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BRIEF COMMUNICATION

Alpha-Methyltyrosine Blocks the Expression of Rotation Classically Conditioned With Apomorphine

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SILVERMAN, P. B. *Alpha-methyltyrosine blocks the expression of rotation classically conditioned with apomorphine.* PHARMACOL BIOCHEM BEHAV **39**(4) 1033–1035, 1991.—Rats with unilateral 6-hydroxydopamine lesions of substantia nigra rotate (circle) when placed, undrugged, in the environment in which they have previously been treated with apomorphine. This conditioned rotation, like the unconditioned rotation which acutely follows the administration of apomorphine, is directed away from the side with the lesion, i.e., the rotation is contralateral. Here, rats that had been administered apomorphine weeks earlier were tested, in a crossover design, for the expression of conditioned rotation following treatment with saline and with alpha-methyltyrosine. When administered four hours prior to testing, 100 mg/kg alpha-methyltyrosine significantly antagonized the expression of classically conditioned rotation. In a second group of animals, alpha-methyltyrosine had no effect on the unconditioned rotation induced by 0.05 mg/kg apomorphine.

Rotational behavior Dopamine 6-Hydroxydopamine Substantia nigra Brain lesions
Conditioned drug effects

STIMULI associated with drug administration can come to elicit drug-like or drug-opposite responses (5,7). There seems to be increasing recognition that these classically conditioned effects may be importantly involved in substance abuse concerns such as craving and relapse (9). Here, an animal preparation in which a persistent drug-like effect can be conditioned with remarkable facility is utilized, and the effect of a monoamine depletor on the expression of the conditioned behavior is tested.

Indirect-acting dopamine (DA) agonists induce rotation directed toward the lesioned side in rats with unilateral 6-hydroxydopamine (6HDA) lesions of substantia nigra (17). This circling behavior presumably results from agonist activity (DA release/reuptake blockade) in only the striatum with intact innervation. Direct-acting dopamine agonists induce rotation directed away from the lesioned side in these animals (16). This contralateral rotation presumably reflects development of denervation supersensitivity in the striatum ipsilateral to the lesion, although examination of the time course of the behavioral response indicates that it actually precedes a demonstrable increase in receptor number on the lesioned side (15). In addition to inducing rotation acutely after administration, treatment with the prototypic direct-acting DA agonist, apomorphine (APO), can result, even after a single administration, in rotation which is conditioned to the environment in which the drug was administered (11). The neural mechanisms involved in this conditioning, which can be demonstrated over a year after APO administration, are obscure. How can the environment associated with APO administration come to elicit a response that ostensibly depends on agonist activity at DA receptors in a denervated brain region? We recently showed that the expression, and presumably the development, of one-trial conditioned rotation can be blocked by treating animals with 2 mg/kg cycloheximide minutes after the conditioning trial (14). The work presented here shows the effects of an acute pretreatment with alpha-methyltyrosine on the expression of the already-conditioned behavior.

METHOD

Sprague-Dawley rats weighing 150–200 g at the time of lesioning were used as subjects. They were lesioned unilaterally by microinjection of 6HDA HBr aimed at substantia nigra. Under pentobarbital anesthesia (40 mg/kg) and stereotaxic control, 4 μl of 6HDA solution (2 μg/μl 6HDA calculated as base in 0.1% ascorbate, 0.9% NaCl) was infused via 27-gauge needle, PE tubing and syringe pump at the rate of 0.33 μl/min. The stereotaxic coordinates were 1.4 L and 4.2 P with respect to bregma and 8.0 ventral from the skull surface, with the incisor bar set at 0.0. After being lesioned, rats were individually housed and maintained on a 12:12 light-dark cycle with lab chow and water freely available.

Eighteen rats in two groups were used in this experiment. All had been lesioned for at least 6 months, and had previously been used in experiments during the course of which they had been administered APO (0.05 to 1.0 mg/kg) one or more times. All actively rotated contralaterally in response to APO treatment. None of the animals had been given any drug treatment or other handling in the 2 weeks immediately preceding this experiment. Tests of rotational behavior in group 1 animals (n = 12) were conducted by placing the animals individually in a clear plastic hemispherical bowl. Complete 360-degree turns in either direction were counted by an observer. Two test sessions were conducted with group 1 animals. Each consisted of placing the animals in the rotation bowl for a 3-min period during which rotations in either direction were counted. Ipsilateral turns were rarely observed; the data are presented as net (contralateral minus ipsilateral) turns per min. While conditioned rotation can be observed and remains relatively consistent in widely spaced undrugged test sessions for over a year after drug administration, the behavior can be rapidly extinguished (with spontaneous recovery) by repeated daily undrugged exposure to the environment (10). Therefore, the two sessions were conducted two weeks apart. In the interval between the two tests, the rats remained undisturbed in their home cages. Prior to the first test, six animals were treated (IP) with 100 mg/kg racemic alpha-methyl-p-tyrosine methyl ester (AMT), and the other six animals were treated with saline. Treatments were given four hours before the rotation test. Prior to the second test, animals that had previously been treated with AMT received saline, and those that had previously been treated with saline were given AMT; treatments again were four hours before behavioral testing.

Group 2 animals (n = 6) were used to test the effect of AMT on unconditioned apomorphine-induced rotation. Four hours after saline or 100-mg/kg AMT pretreatment, they were tested for rotation in response to 0.05 mg/kg apomorphine. As was the case with group 1 rats, the 2 tests were conducted in a counterbalanced design with a 2-week interval between. The test environments were opaque, off-white plastic bowls, the same size and shape as those used for group 1 animals. Prior to each test, animals were marked between the scapulae with black ink. The ink spot was tracked by a video camera and video analyzer (Videomex-V, Columbus Instruments, Columbus, OH) with the number of circles completed in each direction printed out at 1-min intervals for the duration of the 40-min tests.

RESULTS

Pretreatment with AMT significantly blocked expression of rotation conditioned to the rotation environment. A minute-by-minute comparison of rotation following saline and AMT treatment is presented in Fig. 1. When tested four hours after saline administration, rats completed a total of 33.0 ± 5.0 (mean ± SEM) contralateral circles when placed in the rotation bowls for a 3-min test period. Four hours after AMT administration, rats completed 9.5 ± 1.7 (mean ± SEM) contralateral circles in a 3-min test period. This difference is highly significant, paired $t(11) = 4.65$, $p<0.001$. The difference between the first and second tests, without regard to drug treatment, was not significant (means of 20.8 ± 4.5 vs. 21.7 ± 5.7, paired $t = 0.09$, n.s.), indicating that there was no extinction or treatment order effect. Every animal, without exception, completed fewer turns in the test after AMT than in the test after saline.

AMT had no effect on the unconditioned rotation induced by a small dose of apomorphine (Fig. 2). Rats completed a session total of 434 ± 64 net contralateral turns after AMT pretreatment and 460 ± 47 net contralateral turns after saline pretreatment, paired $t(5) = 0.75$, n.s.

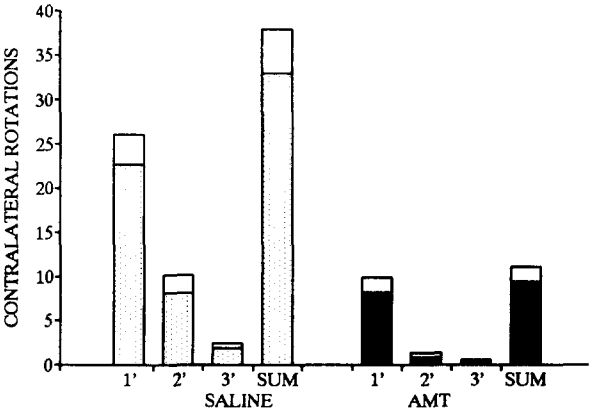

FIG. 1. Effect of AMT on the expression of conditioned rotation. Rats that had previously been treated with APO were tested for expression of rotation conditioned to the rotation environment four hours after treatment with saline or 100 mg/kg AMT. The two trials were two weeks apart with six animals tested first with AMT and six animals tested first with saline. AMT pretreatment significantly blocked conditioned rotation, paired $t(11) = 4.65$, $p<0.001$.

DISCUSSION

The unconditioned effects of stimulant drugs can come to be elicited by stimuli associated with drug administration. Because the unconditioned behavioral effects of apomorphine, amphetamine and cocaine generally can be blocked by pretreatment with dopamine antagonists, it is only logical that the question of whether or not conditioned effects of these drugs could similarly be blocked by dopamine antagonists would be raised. This issue has been addressed in intact animals in several studies. It has been reported that pimozide blocked the conditioned stereotypic effect of apomorphine (6) but not the hyperactivity conditioned by repeated amphetamine (2) or cocaine (3). Pimozide was found to block only incompletely the expression of conditioned amphetamine stereotypy (6). A dose of haloperidol (0.1 mg/kg) that largely blocked the unconditioned stereotypy induced by apomorphine had much less effect on the conditioned stereotypy (19). Poncelet et al. (8) found that, in rats treated for 21 days

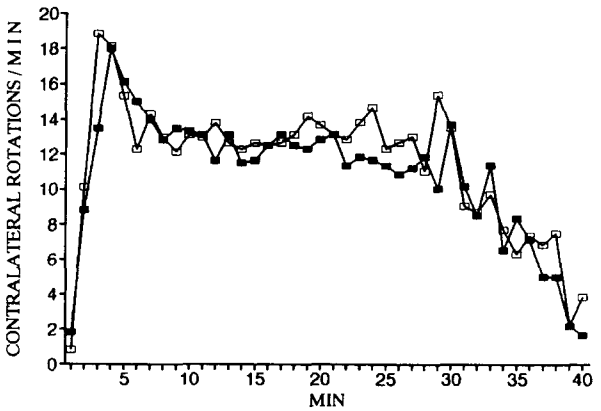

FIG. 2. Effect of AMT on unconditioned rotation. Rats were treated with saline (□) or 100 mg/kg AMT (■) four hours prior to administration of 0.05 mg/kg apomorphine. The two sessions were two weeks apart with three animals tested first with AMT and three tested first with saline. AMT pretreatment had no significant effect on apomorphine-induced rotation, paired $t(5) = 0.74$, n.s.

with amphetamine, the dopamine antagonists haloperidol, sulpiride and pimozide blocked conditioned effects only at doses nearly as high as, or higher than, those that also blocked unconditioned effects. On the other hand, the alpha-noradrenergic blocker, clonidine, as well as lithium, completely blocked the conditioned effects of amphetamine at doses that had no effect on the unconditioned behavior. Thus, while not conclusive, there is at least some evidence that conditioned dopamine agonist-like effects can be expressed even when dopamine receptors are blocked, but that noradrenergic antagonists block expression of the conditioned effects.

Apomorphine conditioned rotation differs in a number of particulars from conditioning with stimulants in intact animals. The conditioned effect can be accomplished in a single treatment, can be demonstrated months after treatment, is more apparent after low rather than high doses, and requires an incubation period between conditioning trial and test trial (11). Nonetheless, it is reasonable to assume until the contrary is established that similar biochemical processes are at work which perhaps are greatly potentiated in the denervated preparation. The question of the requirement of dopaminergic receptor activation for the expression of conditioned behaviors takes on an added dimension in the unilaterally lesioned animals. Here, the unconditioned effect of apomorphine depends upon significant destruction of the dopaminergic input to the ipsilateral striatum. This lack of dopamine input makes at least one means by which stimuli associated with previous apomorphine administration might elicit rotation, i.e., by release of dopamine and its subsequent activity at dopamine receptors, highly unlikely. There is one recent report of testing the effect of dopamine receptor blockade on unconditioned and conditioned apomorphine rotation. Carey (4) found that combined treatment with haloperidol and SCH 23390 effectively blocked apomorphine-induced rotation, but did not affect rotation elicited by the environment in which apomorphine had been administered. The data presented here show that an AMT treatment blocks the expression of conditioned rotation at a dose that has little sedative effect (18), and that has no effect on the unconditioned rotation induced by a small dose of apomorphine. At doses such as the 100 mg/kg used here, AMT does block locomotor stimulation by amphetamine, which is dependent on the release of newly synthesized catecholamines (18). These results suggest that, although dopamine receptor stimulation may play little role in the expression of the conditioned effect, there may nonetheless be catecholamine involvement. A major role for norepinephrine in the expression of conditioned stimulant effects would be consistent with the data of Poncelet et al. (8). That norepinephrine is importantly involved in behavioral effects of drugs and other stressors that persist and even grow over time has been previously noted [see (1) for a review].

An alternative explanation for the ability of AMT to block the expression of conditioned rotation concerns stimulus control of the behavior. Just as the external environment associated with apomorphine administration can come to elicit rotational behavior, so can drugs paired with apomorphine (13,14). Thus stimulus control can be shifted from one stimulus to another, and expression of the conditioned effect is dependent on stimulus conditions being similar in conditioning and test trials. If AMT, even at the modest dose and long pretreatment interval used here, exerts an appreciable stimulus effect, it may be that stimulus conditions in the conditioning trials (absent AMT) and test trial (with AMT present) were sufficiently different as to limit expression of the conditioned behavior.

ACKNOWLEDGEMENTS

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