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Effects of Scopolamine on the Rewarding and Seizure-Inducing Properties of Amygdaloid Stimulation

PATRICK D. BROPHY, THOMAS B. BOROWSKI AND LARRY KOKKINIDIS[1]

Department of Psychology, University of Saskatchewan, Saskatoon, Saskatchewan S7N 0W0, Canada

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BROPHY, P. D., T. B. BOROWSKI AND L. KOKKINIDIS. *Effects of scopolamine on rewarding and seizure-inducing properties of amygdaloid stimulation.* PHARMACOL BIOCHEM BEHAV **44**(2) 425–428, 1993.—The relationship between amygdaloid brain-stimulation reward and the evolution of seizure activity was evaluated in this study. Current levels that maintained optimal intracranial self-stimulation (ICSS) rates were found to be lower than the minimal current intensity required to elicit an afterdischarge (AD) from the central nucleus of the amygdala. After the ICSS session, AD thresholds (ADTs) were reduced to the same levels of current used to support ICSS. Assessment of seizure stage development during ICSS testing revealed that the emergence of early-stage epileptiform events following repeated amygdaloid stimulation suppressed ICSS performance. While administration of the antimuscarinic scopolamine did not prevent the stimulation-elicited reduction in AD thresholds, it was observed to inhibit seizure progression and increase ICSS rates. These results are consistent with the excitatory function of acetylcholine in epileptogenesis and were related to the possibility that different mechanisms underlie the rewarding and seizure-inducing properties of amygdaloid stimulation.

Brain-stimulation reward Amygdala Epileptogenesis Scopolamine Acetylcholine Dopamine

THE amygdaloid complex is a component of the mesolimbic system and receives dopamine (DA) projections from the A10 cell grouping of the ventral tegmental area (VTA), with the densest aggregation of DA fibers and terminals located in the central, basolateral, and intercalated nuclei (15). As is the case with the nucleus accumbens (18,19), recent work has shown that with the exception of the lateral nucleus all regions of the amygdala support intracranial self-stimulation (ICSS) (12,18). Earlier research found that of the amygdaloid nuclei stimulation of the central nucleus resulted in the highest levels of ICSS (27), an observation consistent with this region's significant content of DA (15).

Repeated intermittent electrical stimulation of the amygdala results in a kindling-associated development of electrophysiological and behavioral seizure activity through five defined stages, culminating in bilateral clonic convulsions (9,21). Amygdaloid ICSS also elicits epileptiform events (12), suggesting a kindling-like process; however, there is a paucity of empirical information concerning the relationship between the rewarding properties of amygdaloid ICSS and epileptogenesis. The purpose of this study was to assess the various stages of kindling development in relation to ICSS performance and to evaluate the association between current intensities necessary to induce afterdischarges (ADs) in the amygdala and current levels that support ICSS. In addition, it is known that the anticholinergic, scopolamine, retards kindling acquisition (13,24), and a further aim of this research was to determine whether scopolamine administration would influence ICSS-related seizure activity and responding for brain-stimulation reward.

METHOD

Sixteen male Wistar rats (250–300 g) were individually housed under standard laboratory conditions and provided free access to food and water throughout the experiment. Animals were maintained on a 12 L : 12 D cycle and behavioral testing was conducted during the light portion of the cycle. Subjects were anesthetized with sodium pentobarbital (60.0 mg/kg) and a bipolar nichrome stimulating electrode (MS-303/1, Plastics One) was stereotaxically implanted in the central nucleus of the amygdala (0.5 mm posterior to bregma, 4.5 mm lateral to the midline suture, and 8.5 mm below 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 posterior and anterior portions of the skull. A jeweler's screw was mounted in the frontal pole and served as the indifferent lead during electroencephalographic (EEG) recording.

Seven days postoperatively, AD thresholds (ADTs) were

[1] To whom requests for reprints should be addressed.

determined for each animal. This involved placing animals in a clear Plexiglas box (20 × 10 × 20 cm) and recording EEG activity (Grass Instruments polygraph, Model 79E, Grass Instruments, Quincy, MA) following amygdaloid stimulation. Electrical stimulation (100-Hz monophasic square wave, 0.1-ms pulse duration, 2.0-s train duration from a constant-current stimulator) was administered in incremental 5-μA steps every 60 s, starting at 5 μA (rms) until an AD was observed.

The next day, one half of the animals were treated with an IP injection of scopolamine (10.0 mg/kg) and the remaining half received saline. This dose of scopolamine was previously shown to be effective in retarding the development of kindled seizures from the amygdala (13). Immediately after drug administration, animals were placed in the ICSS apparatus (black Plexiglas box, 60 × 50 × 35 cm), which had two holes, 10 cm apart, situated in the center of the floor. A ring of lights embedded in the black Plexiglas floor with a translucent cover (2 cm in width) surrounded each hole. Three infrared photobeam units were mounted in each hole (0.5 cm from the top) and disruption of the photobeams by a nose-poke response resulted in electrical brain stimulation. A constant-current stimulator delivered brain stimulation through a mercury-filled commutator. The stimulation consisted of a positive monophasic square wave with a pulse duration of 0.1 ms and a pulse frequency of 100 Hz. The light was associated with only one of the holes and a nose-poke response into the signaled hole resulted in brain stimulation (0.5-s duration), whereas responding into the unsignaled hole was not reinforced. The number of nose-poke responses into the signaled and unsignaled holes was recorded over a 1-h ICSS test session.

ICSS was initiated by giving animals several priming stimulations at 40 μA (rms), and after animals exhibited nose-poke responding the current intensity was set at a level that supported optimal rates of ICSS for each animal. Animals quickly learned to nose-poke into the signaled hole for brain-stimulation reward. During the 1-h ICSS session, animals were observed and the progression of seizure stage development recorded. As described by Racine (21), the seizure stages are characterized by rhythmic facial movements (stage 1), head-bobbing (stage 2), bilateral forelimb clonus (stage 3), bilateral forelimb clonus and rearing (stage 4), and convulsions with the loss of postural control (stage 5). Twenty-four hours following ICSS testing, ADTs were again determined for each animal using the procedure described earlier. At the termination of the experiment, subjects were deeply anesthetized with sodium pentobarbital and perfused intracardially with saline followed by 10% formalin. The brains were removed, sliced in 40 μm coronal sections, and were stained with thionine. Electrode placements were verified to be in the central nucleus of the amygdala.

RESULTS AND DISCUSSION

Figure 1 depicts the mean (±SEM) rates of ICSS (insert) and seizure stage development as a function of drug treatment. Saline-treated subjects showed stable levels of responding for brain-stimulation reward; however, maximal rates over the ICSS session were low. The analysis of seizure stage evolution indicated that ICSS performance was constrained by the emergence of convulsive activity. As seizures developed, animals would retreat into the corners of the ICSS chamber, and while subjects would intermittently revisit the signaled hole the inimical properties of amygdaloid stimulation eventually

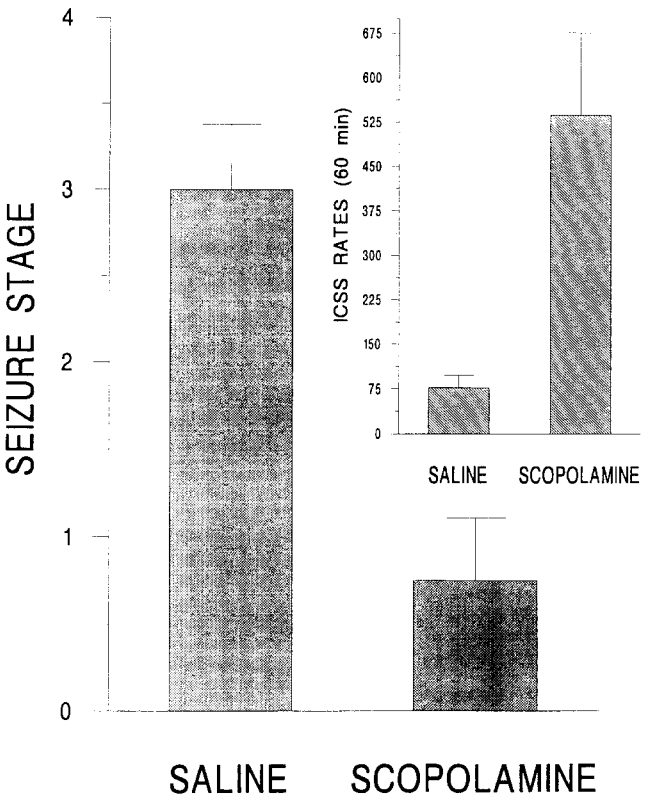

FIG. 1. Mean (±SEM) seizure stage development and ICSS rates (insert) seen during amygdaloid self-stimulation as a function of saline and scopolamine (10.0 mg/kg) treatment.

resulted in a complete cessation of ICSS behavior. In terms of seizure stage development, three rats stopped responding after stage-2 seizures, three showed ICSS inhibition at the stage-3 level, one continued to self-stimulate until stage 4, and the remaining subject did not show ICSS cessation until a stage-5 seizure evolved. These results confirm that the genesis of early-stage kindled seizures can interfere with brain-stimulation reward from the amygdala, and in only a few subjects will the convulsions be advanced enough to be outwardly apparent (rearing and loss of postural control). Unless seizure stage activity is monitored during ICSS testing, the effects on reinforced responding might well be overlooked and could contaminate measures of reward performance, in particular from the limbic system and related regions.

Scopolamine treatment resulted in a marked increase in ICSS, $t(14) = 3.39$, $p < 0.05$. This effect was specific to brain-stimulation reward because responding into the unsignaled hole was not evident from animals in either treatment condition. As can be seen in Fig. 1, the enhanced ICSS rates coincided with a scopolamine-induced inhibition of epileptogenesis, $t(14) = 4.27$, $p < 0.05$. Three animals injected with this anticholinergic exhibited stage-2 behaviors and inhibition of ICSS, whereas the remaining five showed intermittent nose-poke responding for brain-stimulation reward during the entire ICSS session with no overt behavioral signs of seizure activity. These results are consistent with previous reports involving the effects of scopolamine on kindling acquisition (13,24), indicating that scopolamine suppresses the progres-

sion of seizure development following repeated electrical stimulation of the amygdala.

ICSS current intensities and the pre- and post-ICSS AD threshold data are depicted in Fig. 2. The analysis of variance (ANOVA) involved a two (drug) × three (current) design with repeated measures on the latter factor and yielded a significant main effect for current, $F(2, 28) = 22.11, p < 0.001$. There was no significant effect for scopolamine treatment, $F(1, 14) = 2.24, p > 0.05$. Subsequent Newman-Keuls multiple comparisons ($\alpha = 0.05$) revealed that pre-ICSS ADTs were significantly higher than ICSS current intensities; however, after the ICSS session ADTs decreased and were comparable to the ICSS current levels regardless of drug treatment. To ensure that the lower ADTs were the result of repeated amygdaloid stimulation during ICSS and not due to the initial ADT test, we tested the ADTs of a group of naive amygdaloid-implanted animals ($n = 5$) twice, with 48 h separating the two ADT sessions. ADTs were somewhat lower on the second ADT determination (52.5 ± 5.3) as compared to the first test (57.8 ± 11.3); however, this difference was not statistically significant.

GENERAL DISCUSSION

Taken together, the results of this study indicate that nosepoke responding seen early in the ICSS test session reflects the rewarding aspects of brain stimulation in the absence of stimulation-induced epileptiform events. As ICSS progresses, ADTs are lowered and the resulting ictal activity negatively impacts on ICSS performance. It is important to note that while scopolamine inhibited seizure development it did not influence ADTs following amygdaloid ICSS. This was not entirely unexpected because scopolamine has been shown to retard kindling acquisition without affecting ADTs or AD durations in a kindling paradigm (13,24).

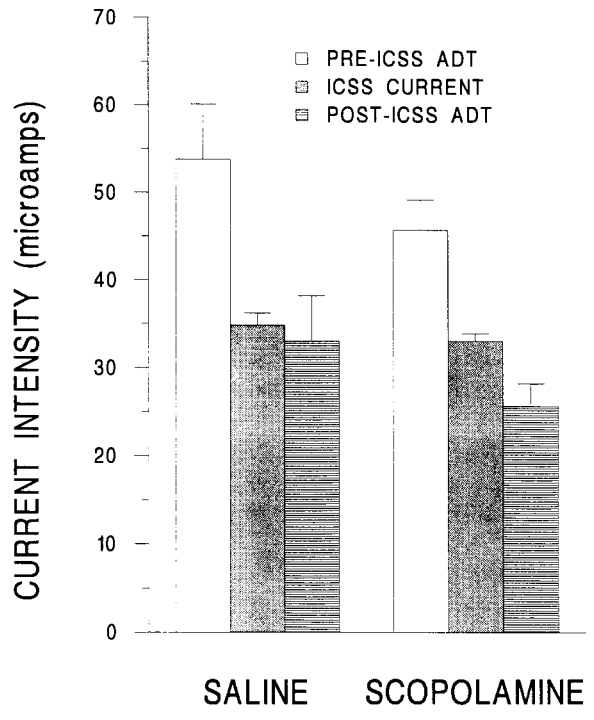

FIG. 2. Mean (±SEM) pre-ICSS afterdischarge thresholds (ADTs), intracranial self-stimulation (ICSS) current intensities, and post-ICSS ADTs for the saline and scopolamine (10.0 mg/kg) groups.

The reduction in threshold as a function of ICSS for the scopolamine group (20.0 μA) was comparable to that seen in saline animals (20.8 μA). A larger change in ADTs would have been expected after scopolamine treatment given the higher levels of amygdaloid stimulation. Racine (20) reported that the neural events associated with repeated electrical stimulation, and not AD activity, are critical for decreasing ADTs in the amygdala. In addition to the number of stimulations, however, the current intensity and the interval between stimulations are also important variables in determining threshold changes. While intermittent subthreshold stimulations were more effective than suprathreshold stimulations in reducing ADTs, massed presentation of suprathreshold stimulations was observed to increase threshold levels in some animals, and this effect was attenuated when the interval between threshold tests was extended. Given these factors, the ADT-lowering potential of repeated electrical stimulation in scopolamine-treated animals might well have been limited by the high number of stimulations received during a relatively short period of time in the ICSS session.

The results of this study extend the findings of previous reports showing that the central nucleus of the amygdala supports ICSS (12,18,27) in so far as the rewarding stimulation is at a current intensity lower than that required to induce an AD in the amygdala. The important element in relation to the positive consequences of scopolamine on ICSS performance involves the ability of this anticholinergic to suppress the behavioral expression of seizure activity after repeated stimulation of the amygdala. The finding that scopolamine increases ICSS rates while arresting seizure stage development demonstrates an inverse relationship between ictal events and reward system functioning, an observation consistent with recent conclusions concerning the positive influence of diazepam on brain-stimulation reward in seizure-prone animals (10).

While the functional significance of the interaction between epileptiform activity and reward functioning remains to be determined, there are several possibilities that require consideration. The first and most obvious option is that the sensorimotor effects of electrical seizure activity influence ICSS. Indeed, in man epileptic amygdaloid discharge often results in sensory illusions and mental confusion (11,14). The observation, however, that some animals continued to self-stimulate until late-stage generalized seizures developed suggests that other effects of amygdaloid stimulation might also play a role in disrupting ICSS.

It is known that activation of the amygdala causes fearful emotions in man, and animal research in a variety of species has shown that amygdaloid stimulation elicits unconditioned fear responses (8). Moreover, it has been demonstrated in man (2) and animals (26) that stimulation of the amygdala can have positive and negative consequences on behavior, and this limbic system structure is considered a reward and punishment area (8). Based upon this evidence, it is possible that the ictal events associated with continued amygdaloid stimulation might have aversive repercussions on behavior interfering with active ICSS responding.

With respect to scopolamine, it cannot be ruled out from our data that the increases in ICSS were the result of the effects of this anticholinergic on reward processes. Recent studies have shown, however, that peripheral scopolamine treatment does not affect brain-stimulation reward (1,4). Moreover, while the involvement of DA in amygdaloid ICSS

has not yet been demonstrated DA has a modulatory role on mesolimbic ICSS (6,17), and it is noteworthy that in contrast to the reward depression seen after administration of DA receptor antagonists (5,25) pretreatment with either haloperidol or pimozide does not influence kindling evolution (3,13,23). There is evidence to suggest that low but not high doses of haloperidol increase kindling rate (7,22); however, these alterations are more consistent with the effects of haloperidol on norepinephrine neuronal dynamics than DA activity (16). Together, these observations raise the possibility that DA and acetylcholine might function independently in the rewarding and seizure-inducing properties of amygdaloid stimulation.

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