amount of corticosterone and the highest dose has been shown to reproduce the basal diurnal levels of the hormone (Meyer *et al.*, 1979; Marinelli *et al.*, 1994). *ADX* animals undergoing these treatments will be referred to as *Pellet 0 mg*, *Pellet 3.125 mg*, *Pellet 12.5 mg* and *Pellet 50 mg* groups, respectively. One week after the surgeries, animals in the *Sham* ($n = 8$), *Pellet 0 mg* ($n = 7$), *Pellet 3.125 mg* ($n = 8$), *Pellet 12.5 mg* ($n = 8$) and *Pellet 50 mg* ($n = 9$) groups were tested for their locomotor response to 20 mg/kg of cocaine by the protocol described in experiment 1.

To determine the actual plasma levels of corticosterone in the various groups, all animals were sacrificed by decapitation at 11:00 A.M. the day after the locomotor test, and trunk blood was collected

for assay. We trust that these values correspond to those at the moment of the cocaine injection because these animals are adrenalectomized (thus unable to secrete corticosterone), and their circulating levels of the hormone uniquely depend on the corticosterone pellets which release a stable and constant amount of the hormone (Meyer *et al.*, 1979).

Experiment 4: Influence of corticosterone on locomotor activity. To test the effects of corticosterone itself on locomotor activity, a separate experiment was performed. Animals were implanted with an intravenous catheter, and locomotor response to i.v. corticosterone was studied 10 to 12 days later. The i.v. procedure was chosen to avoid possible stress effects caused by manipulations associated with i.p. or s.c. injections. Animals ($n = 7-9$ per group) were placed in the activity cages at 9:00 A.M. Two hours later, their catheter was connected, *via* a polyethylene tube (40 cm long), to a pump-driven syringe which delivered the assigned dose of corticosterone (0, 0.375, 0.75, 1.5, 3 mg/kg in a 100 μl /kg volume) over a 10-sec period. The catheter was then disconnected, and locomotor activity recorded for 2 h over 10-min intervals. Both the crossings and total counts were registered.

Experiment 5: Influence of plasma concentrations of corticosterone at the time of adrenalectomy on cocaine-induced locomotion. To determine whether plasma corticosterone concentrations at the moment of adrenalectomy had dose-dependent long-lasting influences on the subsequent locomotor response to cocaine, animals were adrenalectomized (or received a sham operation) under one of four conditions, which resulted in different plasma levels of corticosterone at the time of surgery. The first group (*morning* group) underwent surgery in the morning when corticosterone levels are low (Akana *et al.*, 1986), and surgery was performed under ether anesthesia, within 2.5 to 3.5 min after removal from the animal room (as for the previously described experiments). The second group (*pentobarbital* group) also underwent surgery in the morning, but was anesthetized with sodium pentobarbital (60 mg/kg i.p.). Because of the longer induction of anesthesia with barbiturates, adrenals were not removed until 7 to 10 min from when the animal was removed from the colony room, a time long enough for corticosterone levels to rise. The third group of rats (*night* group) underwent surgery under ether anesthesia within 2.5 to 3.5 min after removal from the animal room, but between 8:00 and 9:00 P.M., *i.e.*, when plasma corticosterone concentrations reach their daily peak (Akana *et al.*, 1986). Finally, the fourth group (*corticosterone* group) underwent surgery in the morning under ether anesthesia as above, but 1 h after having received a s.c. injection of corticosterone (10 mg/kg suspended in sesame oil). This dose of corticosterone increases plasma levels of the hormone to levels similar to those observed after stress. One week later, *Sham* and *ADX* rats from the *morning* group ($n = 17$ and $n = 15$, respectively), *pentobarbital* group ($n = 8$ each), *night* group ($n = 8$ each) and *corticosterone* group ($n = 8$ each) were tested for their locomotor response to 15 mg/kg of cocaine with the protocol described in experiment 1. The following day, the same animals received the assigned cocaine injection in their home cage and were sacrificed by decapitation 20 min later to collect blood for corticosterone assay to verify that the adrenalectomies were successful.

To determine corticosterone levels at the moment of adrenalectomy, separate groups of animals underwent surgical procedures identical with the rats in the *morning*, *pentobarbital*, *night* and *corticosterone* groups ($n = 6$ per group), but were sacrificed by decapitation instead of removing the adrenal glands, and blood was collected for corticosterone determination. These animals were run at the same time as those undergoing adrenalectomy or sham operations. Thus for each experimental group (*morning*, *pentobarbital*, *night* and *corticosterone*) some animals underwent adrenalectomy, others were sham operated and others were sacrificed for blood sampling.

Statistical analysis

Locomotor activity and brain levels of cocaine were analyzed with use of parametric statistics including analyses of variance and Newman-Keuls tests for pairwise comparisons where appropriate. Because the presence of corticosterone was nondetectable in all *ADX* rats, whenever *ADX* rats were included in the statistics, plasma levels of the hormone were analyzed by nonparametric tests (Kruskall Wallis test or Mann-Whitney *U* test, according to the experiment). Plasma levels of the hormone were otherwise analyzed by analysis of variance.

Results

Experiment 1: Influence of adrenalectomy on the dose-response curve of cocaine-induced locomotion.

As figures 1 and 2a show, increasing doses of cocaine produced a progressive increase in locomotor activity [Dose effect, $F(5,82) = 19.19$, $P < .001$] reaching maximal locomotor activation at a dose of 30 mg/kg in both *Sham* and *ADX* animals. Adrenalectomy significantly decreased the locomotor response to cocaine [Treatment effect, $F(1,82) = 11.88$, $P < .001$] and this effect was dependent on the dose of cocaine [Treatment \times dose interaction, $F(5,82) = 3.03$, $P < .02$]. Thus, *ADX* rats did not differ from *Sham* controls in their response to a saline injection [$F(1,14) = 0.32$, $P > .58$] or for the locomotor response to the lower doses of cocaine (3.75, and 7.5 mg/kg) [$F(1,12) = 0.04$, $P > .83$ and $F(1,13) = 0.08$, $P > .77$, respectively]. In contrast, at all of the higher doses (15, 30 and 60 mg/kg) *ADX* rats showed a lower locomotor response than *Sham* animals [$F(1,15) = 4.61$, $P < .05$; $F(1,15) = 6.01$, $P < .03$; and $F(1,13) = 4.72$, $P < .05$, respectively]. The maximal effect of the drug was reduced by approximately 60% by adrenalectomy.

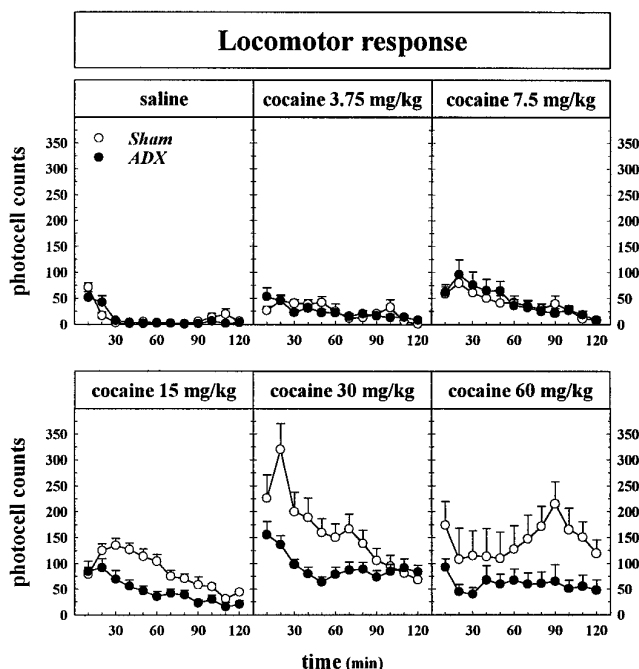


Fig. 1. Effects of adrenalectomy on the dose-response curve of cocaine-induced locomotion. Each point represents the mean \pm S.E.M. locomotor score accumulated over 10 min. Cocaine produced a dose-dependent increase in locomotion in both *Sham* and *ADX* rats. *ADX* rats showed a lower locomotor response than that of *Sham* animals for doses of cocaine between 15 and 60 mg/kg.

Figure 2, a and b, shows cocaine-induced locomotion (accumulated over 2 h) and cocaine-induced corticosterone secretion in *Sham* and *ADX* rats. All *ADX* rats, at all doses of cocaine, had nondetectable levels of corticosterone which significantly differed from those of *Sham* animals (U values = 3.1–3.7; P values $< .01$). In *Sham* rats, cocaine produced a dose-dependent increase in plasma corticosterone concentration [Dose effect, $F(5,42) = 13.25$, $P < .001$], with a maximal effect at 60 mg/kg (fig. 2b). This dose-effect curve in *Sham* rats paralleled the one obtained for locomotion (fig. 2a).

Figure 2c shows that brain levels of cocaine increased with the increase in drug doses [Dose effect, $F(4,68) = 36.93$, $P < .001$]. The increase in brain levels of cocaine over the doses, paralleled the one observed for locomotor activity and corticosterone levels up to the 30 mg/kg dose. However, although the 60 mg/kg dose induced a further increase in brain concentrations of cocaine, this dose did not further increase locomotion or corticosterone levels. This result confirms that the maximal locomotor response to cocaine was reached for the 30 mg/kg dose. Adrenalectomy induced a slight increase in brain concentrations of cocaine [Treatment effect, $F(1,68) = 4.37$, $P < .05$] independently from the dose of cocaine [Treatment \times dose effect, $F(4,68) = 0.91$, $P > .46$].

Experiment 2: Influence of corticosteroid receptor antagonists on cocaine-induced locomotion. Figure 3 shows that corticosteroid receptor blockade, as adrenalectomy, had no effects on the locomotor response to a saline injection [Treatment effect, $F(1,11) = 0.44$, $P > .52$], but reduced of about 60% the response to 15 mg/kg of cocaine [Treatment effect, $F(1,11) = 22.56$, $P < .001$].

Experiment 3: Influence of plasma concentrations of corticosterone on cocaine-induced locomotion. Figure 4b shows that the increase in corticosterone pellet concentration produced a dose-dependent increase in plasma levels of corticosterone [Dose effect, $H(3) = 24.76$, $P < .001$] that reached levels comparable to those observed in *Sham* rats (shaded area in the figure) for the highest pellet concentration (50 mg). These changes in plasma concentrations of corticosterone had no effects on the locomotor response to the injection of saline [Dose effect, $F(3,28) = 1.20$, $P > .33$, data not shown], but were paralleled by changes in the locomotor response to cocaine. Thus, as figure 4a shows, cocaine effects dose-dependently increased with corticosterone replacement [Dose effect, $F(3,28) = 5.85$, $P < .01$]. When corticosterone levels were nondetectable (*Pellet 0 mg*), the locomotor response to cocaine (20 mg/kg) was significantly lower ($P < .01$) than the one observed in *Sham* rats (shaded area in the figure). Only when corticosterone levels reached those observed in *Sham* animals (*Pellet 50 mg*) were the behavioral effects of cocaine completely restored. Figure 4c shows the dose-response relationship between circulating levels of glucocorticoids and the locomotor response to cocaine.

Experiment 4: Influence of corticosterone on locomotor activity. Figure 5 shows that corticosterone itself does not modify locomotor activity. Thus, after i.v. administration of the different doses of corticosterone, all animals showed similar locomotor activation [Dose effect, $F(4,35) = 0.48$; $P > .74$ and $F(4,35) = 0.64$; $P > .64$ for the crossings and total counts, respectively; data shown for the crossings only].

Experiment 5: Influence of plasma concentrations of corticosterone at the time of adrenalectomy on cocaine-induced locomotion. The conditions at the time of

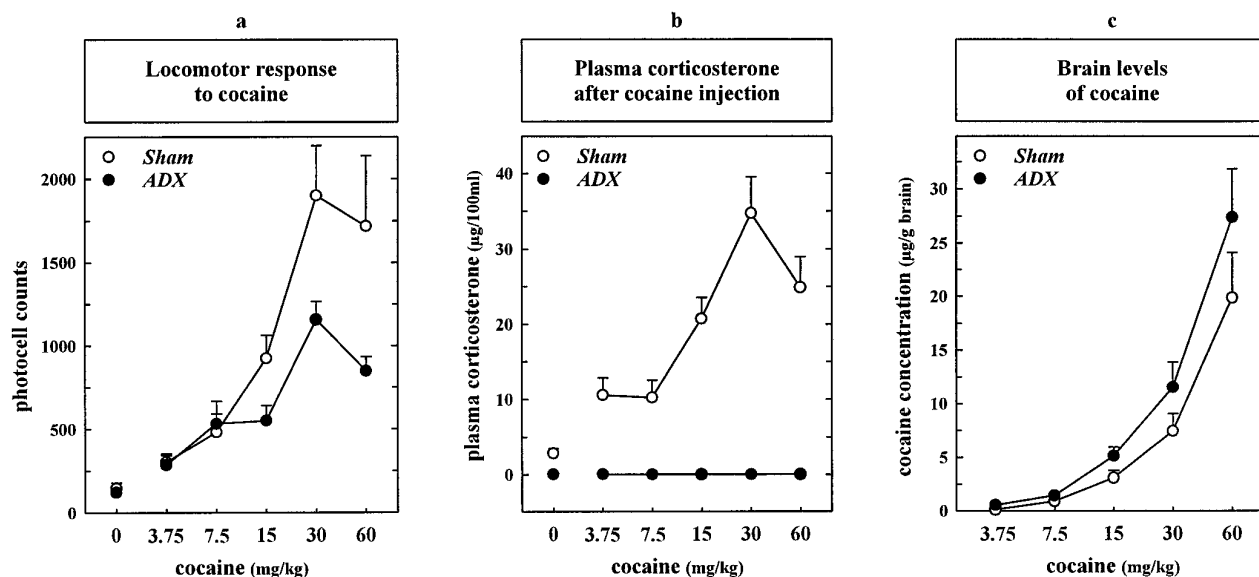


Fig. 2. Effects of adrenalectomy on the dose-response curve of: (a) cocaine-induced locomotion, (b) cocaine-induced corticosterone secretion and (c) brain levels of cocaine. (a) Each point represents the mean \pm S.E.M. total locomotor activity accumulated over 2 h. Adrenalectomy decreased the maximal locomotor response to cocaine. (b) Each point represents the mean \pm S.E.M. of corticosterone levels 20 min after the cocaine injection. All ADX animals had nondetectable levels of corticosterone. In Sham rats, cocaine produced a dose-dependent increase in corticosterone levels. (c) Each point represents the mean \pm S.E.M. of brain levels of cocaine 20 min after the cocaine injection. Cocaine produced a dose-dependent increase in brain levels of the drug. Brain cocaine concentrations were slightly higher in ADX rats.

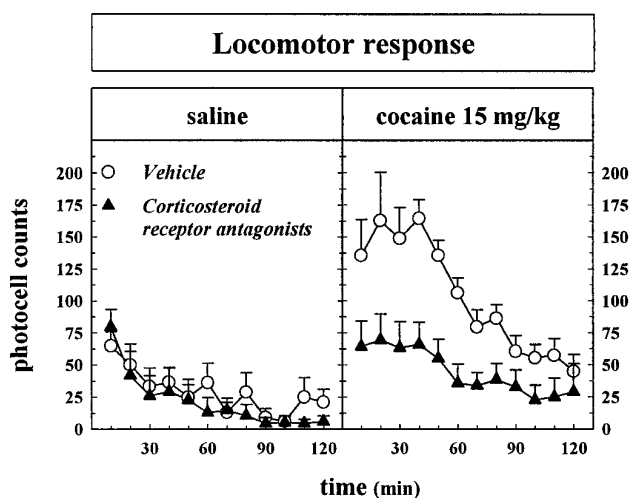


Fig. 3. Effects of corticosteroid receptor antagonists on the locomotor response to saline (left) and cocaine (right). Each point represents the mean \pm S.E.M. locomotor score accumulated over 10 min. The combined i.c.v. administration of type I and II antagonists (100 ng each, in 3 μ l) decreased the locomotor response to cocaine (15 mg/kg).

surgery had no effect on the locomotor response of Sham animals [Treatment effect, $F(3,37) = 0.128$, $P > .9$]; therefore, these groups were pooled (shaded area in fig. 6a) for comparison with the different ADX groups. Figure 6, a and b, shows that corticosterone levels at the time of surgery dose-dependently increased the locomotor response to cocaine of ADX animals. Thus, only rapid morning adrenalectomy significantly reduced the locomotor response to cocaine relative to Sham rats [$F(1,54) = 6.37$, $P < .02$], and there was a progressive decrease in the effect of adrenalectomy when surgeries were performed under *pentobarbital* [$F(1,47) = 1.19$, $P > .28$], at *night* [$F(1,47) = 1.22$, $P > .72$], or after a *corticosterone* injection [$F(1,47) = 0.032$, $P > .85$]. Figure 6b shows that these changes in the response of ADX animals

were perfectly paralleled by differences in the amount of circulating corticosterone at the time of surgery [Treatment effect, $F(3,20) = 17.38$, $P < .001$]. Rats in the *pentobarbital* group had higher levels of the hormone than those in the *morning* group ($P < .05$). Rats undergoing *night* surgeries had higher corticosterone levels than those in the *pentobarbital* ($P < .05$) and *morning* ($P < .001$) groups. Rats operated on 1 h after having received a corticosterone injection (*corticosterone* group) showed higher levels of the hormone than all other groups (*night*, $P < .05$; *pentobarbital*, $P < .001$; *morning*, $P < .001$). These results are summarized in figure 6c which shows that there is a dose-response relationship between corticosterone levels at time of adrenalectomy and the amount of cocaine-induced locomotion 1 week later.

In response to the cocaine injection, all ADX rats, in all the experimental groups, had nondetectable levels of corticosterone, and the levels of the hormone were similar in all groups of Sham rats [Treatment effect, $F(3,37) = 1.24$, $P > .3$]. (Corticosterone levels = 20.6 ± 1.8 , 26.97 ± 3.6 , 27.51 ± 4.0 , 25.0 ± 4.6 μ g/100 ml, for the *morning*, *pentobarbital*, *night* and *corticosterone* Sham groups, respectively; data not shown). As expected, these levels of corticosterone in Sham animals were significantly higher than those in ADX animals for all the conditions studied ($U = 5.08$, $P < .001$ morning; $U = 3.59$, $P < .001$ for all the other groups).

Discussion

The results of the first series of experiments confirm and extend previous data showing that glucocorticoids facilitate the psychomotor effects of cocaine. Three main findings can be identified.

First, suppression of endogenous glucocorticoids induces a vertical shift in the dose-response function of cocaine-induced locomotion. This gives an insight into the type of interactions between glucocorticoids and the behavioral effects of cocaine.

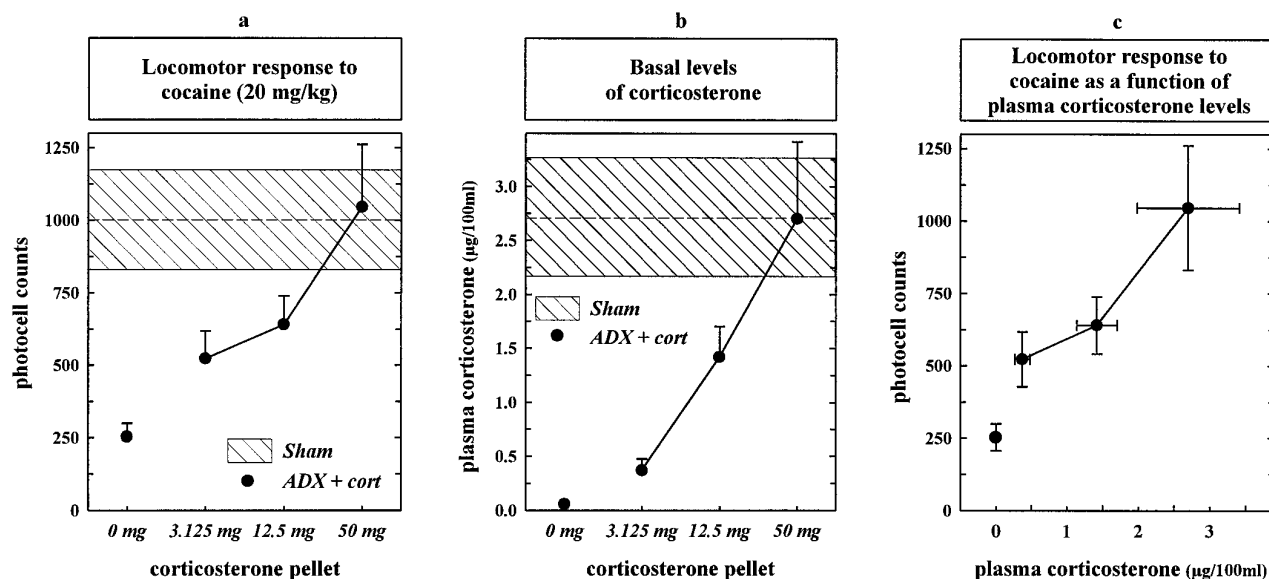


Fig. 4. Effects of corticosterone pellets in ADX rats on: (a) the locomotor response to cocaine (20 mg/kg); (b) plasma levels of corticosterone; (c) relationship between these two factors. (a) Each point represents the mean \pm S.E.M. of the total locomotor activity accumulated over 2 h of testing in ADX rats with corticosterone replacement (ADX + cort). The shaded area represents the mean \pm S.E.M. response of Sham rats. Exogenous administration of corticosterone by pellet implantation induced a dose-dependent increase in the locomotor response to cocaine. (b) Each point represents the mean \pm S.E.M. of plasma corticosterone levels in ADX rats with corticosterone replacement (ADX + cort). The shaded area represents the mean \pm S.E.M. of the level of the hormone in Sham rats. Exogenous administration of corticosterone by pellet implantation induced a dose-dependent increase in the plasma levels of the hormone. (c) Each point represents the mean \pm S.E.M. of corticosterone levels and total locomotor activity for the different groups of adrenalectomized animals.

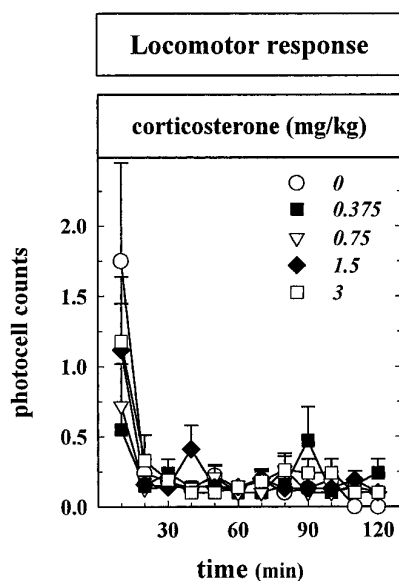


Fig. 5. Effects of corticosterone on locomotor activity. Each point represents the mean \pm S.E.M. locomotor score (crossings) accumulated over 10 min. Corticosterone administration (i.v.) did not modify locomotor activity.

Vertical shifts in dose-response curves are principally indicative of changes in drug efficacy. Changes in efficacy suggest that glucocorticoids impair the functional activity of the neural substrate of the locomotor effects of cocaine. The effects of glucocorticoids on the dopaminergic projection to the nucleus accumbens, one of the major neural substrates of cocaine-induced locomotion (for review, see Fibiger and Phillips, 1988; Wise and Rompré, 1989), support this idea. We have recently shown (Piazza *et al.*, 1996a) that adrenalectomy reduces basal dopaminergic release in the nucleus accumbens

and the release induced by a depolarizing stimulus such as the injection of the opiate morphine.

Second, cocaine-induced locomotion depends on basal diurnal levels of glucocorticoids but not on a drug-induced increase in these levels. This statement is supported by the observation that the effect of adrenalectomy on cocaine-induced locomotion was dose-dependently compensated by corticosterone concentrations that were in the range of basal diurnal levels, *i.e.*, well below the ones induced by cocaine. Indeed, a full recovery of the effects of adrenalectomy on cocaine response was observed for plasma levels of corticosterone reaching $2.7 \mu\text{g}/100 \text{ ml}$. This concentration is at least eight times lower than that (between 20 and $40 \mu\text{g}/100 \text{ ml}$) observed in intact rats after the drug injection. Thus, although cocaine induced a dose-dependent increase in corticosterone levels, this effect of the drug does not seem to participate in mediating its locomotor effects.

Third, the action of glucocorticoids on cocaine-induced locomotion is not caused by nonspecific effects such as a general alteration of motor responses or drug metabolism. Thus, neither adrenalectomy nor corticosterone replacement treatment modified the locomotor response to a saline injection and the administration of glucocorticoids, over a large range of doses, did not increase locomotion. Furthermore, adrenalectomy slightly increased brain concentrations of cocaine, an effect that cannot account for the decrease in the locomotor effects of the drug induced by adrenalectomy. The effect of adrenalectomy on brain cocaine concentrations is not surprising in the light of the influence of glucocorticoids on blood-brain barrier permeability (Long and Holaday, 1985). Finally, the effects of adrenalectomy were specific of a suppression of corticosterone action because they were fully replicated by the acute central i.c.v. infusion of corticosteroid

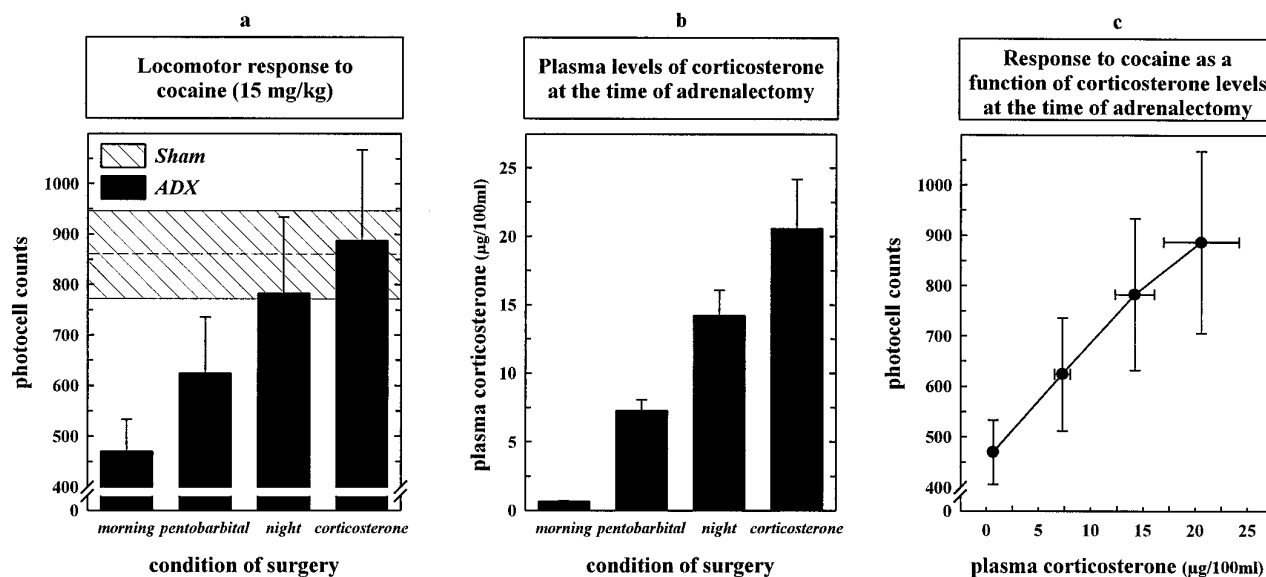


Fig. 6. Effects of different conditions of surgery at the time of adrenalectomy on: (a) cocaine-induced locomotion; (b) corticosterone levels; and (c) relationship between these two factors. (a) Each bar represents the mean \pm S.E.M. of the total locomotor activity score accumulated over the 2 h of testing in the different conditions of surgery. The shaded area represents the mean \pm S.E.M. response of *Sham* rats. There was a progressive decrease in the effects of adrenalectomy on the locomotor response to cocaine (15 mg/kg) according to the condition of surgery. (b) Each bar represents the mean \pm S.E.M. of plasma levels of corticosterone at the moment of adrenalectomy in the different surgical conditions. (c) Each point represents the mean \pm S.E.M. levels of corticosterone at the time of surgery and of drug-induced locomotion for the different surgical conditions. Corticosterone concentrations at the time of surgery dose-dependently increased the response to cocaine of adrenalectomized rats.

receptor antagonists. Again, this manipulation did not modify saline-induced locomotion.

As mentioned previously, the effects of glucocorticoids on cocaine-induced locomotion are probably mediated by functional changes induced by these hormones in the activity of the dopaminergic projection to the nucleus accumbens. This idea is also supported by the effects of adrenalectomy on other drugs whose locomotor effects depend on this neural system. Indeed it has been shown previously that adrenalectomy, similar to what is observed for cocaine here, also reduces the locomotor response to the psychostimulant amphetamine (Cador *et al.*, 1993) and the opioid morphine (Marinelli *et al.*, 1994). Furthermore, reduction by adrenalectomy of the locomotor effects of psychostimulants and opioids are also observed when these drugs are directly injected in the nucleus accumbens (cocaine or amphetamine) or in the ventral tegmental area (morphine) (Marinelli *et al.*, 1994; Deroche *et al.*, 1995). The ability of psychostimulants and opioids to induce locomotion when injected into these brain structures depends on the activation of the mesencephalic DA transmission (Kelly and Iversen, 1976; Delfs *et al.*, 1990).

The action of glucocorticoids on the psychomotor-activating effects of cocaine could also involve other neurotransmitters. In particular opioids, serotonin (Biegon *et al.*, 1985; De Kloet *et al.*, 1986; Martire *et al.*, 1989), γ -aminobutyric acid (Majewska *et al.*, 1986; Sutanto *et al.*, 1989) and excitatory amino acids (Tischler *et al.*, 1988; Sapolsky, 1990) are all influenced by glucocorticoids and can modulate dopamine-mediated responses to psychostimulants (Scheel-Krüger *et al.*, 1981; Kalivas *et al.*, 1989; Kelland *et al.*, 1990; Pulvirenti *et al.*, 1991).

The second series of experiments indicates that experimental manipulations at the time of adrenalectomy can profoundly modify the outcome of this condition. In particular, if

adrenalectomy was performed in situations in which corticosterone levels were high, the reduction in cocaine-induced locomotion induced by adrenalectomy was no longer present. Different lines of data suggest that the level of corticosterone at the time of adrenalectomy were responsible for the effect of the different manipulations tested. First, independent from the type of manipulation, there was a very good dose-response relationship between corticosterone levels at the time of surgery and the locomotor response of adrenalectomized animals 1 week later. Second, the injection of corticosterone alone before adrenalectomy was also able to suppress the effects of adrenalectomy. These results, although surprising, seem to reflect a robust phenomenon; thus a very similar finding has already been reported by Ratka and colleagues (1988) for opioids. These authors showed that a rise in corticosterone levels at the time of adrenalectomy decreases the effects of this manipulation on the analgesic response to morphine and opioid receptors, even if adrenalectomized rats are tested 2 weeks later.

The possible mechanisms underlying these long-lasting effects of glucocorticoids in adrenalectomized animals deserve some discussion. The first important observation is that they do not occur in animals with an intact corticosterone secretion. Thus, corticosterone levels at the time of surgery did not modify the subsequent locomotor response of *Sham* rats to cocaine. This suggests that control animals are able to reverse the long-term effects of corticosterone, whereas adrenalectomized rats cannot. It is then possible that the removal of the adrenal glands suppresses some adrenal factor necessary to reverse the effects of an increase in corticosterone secretion. Glucocorticoids themselves might be this factor. Indeed, it has been shown that these hormones can initially potentiate a response, and, with delay, inhibit it (McEwen *et al.*, 1992). For example, adrenal steroids produce an initial increase in neuronal excitability, but subsequently

depress this response (Joëls *et al.*, 1991; Joëls and De Kloet, 1989, 1990). Also, adrenal steroids secreted during stress initially facilitate serotonin turnover, and then suppress postsynaptic sensitivity to serotonin (Azmitia and McEwen, 1974; Biegon *et al.*, 1985; De Kloet *et al.*, 1986). It is therefore possible that a rise in corticosterone concentrations *turns on* some neural response and that the subsequent absence of corticosterone produced by the adrenalectomy does not allow the steroid to *turn off* the response. In other words, as suggested by Ratka and co-workers (1988) "adrenalectomy, which removes circulating corticosterone, in fact arrests a neuroendocrine state that persists. . ."

The long-lasting effect of an increase in corticosterone concentrations on the subsequent response of adrenalectomized animals also raises some important methodological considerations. Indeed, when corticosterone concentrations at the time of surgery reached levels similar to those observed during stress, the effects of adrenalectomy were totally suppressed. Consequently, to reveal the effect of a suppression of endogenous glucocorticoids by adrenalectomy, the entire surgical procedure must be performed at the beginning of the light cycle, rapidly and with extreme care, avoiding all those manipulations that might increase corticosterone secretion. Some manipulations that are most currently used in laboratory practice should be avoided; for example, 1) food-restricting the animals before surgery; 2) bringing the animals into the surgery room ahead of time; 3) housing conditions that, to remove one cage from the animal rack, determine that all the animals that will subsequently undergo surgery are disturbed. All these situations increase corticosterone secretion and can also be considered as models of stress. Finally, the anesthetic should allow a rapid anesthesia. Pentobarbital, for example, although commonly used, seems poorly adapted in this case.

In conclusion, the results of the present experiments indicate that glucocorticoids facilitate the locomotor response to cocaine. In particular glucocorticoids, in the range of basal diurnal concentrations, modify the maximal response to cocaine which indicates that these hormones probably modify the efficacy of the drug. These effects of glucocorticoids involve central corticosteroid receptors, because suppression of the endogenous source of these hormones or central (i.c.v.) infusion of corticosteroid antagonists had similar effects. Furthermore, the glucocorticoid status at the moment of adrenalectomy has the capacity to modify the outcome of this manipulation. In particular high levels of glucocorticoids were able to suppress the effects of adrenalectomy even if the test was performed 1 week later. This result suggests that glucocorticoids can have complex and long-lasting influences on brain plasticity, although the mechanisms of these effects remain to be unraveled.

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