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Effects of Repeated Administration of Corticotropin-Releasing Factor on Schedule-Controlled Behavior in Rats

STEPHEN T. AHLERS¹ AND MARY K. SALANDER

*Neurochemistry Division, Thermal Stress/Adaptation Program,
Naval Medical Research Institute, Bethesda, MD 20889-5055*

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AHLERS, S. T., AND M. K. SALANDER. *Effects of repeated administration of corticotropin-releasing factor on schedule-controlled behavior in rats.* PHARMACOL BIOCHEM BEHAV 44(2) 375-380, 1993. — To examine the effects of repeated administration of corticotropin-releasing factor (CRF) on behavior, rats were administered ICV injections of either CRF or saline on alternate days for 10 days prior to performing on a multiple fixed-interval (FI) 60 s/fixed-ratio (FR) 20 schedule for food reinforcement. A daily session consisted of 10 components of each schedule that alternated, starting with the FI component. CRF doses were individually determined for each rat and were either 1.0, 3.0, or 10 μ g CRF based upon the dose that occasioned more than a 50% reduction in the rate of responding. Acute administration of CRF decreased the rate of responding in both components well below control rates; this decrease in responding was associated with a 20 or 50% decrease in the number of earned reinforcements in the FI and FR components, respectively. With repeated administration, CRF-induced suppression of responding was attenuated, although CRF continued to decrease response rate. Despite the continued reduction in response rate, subsequent CRF injections did not result in a loss of reinforcements in the FI component, whereas rats continued to lose 20% of the reinforcers in the FR component. After an 18-day hiatus in which no CRF was administered, the baseline rate of responding on the multiple schedule increased, in particular in the FI component. When CRF was readministered, response rates were slightly suppressed relative to a reestablished saline control but significantly higher than CRF-induced suppression on the last day of the chronic regimen. These data demonstrate that with repeated administration tolerance develops to CRF-induced suppression of responding in rats.

Corticotropin-releasing factor Stress Schedule-controlled behavior Adaptation Tolerance Chronic

ACUTE administration of corticotropin-releasing factor (CRF) has been shown to produce a variety of behavioral and physiological effects similar to those observed when animals are exposed to physical stressors (12,22). CRF produces stress-like release of adrenocorticotropin hormone (ACTH) and β -endorphin (24), and release of central (4,11,19,20) and peripheral catecholamines (8), as well as increases in oxygen consumption, heart rate, and mean arterial pressure (9). Administration of CRF also produces behavioral effects such as decreased food intake (21), increased locomotor activity (27), increased emotionality in a novel environment (6), and impairment of performance in a variety of conditioned behavioral paradigms (2-5,7,14,15,23).

When administered repeatedly, studies have shown that the effects of CRF are either unchanged (1,27), increased (1,15), or decreased (1,3,4,10,18,28). Tolerance to the effects of repeated administration of CRF has been observed with CRF-induced anorexia (18), suppression of schedule-controlled responding (3,4), release of norepinephrine and corticosterone

(10), and CRF-induced seizure activity (28). On the other hand, Glowa and Gold (15) observed sensitization to the disruption of schedule-controlled responding after CRF was administered chronically while Sutton et al. (27) found no change in CRF's locomotor-activating effects when given repeatedly. Abreu et al. examined the effects of repeated CRF administration on several behavioral and neurohumoral indices, as well as on brain CRF receptors in the rat (1). Chronic CRF administration decreased CRF receptors in the pituitary and cortex, sensitized rats in terms of foot-shock-induced freezing, but substantially decreased CRF-induced grooming and plasma ACTH release after a CRF injection.

The purpose of the present experiment was to examine the effects of repeated CRF administration in rats performing on a multiple schedule similar to the design employed previously in which pigeons developed tolerance to the rate-decreasing effects of CRF (3,4). Given the differences in effects with chronic CRF between the pigeon and the monkey with schedule-controlled behavior, and the diversity of physiological ac-

¹ To whom requests for reprints should be addressed.



tions observed with chronic CRF administration in other paradigms, it was important to determine how a similar behavioral baseline in another species is affected by chronic CRF. In the present study, prior to implementing the chronic CRF regimen dose-response manipulations determined the dose of CRF that decreased response rates on a multiple-fixed interval (FI) 60 s fixed ratio (FR) 20 schedule below 50% of control responding. During this phase of the experiment, only one or two injections of CRF were given per week because previous data from our laboratory had indicated that tolerance to the rate-decreasing effects of CRF does not occur under these circumstances (2,4). Once the optimal dose was determined for each animal, the chronic regimen was begun in which rats received saline and the dose of CRF that impaired performance. During this phase of the experiment, rats received five saline and five CRF injections on alternate days for 10 days. After the fifth CRF injection, rats were run on the multiple baseline for 10 sessions over an 18-day period during which no CRF or saline was administered. Thereafter, rats were re-administered saline and CRF to determine whether tolerance to the rate-suppressing effects of CRF endured.

METHOD

Subjects

Six Long-Evans rats maintained at 85% of their free-feeding body weight of approximately 325 g served as subjects. Rats were individually housed in hanging cages in an air-controlled unit. Water was available continuously in the home cage. Rats were maintained on a 12 L : 12 D cycle (lights on at 0600 h).

Apparatus

Subjects performed in a standard two-lever operant chamber 24.1 × 30.4 × 26.6 cm. Two response levers were mounted on the front wall, 5.0 cm above the grid floor and 3.8 cm from either of the side walls. A food tray was mounted 1.2 cm above the grid floor and in the center of the front wall equidistant from each of the levers. The tray was connected by a short tube to a pellet feeder located behind the front wall that could dispense 45-mg (Bio-Serv, Inc., Frenchtown, NJ) food pellets. A small light with a white lens cover was mounted 5.0 cm above both the right and left levers. A houselight was mounted on the top of the front wall. The experimental chamber was placed within a larger sound- and light-attenuating enclosure that was provided with white noise to mask extraneous sounds and a fan for adequate ventilation. Experimental events were controlled and recorded by a micro-computer system.

Procedure

Animals were trained to lever press for food presentation by the method of successive approximations. Once lever-pressing behavior was established, rats were gradually shaped to respond on a multiple schedule of reinforcement with an FR 20 schedule programmed on the left lever and an FI 60-s schedule programmed on the right lever. A light located directly above each lever was illuminated when the respective schedules were operative. A daily session consisted of exposure to 10 components of each of the two schedules. The components alternated regularly, starting with the FI schedule, and were separated by a 30-s period during which all lights were extinguished and lever pressing had no scheduled

consequences (timeout). Each schedule component was required to be completed within a 2-min period (limited hold); if the schedule requirement was not met within that time, the component terminated and the schedule alternated into the next component in the session after the 30-s timeout.

Surgical Procedure

Once stable performance on the multiple schedule was reached and maintained for several weeks, rats were implanted with a chronic cannula placed into the lateral ventricle. Rats were anesthetized with pentobarbital sodium (50.0 mg/kg, IP) and placed in a stereotaxic apparatus. A 22-ga guide cannula (Plastics One, Roanoke, VA) was implanted into the lateral ventricle (AP = -0.8, L = +1.3 from bregma) using stereotaxic coordinates from Paxinos and Watson (24). The depth or vertical location of the cannula was determined individually with each rat based upon a sudden drop in the fluid level (phosphate-buffered saline solution, Sigma Chemical Co., St. Louis, MO) in a piece of 20-cm tubing attached to the guide cannula as it was being slowly lowered into the ventricle. The guide cannula was anchored in place by cranioplastic cement that surrounded the guide cannula and four stainless steel screws threaded into the skull. At all times other than during injection, the guide cannula was sealed with a dummy cannula (Plastics One). Drug studies were undertaken no sooner than 2 weeks after implantation of the cannula.

Drug Administration

CRF, obtained from Peninsula Laboratories (San Carlos, CA), was dissolved in sterile 0.9% saline and injected as a freshly prepared solution. CRF or saline were injected ICV through a 28-ga injector cannula that, when inserted, extended 1 mm beyond the tip of the guide cannula into the ventricle. The injector cannula was connected to a Hamilton microliter syringe (Hamilton Co., Reno, NV) with approximately 30 cm of polyethylene tubing. A Harvard microsyringe pump (Model 22, Harvard Apparatus, South Natick, MA) was programmed to deliver the solution through the injector at a flow rate of 10 μ l/min. Injections of saline or CRF (1.0, 3.0, or 10 μ g) were given in a volume of 5 μ l 60 min before the session.

Rats were given different doses of CRF based upon the dose of CRF that would suppress responding to greater than 50% of baseline performance levels on the multiple schedule. Our experience with CRF has shown that there are substantial individual differences in rats in terms of dose required to produce CRF-induced disruption of schedule-controlled behavior. For these reasons, four rats received 1.0 μ g CRF during the chronic phase of the experiment while one was given 3.0 μ g and another 10.0 μ g CRF chronically. Dose-response determinations with saline and CRF were conducted prior to the beginning of chronic regimen. During this phase of the experiment, injections of CRF or saline were administered on either Tuesday or Friday until the criterion of 50% suppression was reached. Under conditions in which animals (both rats and pigeons) are administered biweekly injections of CRF, previous research has shown that tolerance to the effects of CRF on responding does not occur (2,4). Once the effective dose of CRF was determined for each rat, the chronic manipulation was undertaken starting with an ICV injection of saline. During the chronic phase of the experiment, rats received five saline and five CRF injections on alternate days for a 10-day period. The alternating-day CRF regimen was employed in the present experiment because it was noted in previous experiments with pigeons that CRF given on consecutive days pro-

duced a transient reduction in body weight that was compensated for by increasing the amount of food to each animal (3,4). By alternating CRF every other day, it was hoped that supplemental feeding would not be necessary with rats. Baseline performance consisted of the average of five nondrugged sessions just prior to the beginning of the experiment.

After an 18-day period in which no CRF or saline was administered, and during which animals performed 10 sessions on the multiple schedule, the effects of saline and CRF on the operant baseline were reassessed in five of the six subjects. One subject died 1 week after termination of chronic CRF from unknown causes so redetermination included only five subjects.

During the course of the chronic study, rats were maintained at the same weight. There were no differences in body weight throughout the course of the study nor was there a significant change in the amount of food required to maintain the stable body weight.

Data Analysis

Overall group differences were determined by analysis of variance (ANOVA) with repeated measures. Pairwise comparisons were accomplished using a paired *t*-test (two tailed). The level of statistical significance was $p < 0.05$.

RESULTS

FI Response Rate

The baseline (B) control rate, the average of five nondrugged sessions prior to the beginning of the experiment, is indicated by the open square on the left portion of the left panel of Fig. 1. Statistical analysis of the FI response rate between the saline and CRF injection conditions indicated that response rate changed significantly across sessions, $F(4, 20) = 6.90$, $p < 0.01$, and that there was a significant main effect of CRF vs. saline, $F(1, 5) = 6.35$, $p < 0.05$. Also, there was a highly significant treatment $\times$ session interaction, $F(4, 20) = 7.72$, $p < 0.001$. Subsequent pairwise analysis indicated that administration of CRF in Session 1 produced a significant reduction in the rate of responding in the FI component relative to the first saline injection, $t(5) = 8.46$, $p < 0.001$, and to baseline performance, $t(5) = 7.78$, $p < 0.001$. When CRF was administered the second time, the magnitude of response rate suppression was significantly decreased, $t(5) = 5.39$, $p < 0.01$, relative to the first CRF injection. The response rate increased slightly during the third injection of CRF but was not observed to change after the fourth or fifth CRF injections. There were no statistically significant differences in the rate of FI responding in rats between CRF injections 2-5, and these were not statistically different from the corresponding saline controls. Repeated injections of saline did not alter FI response rate.

FR Response Rate

The effects of chronic CRF administration on responding in the FR component are depicted in the middle panel of Fig. 1. Overall analysis indicated significant differences in response rate across sessions, $F(4, 20) = 4.46$, $p < 0.01$, but a nonsignificant main treatment effect, $F(1, 5) = 0.81$, $p > 0.1$. However, there was a highly significant treatment $\times$ session interaction, $F(4, 20) = 9.93$, $p < 0.001$. Pairwise comparisons revealed that the first injection of CRF produced significant suppression of responding compared to the first saline

injection, $t(5) = 10.34$, $p < 0.001$, and to baseline performance, $t(5) = 4.73$, $p < 0.01$. Although responding was slightly decreased after the initial injection of saline, this was not statistically significant, $t(5) = 1.17$, $p > 0.1$. Subsequent injections of saline did not systematically decrease FR response rate. The second injection of CRF decreased FR response rate compared to the second saline injection, $t(5) = 3.07$, $p < 0.05$ and baseline performance, $t(5) = 2.79$, $p < 0.05$. The rate of FR responding after the second CRF injection was not significantly less than the suppression of responding observed with the first CRF injection, $t(5) = 1.88$, $p > 0.1$. The rate of FR responding after the third CRF injection was significantly higher than the first injection, $t(5) = 3.31$, $p < 0.05$, but was not higher than the level of responding after the second CRF injection. Response rates after the second through fifth injections of CRF were not significantly different from each other. FR response rates after the third, fourth, and fifth CRF injections were not significantly different from corresponding saline injections.

Earned Reinforcers

During baseline and saline control sessions, rats earned all the available reinforcements by meeting the respective schedule requirements. In the right panel of Fig. 1, the total number of reinforcements obtained after acute and chronic administration of CRF for the FI and FR components is depicted. The analysis indicated that there were no significant differences in the effects of CRF across sessions. There were also no significant differences between the FI and FR components after CRF administration. However, there was a significant session $\times$ component interaction, $F(4, 20) = 4.07$, $p < 0.02$, indicating there were differences between the components during the course of chronic CRF administration. Analysis of each component in terms of the number of reinforcers earned after CRF and saline indicated that there were significant differences between the saline (data not shown) and CRF conditions across sessions. In the saline condition, there was essentially no variance as subjects earned all the available reinforcers.

Compared to the first saline injection, the number of reinforcements obtained after CRF administration was significantly decreased in the FR component, $t(5) = 2.76$, $p < 0.05$, and marginally decreased in the FI component, $t(5) = 2.27$, $p = 0.07$. There were no significant differences in earned reinforcements between the FI and FR components after the first CRF injection, $t(5) = 1.56$, $p > 0.1$. With subsequent CRF injections, the number of earned reinforcements remained at approximately 80% in the FR component. In the FI component, rats obtained nearly all the available reinforcements when CRF was administered chronically.

Readministration of CRF After 18-Day Hiatus

After the brief cessation of chronic CRF, response rates in both components were observed to increase slightly during the 18-day hiatus; a second baseline response rate was redetermined from the last five nondrugged sessions of the 18-day interim period. The mean baseline rate in the FI component was observed to increase from 0.54 (SEM $\pm$ 0.06) to 0.61 ($\pm$ 0.06) responses per second. In the FR component, the increase was from 1.65 ($\pm$ 0.18) to 1.82 ($\pm$ 0.33) responses per second. These increases in response rates were not significantly different from initial baseline performance. Likewise, when rats were readministered saline the rate of responding in both components was higher than previous saline determinations, especially in the FI component (see right portion of the

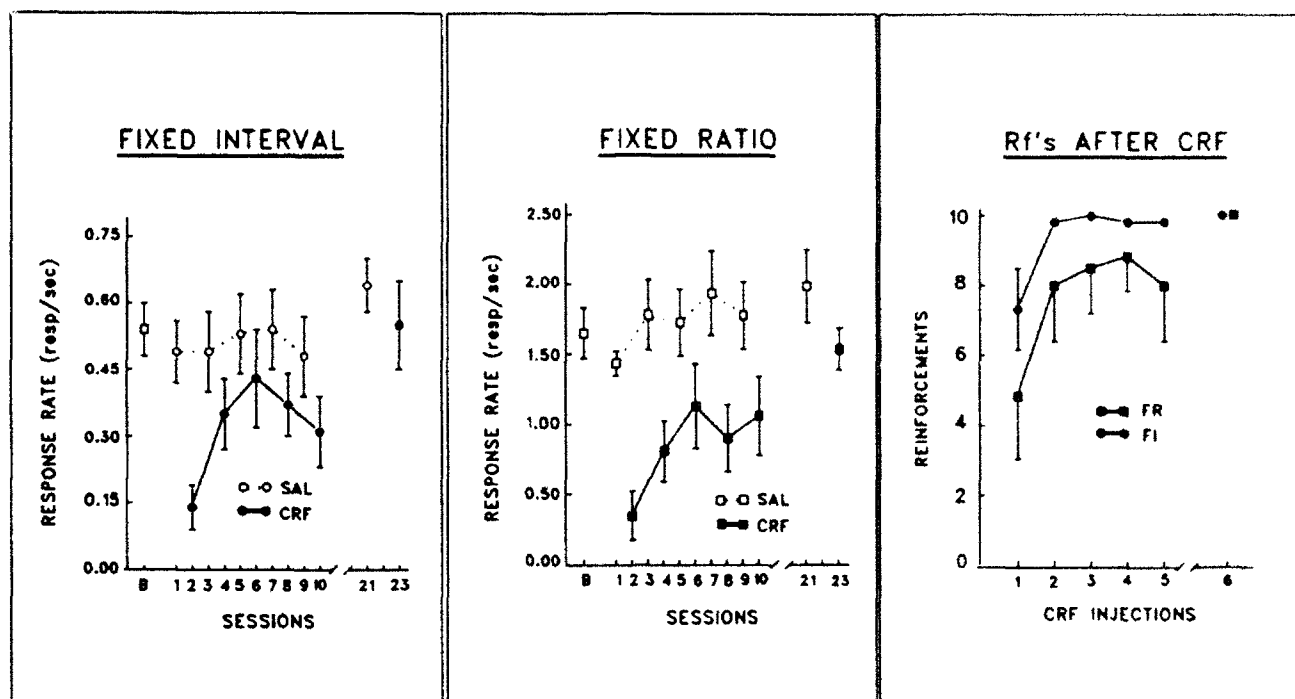


FIG. 1. Left: Effects of acute and chronic corticotropin-releasing factor (CRF) on responding during a fixed-interval 60-s schedule. Data points show the mean ($\pm$ SEM). Middle: Effects of CRF on a fixed-ratio 20 schedule. Right: Mean ($\pm$ SEM) number of reinforcements (Rf's) earned after CRF was injected. A single session consisted of 10 components of each schedule component, which alternated. CRF or saline were administered 60 min prior to the session. CRF doses were determined individually for each animal and were either 1.0, 3.0, or 10.0 μ g CRF based upon the dose that occasioned at least a 50% reduction in the rate of responding in both components. Between Sessions 10 and 21, a period of 18 days, rats performed on the multiple schedule but were not injected.

panel in Fig. 1). This increased rate of responding after saline administration was not significantly different from any previous saline determination.

When CRF was again administered during Session 23, the rate of responding in the FI component was slightly, but not significantly, decreased relative to the reestablished saline or baseline control levels. The rate of responding in the FI component after the sixth CRF injection (Session 23) was significantly higher than the level of responding during the fifth CRF (Session 10), $t(4) = 4.57$, $p < 0.01$. The increase in the rate of responding after CRF in the FR component observed during the 23rd session was not significantly different than after the 5th CRF injection. In both FI and FR components, rats earned all the available reinforcements after the sixth injection of CRF. The number of earned reinforcements in the FI and FR components was not significantly different from the number obtained after the fifth CRF injection.

DISCUSSION

Acute administration of CRF substantially decreased the rate of responding in both components of the multiple schedule. This nonselective suppression of schedule-controlled responding is similar to the effects of CRF observed in several species utilizing a variety of schedule parameters (2-5,7, 14,15,23). At doses that produced greater than 50% decrease in responding, acute administration of CRF decreased the number of reinforcements in both components with a slightly greater loss of reinforcements in the FR component. As would be expected, the reduction in reinforcements was more pro-

nounced in the FR than in the FI schedule because the FR schedule is more affected by an overall decrease in the reinforcement rate. In the FI time-based schedule, the rat must make only a single response after a 1-min period to meet the schedule requirement and obtain the food reinforcer.

Repeated administration of CRF resulted in an attenuation of the rate-decreasing effects and an increase in the number of reinforcements earned. Despite a continued reduction in the rate of responding to approximately 75% of control rates, rats earned the maximum number of reinforcers in the FI component after the second CRF injection. In the FR component, the attenuation of CRF-induced response rate suppression was more gradual and performance after the third CRF injection remained at approximately 60% of control rates. This continued suppression of response rate in the FR component was associated with a 20% loss in earned reinforcers. Overall, the development of tolerance to the rate-decreasing effects of CRF enabled rats to earn more reinforcers in both components of the multiple schedule, data that would indicate that reinforcement loss influenced tolerance to CRF in the rat. Studies have shown that it is necessary for loss of reinforcement to occur for tolerance to develop in schedule-controlled behavior (26).

The pattern of results indicates that tolerance of CRF is not rate dependent, that is, both components of the multiple schedule showed a similar pattern despite rather large differences in the baseline response rates in the FI and FR components, respectively. However, evidence suggesting that the baseline rate of responding did influence tolerance to CRF was observed when the chronic CRF regimen was temporarily

suspended. During the 18-day period, response rates increased substantially and appeared to contribute to greater attenuation of CRF's effects on behavior. The net effect of the response rate increases, although relatively small in the FR component compared to the FI component, was to increase rats' ability to earn all the reinforcers after subsequent injections of CRF. These effects are similar to the persistent rate increases observed after the development of amphetamine tolerance (13).

Previous studies from our laboratory (2) and from Britton and Koob (5) have shown CRF-induced suppression of responding occurs without necessarily decreasing reinforcement rate. In the present study, a similar finding was also shown in that response rates after the second CRF injection in the FI component continued to be suppressed but rats earned all the available reinforcers. In situations where response rate and rate of reinforcement are both decreased, the effects of CRF on responding may be due to an anorexigenic effect of CRF (14,15). However, it would appear that CRF-induced suppression of responding is not completely explicable in terms of anorexigenic mechanisms because responding in the FI component continued to be suppressed but not the number of reinforcements earned. In support of this, Krahn et al. has shown that the acute anorexigenic effects of CRF in the rat are decreased with repeated administration (18).

Tolerance to the rate-decreasing effects of CRF occurred despite fairly large individual differences between rats in the dose of CRF required to occasion more than a 50% reduction in the rate of responding. These differences in rats occurred irrespective of differences in their baseline rates of responding. Although we did not measure the plasma corticosterone response to gauge the efficacy of CRF using another dependent measure, we believe these individual differences in susceptibility to CRF dose would appear to reflect some unique characteristic in each animal in response to CRF rather than a pharmacokinetic variable owing to cannula placement, etc.

The observed tolerance to the rate-decreasing effects of CRF is consistent with two previous studies in which the suppression of schedule-controlled responding in pigeons was attenuated with repeated dosing (3,4). In both rats and pigeons, tolerance to the rate-decreasing effects of CRF on a multiple FI/FR schedule occurred within three to four injections of CRF. These effects appeared to be fairly nonspecific because the decreased efficacy was generalized to both components of the multiple schedule.

Although rats and pigeons are similar in terms of the development of CRF tolerance over three to four injections, there are some key differences between them in terms of CRF tolerance. Tolerance to CRF in the pigeon was complete in that response rates by the fourth day of chronic administration were at baseline control levels. In the rat, the rate of responding when given several CRF injections was still below baseline performance. These differences between the rat and pigeon may result from a variety of reasons. For example, pigeons in general require a higher dose of CRF to reliably disrupt performance on a multiple schedule. In addition, the spacing of injections—alternate days for the rat and consecutive days in the pigeon—may influence the development of tolerance. Previous research has demonstrated that the spacing of CRF injections influences the level of observed tolerance in both species. Dose-response determinations with CRF in which rats or pigeons received one or two injections of CRF during the week indicated no change in the dose-response curve over the course of several months (2,4). Clearly, additional study is needed using different dosing and spacing parameters to deter-

mine how these influence tolerance to CRF's effects on schedule-controlled behavior.

Still another difference in pigeons and rats in terms of the development of tolerance to CRF is the effect of suspending the chronic treatment for a short period and then readministering CRF. In the rat, there was no loss of tolerance to CRF-induced suppression of responding as was observed in the pigeon (3,4). On the contrary, temporarily suspending CRF administration appeared to strengthen tolerance to CRF. This further increase in tolerance to CRF would appear to be explained by the increase in response rates after the chronic CRF was terminated. Because body weights of rats were kept constant throughout the course of the entire experiment, the mechanism underlying this behavioral effect is unclear. In contrast with the rat, suspension of CRF administration in pigeons had an altogether opposite effect. After a similar 2-week hiatus, the acute effects of CRF were recovered in the pigeon (3,4). In addition, baseline rates of responding were unchanged. It would thus appear as if there are distinct species differences in terms of the durability of tolerance to the rate-decreasing effects of CRF.

Tolerance to the rate-decreasing effects of drugs on multiple schedules has been well studied. In general, the development of tolerance is considered "contingent tolerance" if no decrease in drug efficacy occurs when repeated administration of the drug is given away from the behavioral test situation, that is, tolerance only occurs if animals "experience" the drug in the test situation [see (16) for a review]. As this was not tested in the present experiment with rats, it is uncertain whether the animal must experience CRF in the test context for tolerance to CRF to occur. In the pigeon, tolerance to the rate-decreasing effects on an FR 30 schedule has been shown to be noncontingent in that animals given daily CRF after the session developed tolerance to the rate-decreasing effects of CRF (3). While this would suggest that noncontingent tolerance to the rate-decreasing effects of CRF might also apply to the rat, the apparent species differences between rats and pigeons necessitate caution in generalizing from one species to another.

The decrease in the magnitude of CRF effects on schedule-controlled responding may involve behavioral changes or physiological alterations in CRF receptors or downstream neurotransmitter systems. For example, Cunningham et al. (10) demonstrated that delivery of CRF into the ventricles of rats using an osmotic minipump produced an increase in corticosterone and norepinephrine. After the initial increases, however, continuous delivery of CRF over several days resulted in norepinephrine and corticosterone returning to basal levels. These physiological changes, as well as the diminution of CRF's effects on schedule-controlled responding and anorectic actions, may result from downregulation of CRF receptors as shown by Abreu et al. (1). Further research is clearly needed to fully characterize the effects of repeated CRF administration to understand how various behavioral and physiological changes might contribute to the development of tolerance to CRF on schedule-controlled behavior. Elucidation of these changes may hold some promise for understanding the underlying processes involved in pathologic conditions in which dysregulation of endogenous CRF release has been implicated [reviewed in (22)].

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according to the principles set forth in the Guide for the Care and Use of Laboratory Animals, Institute of Laboratory Animal Resources, National Research Council, DHHS Publication (NIH) 86-23-1985.

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