Pharmacology Biochemistry and Behavior, Vol. 43, pp. 657–660, 1992
Printed in the U.S.A.

0091-3057/92 $5.00 + .00

BRIEF COMMUNICATION

Scopolamine Increases Nonreinforced Behavior in an Intracranial Self-Stimulation Discrimination Paradigm

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Received 14 November 1991

AGARS, K. AND L. KOKKINIDIS. *Scopolamine increases nonreinforced behavior in an intracranial self-stimulation discrimination paradigm.* PHARMACOL BIOCHEM BEHAV 43(2) 657–660, 1992.—The effects of several doses of systemic scopolamine administration on brain-stimulation reward from the A10 nucleus of the ventral tegmental area (VTA) were evaluated. The intracranial self-stimulation (ICSS) task involved a two-hole nose-poke procedure allowing for the assessment of both reinforced (correct) and nonreinforced (incorrect) performance levels as a function of varying current intensities. Scopolamine (0.75, 1.5, and 3.0 mg/kg) was found not to alter the rate-intensity functions derived from descending and ascending presentation of seven current levels. However, when nonreinforced behavior was considered significant increases in error responding were evident following scopolamine injection. These results are consistent with the known disinhibitory and perseverative properties of scopolamine, and indicate that the previously reported positive actions of peripheral administration of anticholinergic drugs on ICSS likely involved a drug-induced rate-enhancement of reward-unrelated performance variables.

ICSS VTA Scopolamine Acetylcholine Reward Error responding Disinhibition

RECENT research has implicated a role for acetylcholine (ACh) in modulating brain-stimulation reward. Microinfusion of antimuscarinic drugs (scopolamine and atropine) into the ventral tegmental area (VTA) increase reward thresholds for medial forebrain intracranial self-stimulation (ICSS) (9,24). Given the high concentrations of muscarinic receptors in the region surrounding the A10 cell group of the VTA (20), these results indicate an excitatory role for ACh on reward processes (9). In marked contrast to these data, Stephens and Herberg (21) found that injection of scopolamine directly into the nucleus accumbens reversed the depressing effects of spiroperidol on lateral hypothalamic ICSS, indicating a positive influence for this anticholinergic on mesolimbic reward system functioning. In agreement with the latter finding, systemic injections of scopolamine facilitate ICSS (17,19), whereas cholinergic agonists attenuate ICSS (17,18).

One explanation that has been offered for the beneficial actions of scopolamine on ICSS incorporates drug-elicited changes in reward-unrelated performance factors (16). Thus, for example, Edmonds and Gallistel (7) found high doses of atropine not to influence reward thresholds and, more recently, Druhan et al. (6) reported that whereas scopolamine in low doses increased ICSS rates reward thresholds were not altered by anticholinergic treatment. The present study was designed to evaluate more directly the reward and performance-enhancing properties of peripheral scopolamine administration. This was accomplished by using a two-hole nose-poke discrimination procedure to assess ICSS. In this ICSS paradigm, a neutral stimulus (light) is associated with rewarding brain stimulation following a nose-poke response. At predetermined intervals, the conditioned stimulus is alternated between two holes situated in the floor of the ICSS apparatus and the number of correct (reinforced) and incorrect (nonreinforced) responses are recorded during ICSS testing. Previous work from this laboratory has found that examination of reinforced and error responding provides a sensitive analysis of the reward and performance effects induced by drug treatments (13–15).

METHOD

Subjects

Fifteen male Wistar rats (250–300 g) were individually housed in a temperature-controlled room and provided free access to food and water throughout the duration of the experiment. Animals were maintained on a 12 L : 12 D cycle and behavioral testing was conducted during the light portion of the cycle.

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Apparatus

The ICSS apparatus consisted of four identical black Plexiglas boxes (60 × 50 × 35). Two holes, 4 cm in diameter and 10 cm apart, were located in the center of the black Plexiglas floor of each ICSS chamber. A ring of lights embedded in the floor of each ICSS box with a white translucent cover (2 cm in width) surrounded the perimeter of each hole. Three infrared photobeam units were mounted in each hole 0.5 cm from the surface, and disruption of the photobeams by a nose-poke response resulted in delivery of electrical brain stimulation through a mercury-filled commutator. A constant-current stimulator delivered a monophasic square wave with a pulse duration 0.1 ms and a frequency of 100 Hz. Once initiated, the electrical stimulation had a duration of 0.5 s. The ICSS chambers were interfaced to a Commodore 64 computer whose software controlled the presentation and the intensity of electrical simulation, the discrimination procedure that involved alternating the light onset between holes at predetermined intervals, as well as recording the number of nose-poke responses in each hole during ICSS testing.

Procedure

Surgery. Subjects were anesthetized with sodium pentobarbital (60.0 mg/kg) and a bipolar electrode (MS-303/1, Plastics One, Roanoke, VA) was stereotaxically implanted in the VTA (AP −2.8 mm from bregma, L ± 1.4 mm from the midline suture, and V −8.6 mm from the skull surface). Electrodes were implanted perpendicular to the horizontal plane and the incisor bar was adjusted for each animal such that the horizontal plane was level for the anterior and posterior portions of the skull.

Discrimination training. Seven days postoperatively animals were trained for ICSS. During the daily ICSS sessions the light around one of the holes remained on and a nose-poke into the signaled hole resulted in brain stimulation. Responding into the nonsignaled hole was not reinforced. Once stable ICSS rates were established at a current intensity that was adjusted for each animal to elicit optimal levels of responding, discrimination training was initiated. Light onset was alternated between holes every 30 s for a 5-min ICSS session. Animals received brain-stimulation reward only when nose-poke responding was directed into the signaled hole. When subjects performed correctly on at least 90% of the total responses made during each training session, the alternation time for switching the light onset between holes was reduced to 20 s and the ICSS test duration was decreased to 4 min. This training procedure was continued until animals developed stable rates of responding with an alternation time of 10 s and ICSS trial duration of 2 min.

Rate-intensity functions. After animals mastered the discrimination task, descending and ascending rate-intensity functions were determined. At the outset of each daily ICSS test session, subjects were allowed to respond for brain stimulation at their individual training current intensities for a 5-min period. Current level was then decreased by 4-μA steps in a descending fashion starting at 40 μA (RMS). Correct and error responding were recorded for 2 min at each of seven current levels (40, 36, 32, 28, 24, 20 and 16 μA). After completion of the descending mode of current presentation, current intensity was increased by 4-μA steps, and reinforced and incorrect responding were recorded for 2 min at each level of the ascending phase of the ICSS test session.

Drug treatments. Once baseline rate-intensity functions stabilized, animals were treated with an IP injection of either saline or one of three doses of scopolamine hydrobromide (0.75, 1.5, and 3.0 mg/kg). All animals were tested with these doses and behavioral testing was initiated 10 min after drug injection. The order of drug administration was randomized for each animal. Three days were allowed between drug treatments to minimize carryover effects. During this period, animals were tested daily for ICSS and baseline rate-intensity functions remained stable.

Histology. Upon completion of the experiment, animals were deeply anesthetized with an overdose of sodium pentobarbital and perfused intracardially with saline followed by a 10.0% formalin solution. Brains were removed, sliced in 40-μm coronal sections, and stained with thionine for verification of electrode tracts. The data from two animals in which electrode placements were located outside the A10 region of the VTA were excluded from the statistical analyses.

RESULTS AND DISCUSSION

Because scopolamine administration did not differentially modify reinforced and nonreinforced performance with respect to the descending and ascending rate-intensity functions, ICSS and error rates were averaged over the descending and ascending current presentation modes. Rate-intensity functions for reinforced nose-poke responding are depicted in Fig. 1. A four (drug treatment) × seven (current intensity) analysis of variance (ANOVA) with repeated measures on both factors yielded a significant main effect for current intensity, $F(6, 72) = 56.51$, $p < 0.0001$. As shown in Figure 1, animals showed typical rate-intensity functions, and a shift in the current-response curve was not evident after administration of any of the doses of scopolamine, $F(3, 36) = 0.62$, $p > 0.1$.

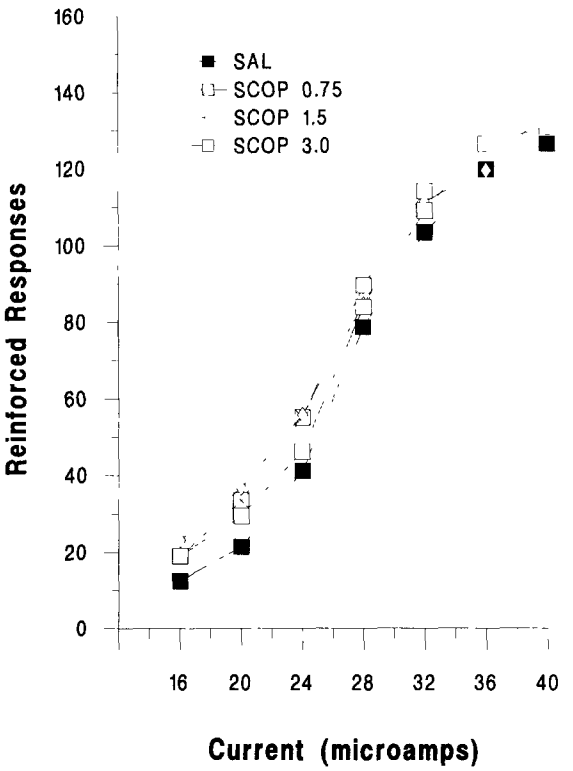

FIG. 1. Mean number of reinforced (correct) responses as a function current intensity and drug treatment (saline and 0.75, 1.5, and 3.0 mg/kg scopolamine HBr).

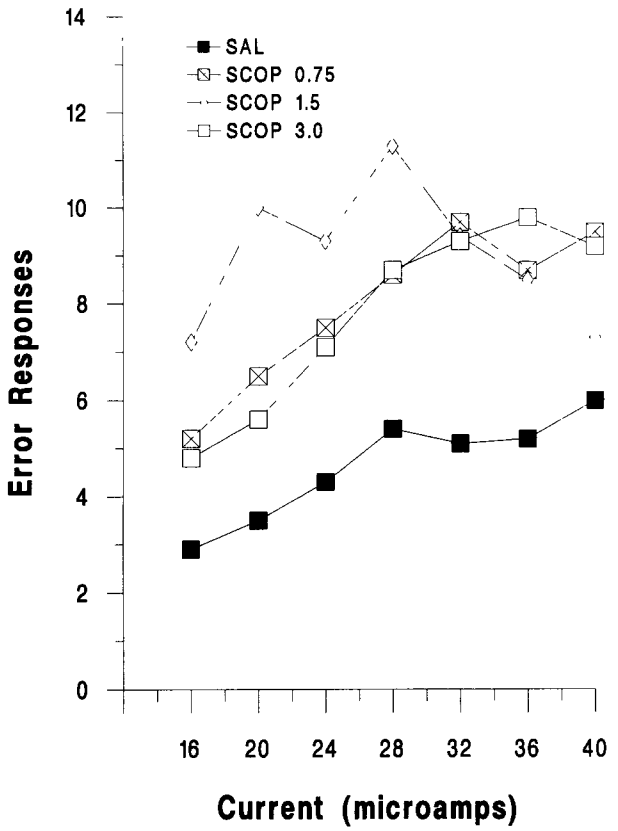

FIG. 2. Mean number of incorrect (nonreinforced) responses as a function of current intensity and drug treatment (saline and 0.75, 1.5, and 3.0 mg/kg scopolamine HBr).

Reward thresholds were determined for each animal using a constant value of 40 responses/min and current thresholds were not significantly modified by scopolamine administration, $F(3, 36) = 0.59$, $p > 0.1$. The mean (±SEM) thresholds for the saline and 0.75, 1.5, and 3.0 mg/kg scopolamine treatments were 30.9 ± 1.5, 27.9 ± 1.5, 27.8 ± 1.9, and 29.4 ± 1.4, respectively.

Figure 2 depicts the mean error responding into the nonsignaled hole as a function of current level during each 2-min ICSS test interval. Consistent with previous reports, error rates were observed to increase with current intensity, $F(6,72) = 3.72$, $p < 0.01$ (13–15), and scopolamine injection had a pronounced influence on incorrect responding in the ICSS task, $F(3, 36) = 3.54$, $p < 0.01$. Following scopolamine treatment, animals exhibited significantly higher rates of nose-poke responses into the nonsignaled hole than control animals.

The results of this experiment reveal that scopolamine does not affect ICSS supported by the A10 cell grouping of the VTA. This observation is consistent with previous reports that found peripheral injection of scopolamine (6), and atropine (7), not to alter thresholds for brain-stimulation reward. The utility of the discrimination paradigm in ferreting out the rate-enhancing performance effects of scopolamine from reinforced behavior shows that while scopolamine did not induce a shift in the rate-intensity function when responding to the signaled hole was evaluated, the magnitude of nonreinforced responding was significantly increased.

Considerable research has demonstrated that many of the behavioral effects of cholinergic antagonists are associated with changes in central inhibitory processes (1,3–5). For example, scopolamine increases locomotor activity (2) and produces substantial deficits in spontaneous alternation behavior (10,11). Conversely, inhibition of acetylcholinesterase by physostigmine decreases locomotor activity and enhances spontaneous alternation behavior (12,22). It has been argued that the disruption of the spontaneous alternation tendency after scopolamine injection is associated with the animal's inability to inhibit responding to previously visited stimulus cues resulting in random alternation levels (1,10). Similar disinhibitory effects have been reported in a two-lever double-alternation paradigm, in which scopolamine increased the number of errors in a dose-dependent fashion (23). As well, in a discrete trial two-lever delayed spatial alternation setting scopolamine treatment was found to elicit a substantial increase in error responding (8).

The elevation of nonreinforced behavior seen in this experiment following scopolamine injection is consistent with the disinhibitory properties of this drug. One robust effect we observed in experiments using the two-hole nose-poke discrimination ICSS paradigm is that error responding increases as a function of current (13–15). Thus, as the reward value of brain stimulation is enhanced animals have more difficulty terminating responding to the nonsignaled hole, resulting in a small but significant increase in nonreinforced performance levels. It would appear from our data that scopolamine elicits a positive influence on this reward-unrelated component of ICSS, possibly by reducing the animal's capacity to inhibit responding to the previously signaled hole.

In summary, the results of this study indicate that scopolamine's enhancing effect on ICSS performance (17,19) is not related to specific changes in the rewarding value of brain stimulation (6) but rather involves a drug-elicited increase in nonreinforced behavior. While the results of this experiment do not provide any new information concerning the role of central ACh in modulating reward processes, they do suggest that systemic administration of cholinergic antagonists is not a good avenue for exploring this issue.

ACKNOWLEDGEMENT

This research was supported by Grant A7042 from the Natural Sciences and Engineering Council of Canada.

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