Pharmacology Biochemistry and Behavior, Vol. 64, No. 1, pp. 65–73, 1999

0091-3057/99/$–see front matter

PII S0091-3057(99)00090-8

Ketoconazole Does Not Block Cocaine Discrimination or the Cocaine-Induced Reinstatement of Cocaine-Seeking Behavior

JOHN R. MANTSCH*[1] AND NICK E. GOEDERS†[2]

*Departments of *Pharmacology & Therapeutics and †Psychiatry, Louisiana State University Medical Center, P.O. Box 33932, Shreveport, LA 71130*

Received 13 November 1998; Revised 9 February 1999; Accepted 25 February 1999

MANTSCH, J. R. AND N. E. GOEDERS. *Ketoconazole does not block cocaine discrimination or the cocaine-induced reinstatement of cocaine-seeking behavior.* PHARMACOL BIOCHEM BEHAV **64**(1) 65–73, 1999.—Ketoconazole is an FDA-approved antifungal agent that also blocks the synthesis of adrenocorticosteroids and functions as a glucocorticoid receptor antagonist. It has been previously demonstrated that this drug blocks the stress-induced reinstatement of cocaine-seeking behavior and reduces low-dose cocaine self-administration in rats. In the present experiments, the effects of ketoconazole on the cocaine-induced reinstatement of extinguished cocaine-seeking behavior and on cocaine discrimination were investigated in male Wistar rats. In rats trained to self-administer cocaine (0.5 mg/kg/infusion) by pressing a lever under a fixed-ratio 4 schedule of reinforcement, cocaine (5–20 mg/kg, IP) dose dependently reinstated cocaine-seeking behavior following at least 10 days of extinction, during which responding on the cocaine lever resulted in no programmed consequences. Ketoconazole (50 mg/kg, IP) failed to block cocaine-induced reinstatement despite blocking cocaine-induced increases in plasma corticosterone. Ketoconazole (25 or 50 mg/kg) also failed to block cocaine discrimination in rats trained to discriminate 10 mg/kg cocaine from saline. In these rats, generalization to the training dose of cocaine was observed in the absence of increases in plasma corticosterone. The results of these experiments indicate that corticosterone may mediate the effects of stressors on cocaine-seeking behavior but not the direct effects of cocaine itself. © 1999 Elsevier Science Inc.

Cocaine Ketoconazole Drug discrimination Self-administration Reinstatement Adrenocorticosteroid Rat

AN accumulating body of preclinical evidence suggests that the hypothalamo–pituitary–adrenal (HPA) axis is a substrate through which exposure to stressful environmental stimuli can influence individual predisposition to engage in compulsive drug-seeking behavior (17,32). Interestingly, much evidence also indicates that the ability of cocaine itself to stimulate corticosterone secretion (29,35) may be required for its establishment as a positive reinforcer in the absence of stress. It has been demonstrated that inhibitors of corticosterone synthesis attenuate ongoing cocaine self-administration in rats (15,18,31), and that adrenalectomized rats do not acquire cocaine self-administration over a wide range of doses (8,15). Additionally, the inhibition of corticosterone synthesis has been shown to prevent the spontaneous reacquisition of extinguished cocaine self-administration in rats (31). These findings suggest that corticosterone may be an important mediator of cocaine's reinforcing effects. Consistent with this scenario, it has been reported that rats will actually self-administer corticosterone under certain conditions (7,30). However, although self-administration is a useful tool for the study of drug reinforcement, the characterization of the role of corticosterone in cocaine-seeking behavior will require further examination and validation using additional preclinical models of cocaine addiction.

Drug discrimination provides a sensitive, reliable, and specific assay for the determination of the pharmacological profiles of drugs based on their abilities to produce discriminable interoceptive states (2). Additionally, this method has allowed

[1]Current address: Laboratory of the Biology of Addictive Diseases, The Rockefeller University, 1230 York Ave., New York, NY 10021.
[2]Requests for reprints should be addressed to Nick E. Goeders, Ph.D., Department of Pharmacology & Therapeutics, LSU Medical Center, P.O. Box 33932, 1501 Kings Highway, Shreveport, LA 71130-3932.

the identification of many of the neuronal mechanisms underlying the in vivo effects of cocaine (5). Surprisingly, a role for corticosterone in the discriminative stimulus effects of cocaine has not been established. However, it was demonstrated recently that exposure to a stressor (i.e., restraint) produces a discriminative stimulus that generalizes to cocaine in rats trained to discriminate the drug from saline, presumably as a result of the activation of common pharmacological effector systems (25). Because stressors and cocaine both stimulate plasma corticosterone secretion, a logical assumption is that this hormone may also be involved in the production of cocaine's discriminative cue.

Experiments designed to measure the reinstatement of responding previously reinforced by cocaine following extended periods of extinction have been regarded as preclinical models for the investigation of drug "craving" and the relapse of drug self-administration in abstinent users (27). Using these models, it has been demonstrated that cocaine-seeking behavior is reinstated by priming injections of cocaine (10,14,36) and by exposure to electric footshock [EFS; (1,11,24)]. The reinstatement of cocaine-seeking behavior by EFS is mimicked by the administration of exogenous corticosterone (8), and is blocked by adrenalectomy (12) or by the pharmacological inhibition of corticosterone synthesis (24). In contrast, it has been reported that adrenalectomy fails to block the reinstatement of extinguished cocaine-seeking behavior by priming injections of cocaine (12), suggesting that corticosterone mediates the reinstating effects of stressors but not cocaine itself.

Ketoconazole is an oral antimycotic agent currently approved by the FDA for the treatment of fungal disease (3). The drug blocks the synthesis of adrenocorticosteroids (37) and may also function as a glucocorticoid receptor antagonist (23). It has been reported that ketoconazole attenuates low-dose cocaine self-administration (18) and prevents the EFS-induced reinstatement of cocaine-seeking behavior in rats (24). In the present study, this drug was used to further investigate the potential role of corticosterone in the cocaine-induced reinstatement of cocaine-seeking behavior and in cocaine discrimination.

METHODS

Subjects

Thirty-six male Wistar rats (Harlan–Sprague–Dawley, Indianapolis, IN), 80 to 100 days old at the start of the experiments, were used. All rats were housed individually in cages equipped with a laminar flow unit and air filter in a temperature- and humidity-controlled, AAALAC-accredited animal care facility on a reversed 12L:12D cycle (lights on at 1800 h). Rats were maintained at 85 to 90% of their preexperimental free-feeding body weights by presentations of food pellets (P. J. Noyes, Lancaster, NH; 45 mg) during the behavioral sessions when applicable and/or by supplemental feeding (Purina Rat Chow) and had access to water ad lib. All procedures were carried out in accordance with the NIH Principles of laboratory animal care (NIH publication No. 85-23).

Drugs

Cocaine HCl was provided by the National Institute on Drug Abuse (Research Triangle Park, NC). For the self-administration experiments, cocaine was dissolved in bacteriostatic, heparinized 0.9% saline, and was self-administered at a dose of 0.5 mg/kg/infusion in a volume of 200 μl delivered over 5.6 s. During the drug discrimination and reinstatement experiments, cocaine (0, 1.25, 2.5, 5.0, 10.0, or 20.0 mg/kg) was dissolved in 0.9% bacteriostatic saline and administered intraperitoneally in a volume of 1 ml/kg. Ketoconazole (KETO) was purchased from Research Biochemicals International (Natick, MA), and was administered as a suspension in 5% emulphor dissolved in 0.9% saline. KETO (25 and 50 mg/kg) was also administered intraperitoneally in a volume of 1 ml/kg.

Experimental Apparatus

Modified plastic and stainless steel operant conditioning chambers contained in sound-attenuating cubicles (Med-Associates, Inc., Lafayette, IN) were used for all of the experiments. The operant chambers were equipped with two retractable response levers, with stimulus lights located above each lever. For the cocaine self-administration experiments, one lever was mounted on the front and the other on the back wall of the chamber. For the drug discrimination experiments, the levers and their respective stimulus lights were mounted on the front wall of the chamber on either side of a food pellet dispenser. The cubicles were equipped with an exhaust fan that supplied ventilation and white noise to mask extraneous sound. Programming and data collection were performed using Med-PC software and an IBM-compatible personal computer and interface system (Med-Associates, Inc.).

Venous Catheterization and Drug Delivery

For the cocaine self-administration experiments, each rat was implanted with a chronic indwelling catheter under sodium pentobarbital anesthesia (50 mg/kg, IP) with methylatropine nitrate pretreatment (10 mg/kg, IP) using previously reported procedures (21,26). A silicon catheter (0.2 mm o.d. × 0.037 mm i.d.) was inserted into the right posterior facial vein and pushed down into the jugular vein so that it terminated outside the right atrium. The catheter was sutured to tissue surrounding the vein, and continued subcutaneously to the back where it exited just posterior to the scapulae via a Marlex® mesh/dental acrylic/22-gauge guide cannula (Plastics One, Inc., Roanoke, VA) assembly. This assembly was anchored to tissue under the skin for attachment of a stainless steel spring leash (Plastics One, Inc.), which was connected to a 20-ml syringe in a motor-driven pump (Razel, Stamford, CT) via a leak-proof fluid swivel suspended above the chamber to allow the delivery of drug solution. The swivel and leash assembly was counterbalanced to permit relatively unrestrained movement of the animal. Rats were injected with sterile penicillin G procaine suspension (75,000 units, IM) immediately prior to surgery, and were allowed at least 5 days to recover subsequent to implantation. The swivel and leash assembly was always connected during the behavioral sessions. Following each session, the leash was disconnected, the catheter filled with Urokinase solution (250,000 IU, Abbott Laboratories) to eliminate blood clots, and a dummy cannula was inserted into the guide before rats were returned to their home cages. Catheter patency was tested regularly by obtaining blood from the catheter. If blood could not be obtained, methohexital sodium (1.5 mg) was infused through the catheter. In this case, a functional catheter was indicated by an immediate, light anesthesia.

Cocaine Self-Administration, Extinction, and Reinstatement

During the self-administration sessions, the cocaine (back) lever was extended into the experimental chamber and the

corresponding stimulus light illuminated. A second (front) inactive lever was also extended into the chamber and functioned as a measure of nonspecific responding during the course of the experiment. Rats (n = 20) were trained to self-administer cocaine (0.5 mg/kg/infusion) by pressing the lever under a fixed-ratio 4 (FR4) schedule of reinforcement with a 90-s limited hold during daily 2-h sessions. Under this schedule, rats were required to press the cocaine lever four times within 90 s to receive an infusion, or else the schedule requirements were reset. Each infusion was followed by a 20-s time-our period, during which the lever was retracted and the stimulus light darkened. During training, rats initially learned to self-administer cocaine under a continuous schedule of reinforcement (FR1 schedule). The schedule requirements were gradually increased to FR2 and then to FR4 as stable responding was observed (i.e., three consecutive sessions during which the total number of infusions varied within 10% of the mean). Rats were allowed to self-administer cocaine under the FR4 schedule for 15 consecutive sessions before self-administration was extinguished. During extinction, conditions were identical to those during self-administration except that responding on the cocaine lever did not result in any programmed consequences. After 9 days of extinction (i.e., on extinction day 10), the ability of an injection of saline to induce reinstatement following pretreatment with the KETO vehicle (VEH) was determined in all 20 rats. Rats were pretreated with VEH and injected with saline 30 min later. Fifteen minutes following the saline injection, these rats were placed into the operant chambers and responding was recorded. The ability of cocaine in combination with KETO (or VEH) to induce reinstatement was then determined on days 11 and 13. Rats (n = 6/treatment combination) were pretreated with KETO (50 mg/kg, IP) or VEH and were injected with cocaine (5.0, 10.0, or 20.0 mg/kg, IP) 30 min later. After 15 min, the rats were placed into the operant chambers and reinstatement was measured as responding on the cocaine lever. These two reinstatement sessions were separated by an extinction session on day 12. All reinstatement test sessions were identical to the extinction sessions except that they were preceded by drug treatments. Sixteen rats were tested twice for cocaine-induced reinstatement. The remaining four rats were tested for reinstatement once following an injection of cocaine in combination with VEH or KETO and once following an injection of saline in combination with Keto (n = 4). The sequence of dosing was counter-balanced so that no rat received the same test dose of cocaine twice, and each rat was tested once with KETO and once with VEH, except for two rats that were tested with KETO twice: once in combination with cocaine, and once with saline. Likewise, the order of testing with the three doses of cocaine was counterbalanced using a Latin square design. During self-administration, the total number of cocaine infusions and responses per session were recorded. The total number of nonreinforced responses per session on the cocaine lever were recorded during extinction and reinstatement. Additionally, responding on the inactive lever was measured during all phases of the experiment (i.e., during self-administration, extinction, and reinstatement test sessions).

Drug Discrimination

Rats (n = 16) were trained to discriminate cocaine (10 mg/kg, IP) from saline (0.9% NaCl) using a two-lever operant, food-reinforced drug discrimination design modified (25) from that previously described (4). The drug- and saline-appropriate levers were randomly assigned for individual rats. During the first phase of training (i.e., errorless training), rats were injected with either cocaine or saline prior to each training session, and were then returned to their home cages. After 15 min, these rats were placed into the operant chambers and training sessions were conducted. During errorless training, only the treatment-appropriate lever (i.e., cocaine or saline) was extended, and the corresponding stimulus light illuminated. Each session lasted 30 min or until 100 reinforcers were delivered. Training began under an FR1 schedule of food (45 mg pellets) reinforcement, and this ratio was gradually increased until stable responding under an FR20 schedule was observed after both cocaine and saline treatments. Stable responding was defined as the successful completion of at least 10 consecutive sessions with less than 10% variation in response rate following each treatment. Once this criterion was met, discrimination training began. During discrimination training sessions, both levers were extended and both stimulus lights illuminated, but only responding on the treatment-appropriate lever was reinforced. There were no programmed consequences for responding on the inappropriate lever. Response levers were cleaned with 90% ethanol prior to each session to reduce any potential response bias stemming from olfactory cues (13). Discrimination training continued until at least 85% of all responses prior to the delivery of the first reinforcer were on the treatment-appropriate lever for at least 10 consecutive sessions (5 cocaine and 5 saline), at which time generalization testing began. Throughout training, sessions were conducted daily, 6 days per week (Mon.–Sat.), and neither treatment (cocaine or saline) was administered for more than two consecutive training sessions. Generalization testing was performed on Tuesdays and Fridays, and each test session was separated by at least one cocaine and one saline pretreated training session. Testing was only conducted in rats displaying at least 85% treatment-appropriate responding during the previous three training sessions. Test sessions were terminated immediately following 20 total responses on either lever (combined), which resulted in the delivery of a single food pellet, or after 20 min had elapsed. Generalization was defined as the percent of total responses (i.e., 20) on the cocaine-appropriate lever. The effects of KETO or VEH on cocaine discrimination were determined by administering these treatments in combination with various doses of cocaine (0.0, 1.25, 2.5, 5.0, 10.0, or 20.0 mg/kg, IP), and then testing for generalization. During generalization testing, rats were pretreated with KETO (25 or 50 mg/kg, IP) or VEH and then were returned to their home cages. Thirty minutes later, the rats were injected with the test doses of cocaine, and generalization was determined as described above. Each rat was treated with each dose of cocaine in combination with the two doses of KETO and VEH. The sequence of treatment administration was determined using a Latin square design.

Plasma Corticosterone Determination

The effects of pretreatment with KETO (50 mg/kg, IP) or VEH on the plasma corticosterone response to IP injections of cocaine or saline were determined in seven of the reinstatement rats. These rats were pretreated with KETO or VEH, returned to their home cages, and then injected with cocaine (0.0, 5.0, 10.0, or 20.0 mg/kg, IP) 30 min later. Each of these seven rats was tested with every dose of cocaine. Blood for the measurement of plasma corticosterone was obtained from the rats' catheters 15 min following the cocaine or saline injections. Additionally, blood for the determination of plasma

corticosterone was obtained via the implanted catheters in all reinstatement rats before and after the second to last cocaine self-administration session prior to extinction, before and after the first extinction session, and before and after the session on extinction day 9. For the drug discrimination experiments, blood was obtained from the tail vein of conscious rats immediately after the generalization sessions, as described previously (24). Following a week of daily habituation, rats were wrapped in a hand towel, and 1–2 mm was cut from the tip of the tail to allow the collection of blood for the measurement of corticosterone. All blood sampling was performed between 0900 and 1600 h. Blood (approximately 500 μl) was collected into preheparinized tubes, placed on ice, and centrifuged to allow separation of plasma, which was collected and frozen at −20°C until assayed. Plasma corticosterone was measured using the Immunochem™ Double Antibody Corticosterone assay kit (ICN Biomedical, Irvine, CA).

Statistical Analysis

During each self-administration, extinction, and reinstatement session, the total numbers of responses on the cocaine lever and on a second, inactive lever were recorded. For all analyses, post hoc testing was performed using the Fisher's Protected Least Significant Difference (PLSD) Test. During extinction, the significance of the differences between the mean number of responses/session emitted during the self-administration and extinction sessions was determined using a one-way analysis of variance (ANOVA). Repeated-measures ANOVA was used to determine the significance of the differences between pre- and postsession plasma corticosterone (ng/ml) during self-administration and extinction sessions, as well as the differences between sessions. The significance of the differences in responding on the drug and inactive levers between baseline extinction sessions (day 9), reinstatement sessions conducted following pretreatment with saline in combination with VEH (day 10), and those conducted following pretreatment with cocaine in combination with VEH or KETO (day 11 or 13) were determined using repeated-measures ANOVAs calculated for each individual dose of cocaine. The significance of the differences between the corticosterone (ng/ml) responses to cocaine and basal corticosterone concentrations in the drug treatment groups was determined using a one-way ANOVA. During drug discrimination generalization testing, generalization was defined as the percent responding on the cocaine-appropriate lever out of 20 total responses. Effective dose 50 (ED50) values for cocaine generalization were estimated using Pharmacological Calculation System software (PCS; ver. 4.0). Repeated-measures ANOVA was used to determine the significance of the differences in response rate (responses/min) and plasma corticosterone (ng/ml) between doses of cocaine and treatment conditions in the drug discrimination experiment. For all analyses, alpha was set at $p < 0.05$.

RESULTS

Rats rapidly acquired cocaine self-administration and began responding under an FR4 schedule of reinforcement after a mean of 11.26 (±0.66) sessions. The extinction of cocaine self-administration is shown in Fig. 1. A one-way ANOVA revealed a significant extinction effect, $F(9, 162) = 59.568$, $p < 0.0001$. The total numbers of responses emitted during all extinction sessions were significantly less than during the final self-administration session prior to extinction ($p < 0.001$). Additionally, the total number of responses emitted during

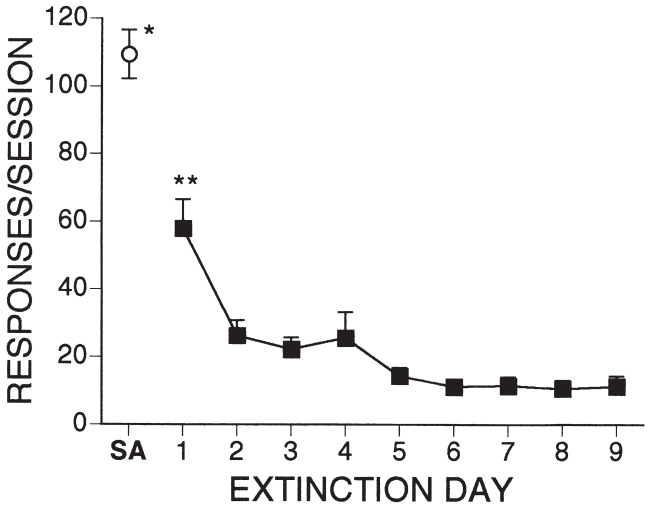

FIG. 1. Extinction of cocaine self-administration. Data represent the mean number of responses per session (±SEM) during the final 2-h self-administration session prior to extinction (SA) and during the nine consecutive daily 2-h extinction sessions in rats trained to self-administer cocaine (0.5 mg/kg/infusion). *$p < 0.001$ vs. all extinction sessions; **$p < 0.001$ vs. extinction sessions 2 through 9.

the extinction sessions on days 2 through 9 were significantly less than on extinction day 1 ($p < 0.001$). Plasma corticosterone measured before and after the second to last self-administration session prior to extinction (SA) and before and after the first (EXT DAY 1) and last (EXT DAY 9) extinction sessions is represented in Fig. 2. Significant main effects of time [pre- vs. postsession; $F(1, 35) = 5.008$, $p < 0.05$] and session [SA vs. EXT DAY 1 vs. EXT DAY 9; $F(2, 70) = 6.553$, $p < 0.005$] and an interaction between time and session, $F(2, 70) = 4.189$, $p < 0.05$, were observed. On day 9 of extinction, postsession plasma corticosterone was significantly reduced below presession concentrations ($p < 0.01$). In contrast, no significant differences between pre- and postsession corticosterone were observed during self-administration or on day 1 of extinction. No statistically significant differences in presession corticosterone were observed between any of the self-administration and extinction sessions. However, on days 1 and 9 of extinction, postsession plasma corticosterone was significantly less than corticosterone measured following the self-administration session ($p < 0.01$ for both). No significant differences in postsession corticosterone were observed between extinction days 1 and 9.

The ability of IP injections of various doses of cocaine (0.0, 5.0, 10.0, and 20.0 mg/kg) to reinstate cocaine-seeking behavior following pretreatment with KETO (50 mg/kg, IP) or VEH is depicted in Fig. 3A. KETO did not significantly affect the ability of cocaine to reinstate responding at any dose. Significant main effects of cocaine treatment were observed at the 10.0, $F(2, 20) = 28.266$, $p < 0.0001$, and 20.0 mg/kg, $F(2, 20) = 6.819$, $p < 0.01$, doses. Cocaine (10 mg/kg, IP) significantly increased responding on the cocaine lever during reinstatement testing compared to the baseline extinction and saline-pretreated sessions ($p < 0.01$ for all comparisons), regardless of whether the rats had been treated with KETO or VEH. In rats pretreated with KETO, 20 mg/kg cocaine induced significant reinstatement, as observed by increased co-

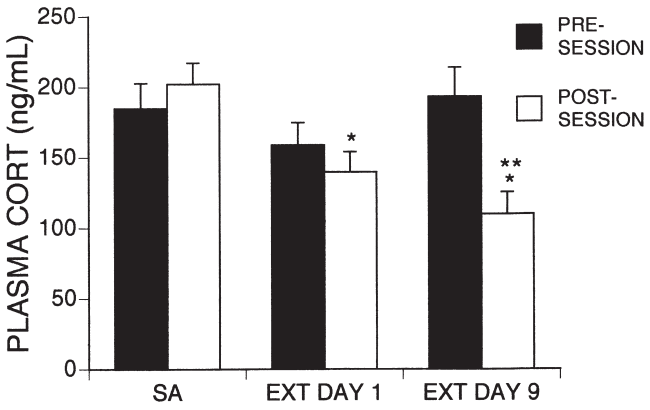

FIG. 2. Effects of cocaine self-administration and extinction on plasma corticosterone. Data represent the mean plasma corticosterone concentrations (ng/ml ± SEM) before (PRESESSION) and after (POSTSESSION) the second to last cocaine self-administration session prior to extinction (SA) and the first (EXT-DAY 1) and last (EXT-DAY 9) extinction sessions. *$p < 0.01$ vs. SA postsession; **$p < 0.01$ vs. presession.

caine-lever responding during reinstatement testing ($p < 0.01$). Although rats treated with 20 mg/kg cocaine in combination with VEH emitted a mean of 108.00 (±48.89) responses on the cocaine lever during reinstatement, this was not significantly different from the extinction baseline or saline-pretreated sessions due primarily to the large variability in responding observed following treatment with this dose of cocaine (range of 8 to 391 responses/session). No significant effects of cocaine treatment were observed at the 0.0 or 5.0 mg/kg doses. The effects of the various drug treatment combinations on responding on the inactive response lever are shown in Fig. 3B. Cocaine failed to produce significant increases in responding on this lever at any dose, and no significant effects of KETO treatment were observed.

Figure 3C represents the mean plasma corticosterone response (ng/ml ± SEM) to various doses of cocaine (0.0, 5.0, 10.0, and 20.0 mg/kg, IP) in rats pretreated with VEH or KETO. A significant main effect of cocaine dose was observed on plasma corticosterone, $F(3, 36) = 5.853$, $p < 0.01$. In VEH-pretreated rats, plasma corticosterone was significantly elevated following injections with 10 and 20 mg/kg cocaine compared to rats injected with saline or 5.0 mg/kg cocaine ($p < 0.05$ for all comparisons). No significant differences between the corticosterone response to 10 and 20 mg/kg cocaine were observed. In contrast, cocaine failed to produce statistically significant increases in plasma corticosterone at any dose tested in KETO-pretreated rats. It should also be noted, however, that no specific main effect of KETO treatment was observed, $F(1, 12) = 4.102$, $p = 0.0657$.

The effects of treatment with KETO (25 or 50 mg/kg, IP) or VEH on the generalization of various of doses of cocaine (0.0, 1.25, 2.5, 5.0, 10.0, and 20 mg/kg, IP) to the training dose in rats trained to discriminate 10 mg/kg cocaine from saline are shown in Fig. 4A. In all three treatment groups, cocaine dose dependently generalized to the 10 mg/kg training dose. No differences in cocaine generalization were observed following treatment with either dose of KETO relative to VEH-treated controls. The ED50s for cocaine generalization in the VEH, 25 mg/kg KETO, and 50 mg/kg KETO treatment

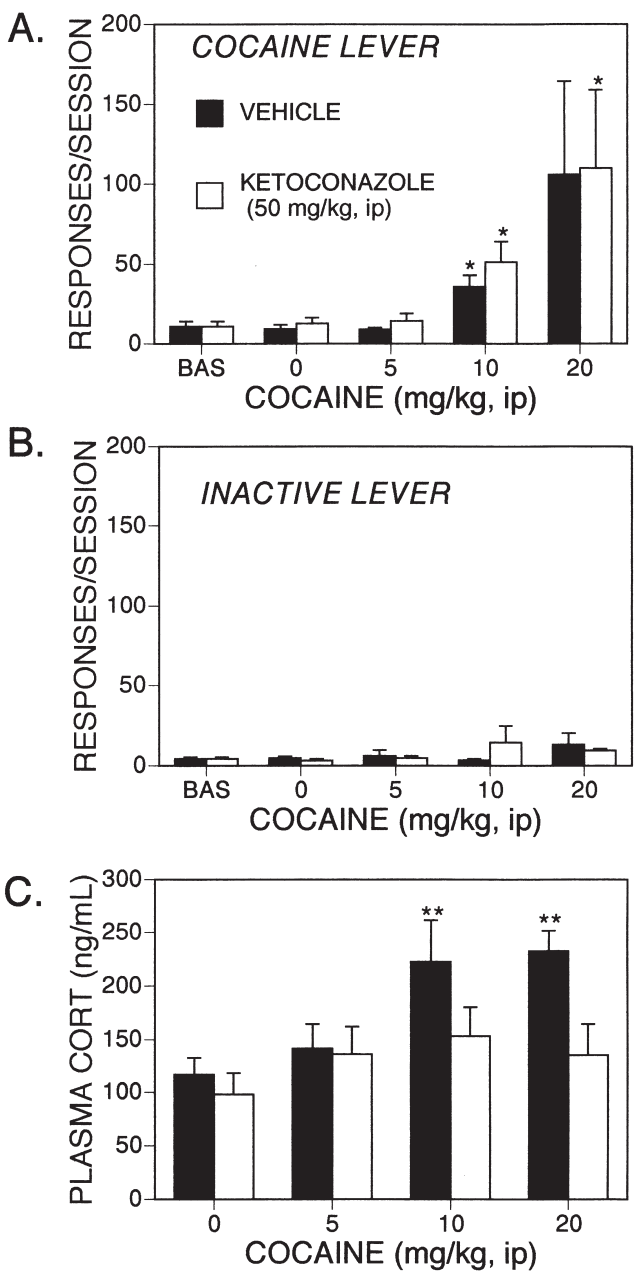

FIG. 3. Cocaine-induced reinstatement of extinguished cocaine-seeking behavior following pretreatment with ketoconazole (KETO) or vehicle (VEH). Data in A and B represent responding on the cocaine or inactive response lever (±SEM) during the extinction sessions prior to reinstatement testing (BAS) or during reinstatement test sessions 15 min after injections with cocaine (0.0, 5.0, 10.0, or 20.0 mg/kg, IP) in rats pretreated with KETO (50 mg/kg, IP) or VEH (5% emulphor in 0.9% saline; 30 min). BAS and saline (i.e., 0.0 cocaine)/VEH data represent means determined from all 20 rats. All other data were obtained from six rats/treatment combination except for the saline/KETO treatment combination data, which were determined from four rats. Data in C represent the mean plasma corticosterone concentrations (ng/ml; ±SEM) 15 min after injection with cocaine (0.0, 5.0, 10.0, or 20.0 mg/kg, IP) in rats pretreated with KETO or VEH. *$p < 0.05$ vs. BAS and 0.0 mg/kg cocaine; **$p < 0.05$ vs. 0.0 and 5.0 mg/kg cocaine.

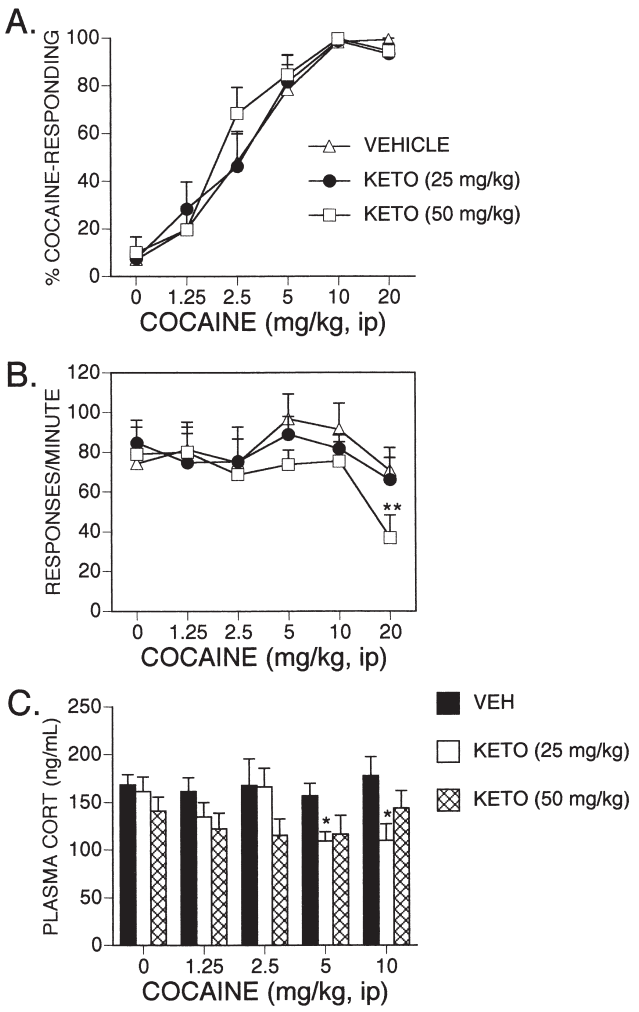

FIG. 4. Cocaine discrimination in rats pretreated with ketoconazole or vehicle. Data represent the mean percent responding on the cocaine-appropriate lever (±SEM; A), response rates (responses/min ± SEM; B), and plasma corticosterone concentrations (ng/ml ± SEM; C) following injections with cocaine (0.0–20.0 mg/kg, IP; 15 min) in rats ($n = 16$) pretreated with ketoconazole (KETO, 25 or 50 mg/kg, IP) or vehicle (VEH, 5% emulphor in 0.9% saline; 30 min). Plasma corticosterone was not measured following injection with 20 mg/kg cocaine. *$p < 0.05$ vs. cocaine 0.0–10.0 mg/kg; **$p < 0.05$ vs. VEH.

groups were 2.70, 2.37, and 2.05 mg/kg, respectively. The effects of the treatment combinations on response rate (responses/minute) are shown in Fig. 4B. A significant main effect of dose on response rate was observed, $F(5, 165) = 3.547$, $p < 0.01$. When rats were pretreated with 50 mg/kg KETO in combination with 20 mg/kg cocaine, the rate of responding was significantly less than that observed following pretreatment with the same dose of KETO in combination with each of the lower doses of cocaine. No significant effects of dose were observed in the VEH or 25-mg/kg KETO treatment groups. There was also no significant main effect of KETO treatment. The effects of cocaine (0.0, 1.25, 2.5, 5.0, and 10.0 mg/kg, IP) on plasma corticosterone in rats treated with VEH or KETO (25 or 50 mg/kg, IP) are depicted in Fig. 4C. Tail blood for plasma corticosterone measurements was not obtained from rats treated with 20 mg/kg cocaine due to the high degree of vasoconstriction resulting from the administration of this dose. No significant main effect of cocaine dose on plasma corticosterone was observed in these rats, $F(4, 124) = 1.212$, $p = 0.3090$. However, a significant effect of KETO treatment was observed, $F(2, 31) = 5.293$, $p < 0.0105$. Interestingly, pretreatment with 25 but not 50 mg/kg KETO significantly decreased plasma corticosterone following injections with 5 or 10 mg/kg cocaine relative to VEH-treated rats ($p < 0.05$ for both). No other significant effects of KETO on plasma corticosterone were observed in these rats.

DISCUSSION

In the present experiments, KETO failed to block the cocaine-induced reinstatement of extinguished cocaine-seeking behavior, and did not attenuate cocaine discrimination in rats trained to discriminate the drug from saline. The doses of KETO used in these experiments (i.e., 25 and 50 mg/kg) have previously been demonstrated to block EFS-induced reinstatement (24) and to reduce low-dose cocaine self-administration in rats (18). Together, these findings indicate that there is a distinction between the mechanisms underlying low-dose cocaine self-administration and the effects of stressors on cocaine reinforcement and those mediating high-dose self-administration and the discriminative stimulus and response-reinstating effects of the drug.

More importantly, the present findings suggest that corticosterone may not be directly involved in the cocaine-induced reinstatement of cocaine-seeking behavior or in the discriminative stimulus effects of the drug. These conclusions can be reached based on two pieces of evidence. First, KETO is a putative inhibitor of adrenocorticosteroid synthesis (37), and has been reported to function as a glucocorticoid receptor antagonist (23). In the present experiment, cocaine (10 and 20 mg/kg) reinstated extinguished cocaine-seeking behavior while simultaneously increasing plasma corticosterone. Interestingly, no significant differences between the plasma corticosterone responses to 10 and 20 mg/kg cocaine were observed, suggesting that, under the present conditions, cocaine-induced increases in corticosterone secretion may have represented an "all-or-nothing" phenomenon. Pretreatment with KETO attenuated these cocaine-induced increases in plasma corticosterone without affecting cocaine-induced reinstatement. However, the effects of KETO on plasma corticosterone were very modest, and such small reductions in plasma corticosterone may have been insufficient to disrupt cocaine's reinstating effects. Secondly, cocaine generalized to the 10 mg/kg training dose of cocaine without increasing plasma corticosterone in rats trained to discriminate the drug from saline. In these rats, pretreatment with KETO also failed to attenuate cocaine discrimination.

In the reinstatement experiment, intraperitoneal injections of cocaine dose dependently reinstated behavior previously reinforced by cocaine following at least 10 days of extinction without increasing responding on a second, inactive response lever. These findings are consistent with those of other groups who have reported that "priming" injections of cocaine will reinstate extinguished responding in primates (14,36) and rats (6,10,11). It has also been reported that cocaine administration precipitates "craving" in experienced cocaine users (20), lending support to the use of reinstatement methods as valid preclinical models for relapse in humans (27). Significant reinstatement was observed in VEH- and KETO-pretreated rats

following injection with 10 mg/kg cocaine. Although the mean number of reinstatement responses observed following injections with 20 mg/kg cocaine was much greater than that observed at the 10 mg/kg dose, statistically significant differences from baseline extinction and saline pretreatment were not observed following injections with 20 mg/kg cocaine in VEH-pretreated rats primarily due to the large amount of variability in responding (range 8 to 391). This was likely due in part to the emergence of competitive behaviors (e.g., stereotypy), which may have interfered with operant responding. KETO (50 mg/kg, IP) failed to block cocaine-induced reinstatement, despite preventing statistically significant cocaine-induced increases in plasma corticosterone.

KETO also failed to block cocaine discrimination in rats trained to discriminate 10 mg/kg cocaine from saline. Interestingly, cocaine (1.25 to 10.0 mg/kg) by itself did not significantly increase plasma corticosterone in these rats. These findings are in contrast to those from the reinstatement experiment as well as to findings previously reported (25) demonstrating that 10 mg/kg cocaine (IP) significantly increases plasma corticosterone. Because drug discrimination training and testing require the repeated administration of relatively high doses of cocaine over extended periods of time, it is possible that this may be the result of the development of tolerance or a habituation to the effects of cocaine on corticosterone secretion. Accordingly, tolerance to cocaine's effects on the HPA axis has been observed in rats (38) and humans (28) under certain conditions. Regardless of the reason for the observed lack of corticosterone responsiveness, these experiments clearly demonstrate a separation of the effects of cocaine on plasma corticosterone from the ability of this drug to produce a discriminative cue.

It has previously been demonstrated that KETO, at the doses used in these experiments, blocks EFS-induced reinstatement using a model identical to the one used in the present study (24), and also reduces low-dose cocaine self-administration in rats (18). In consideration of these findings and others implicating corticosterone in the acquisition (8,16,26), maintenance (15,31), and reinstatement (8) of cocaine-seeking behavior, the reported lack of involvement of corticosterone in the discriminative stimulus and response-reinstating effects of cocaine appears to be at odds with the putative involvement of the hormone in cocaine reinforcement (32), and may require a reassessment of the role of corticosterone in cocaine-seeking behavior. However, this interpretation should be made cautiously, in consideration of the modest effects that KETO had on plasma corticosterone in the present experiments. Additionally, a relatively high training dose of cocaine (i.e., 0.5 mg/kg/infusion) was used in these experiments. In light of earlier findings that KETO attenuates low-dose (e.g., 0.125 and 0.25 mg/kg/infusion), but not high-dose (e.g., 0.5 and 1.0 mg/kg/infusion), cocaine self-administration (18), it is possible that greater effects of KETO on cocaine-induced reinstatement would have been observed if rats had self-administered a lower dose of cocaine.

An additional explanation for this apparent discrepancy is that corticosterone provides a substrate through which external factors (e.g., stressors) can influence cocaine sensitivity but is not required for the direct effects of cocaine. This is best illustrated by the ability of KETO to block reinstatement by EFS (24) but not cocaine itself. This is also supported by findings that corticosterone can exert EFS-like effects on the acquisition of cocaine self-administration (26) and can reinstate extinguished cocaine-seeking behavior (8) at doses that produce plasma concentrations within the EFS-induced range. Furthermore, the selective blockade of stressor- but not drug-induced reinstatement is consistent with recent findings that ADX blocks the reinstatement of extinguished cocaine-seeking behavior by EFS but not cocaine (12).

It has been proposed that the interaction between adrenocorticosteroids and cocaine-seeking behavior occurs at the level of the mesocorticolimbic dopaminergic system (32). The ability of cocaine to block the reuptake of dopamine into nerve terminals within this system, most notably in the nucleus accumbens, has been implicated in its reinforcing effects (22). Type II glucocorticoid receptors are localized in the cell bodies of mesocorticolimbic dopamine neurons in the ventral tegmental area (19), and corticosterone, in the stress-induced range, has been reported to increase dopamine overflow in the nucleus accumbens (33). Thus, through their effects on corticosterone secretion, stressors may enhance mesocorticolimbic dopaminergic neurotransmission and thereby facilitate cocaine reinforcement or produce cocaine-like responding in the absence of the drug. In contrast, cocaine itself primarily exerts direct effects on synaptic dopamine through its interaction with dopamine transporters. However, because cocaine also stimulates the release of corticosterone (34), the drug may exert additional indirect effects on mesocorticolimbic dopamine through the release of this hormone. With higher cocaine doses, it is likely that the relative contribution of corticosterone to cocaine-induced increases in mesocorticolimbic dopamine is minimal. This would explain why the attenuation of corticosterone synthesis by ketoconazole blocks the EFS-induced (24), but not the cocaine-induced reinstatement, of extinguished cocaine-seeking behavior. However, at lower doses, the effects of cocaine on corticosterone may play a much greater role in cocaine reinforcement, as illustrated by the ability of KETO to attenuate low- but not high-dose cocaine self-administration (18) and by findings that the facilitation of cocaine self-administration by corticosterone pretreatment is only observed on the ascending limb of the self-administration dose–response curve (26). The determination of the potential role of corticosterone–dopamine interactions in cocaine reinforcement will require further investigation.

In the present study, pre- and postsession plasma corticosterone was measured during self-administration and over the course of extinction. No differences in plasma corticosterone were observed prior to the self-administration and the first or last extinction sessions. Interestingly, plasma corticosterone after the 2-h cocaine self-administration session was not significantly elevated compared to the presession value, but was greater than after the first and last extinction sessions. When postsession corticosterone was compared to presession, no significant differences were observed except on extinction day 9, when postsession corticosterone was reduced to approximately 60% of its presession value. The persistent elevation of plasma corticosterone during the first, but not last, extinction session appears to parallel the increased responding on the cocaine lever in the absence of the drug, and may be related to the reported ability of corticosterone administration to reinstate extinguished cocaine-seeking behavior (8). Furthermore, because plasma corticosterone was elevated at the end of the initial extinction session even though the rats did not receive cocaine infusions, it is likely that the effects of cocaine on plasma corticosterone were conditioned. Conditioned effects on corticosterone may also explain why significant differences between pre- and postsession corticosterone were not observed during the self-administration or initial extinction session. In these cases, simply placing the rats into the experimental chambers may have been sufficient to elevate

plasma corticosterone. Interestingly, the inhibition of corticosterone synthesis by KETO has also been found to attenuate the increased responding on the cocaine-lever observed during the acute extinction of cocaine self-administration (unpublished observations), suggesting that the conditioned effects of cocaine on plasma corticosterone may be responsible for the perseverance of responding in the absence of the drug. Consistent with this scenario, it has been reported that exposure to contextual stimuli paired with the administration of cocaine can elicit increases in plasma corticosterone (9).

In summary, the results from these experiments show that KETO does not block the discriminative stimulus or response-reinstating effects of cocaine in rats. The findings indicate that cocaine-induced increases in corticosterone may not be required for the production of its discriminative stimulus effects or for the cocaine-induced reinstatement of extinguished cocaine-seeking behavior. These data provide a better understanding of the role of adrenocorticosteroids in cocaine abuse, and suggest that corticosterone is more important as a substrate through which environmental stimuli may influence cocaine-seeking behavior rather than as a mediator of the direct effects of cocaine itself.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge G. F. Guerin for his expert technical assistance and invaluable advice. This work was supported by United States Public Health Service grants DA06013 and DA05836 from the National Institute on Drug Abuse.

REFERENCES

1. Ahmed, S. H.; Koob, G. F.: Cocaine- but not food-seeking behavior is reinstated by stress after extinction. Psychopharmacology (Berlin) 132:289–295; 1997.
2. Barry, H., III.: Classification of drugs according to their discriminable effects in rats. Fed. Proc. 33:1814–1824; 1984.
3. Bennett, J. E.: Antifungal agents. In: Hardman, J. G.; Limbird, L. E., eds. Goodman & Gilman's The pharmacological basis of therapeutics, 9th ed. New York: McGraw-Hill; 1996:1175–1190.
4. Callahan, P. M.; Appel, J. B.; Cunningham, K. A.: Dopamine D1 and D2 mediation of the discriminative stimulus properties of *d*-amphetamine and cocaine. Psychopharmacology (Berlin) 103:50–55; 1991.
5. Callahan, P. M.; De La Garza, R., II; Cunningham, K. A.: Mediation of the discriminative stimulus properties of cocaine by mesocorticolimbic dopamine systems. Pharmacol. Biochem. Behav. 57:601–607; 1997.
6. Comer, S. D.; Lac, S. T.; Curtis, L. K.; Carroll, M. E.: Effects of buprenorphine and naltrexone on reinstatement of cocaine-reinforced responding in rats. J. Pharmacol. Exp. Ther. 267:1470–1477; 1993.
7. Deroche, V.; Piazza, P. V.; Deminiére, J.; Le Moal, M.; Dimon, H.: Rats orally self-administer corticosterone. Brain Res. 622: 315–320; 1993.
8. Deroche, V.; Marinelli, M.; Le Moal, M.; Piazza, P. V.: Glucocorticoids and behavioral effects of psychostimulants. II.: Cocaine intravenous self-administration and reinstatement depend on glucocorticoid levels. J. Pharmacol. Exp. Ther. 281:1401–1407; 1997.
9. De Vries, B. G.; Taymans, S. E.; Sundstrom, J. M.; Pert, A.: Conditioned release of corticosterone by contextual stimuli associated with cocaine is mediated by corticotropin-releasing factor. Brain Res. 786:39–46; 1998.
10. de Wit, H.; Stewart, J.: Reinstatement of cocaine-reinforced responding in the rat. Psychopharmacology (Berlin) 75:134–143; 1981.
11. Erb, S.; Shaham, Y.; Stewart, J.: Stress reinstates cocaine-seeking behavior after prolonged extinction and a drug-free period. Psychopharmacology (Berlin) 128:408–412; 1996.
12. Erb, S., Shaham, Y., Stewart, J.: The role of corticotropin-releasing factor and corticosterone in stress- and cocaine-induced relapse to cocaine seeking in rats. J. Neurosci. 18:5529–5537; 1998.
13. Extance, K.; Goudie, A. J.: Inter-animal olfactory cues in operant drug discrimination procedures in rats. Psychopharmacology (Berlin) 73:363–371; 1981.
14. Gerber, G. J.; Stretch, R.: Drug-induced reinstatement of extinguished self-administration behavior in monkeys. Pharmacol. Biochem. Behav. 3:1055–1061; 1975.
15. Goeders, N. E.; Guerin, G. F.: Effects of surgical and pharmacological adrenalectomy on the initiation and maintenance of intravenous cocaine self-administration in rats. Brain Res. 722:145–152; 1996.
16. Goeders, N. E.; Guerin, G. F.: Role of corticosterone in intravenous cocaine self-administration. Neuroendocrinology 64:337–348; 1996.
17. Goeders, N. E.: A neuroendocrine role in cocaine reinforcement. Psychoneuroendocrinology 22:237–259; 1997.
18. Goeders, N. E.; Peltier, R. L.; Guerin, G. F.: Ketoconazole reduces low dose cocaine self-administration in rats. Drug Alcohol Depend. 53:67–77; 1999.
19. Härfstrand, A.; Fuxe, K.; Cintra, A.; Agnati, L. F.; Zini, I.; Wikstrom, A.-C.; Okret, S.; Yu, Z.-Y.; Goldstein, M.; Steinbusch, H.; Verhofstad, A.; Gustafsson, J. Å.: Glucocorticoid receptor immunoreactivity in monoaminergic neurons of rat brain. Proc. Natl. Acad. Sci. USA 83:9779–9783; 1986.
20. Jaffe, J. H.; Cascella, N. G.; Kumor, K. M.; Sherer, M. A.: Cocaine-induced cocaine craving. Psychopharmacology (Berlin) 97:59–64; 1989.
21. Koob, G. F.; Goeders, N. E.: Neuroanatomical substrates of drug self-administration. In: Liebman, J. M.; Cooper, S. J., eds. Oxford reviews in psychopharmacology, vol. 1, Neuropharmacological basis of reward. London: Oxford University Press; 1989:214–263.
22. Koob, G. F.: Drugs of abuse: Anatomy, pharmacology and function of reward pathways. Trends Pharmacol. Sci. 13:177–184; 1992.
23. Loose, D. S.; Stover, E. P.; Feldman, D.: Ketoconazole binds to glucocorticoid receptors and exhibits antagonist activity in cultures cells. J. Clin. Invest. 72:404–408; 1983.
24. Mantsch, J. R.; Goeders, N. E.: Ketoconazole blocks the stress-induced reinstatement of cocaine-seeking behavior in rats: Relationship to the discriminative stimulus effects of cocaine. Psychopharmacology (Berlin) 142:399–407; 1999.
25. Mantsch, J. R.; Goeders, N. E.: Generalization of a restraint-induced discriminative stimulus to cocaine in rats. Psychopharmacology (Berlin) 135:423–426; 1998.
26. Mantsch, J. R.; Saphier, D.; Goeders, N. E.: Corticosterone facilitates the acquisition of cocaine self-administration in rats: Opposite effects of the Type II glucocorticoid receptor agonist, dexamethasone. J. Pharmacol. Exp. Ther. 287:72–80; 1998.
27. Markou, A.; Weiss, F.; Gold, L. H.; Caine, S. B.; Schulteis, G.; Koob, G. F.: Animal models of drug craving. Psychopharmacology (Berlin) 112:163–182; 1993.
28. Mendelson, J. H.; Sholar, M.; Mello, N. K.; Teoh, S. K.; Sholar, J. W.: Cocaine tolerance: Behavioral, cardiovascular, and neuroendocrine function in men. Neuropsychopharmacology 18:262–271; 1998.
29. Moldow, R. L.; Fischman, A. J.: Cocaine induced secretion of ACTH, beta-endorphin, and corticosterone. Peptides 8:819–822; 1987.
30. Piazza, P. V.; Deroche, V.; Deminiére, M. M.; Maccari, S.; Le Moal, M.; Simon, H.: Corticosterone in the range of stress-induced levels possesses reinforcing properties: Implications for sensation seeking behaviors. Proc. Natl. Acad. Sci. USA 90: 11738–11742; 1993.
31. Piazza, P. V.; Marinelli, M.; Jodogne, C.; Deroche, V.; Rougé-

Pont, F.; Maccari, S.; Le Moal, M.; Simon, H.: Inhibition of corticosterone synthesis by metyrapone decreases cocaine-induced locomotion and relapse of cocaine self-administration. Brain Res. 658:259–264; 1994.
32. Piazza, P. V., Le Moal, M.: Pathophysiological basis of vulnerability to drug abuse: Role of an interaction between stress, glucocorticoids, and dopaminergic neurons. Annu. Rev. Pharmacol. Toxicol. 36:359–378; 1996.
33. Piazza, P. V.; Rougé-Pont, F.; Deroche, V.; Maccari, S.; Simon, H.; Le Moal, M.: Glucocorticoids have state-dependent stimulant effects on the mesencephalic dopaminergic transmission. Proc. Natl. Acad. Sci. USA 93:8716–8720; 1996.
34. Rivier, C.; Vale, W.: Cocaine stimulates adrenocorticotropin (ACTH) secretion through a corticotropin-releasing factor (CRF)-mediated mechanism. Brain Res. 422:403–406; 1987.
35. Saphier, D.; Welch, J. E.; Farrar, G. E.; Goeders, N. E.: Effects of intracerebroventricular and intrahypothalamic cocaine administration on adrenocortical secretion. Neuroendocrinology 57:54–62; 1993.
36. Slikker, W., Jr.; Brocco, M. J.; Killam, K. F., Jr.: Reinstatement of responding maintained by cocaine or thiamylal. J. Pharmacol. Exp. Ther. 228:43–52; 1984.
37. Sonino, N.: The use of ketoconazole as an inhibitor of steroid production. N. Engl. J. Med. 317:812–818; 1987.
38. Torres, G.; Rivier, C.: Cocaine-induced stimulation of the rat hypothalamic–pituitary–adrenal axis is progressively attenuated following hourly-interval regimens of the drug. Life Sci. 51:1041–1048; 1993.