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Suppressive Effects of Intraperitoneal and Intraventricular Injections of Nicotine on Muricide and Shock-Induced Attack on Conspecifics[1,2]

ROBERT J. WALDBILLIG

Department of Psychology, University of Florida, Gainesville, FL 32611

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WALDBILLIG, R. J. *Suppressive effects of intraperitoneal and intraventricular injections of nicotine on muricide and shock-induced attack on conspecifics.* PHARMAC. BIOCHEM. BEHAV. 12(4) 619–623, 1980.—Rats were used to investigate the effect of nicotine on mouse-killing and foot shock-induced attack on conspecifics. It was found that intraperitoneal injections of nicotine (100–1000 μg/kg) suppressed mouse-killing in a dose dependent manner. The suppression of mouse-killing by nicotine was not blocked by hexamethonium (30 mg/kg), a peripheral nicotinic receptor blocking agent. Mecamylamine (30 mg/kg), a nicotinic blocking agent with central effects, did reduce the inhibition of attack produced by nicotine. Both intraperitoneal and intraventricular injections of nicotine suppressed shock-induced attack on conspecifics. Shock-elicited flinch, vocalization, and escape were not influenced by nicotine injections. These findings give further support to the view that muscarinic and nicotinic compounds produce antagonistic effects on certain types of attack behavior.

Shock-induced attack Mouse-killing Nicotinic suppression Intraventricular Intraperitoneal

THE pharmacological aspects of attack behavior have been receiving increasing attention. For example, it has been shown that cholinomimetic drugs decrease the latency of mouse-killing (muricide) in spontaneously killing rats [10] and can initiate attack in nonkilling rats [11]. Work has been conducted on the possible brain mechanisms that mediate such effects. Local implantation of eserine, a cholinesterase inhibitor, into the amygdala can induce rats to kill mice [10]. Administration of cholinomimetics to hypothalamic and midbrain sites also decrease the latency to kill [1, 2, 3]. Because killing elicited by cholinergic stimulation of the hypothalamus can be blocked by antimuscarinic drugs it seems likely that the muscarinic postsynaptic receptor is predominant in the elicitation of attack. The recent finding that activation of hypothalamic adrenergic alpha and beta receptors produce reverse effects on ingestive behavior [9,10] raises the possibility that such a duality might exist in the cholinergic mediation of attack behavior. That is, nicotinic and muscarinic receptors might mediate opposite effects on attack behavior. Reported here is a series of experiments designed to test the hypothesis that activation of nicotinic receptors would suppress attack behavior.

EXPERIMENT 1

METHOD

Ten mouse-killing male Long-Evans rats weighing between 350–450 g were individually housed and maintained on ad lib tap water and Purina lab chow. To be classified as an attacker, an animal had to kill mice in 10-min tests on each of four successive days. To test the effects of nicotine, animals received two 10-min muricide tests a day. The tests were separated by a 60-min interest interval. Each test consisted of placing a live white mouse into the home cage of the rat. The latency to attack was determined with a stop watch. The behavior of the animals was observed for signs of sedation or debilitation. Fifteen minutes prior to the first attack test, animals received an IP injection of isotonic saline. Fifteen minutes prior to the second test animals received an injection of either saline or nicotine dissolved in saline. Although for all animals nicotine doses began at 50 μg/kg, dose response curves were determined individually for each animal. Tests were conducted on alternate days, except for weekends, for periods of up to nine months. Each dose was given at least

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[2]Reprint Requests to: Dr. Robert J. Waldbillig, Department of Psychology, University of Florida, Gainesville, Florida 32611

six times in ascending and descending order. Statistical analyses compared latencies to attack and were conducted with a one-tail correlated t-test.

RESULTS AND DISCUSSION

There were no reliable changes in the first test latency (to attack) across the duration of testing. The latency for the first five such tests was 33.4±11.36 sec (mean±SEM). For the last five such tests the latency was 34.1±11.8, $t(9)=0.72$, $p>0.05$. Also there were no differences between the first and second attack test when saline was injected in the second test. The first test mean for all such tests was 30.0±10.1 seconds; the mean for the second test was 29.3±9.8 seconds, $t(9)=1.28$, $p>0.05$.

Figure 1 shows the relationship between the average latency to attack (in seconds) and nicotine does (μg/kg) for the individual animals. It can be seen that for each of the ten attackers, nicotine inhibited mouse-killing in a dose dependent manner. For each animal a dose was found that would nearly completely suppress attack behavior. At the highest dose of nicotine there was statistically significant suppression of mouse-killing, $t(9)=4.9$, $p<0.001$. While there appear to be large individual differences in the sensitivity to nicotine, eight of ten animals show marked suppression in the range of 200–500 μg/kg. There was no apparent adaptation to the suppressive effects of nicotine. There were no differences in the suppressive effect of nicotine comparing the first three injections of the highest dose to the last three injections, $t(9)=0.83$, $p>0.05$. The behavioral protocols indicate that when injected with nicotine the rats repeatedly approach and sniff the mice. Nicotine injected rats also occasionally lunged at the mice without executing a penetrating bite. While not killing mice, nicotine injected rats were observed to groom, eat and drink during the test in the normal manner.

The data presented above indicates that mouse-killing in rats is a highly reliable behavior that is inhibited in a dose-related manner by intraperitoneal injections of nicotine. Observations of the behavior of the animals indicates, as well as such nonquantitative measures can, that there was lack of debilitation or generalized sedation during the test.

EXPERIMENT 2

In a preliminary attempt to determine whether the nicotine inhibition of mouse-killing was due to actions in the central or peripheral nervous system, various antagonists of nicotinic receptors were employed. Specifically, mecamylamine was used to block nicotinic receptors in both the central and peripheral nervous system [7]. Hexamethonium was used to block peripheral receptors while leaving central receptors free to interact with nicotine [7].

METHOD

Five natural mouse-killers from Experiment 1 were tested for attack behavior according to the two tests/day procedures outlined in Experiment 1. On some days animals received additionally an injection of nicotinic blocker prior to the second attack test. For each animal the nicotine dose used was at the midpoint of its individual dose response curve (see Fig. 1). For three animals the nicotinic receptor blocking agent was mecamylamine (30 mg/kg). The other two animals received injections of hexamethonium (30 mg/kg). For each animal the effect of the blocking agent on the nicotine-induced suppression of attack was determined at least ten times. The latency to attack following injections of nicotine with a nicotinic blocking agent was compared to the latency following just nicotine.

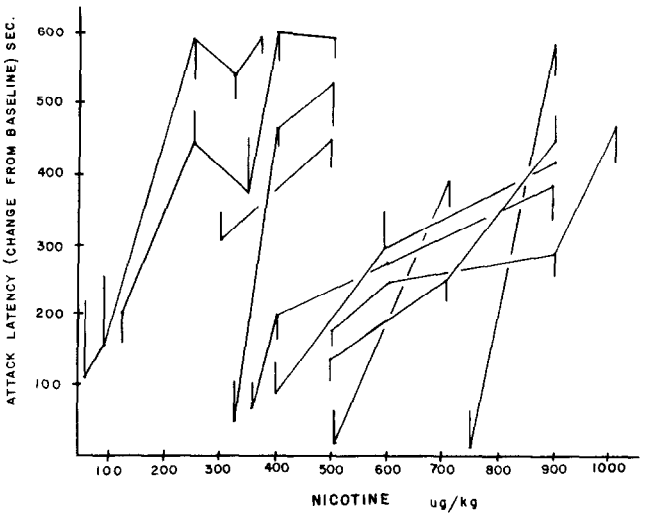

FIG. 1. Changes in the latency to attack mice following intraperitoneal injection of nicotine (mean and standard error for individual animals).

RESULTS AND DISCUSSION

Hexamethonium failed to inhibit the suppressive effects of nicotine on attack in the two animals receiving this drug. For these two animals the latencies to attack following saline injections were 10.6±26.1 and 13.9±27.3 (mean±SEM) sec. Following nicotine injections (220 μg/kg and 550 μg/kg) their respective attack latencies were 520.1±50.1 and 264.3±84.3. Following injections of both nicotine and hexamethonium the attack latencies were not greatly changed, being 490.8±48.3 and 298.0±83.3 sec respectively. When hexamethonium was paired with saline injections there was no suppression of attack latency.

In contrast to the ineffectiveness of hexamethonium, mecamylamine markedly reduced the suppressive effects of nicotine. For the three animals receiving this compound, the latencies to attack following saline injections were 21.0, 9.6 and 10.6 sec. Nicotine prolonged the latencies to 232.2, 282.0 and 550.0 sec. Mecamylamine reduced these latencies approximately 50% to 122.2, 140.5, and 336.2 sec respectively. When mecamylamine was paired with second test saline injections there was no clear change in the attack latencies.

EXPERIMENT 3

To test the generality of the suppressive effects of nicotine on attack behavior an experiment was conducted to determine the effect of intraperitoneal nicotine on the attack on other rats (conspecifics) induced by tail shock.

METHOD

Ten adult Long-Evans male rats were divided into five pairs, matched by weight, and tested for shock-induced attack. For the test, two animals were placed facing each other in a 10″×10″ test area. Animals were held in this position by passing the tail through a hole cut in the wall behind the animal. The portion of the tail that was outside the wall was then slipped through a hole in the center of a disc. The disc

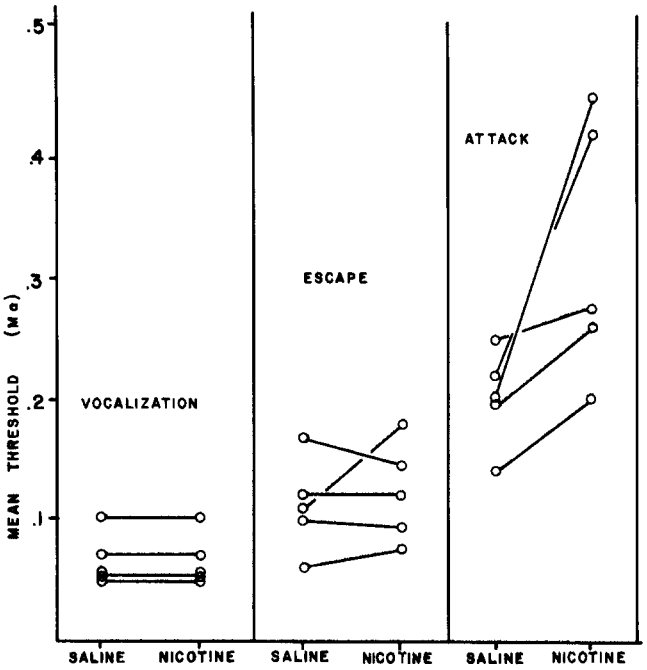

FIG. 2. Thresholds for tail shock-induced vocalization (left panel), escape (center panel), and attack on a conspecific (right panel), following intraperitoneal injections of either nicotine (800 μg/kg) or isotonic saline (means for each pair of rats).

was held to the tail with tape. Electrode paste and electrodes were applied one inch from the tip of the tail.

Animals were given ten trials each at eight levels of tail shock (0.05, 0.10, 0.20, 0.25, 0.30, 0.35, 0.40, 0.45 mA) in ascending and descending order. The shock duration was 0.5 sec and was controlled by conventional electromechanical instrumentation. The intertrial interval was one minute. Each trial was scored for the occurrence of squeaking, escape and attack. If neither animal exhibited these behaviors the trial was scored as negative. If one or both animals exhibited the response the trial was scored as positive. Behavior was categorized in the following way: squeaking was any vocalization detected by the observer; escapes were attempts at locomotion away from the other rat; attack was defined as rearing onto the back legs, facing and striking at the other animal with the paws and/or biting at the other animal. Fifteen minutes prior to each test, animals received an intraperitoneal injection of either nicotine (800 μg/kg) or isotonic saline. Each treatment was repeated at least twice. The observer was unaware of whether nicotine or saline had been injected. The effect of nicotine injection was assessed in terms of changes in the threshold for the elicitation of the response (defined as the current required to elicit the behavior in 20% (2/10) of the trials. Statistical analyses were conducted with one-tail correlated t-tests.

RESULTS AND DISCUSSION

Vocalization (squeaking) had the lowest threshold of the behaviors measured (mean=0.07 mA). Figure 2 (left panel) shows the effect of nicotine on the threshold for the elicitation of squeaking. It can be seen that nicotine had no effect on this behavior, $t(9)=0.99$, $p>0.05$. Nicotine was also without effect on escape behavior (Fig. 2, center panel), $t(9)=0.46$, $p>0.05$, one-tail correlated t-test. Unlike the other behaviors, attack threshold was significantly elevated by nicotine (Fig. 2, left panel), $t(9)=3.60$, $p<0.01$.

It can be seen that nicotine suppresses shock-induced attack on other rats. Unlike Experiment 1, where evidence on the selectivity of the suppression of mouse-killing was obtained by observing the behavior of the animal, here it is shown that nicotine has no suppressive effect on either vocalization or escape behavior. Such a finding indicates that decreases in attack are not likely to be due to a decrease in the reactivity of the animal to the shock.

EXPERIMENT 4

Experiment 4 was conducted to determine whether the suppressive effect of nicotine on shock-induced attack is due to an action of nicotine in the brain. To test this possibility the effects of intraventricular injection of nicotine were examined.

METHOD

Surgery

Fourteen Long-Evans rats were anesthetized with sodium pentobarbital (50 mg/kg) and then unilaterally implanted with 21 ga stainless-steel guide cannulas in the lateral ventricle. Surgery was with the skull horizontal. The cannulas were placed 0.7 mm posterior to Bragma, 1.0 mm lateral to the midline and 4.3 mm below the surface of the cortex. Proper implantation was confirmed by the presence of CSF at the top of the cannula. The guide cannulas were kept free of debris by an inner stylette cut to the same length as the guide cannulas. For the microinfusion the stylettes were removed and replaced with infusion cannulas extending 0.1 mm beyond the tip of the guide cannula.

Procedure

Rats were divided into seven pairs matched by weight. The test for shock-induced attack was conducted in conventional operant conditioning chambers with the door of the sound attenuated shell left open. Both animals were free moving and received pulses of scrambled foot-shock through a floor grid. Pulses had a duration of 0.5 seconds and were delivered at a frequency of 1/sec for ten seconds. There was a two min intertrial interval. In each test, animals received ten trials at each of ten levels of foot-shock (0.1, 0.3, 0.6, 0.9, 1.2, 1.5, 1.8, 2.1, 2.4, 2.7 mA) in repeated ascending order. An observer, unaware of the nature of the intracranial infusion, scored each trial for the occurrence of flinching, vocalization, threat, and attack. Behaviors were categorized in the following manner: vocalization was any vocalization that could be detected by the observer; flinching was any jerk of the limbs or body; threat was defined as rearing on the back legs facing the other animal with the mouth open; attack was defined as lunging or nipping, or biting of the other animals. The trial was scored as positive if either animal emitted these responses and negative if neither animal emitted these responses.

Immediately prior to testing, animals received an intraventricular 5 μl infusion of either saline or 27.0 μg or 55.0 μg of nicotine. The 5 μl volume was injected over a five minute period. Each treatment was delivered at least twice in counterbalanced order. The statistical analysis was performed on the average of the tests using a one-tail correlated t-test.

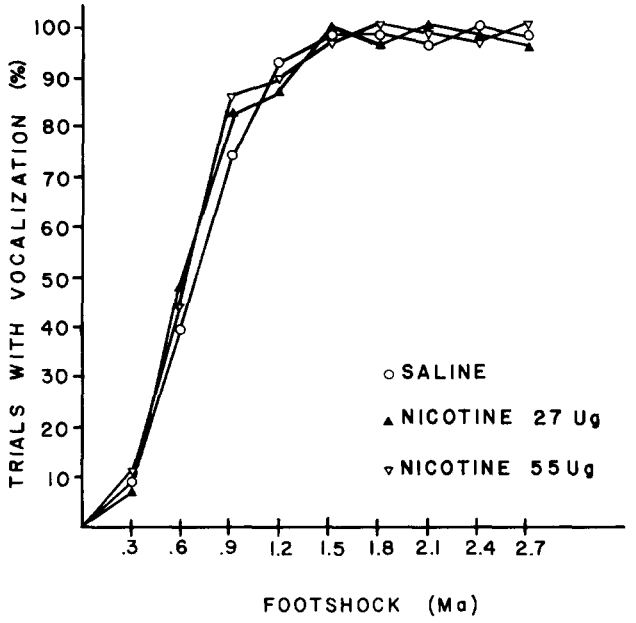

FIG. 3. Percent of trials with foot shock-induced vocalization following intraventricular injections of either saline or nicotine (27 μg and 55 μg).

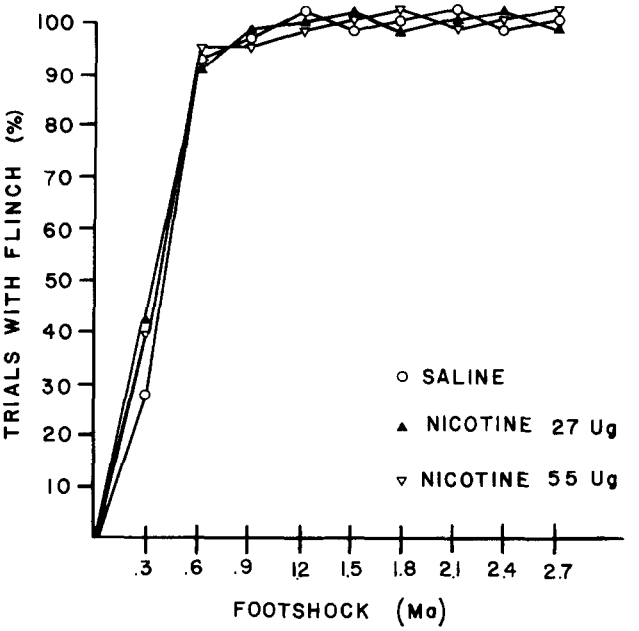

FIG. 4. Percent of trials with foot shock-induced flinch following intraventricular injections of either saline or nicotine (27 μg and 55 μg).

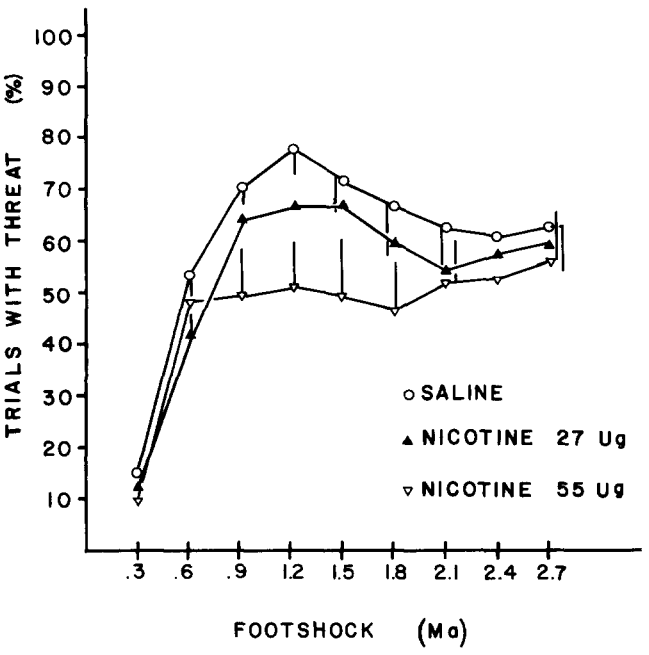

FIG. 5. Percent of trials with foot shock-induced threat following intraventricular injections of either saline or nicotine (27 μg and 55 μg).

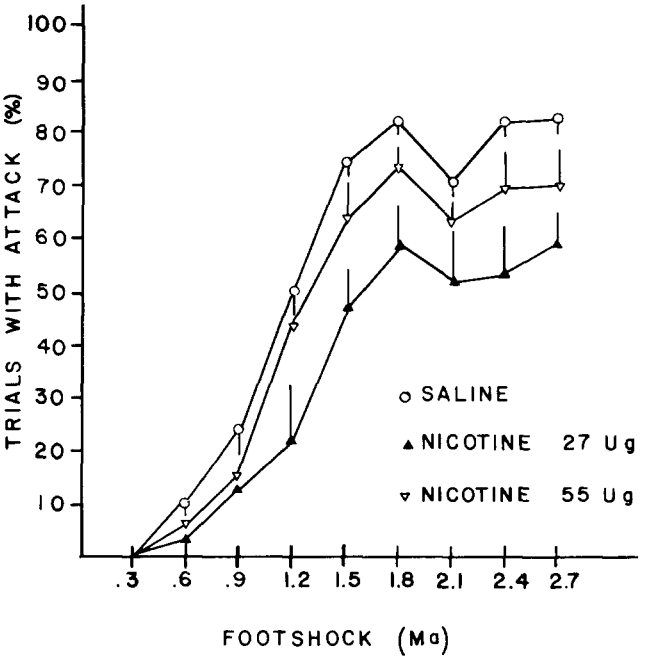

FIG. 6. Percent of trials with foot shock-induced attack on a conspecific following intraventricular injections of either saline or nicotine (27 μg and 55 μg).

RESULTS AND DISCUSSION

The relationship between vocalization and flinching and foot-shock intensity is shown in Figs. 3 and 4 respectively. It can be seen that nicotine had no effect on either shock-induced vocalization or flinching regardless of foot shock value. A statistical analysis confirms this, revealing that for both doses of nicotine the total frequency of flinching and vocalizing was not different than that for saline (vocalization; 27.0 μg, $t(13)=0.63$, $p>0.05$; 55.0 μg, $t(13)=0.81$, $p>0.05$; flinching; 27.0 μg, $t(13)=0.53$, $p>0.05$; 55.0 μg, $t(13)=0.78$, $p>0.05$).

In contrast to vocalization and flinching, threat behavior was suppressed by nicotine (Fig. 5). The total frequency with which threat behavior occurred was significantly suppressed by the 55.0 μg dose of nicotine, $t(13)=3.25$, $p<0.01$. The lower dose of nicotine (27.5 μg) also suppressed threat, however this effect did not reach significance, $t(13)=0.89$, $p>0.05$. The frequency of attack behavior (Fig. 6) was significantly suppressed by both doses of nicotine, for 27.5 μg, $t(13)=3.30$, $p<0.05$; for 55 μg, $t(13)=2.44$, $p<0.05$. Although attack was suppressed by both doses of nicotine according to Fig. 6, the effect does not appear to be as great for 55 μg as it is for 27.5 μg.

The data from Experiment 4 indicates that nicotine can, by an action in the brain, suppress both attack and threat behavior without influencing escape or vocalization.

GENERAL DISCUSSION

The data presented here indicates that in rats, two forms of attack behavior, mouse-killing and attack on a conspecific induced by foot shock can be inhibited by nicotine. The findings are consistent with the hypothesis that activating nicotinic and muscarinic postsynaptic receptors has reverse effects on attack behavior. These data represent further support for the general hypothesis that in some brain regions receptor pairs (eg. nicotinic vs. muscarinic and alpha vs. beta) mediate behaviorally antagonistic effects.

Although a CNS effect seems likely, the exact mechanism of action by which nicotine suppresses attack behavior cannot be determined from the present data. One possible site of action is the midbrain. It has been shown that electrical stimulation of an area adjacent to the mesencephalic central gray elicits mouse-killing in rats [12]. Lesions of the same area have been shown to block both mouse-killing [13] and shock-induced fighting [6]. In the cat, specific midbrain regions have also been shown to be critical for the elicitation of attack by electrical stimulation of the hypothalamus [14]. Alternatively, nicotine may activate serotonergic midbrain mechanisms known to inhibit attack behaviors in rats. It has been shown that lesions of the dorsal but not the median raphe produce mouse-killing in rats that normally do not kill [4]. Because little information is available on the brain synaptic concentration of acetylcholine it is not clear whether the injection of nicotine employed here produces normal levels of postsynaptic activity. It seems clear, however, that not all responses to shock are suppressed by these doses of nicotine. This leads to the view that the effect of nicotine is not due to a general decrease in reactivity to environmental stimuli.

Although nicotine suppressed two forms of attack behavior it does not necessarily follow that those behaviors have a substantially common neural substrate. It should also be pointed out that while mouse-killing occurs without obvious experimental intervention and is widely used as a prototype of attack behavior, it remains to be reported as occurring in natural populations of rats. Although findings similar to those reported here have been reported using cats [5], there may be major species differences in the effects of nicotine. Because of this it is not acceptable to use these data in an attempt to explain smoking behavior in humans.

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