Pharmacology Biochemistry & Behavior, Vol. 37, pp. 231–234. © Pergamon Press plc, 1990. Printed in the U.S.A. 0091-3057/90 $3.00 + .00

Direct Dopamine Agonist-Like Activity Conditioned to Cocaine

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Received 29 January 1990

SILVERMAN, P. B. *Direct dopamine agonist-like activity conditioned to cocaine.* PHARMACOL BIOCHEM BEHAV 37(2) 231–234, 1990.—Rats were lesioned unilaterally by infusions of 6-hydroxydopamine aimed at substantia nigra. In subsequent behavioral testing, apomorphine treatment resulted in rotation (circling) directed contralaterally with respect to the lesion and cocaine treatment induced ipsilaterally directed rotation. When 0.05 mg/kg apomorphine and 10 mg/kg cocaine were administered simultaneously, rotation appropriate for apomorphine resulted. After a number of paired administrations, treatment with cocaine alone resulted in apomorphine-like rotation.

Classical conditioning Substantia nigra lesions Apomorphine 6-Hydroxydopamine Rotational behavior

RATS with unilateral 6-hydroxydopamine lesions of the nigrostriatal pathway exhibit a differential response to direct- and indirect-acting dopamine (DA) agonists. Direct-acting agonists such as apomorphine (APO) induce contralateral rotation ostensibly as a result of having greater activity in the lesioned, thus denervation supersensitive, striatum than in the intact striatum. Indirect-acting DA agonists, their DA release/reuptake-blockade activity limited to the intact striatum, induce ipsilateral rotation. This animal model, originally established by Ungerstedt (15,16), has proven popular in investigations of central DA function and development of dopaminergic agents. In addition to resulting in active acute contralateral rotation, treatment with low doses of APO also results in a striking rotation conditioned to the environment in which the APO was administered (14). This conditioned rotation has a number of extraordinary features: it is best demonstrated after small doses of APO, it results from a single APO treatment, it can be demonstrated unambiguously a year after the single treatment, it does not appear to have an analogous oppositely directed counterpart, it is best manifest only after a latency period of at least two weeks (3,14), and its development can be blocked with cycloheximide (11). These features suggest utility in the study of persistent conditioned drug effects which play a significant role in drug abuse phenomena such as tolerance, craving, and relapse (5,9). In the preliminary work presented here, it is demonstrated that lesioned rats which, consistent with expectations, rotate contralaterally in response to APO and ipsilaterally in response to cocaine, can be conditioned to rotate contralaterally in response to cocaine. In this method cocaine administration serves as a discriminative stimulus and a conditioned stimulus which elicits behavior contrary to its pharmacology.

METHOD

Female Sprague-Dawley rats (Harlan Sprague-Dawley) weighing about 160 g were anesthetized with 40 mg/kg sodium pentobarbital, placed in a stereotaxic frame and unilaterally lesioned by infusion of 4 μl 6HDA HBr solution consisting of 2 μg/μl (base weight) 6HDA in saline containing 0.1% ascorbic acid. The solution was administered at 0.33 μl/min via syringe pump, 10 μl syringe, PE tubing and 27-gauge needle. Stereotaxic coordinates, with incisor bar set at zero, were 1.4 L and 4.2 P with respect to bregma, and 8.0 ventral from the skull surface. Animals were individually housed and maintained on a 12:12 light cycle with ad lib food (Purina Lab Chow) and water throughout the experiment. When administered, APO (hydrochloride salt in 0.1% ascorbate) was given subcutaneously at a dose of 0.05 mg/kg in a volume of 1 ml/kg and cocaine (HCl) was given intraperitoneally at 10 mg/kg in the same volume.

Sessions were conducted by placing individual animals in a clear plastic hemispherical bowl approximately 25 cm in diameter. Prior to the conditioning experiment animals were tested with APO alone to ensure that they were appropriately lesioned (4) and with cocaine alone four times to ensure that their response to it was consistent. Animals were first tested with APO, one week after they were lesioned. The session began with a 3-minute acclimation period prior to drug administration. Observation was made and turns counted in the interval from 10 to 15 minutes after APO administration. Animals were subsequently tested with cocaine on four occasions at one-week intervals. In these sessions, which also began with a 3-minute acclimation period, observations of rotation were made in 3-minute bins beginning 0, 15 and 30 minutes after cocaine was administered. About 3 months after the last of these cocaine sessions, the conditioning experiment proper began. In the conditioning sessions APO, after the acclimation period, and cocaine were administered, as nearly as possible, simultaneously (APO first, followed immediately by cocaine). The first three conditioning trials, conducted on days 1, 2, and 3, were followed by a test trial on day 10 in which cocaine alone was given. This was followed by another set of three conditioning trials on days 15, 16 and 17, a second cocaine test on day 31 and a third and final test on day 45. Thus, cocaine was tested before being paired with APO, one week after being paired three times with APO, and two weeks and four weeks after being paired six times with APO. In the first three conditioning trials, rotation was counted in the

TABLE 1

ROTATION INDUCED BY 10 mg/kg COCAINE IN UNILATERALLY LESIONED RATS

Trial	Interval 0	15	30	Σ
1	0 ± 2	8 ± 0	7 ± 2	15 ± 2
2	15 ± 5	16 ± 4	9 ± 4	40 ± 13
3	19 ± 4	21 ± 7	18 ± 4	58 ± 14
4	25 ± 6	24 ± 6	18 ± 4	66 ± 15

Scores are mean ± SEM (rounded to whole numbers) net ipsilateral turns made in 3-minute intervals beginning at the indicated times after injection (n = 4). Trials were one week apart. Total observed turns (Σ) increased significantly with repeated administration [repeated measures analysis of variance, $F(3,9) = 9.12$, $p < 0.01$].

interval from 10 to 15 minutes after the drugs were administered. It became apparent that the time of peak drug effect shifted and was occurring earlier than this, so in the last three conditioning trials the observation interval was the first 10 minutes after the drugs were administered. In all three test sessions, turning was counted in the 3-minute acclimation period and in the first 10 minutes after cocaine administration.

RESULTS

When tested one week after being lesioned with 6HDA, the animals turned rapidly contralaterally in the interval from 10 to 15 minutes after APO administration (mean ± SEM = 68 ± 9, range 43 to 85 contralateral turns/5 min). Cocaine alone resulted in ipsilaterally directed rotation which increased significantly upon repeated administration (Table 1). When both APO and cocaine were administered simultaneously, the result was APO-like (contralateral) rotation in 23 of 24 instances. Upon the first paired administration, one animal rotated primarily ipsilaterally in the interval from 10 to 15 minutes after drug, but turned contralaterally in all subsequent conditioning sessions. Unfortunately, because of the change in observation interval in the conditioning trials, it is not possible to fully compare the behavior during these trials. However, in the sixth, and last, conditioning trial the rats made, on the average, 160 ± 15, range 136 to 204, net contralateral turns in the first 10 minutes after administration of the combined drugs. In the acclimation period of this last conditioning trial they made from 0 to −2 net contralateral turns/3 min, indicating that the behavior was not under stimulus control of the rotation environment, alone.

When tested with cocaine alone after having had cocaine paired with APO three times, two of the four animals exhibited considerable contralateral rotation (Fig. 1, top). Notice that two of the animals briefly showed rapid rotation in response to the rotation bowl alone. When tested two weeks after the sixth conditioning trial, all four animals rotated contralaterally in response to cocaine (mean ± SEM = 78 ± 30, range 10 to 147 net contralateral turns/10 min; Fig. 1, bottom). This difference in direction of rotation in response to cocaine before and after its being paired with APO is statistically significant [Cochran Q-test (8), $Q = 16$, $p < 0.01$]. Four weeks after the last conditioning session, rotation in response to cocaine had reverted to ipsilateral in all four animals (mean 52 ± 30, range 3 to 130 net ipsilateral turns/10 min).

DISCUSSION

Aspects of the environment in which drugs are repeatedly

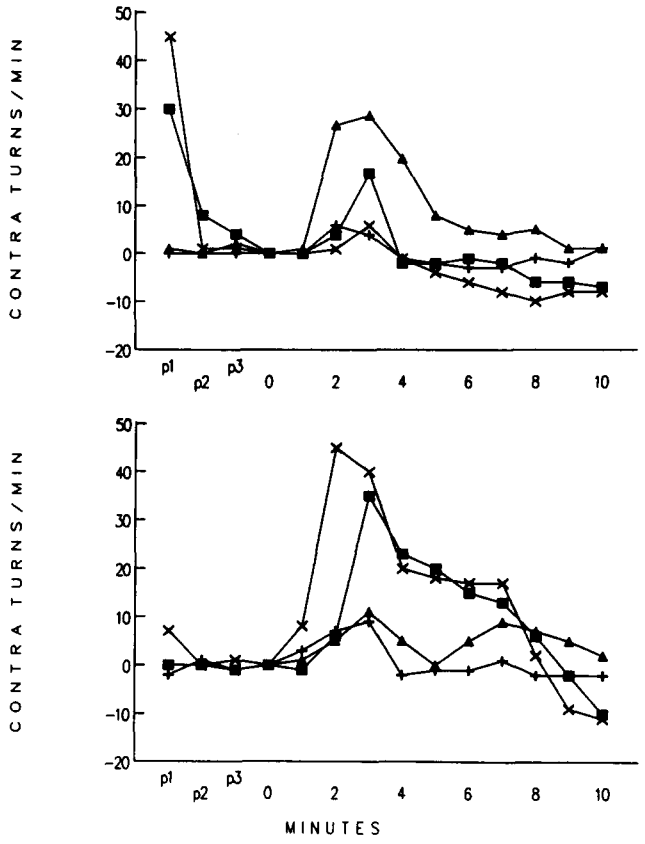

FIG. 1. Rotation induced by 10 mg/kg cocaine in lesioned rats after cocaine had been paired with APO. P1–P3 represent the 3-minute acclimation period prior to cocaine administration. Top: Cocaine test on day 10, one week after the last of three consecutive daily simultaneous administrations of 10 mg/kg cocaine and 0.05 mg/kg APO. Bottom: Cocaine test on day 31, two weeks after the last of six simultaneous administrations of cocaine and APO.

administered can become conditioned stimuli, themselves eliciting drug-like, or, more frequently reported, drug-opposite responses (9). Along these lines, two presumably related conditioning phenomena can be demonstrated following repeated stimulant administration in a given environment. The first of these, sensitization, is demonstrated when successive administrations of the stimulant result in a largely context-dependent, progressively exaggerated response to drug administration (6). An example of this sensitization phenomenon would be the results shown here with repeated cocaine administration (Table 1). A second effect of repeated stimulant administration that may be termed a placebo effect is seen when the environment alone elicits some component of the usual drug response, i.e., the environment in which the stimulant has been repeatedly given will serve as a conditioned stimulus (CS) and will elicit an attenuated stimulant response (1,7). The primary difference in demonstrating sensitization or a placebo effect then is whether or not drug is administered in the test session. By definition, sensitization is measured acutely following drug treatment and a placebo effect is measured with no drug on board.

The novel aspect of the work described here is that cocaine, rather than serving as an unconditioned stimulus (UCS), is used as a CS which comes to elicit the response normally resulting from APO. Advantage is taken of an animal preparation in which a small dose of APO has extraordinary capacity to result in a placebo

effect (to serve as a UCS). A single small dose of APO is all that is required in this preparation to result in a persistent unambiguous drug-like response conditioned to the environment (14). Indeed, the peak rate of conditioned rotation, tested in the absence of APO, usually exceeds the peak rate observed acutely after APO administration (11). Repeated administration of small doses of APO, whether in consecutive daily sessions or in sessions weeks apart, results in an even more marked effect with undrugged animals often completing over 100 circles in a 3-minute test. Larger doses of APO (whether single or repeated) are actually less effective in conditioning rotation to the environment than are small doses (14), perhaps because they induce a compelling interoceptive discriminative stimulus which is conspicuously absent in subsequent test sessions.

Unlike APO, we have been unable to condition to any significant degree the ipsilateral rotation induced by the indirect DA agonists amphetamine (10) or cocaine. Using a variety of testing schedules in which APO effectively conditions contralateral rotation to the test environment, 10 to 20 mg/kg cocaine administered once or repeatedly at various intervals induced acute rotation, but did not result in subsequent conditioned rotation. A typical example: one group of animals (n=4) averaged 3.25 ipsilateral turns in a 3-minute test prior to three consecutive daily administrations of 20 mg/kg cocaine, and 4.75 ipsilateral turns two weeks afterward. The animals thus continued to exhibit only the slight ipsilateral bias characteristic of drug naive 6HDA-lesioned rats (16). Under the same treatment conditions using APO rather than cocaine, explosively rapid contralateral rotation results when the undrugged animal merely is returned to the drug associated environment (10, 11, 14). It should be obvious that this is not to say that rotation *cannot* be conditioned to the indirect agonists under any circumstances. Others have shown, for example, that in intact animals stereotyped behaviors could be elicited by exposure to the distinctive environment in which cocaine (1) or amphetamine (7) had been administered on the immediately preceding 10 consecutive days. Perhaps there are conditions under which repeated administration of cocaine or amphetamine could result in conditioned rotation (e.g., more drug treatments). Nonetheless, under a variety of conditions in which APO is very effective in conditioning rotation, the indirect agonists are not. While treatment with cocaine alone clearly results in sensitization in this preparation (Table 1), it is not a particularly effective UCS and does not result in a clear placebo effect. There is one report of minimal success using amphetamine as a UCS for conditioned rotation (2), but the magnitude of the conditioned behavior, even after repeated conditioning trials, does not approach that seen at an appropriate interval after a single low-dose APO treatment (3, 11, 14). The indirect agonists are highly effective as discriminative stimuli (13) at doses comparable to those used to induce rotational behavior. Perhaps as was suggested above to be the case with large APO doses, the absence of the drug-induced discriminative stimulus in undrugged test sessions limits demonstration of a placebo effect in the rotational model. One result inconsistent with this thinking is that LSD, like APO, can serve as an effective UCS for contralateral rotation, conditioning rotation in a single trial (12). LSD does this at doses that are readily discriminable. This result supports an intriguing hypothesis alternative to the discriminative stimulus hypothesis, i.e., that there is a fundamental advantage to classical conditioning with direct versus indirect agonists in this preparation, and perhaps in denervated preparations, generally. Under this hypothesis, the idiosyncratic dose-response effect for APO conditioning awaits explanation.

The discriminable effect of cocaine repeatedly associated with APO-induced rotation is shown here to come to serve as the CS for contralateral rotation, replacing the environment as such. The conditioned effect was apparent two weeks after the last conditioning trial, and not four weeks after. It is possible that having had four treatments with cocaine alone prior to the conditioning sessions limited the final degree of conditioning achieved. In this early effort, the number of drug pairings, intervals between sessions and session durations were somewhat arbitrary. It is clear that much more work will be required to characterize fully the conditioning phenomenon.

The neural mechanism through which cocaine brings about a direct DA agonist-like effect is obscure, but little more so than the means through which the environment alone can come to serve as a conditioned stimulus for contralateral rotation. In both instances the issue is of how a behavior ostensibly dependent on direct DA agonist activity can be conditoned to occur not only in the apparent absence of such activity, but in a denervated preparation in which it is difficult to envision alternative mechanisms. In related work we have found that ethanol and pentobarbital, which do not by themselves induce rotation in this preparation, also serve as effective conditioned stimuli inducing APO-like rotation after having been paired with APO (in press). Thus, it seems as if rotation in response to low dose APO treatment is a "blank" conditioned response which readily comes under discriminative control of stimuli associated with it. Consistent with our inability to condition substantial cocaine-induced rotation to the environment in which cocaine has been administered, paired administration of pentobarbital and cocaine has thus far failed to result in cocaine-like rotation in response to pentobarbital treatment. We suspect that conditioned rotation will prove to be a useful method for investigation of stimulus effects of centrally active drugs and drug-drug and drug-environment interactions.

ACKNOWLEDGEMENTS

Supported in part by NIDA grant DA 04423. Thanks to Kem Schultz and Chris Orengo for technical assistance.

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