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Evidence of a Central Mechanism Mediating Tolerance to the Discriminative Stimulus Properties of Cocaine

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WOOD, D M , K C RETZ AND M W EMMETT-OGLESBY *Evidence of a central mechanism mediating tolerance to the discriminative stimulus properties of cocaine* PHARMACOL BIOCHEM BEHAV **28**(3) 401–406, 1987 —Rats were trained to detect intraperitoneal (IP) administration of cocaine, 10 0 mg/kg, using a two-lever choice discrimination procedure Following training, cocaine was generalized to the cocaine training stimulus in a dose-dependent manner Subsequently, bilateral cannulae were implanted in the lateral ventricles in ten animals, and intracerebroventricular (ICV) administration of cocaine was also generalized to the IP training dose in a dose-dependent manner with maximum generalization occurring with 80 μg cocaine After baseline testing, training was halted and cocaine, 20 mg/kg/8-hr, was injected chronically in all rats for 6 days, and then the dose-effect curve for generalization of cocaine was redetermined Chronic administration of cocaine significantly shifted the dose-effect curve three-fold to the right for both IP and ICV routes of administration These data suggest that the stimulus properties of cocaine administered centrally are generalized in rats trained by peripheral administration and supports the hypothesis of central mediation of the cocaine stimulus Also, the comparable shift of the cocaine dose-effect curve following chronic cocaine administration suggests that a central pharmacodynamic mechanism mediates tolerance to the discriminative stimulus properties of cocaine

Cocaine Discriminative stimulus Tolerance Rats

COCAINE can serve as a discriminative stimulus in rats [2, 5, 9, 13, 15–17, 28–30]. Using operant conditioning procedures that differentially reinforce selected responses, subjects can be trained to emit one response in the presence of cocaine and to emit an alternate response in the absence of cocaine Following discrimination training, other drugs can be tested for their ability to elicit cocaine-lever responding In such tests, dopamine-receptor agonists such as apomorphine, piribedil, bromocryptine and amantadine, substitute for the cocaine stimulus [5,17], conversely, dopamine-receptor antagonists such as haloperidol and sulpiride, block the cocaine stimulus [2, 5, 13, 17]. These data have been interpreted as evidence that the cocaine stimulus is mediated by dopamine receptors, presumably in dopamine-rich brain areas [17].

Recently, Nielsen and Scheel-Kruger [19] reported that *d*-amphetamine administered directly into the nucleus accumbens generalizes to the stimulus properties of *d*-amphetamine trained by intraperitoneal (IP) injection, demonstrating that the discriminative stimulus properties of amphetamine are centrally mediated Because of the similarities between the stimulus properties of amphetamine and cocaine [6, 9, 10, 12, 28], it is likely that the discriminative stimulus properties of cocaine are also centrally mediated The present experiment tested this hypothesis by training rats to detect the stimulus properties of cocaine administered peripherally and then testing for generalization of this stimulus to cocaine injected intracerebroventricularly

The detection of the stimulus properties of drugs has been proposed as an animal model of human subjective drug effects [1,25] Therefore, studies of tolerance to the stimulus properties of drugs provide a potentially useful procedure for understanding tolerance to their subjective effects. In previous experiments, using rats trained to discriminate 10 0 mg/kg cocaine, when cocaine, 20.0 mg/kg/8-hr, was administered for 6 days, the dose-effect curve for the detection of cocaine significantly shifted to the right. Thus, tolerance developed to the discriminative stimulus properties of the drug [17, 28, 29]; in addition, cross-tolerance was conferred to amphetamine-type drugs [10, 28, 29] In the present experiment, rats were trained to detect cocaine, 10.0 mg/kg, administered IP, and tests were conducted to determine

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whether cocaine given ICV would generalize to the training stimulus Subsequently, subjects were given high-dose cocaine treatments by peripheral administration for 6 days, and dose-effect data for peripherally and centrally administered drug were re-determined.

METHOD

Subjects

Eighteen male Long-Evans hooded rats (Charles River Breeding Laboratories, Willmington, MA) were housed individually in a large room of constant temperature (21±1°C). Body weights were maintained at 320±10 g by limiting daily access to food Water was available ad lib.

Apparatus

Discrimination training was conducted in standard operant chambers (Coulbourn Instruments, Lehigh Valley, PA) Each chamber was housed in a light and sound attenuating box that was fan ventilated. On one wall of the chamber a house light was mounted centrally above a food cup, which was located between two response levers Food reward (45 mg pellets, BioServ, Frenchtown, NJ) was delivered by a pellet dispenser Recording of lever responses and scheduling of reinforcement contingencies were performed through TRS-80 Model-III-microcomputers and printers through LVB interfaces (Med Associates, East Fairfield, VT) using a program developed in this laboratory [26]

Discrimination Training

Using food as a reinforcer, subjects were trained to press a lever, and their behavior was shaped progressively until 10 lever-press responses (FR10) were required to obtain reinforcement Subjects were then trained to press one of the levers following cocaine injection and the other lever following saline injection. For this training, saline or cocaine, 10.0 mg/kg, was injected IP, 15 min prior to each 10 min session Following cocaine injection, only FR10 responses on one of the levers (the cocaine lever) were reinforced, responses on the saline lever were recorded but not reinforced. Similarly, following injection of saline, only FR10 responses on the saline lever were reinforced, and responses on the cocaine lever were recorded but not reinforced There were an equal number of cocaine (C) and saline (S) training sessions, which were presented in the following repeating sequence CSSCSCCSCSSSCCSSCSCC This sequence was selected such that no condition occurred more than 3 training sessions in succession

Only responses emitted prior to obtaining the first reinforcement were used to determine which lever was selected, and the first lever on which 10 responses occurred was considered the selected lever The criterion for learning the discrimination was defined as 10 successive sessions of correct lever selection with ten responses on the lever appropriate to the drug condition (cocaine or saline) with fewer than 10 responses on the incorrect lever. Once this criterion had been achieved, tests were conducted whenever the correct lever was selected for four consecutive sessions.

Generalization Testing

The testing procedure was identical to the training procedure, except that 10 responses on either lever produced a food reinforcer, and sessions were conducted only until one

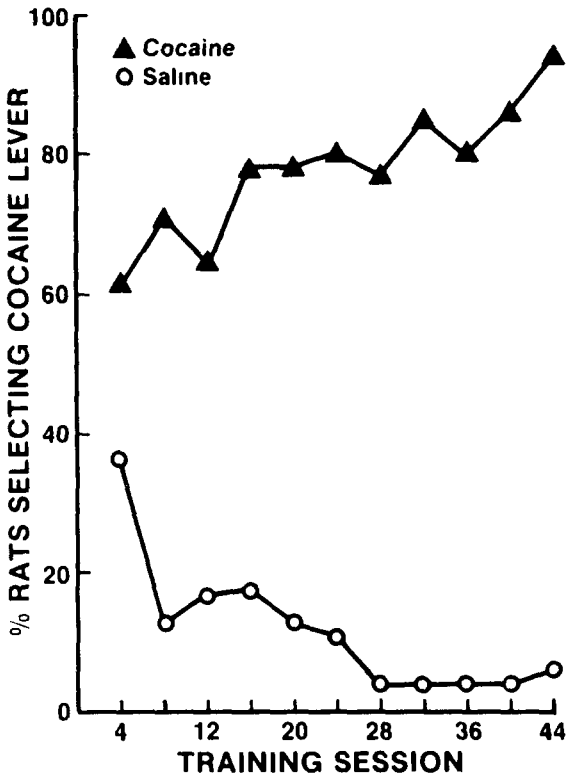

FIG 1 Training data for the acquisition of cocaine as a discriminative stimulus Abscissa, sessions of training on cocaine (10 0 mg/kg) and on 0 9% saline (1 ml/kg) Ordinate, percentage of subjects completing 10 responses on the cocaine lever before receiving reinforcement Each point represents the mean of four administrations of cocaine (▲) or saline (O)

reinforcer was obtained or 10 min had elapsed For generalization testing, cocaine (2.5, 5.0, 10.0, or 20.0 mg/kg) was injected IP), 15 min pretest, and the lever on which 10 responses were first emitted was recorded as the selected lever

Chronic Administration of Cocaine

Following determination of the dose-effect curves for cocaine, repeated administration of cocaine, 20.0 mg/kg/8-hr, was performed for 6 days in a group of eight rats. Subsequently, during days 7, 8, and 9 of chronic treatment the dose-effect curve for cocaine was redetermined 8 hours after a previous dose of 20.0 mg/kg cocaine using the same procedure as above. Doses of cocaine were tested in an irregular order. Immediately following these tests, rats were injected with a supplemental dose of cocaine to maintain a constant level of chronic drug administration. For example, if the chronic dose was 20 0 mg/kg and the test dose was 10.0 mg/kg, rats would receive a supplemental injection of 10.0 mg/kg cocaine

Cannulation Procedure

The 10 rats not used in the above experiment were cannulated using a student stereotaxic instrument (Model 950, David Kopf Instruments, Tujunga, CA) in the lateral ventricles. Bilateral guide cannulae assemblies were made from No. 26 gauge stainless steel hypodermic tubing and 10 mm

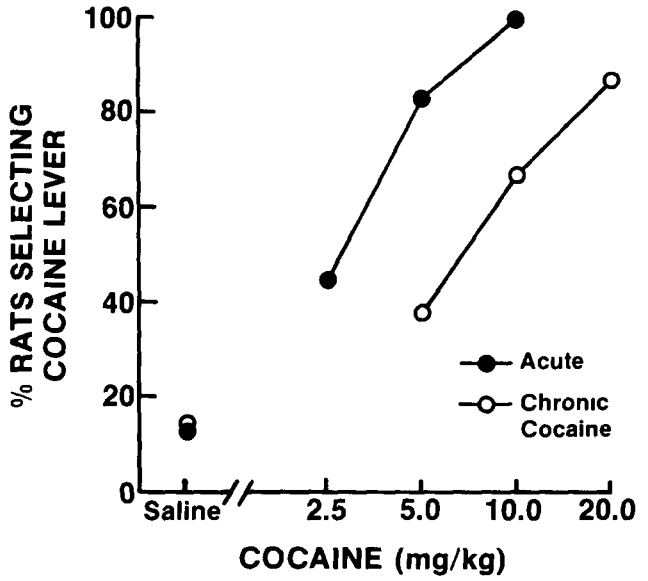

FIG 2 Dose-effect data for the detection of cocaine before (●) and after (○) 6 days of chronic cocaine, 20 mg/kg/8 hr During chronic administration, dose-effect data were redetermined on days 7, 8, and 9 Eight rats tested at all points

teflon molds according to the procedures of Czech and Stein [8]. Cannulae were implanted using coordinates from Paxinos and Watson [22]. Untrained animals were implanted with the bilateral cannulae in the lateral ventricles, and the results verified histologically for accuracy of the coordinates before any implants were made in trained rats From bregma, coordinates used for positioning guide cannulae above the lateral ventricles were A=−0.8, L=1.5, V=3.0

Intraventricular Microinjection Generalization Testing

Microinjections were made using No. 33 gauge cannulae which were inserted inside the guide cannulae. The injection cannulae were attached to No 22 gauge polyethylene tubing using acrylic glue The injection cannulae was cut 1 mm longer than the guide cannulae, to prevent backflow of drug into the guide cannulae. Injections were delivered with a Hamilton syringe over a 30 second period using a volume of 5 μl per side Cocaine (10.0, 20.0, 40.0, 80 0, 160 0 μg) was dissolved in artificial CSF prepared by this laboratory, and cocaine generalization testing was performed similar to the procedure described above Each intracerebroventricular test session was conducted after four training sessions

Preliminary data on the dose and time parameters for the generalization of ICV-administered cocaine for the cocaine stimulus were initially tested. Dose-effect data were determined for 10, 20, 40, and 80 μg doses of cocaine and rats were tested 2, 5, 10, and 20 minutes post injection These doses were selected based on amphetamine doses used in the Nielsen study [18]. The time course for maximum generalization for cocaine occured 5 minutes post ICV injection Based on these results, animals were placed in the operant chambers 5 min after the ICV injection. After complete dose-effect testing and restabilization on the peripheral training stimulus, tolerance was induced by chronic administration of cocaine 20 mg/kg/8-hr for 6 days, and the dose-effect curve for intracerebroventricular administration of cocaine was redetermined. Following testing, rats were sacrificed,

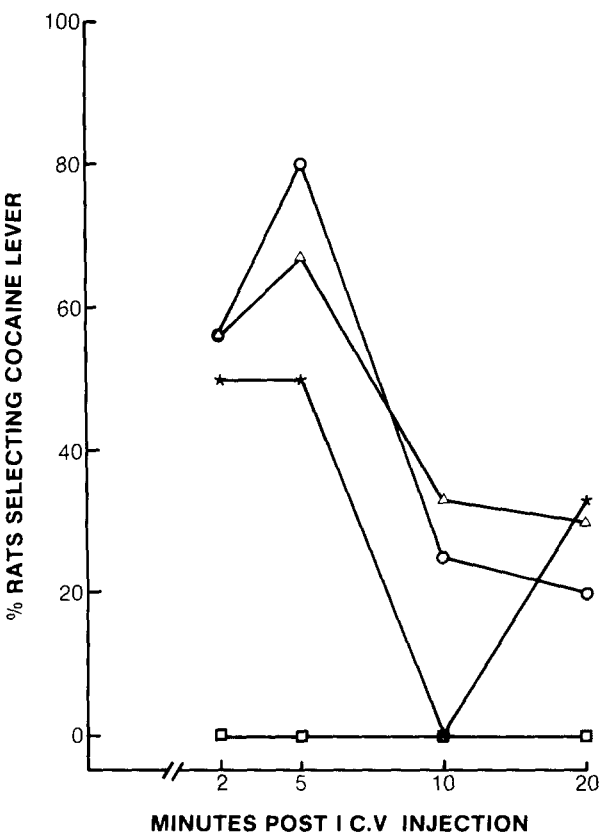

FIG 3 Time course for generalization to the cocaine stimulus for ICV administration of cocaine trained by peripheral administration Abscissa, minutes post ICV injection Ordinate, percentage of subjects completing 10 responses on the cocaine lever Dose-effect data were determined for 80 μg (○), 40 μg (△), 20 μg (*), and 10 μg (□) injections of cocaine Eight rats tested at all points

and cannula placement was verified histologically. One animal died during the experiment and one cannula was implanted superior to the ventricles. Data were tabulated for the 8 subjects which had accurate cannulae placements.

Data Analysis

Data are presented in terms of percentage of subjects selecting the cocaine lever. As shown by Colpaert [4], FR10 discrimination responding produces bimodal data which should be presented and analyzed as quantal data. Tolerance to the discriminative stimulus properties of cocaine was defined as a shift to the right of the dose-effect curve for the detection of the cocaine stimulus [7] of at least two-fold [28]

RESULTS

Subjects required approximately 56 sessions of training to discriminate 10 0 mg/kg cocaine from saline and meet the criterion of selecting the correct lever on ten consecutive sessions After meeting this criterion, animals maintained a high degree of stimulus control with usually no more than 10% of rats selecting the incorrect lever on any given training session (Fig 1).

Prior to chronic administration, cocaine was generalized to the cocaine stimulus in a dose-dependent manner with an approximate ED50 of 3 5 mg/kg (Fig. 2) Following chronic

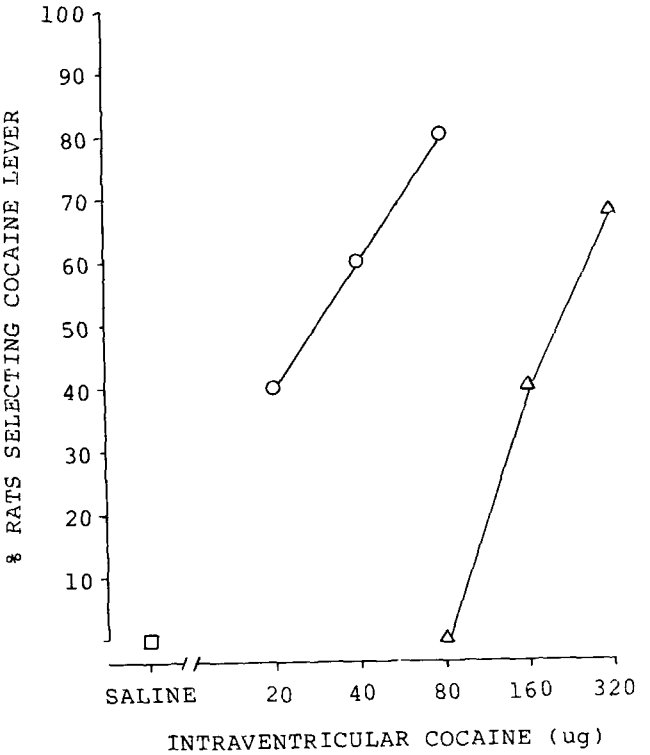

FIG 4 Dose-effect data for the detection of cocaine administered ICV while trained by peripheral injection of 10 mg/kg cocaine Testing occurred 5 min post ICV injection Data were determined (○) before and after (△) chronic administration of 20 mg/kg/8 hr cocaine for 6 days Eight rats tested at all points with at least 5 making a lever selection

administration of cocaine, 20.0 mg/kg/8-hr, for 6–10 days, a two-fold shift to the right of the dose-effect curve was found. Maximum generalization of cocaine occurred with 20 0 mg/kg, with the ED50 shifting to approximately 8 0 mg/kg (Fig 2)

Maximum generalization to the cocaine stimulus occurred 5 minutes after ICV injection (Fig 3) Cocaine lever selection progressively decreased over time beginning approximately 10 minutes post injection Rats given cocaine intracerebroventricularly and tested 5 minutes post injection selected the cocaine lever in a dose-dependent manner with maximum generalization (80%) occurring after 40 μg cocaine injected on each side (total dose=80 μg), with an approximate ED50 of 32 μg (Fig. 4). Doses higher than 80 μg cocaine resulted in no lever selection during the 10 minute test session (Fig 4) Following IP administration of cocaine, 20.0 mg/kg/8-hr, for 6 days, a four-fold shift to the right of the ICV dose-effect curve was produced Maximum generalization of cocaine occurred with 160 μg cocaine per side or 320 μg cocaine total, with the ED50 shifting to 100 per μg per side or 200 μg total (Fig. 4). Doses higher than 320 μg cocaine resulted in no lever selection

DISCUSSION

Cocaine administered intracerebroventricularly (ICV) was generalized to the discriminative stimulus properties of cocaine trained by peripheral administration. However, when measured at the corresponding peak times, cocaine administered ICV was approximately 40-times more potent than by IP administration The most reasonable account for this observation is that cocaine administered ICV is limited only by local diffusion for interaction with receptors in various brain areas, whereas, with IP administration of cocaine, the amount of drug reaching brain receptors is dependent on the absorption, distribution, and metabolism of cocaine peripherally

Data from the present experiment suggest that the discriminative stimulus properties of cocaine are centrally mediated. These results are comparable to those in which *d*-amphetamine administered in the lateral ventricles [23] and nucleus accumbens [19] generalized to the amphetamine stimulus in rats trained by peripheral administration With respect to cocaine, two previous studies [11,23] have failed to demonstrate that cocaine injected ICV will generalize to the cocaine stimulus. The reason for these previous failures is unclear However, as demonstrated by the time course for generalization to the cocaine stimulus for ICV administration (Fig 3), it may be possible that the rapid offset of the cocaine stimulus is responsible for the lack of generalization in the previous experiments since in our experiments we tested the rats 5 min post ICV injections compared to 15 min in the other studies

Tolerance developed to the discriminative stimulus properties of cocaine after IP administration of cocaine, 20 0 mg/kg/8-hr, for 6 days These data agree with previous reports of tolerance to the cocaine stimulus following chronic administration of cocaine [17, 28–30] Furthermore, in subjects trained to detect a peripheral injection of 10 0 mg/kg cocaine and made tolerant by peripheral injection, tolerance also occurred to cocaine administered ICV. Moreover, the tolerance that was produced was of a comparable magnitude for both ventricular and peripheral administration of cocaine. Thus, these data are incompatible with a pharmacokinetic explanation of tolerance, which is consistent with the observation that chronic administration of cocaine produces negligible effects on its rate of metabolism, elimination, or distribution in the brain or plasma [18]. The data are, however, compatible with the hypothesis that tolerance to the discriminative stimulus properties of cocaine is a centrally mediated pharmacodynamic phenomenon As additional support for this hypothesis, in a recent experiment to investigate the role of the dopaminergic system in mediating tolerance to cocaine, we observed that chronic administration of the dopamine receptor agonist, apomorphine, shifted the dose-effect curve for generalization to the cocaine stimulus two-fold to the right [30] Thus, tolerance to the discriminative stimulus properties of cocaine may be mediated by a mechanism involving dopamine receptors

There has been controversy concerning whether tolerance develops to cocaine in the discrimination procedure Colpaert *et al* [2] have suggested that tolerance does not develop to the cocaine stimulus because the discriminability of cocaine did not fade after several months of training. However, training was conducted five days a week with only a maximum exposure of 2–3 cocaine doses per week According to the tolerance theory described by Kalant *et al.* [14], tolerance occurs when a drug is administered repeatedly and in high doses. Thus, one explanation for failure of the Colpaert *et al* study to observe tolerance may be related to a lack of exposure to sufficiently high doses or frequent doses of cocaine Colpaert *et al* [3] have also suggested that "tolerance" in the drug discrimination

paradigm is an artifact This conclusion was based on the observation that tolerance failed to develop to fentanyl when it was administered in high doses while training at lower doses was continued. Colpaert *et al* suggested that terminating training and injecting high doses of the training drug may teach subjects to attend to the higher magnitude of these doses However, this learning hypothesis has not been supported by data from our laboratory as well as data from Schechter's laboratory In those studies [24, 28, 29], when chronic drug administration was terminated, sensitivity to the training stimulus spontaneously recovered This result is consistent with a tolerance hypothesis, but it is inconsistent with a learning hypothesis In addition, Overton [21] has pointed out that chronic administration of high doses in a procedure such as Colpaert *et al* [3] used could result in a stimulus that diminishes progressively, and training during the development of this tolerance teaches subjects to detect lower magnitudes of the stimulus. Thus, tolerance may have occurred in the Colpaert *et al* study, but it would have gone undetected In support of this hypothesis, several studies have shown that subjects can learn to detect progressively lower intensities of drug stimuli using "fading" procedures [20,27]

There have been no studies investigating whether the discriminative stimulus properties of cocaine are mediated at site-specific brain areas. However, since the discriminative stimulus properties of amphetamine and cocaine are similar, it may be possible that the discriminative stimulus properties of cocaine and tolerance to these properties are mediated in specific brain sites Recently, Nielsen and Scheel-Kruger [19] reported that amphetamine administered in the nucleus accumbens generalized to the discriminative stimulus properties of amphetamine trained by peripheral (IP) administration. Therefore, we anticipate that direct administration of cocaine may also be active in the nucleus accumbens

ACKNOWLEDGEMENTS

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