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Nicotine-Induced Taste Aversion: Characterization and Preexposure Effects in Rats

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IWAMOTO, E. T. AND E. C. WILLIAMSON. *Nicotine-induced taste aversion: Characterization and preexposure effects in rats.* PHARMACOL BIOCHEM BEHAV **21**(4) 527–532, 1984.—Rats were trained to drink their 24 hr water intake during a single daily 30 min period. After stabilization, rats were presented with 0.1% (w/v) of sodium saccharin for 30 min. Immediately after removal of the saccharin solution, the animals were injected with saline, mecamylamine hydrochloride or hexamethonium hydrobromide; thirty minutes later, saline or nicotine, 0.05, 0.16, or 0.50 mg/kg were administered. Twenty-four hr later, rats were allowed access to both water and saccharin. Nicotine caused a dose-related decrease in the *proportion* of fluid consumed as saccharin solution during the 30 min testing situation. Neither mecamylamine nor hexamethonium alone decreased saccharin preference; however, 3 mg/kg of mecamylamine blocked the decrease of saccharin preference induced by nicotine. Preexposure of drug-naive rats to 0.5 mg/kg of nicotine for 2 or 4 days abolished the nicotine-induced taste aversions to saccharin when tested one day, or one week, after conditioning.

Saccharin Nicotine-induced taste aversion Conditioned taste-aversion Nicotine Mecamylamine Hexamethonium Two-bottle choice Rats

THE possibility that a drug causes both reward and aversion is unsettling to substance abuse theories that are based solely on the hypothesis of positive reinforcement, but evidence for this possibility was introduced eight years ago [18]. Opioids, amphetamines, alcohol, nitrous oxide, and the benzodiazepines are among the compounds reported to have positive reinforcing effects as assessed by drug self-administration paradigms and by studies involving drug-maintained, schedule controlled behavior [16]. However, these same drugs also have aversive effects as demonstrated in avoidance paradigms [15], and as assessed by their ability to condition taste aversions to solutions of saccharin [2, 5, 6, 10]. Nicotine is a drug known to be self-administered [9,12], to maintain high rates of reinforcement under certain conditions [8,16], and to function as a punisher by suppressing responding [7]. Only recently was it reported that nicotine is aversive as tested by the model of conditioned taste aversion [13].

One characteristic of conditioned taste aversion is the preexposure effect in which the conditioning of the taste aversion is attenuated by prior administration of the unconditioned stimulus, the drug. The preexposure effect has been demonstrated for the benzodiazepines, barbiturates, amphetamine, morphine, ethanol, apomorphine, and lithium chloride [5,17]. A preexposure effect of nicotine has not yet been reported.

The purpose of the present experiments was to confirm the recent report that nicotine could condition taste aversion to solutions of saccharin, to characterize the probable site of action of nicotine-induced taste aversion using mecamylamine (central and peripheral nicotinic antagonist) and hexamethonium (peripheral nicotinic antagonist), and to extend these findings by determining the effects of nicotine preexposure on the conditioned taste aversion induced by nicotine in rats.

METHOD

Animals

Two hundred fifty-six male, adult, Sprague-Dawley rats weighing approximately 250 to 300 g at the time of the experiments were temporarily housed in pairs in a quarantine room for 10 days after delivery from the distributor (Harlan Industries, Indianapolis, IN). The animal facility had automatically controlled conditions (lights on at 0700 and off at 1900) and the rats were given free access to food and water.

Procedure

After quarantine, animals were housed individually with free access to Purina rat chow in ceiling-suspended wire-mesh cages. Water was then withheld for 24 to 36 hours. Access to one bottle of tap water was subsequently limited to one 30 min period per day between 1100 and 1200 hours. Water intake stabilized between 6 to 8 ml of water/100 g of body weight after about 10 days of training. Normally, 32 rats were run at one time (4 groups of 8 rats). Experiments commenced only if the mean water intakes of the 4 groups were within 15% of each other for three consecutive days.

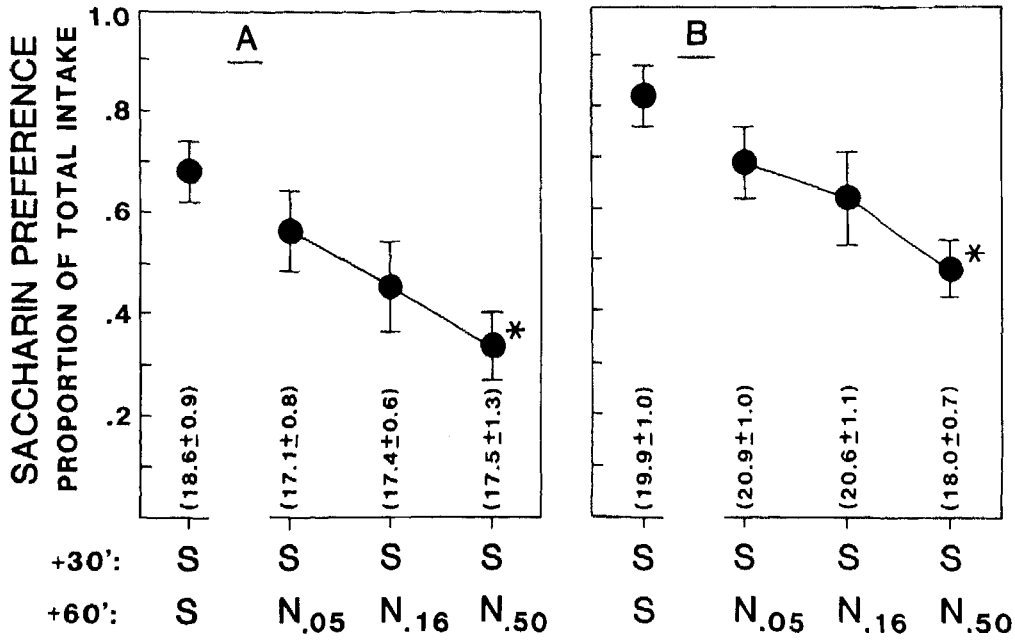

FIG. 1. Effects of nicotine on saccharin preferences in rats. Each point depicts the mean (±1 S.E.) saccharin preference determined on testing day of groups of 8 animals that received an SC injection of saline (S) immediately after removal of the saccharin bottles (t=+30 min) on conditioning day, and injections of either S or nicotine (N, 0.05, 0.16 and 0.50 mg/kg SC) at t=+60 min. Panels A and B represent 2 separate experiments. Saccharin preference is expressed as proportion of total fluid intake. Numbers in parentheses are mean total fluid intakes (±S.E.) for each group on testing day. *Denotes significant difference from S + S control, $p<0.05$, Newman-Keuls multiple comparisons test.

On conditioning day, access to one bottle containing 0.1% (w/v) sodium saccharin was provided during the 30 min drinking session. Immediately after removal of the saccharin solution bottle ("t=+30 min"), saline or various doses (expressed in terms of the salts) of mecamylamine hydrochloride (Sigma Chemical Co.) or hexamethonium hydrobromide (Sigma) were administered subcutaneously (SC). At t=+60 min, saline or logarithmically-spaced doses of nicotine (as nicotine base, Sigma, 0.05, 0.16, 0.5 mg/kg) were administered SC. Twenty-four hr later, on preference testing day, rats were given access to two bottles containing water or saccharin solution for 30 min. "Saccharin preference" was tabulated as: (volume of saccharin solution consumed)/(total volume of fluid consumed).

All drug injections were given in a volume of 1 ml of saline per kg body weight. All testing was performed using two bottle choices between water and 0.1% sodium saccharin. As previously cited [14], two-bottle tests are more sensitive to taste aversions than one-bottle tests [4,11]. During training, the position of the water bottle was alternated between the left and right sides of the front of the cages. During conditioning and testing, the positions of the water bottle and the saccharin solution bottle were randomized between animals in a given group. Fluid intakes were estimated by weighing the water bottle with the curved drink tube on a digital balance before and after the 30 min drinking periods. Animals were used only once.

In experiments testing the effects of nicotine preexposure on nicotine-induced taste aversion, eight groups of 8 rats whose daily water intakes had stabilized were injected SC with either 1 ml/kg of saline or 0.5 mg/kg of nicotine once a day (immediately following the 30 min daily access session to water) for 2 or 4 days. Twenty-four hr, or one week later, access to one bottle of 0.1% saccharin solution was provided for 30 min, and animals injected with either saline or 0.5 mg/kg of nicotine at t=+60 min. Saccharin preference scores and total fluid intakes were calculated as before.

Statistical Analysis

Variability of the saccharin preference scores due to dose effects were analyzed using a one way analysis of variance with regression analysis. Significant differences of mean saccharin preference scores between doses, and differences between means of total fluid intake, were analyzed using the Newman-Keuls multiple comparisons test.

RESULTS

The administration of 0.05, 0.16 or 0.50 mg/kg SC of nicotine on conditioning day caused a dose-related decrease in the proportion of saccharin solution consumed during the preference testing period (Fig. 1A). In the saline-treated group of rats, 68 percent of the total fluid intake was saccharin solution, whereas it was only 33 percent in the group of rats administered 0.5 mg/kg of nicotine. Analysis of variance indicated significant effects of dose, $F(3,28)=4.1$, $p<0.025$, and the slope of the dose-response curve was significant by regression analysis, $F(1,28)=10.2$, $p<0.005$. There were no group differences in total fluid intake. In another experiment (Fig. 1B), nicotine-induced taste aversion was reproduced. Although saccharin preference in the saline-treated group B was about 14% greater than the first experiment, nicotine decreased saccharin preference by the same extent. Again,

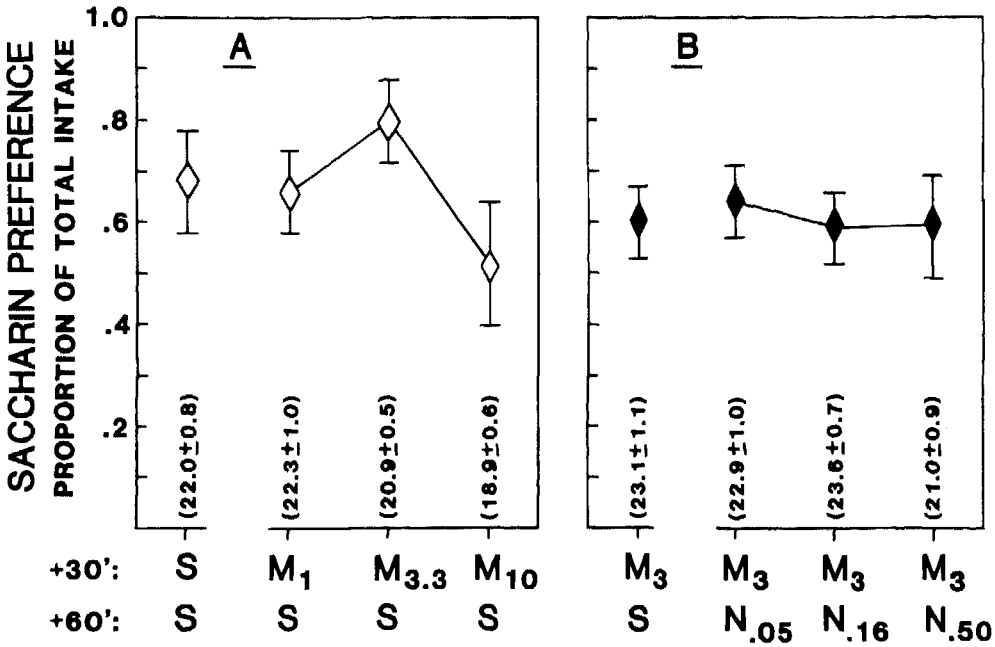

FIG. 2. Effects of mecamylamine on nicotine-induced taste aversion to saccharin in rats. In panel A, each point depicts the mean saccharin preference determined on testing day of groups of 8 animals that received saline (S), or mecamylamine, (M, 1, 3.3 and 10 mg/kg SC) at t=30 min and additional S injection at t=60 min on conditioning day. In panel B, each point depicts the mean saccharin preference of groups of 8 animals that received 3 mg/kg SC of M at t=30 min, and S or nicotine (N, 0.05, 0.16 and 0.50 mg/kg SC) at t=60 min on conditioning day.

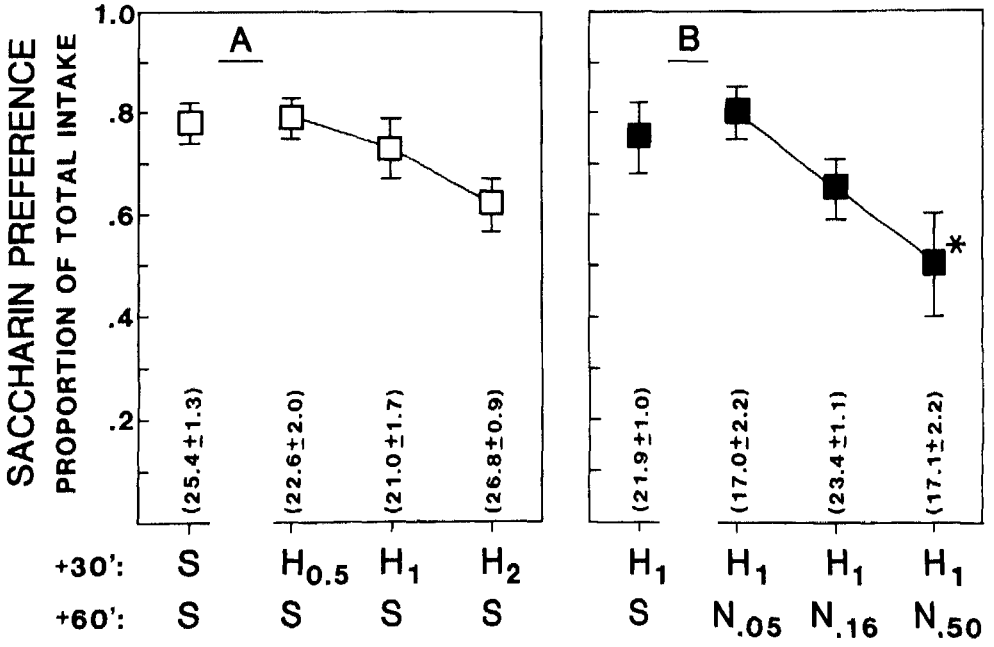

FIG. 3. Effects of hexamethonium on nicotine-induced taste aversion to saccharin in rats. In panel A, each point depicts the mean saccharin preference of groups of 8 animals that received saline (S), or hexamethonium (H, 0.5, 1, and 2 mg/kg SC) at t=30 min and additional S injections at t=60 min on conditioning day. In panel B, each point depicts the mean saccharin preference of groups of 8 animals that received 1 mg/kg SC of H at t=30 min, and S or nicotine (N, 0.05, 0.16 and 0.50 mg/kg SC) at t=60 min on conditioning day.

TABLE 1

THE EFFECT OF PRETREATING RATS WITH NICOTINE ON NICOTINE-INDUCED TASTE AVERSION*

Pretreatment Group	Unconditioned Stimulus: Saline	Unconditioned Stimulus: Nicotine
Saline (4 da)†	0.78 ± 0.05 (25.6 ± 1.3 ml)	0.47 ± 0.08‡ (22.7 ± 1.0 ml)
Nicotine (2 da)†	0.65 ± 0.06 (26.0 ± 0.7 ml)	0.69 ± 0.04 (23.6 ± 1.1 ml)
Nicotine (4 da)†	0.70 ± 0.04 (25.6 ± 0.5 ml)	0.70 ± 0.08 (22.8 ± 1.1 ml)
Nicotine (4 da, conditioned after 1 week)§	0.78 ± 0.04 (24.3 ± 0.7 ml)	0.83 ± 0.06 (22.6 ± 1.1 ml)

*Data represent mean saccharin preference scores (±S.E.M., N=8 rats per group) determined 24 hr after conditioning day. The unconditioned stimuli on conditioning day were saline (1 ml/kg SC) or nicotine (0.5 mg/kg) administered at $t=+60$ min. The numbers in parentheses are mean total volumes of fluid consumed per group ±S.E.M.

†Rats were pretreated with either 1 ml/kg SC saline or 0.5 mg/kg SC of nicotine once a day for 2 or 4 da prior to the conditioning day.

‡Denotes significant difference from saline-pretreated, saline-conditioned group, $p<0.05$, Newman-Keuls multiple comparison test.

§Rats were pretreated with either 1 ml/kg SC saline or 0.5 mg/kg SC of nicotine once a day for 4 days, and conditioned after one week. Saccharin in preference was tested 24 hr later.

the slope of the dose effect was significant, $F(1,28)=11.9$, $p<0.0025$.

Mecamylamine did not systematically alter saccharin preference (Fig. 2A). In groups of rats injected with saline, 1, 3.3, or 10 mg/kg of mecamylamine at t=+30 min on conditioning day, saccharin preference scores on testing day ranged between 0.51 and 0.80. Analysis of variance yielded no dose effects, and the data points deviated significantly from linearity, $F(2,28)=6.3$, $p<0.01$. Total fluid intakes were not altered by the doses of mecamylamine used.

Conditioned taste aversion induced by nicotine was antagonized by mecamylamine (Fig. 2B). In contrast to the decrease caused by nicotine in the amount of saccharin solution consumed (Fig. 1), rats pretreated with 3 mg/kg of mecamylamine still demonstrated a preference for saccharin solution on testing day (Fig. 2B). Analysis of variance did not reveal significant dose effects. Again, total fluid intakes were not altered by any of the treatments. Thus, nicotine-induced conditioned taste aversion was antagonized by pretreating the rats with mecamylamine.

Hexamethonium administration had minimal effects on saccharin preference when given at t=+30 min on conditioning day (Fig. 3A). Although analysis of variance did not reveal significant between treatments variability, analysis of the effects after 0, 0.5, 1 and 2 mg/kg SC of hexamethonium indicated that the data had a linear relationship with significant slope, $F(1,28)=7.4$, $p<0.025$.

Pretreating rats with 1 mg/kg SC of hexamethonium did not antagonize nicotine-induced taste aversion (Fig. 3B). In groups of rats given 1 mg/kg of hexamethonium at t=+30 min and nicotine at t=+60 min on conditioning day, the effect of nicotine dose was significant, $F(3,28)=3.8$, $p<0.025$. The mean saccharin preference scores determined on testing day formed a linear graph with a significant slope as determined by regression analysis, $F(1,28)=10.5$, $p<0.0005$. Thus, hexamethonium, a nicotinic cholinergic receptor blocker which does not cross the blood-brain barrier, does not antagonize nicotine-induced conditioned taste aversion.

The magnitude of nicotine-induced taste aversion was diminished by preexposing the animals to nicotine (Table 1). Three groups of 16 rats were administered saline, or 0.5 mg/kg SC of nicotine, daily for two or four days before conditioning day ("Saline 4 da," "Nicotine 2 da," and "Nicotine 4 da"). Nicotine-induced taste aversion was unaffected by the four day saline preexposure: the proportion of saccharin solution consumed in the saline-preexposed, saline-conditioned group, 78%, was decreased to 47% in the saline-preexposed, nicotine-conditioned group. In contrast, the mean saccharin preference scores of rats conditioned with either saline or 0.5 mg/kg of nicotine were both 0.70 in the nicotine-preexposed group. Similar results were obtained after only pretreating twice ("Nicotine 2 da") with nicotine, and in another group of animals preexposed to nicotine for four days and conditioned one week later (Table 1). The total volumes of fluid consumed were not altered by any of the treatments (Newman-Keuls multiple comparisons test).

DISCUSSION

These data confirm previous findings [13] that SC administered nicotine conditions taste aversions to saccharin in rats in a dose-related manner, and that the site of action for these effects is probably central since mecamylamine-blocked, and hexamethonium did not alter nicotine-induced taste aversion. In addition, our data clearly demonstrate that rats preexposed to nicotine under certain regimens do not exhibit nicotine-induced taste aversion.

In agreement with the recent results of Kumar *et al.* [13] we found that taste aversions could be induced after only one conditioning session with nicotine. It appears that the 30 min conditioning sessions used in this study are more sensitive

than the 15 min sessions used in [13]; 0.5 mg/kg of nicotine was inactive in the study by Kumar whereas we found very significant taste aversions induced by this dose of nicotine in our animals (Fig. 1).

With respect to the nicotine receptor blockers, we found that pretreatment with 3 mg/kg SC of mecamylamine blocked the taste aversion conditioned by 0.05 to 0.5 mg/kg of nicotine in rats (Fig. 2). Kumar *et al.* [13] found that mecamylamine antagonized taste aversion produced by 0.4 mg/kg of nicotine in a dose-related manner from 0.1 to 2 mg/kg. Although these investigators also found that 2 mg/kg of mecamylamine decreased intake of the paired flavored solutions, our data show that mecamylamine at a dose as high as 10 mg/kg does not interfere with saccharin preferences (Fig. 2A). Our results indicating that 1 mg/kg of hexamethonium had no effect on nicotine-induced taste aversion are also in agreement with their study which showed that a high dose of 10 mg/kg of hexamethonium did not modify nicotine flavor aversions. In both of our studies, neither mecamylamine nor hexamethonium induced taste aversions when administered alone. Thus, our present data and those of Kumar *et al.* [13] support the hypothesis that the site of action of nicotine-induced taste aversion is probably the CNS since mecamylamine blocked and hexamethonium had no effect on nicotine's action.

The work by Kumar *et al.* [13] also included another interesting finding: 20 day treatment with mecamylamine before conditioning with nicotine did not alter nicotine-induced taste aversion. They concluded that central nicotinic receptors do not develop increased sensitivity to the effects of nicotine. Thus, the facts that mecamylamine does not induce taste aversions when administered alone (Fig. 2A), and has little effect on nicotine-induced taste aversion after chronic administration [13] suggest that: (1) mecamylamine-induced nicotinic receptor blockade has minimal effects on the mechanisms underlying conditioned taste aversion i.e., mecamylamine has no agonistic effects in this paradigm; and (2) the mechanism(s) underlying the phenomenon of nicotine-induced taste aversion is dependent on the agonistic activity of nicotine in the rat CNS.

One mechanism for conditioned taste aversion has been reviewed [5] which states that the only feature of a drug, the unconditioned stimulus, necessary for conditioning taste aversions is that it induces a "novel" state distinguishable from the drug-naive state. Indeed, some have postulated that all first-time exposures to most drugs are neophobic and aversive [1]. Although the actual mechanism underlying nicotine's induction of this novel state is not known at this time, our data suggests it probably is central in origin (Figs. 2 and 3). Furthermore, our data suggest that tolerance may develop to the "novelty" of the nicotine stimulus, since the ability of nicotine to induce taste aversions diminishes after preexposure to nicotine but not saline injections (Table 1).

Although it now appears that nicotine possesses both rewarding and aversive effects, it is not known if nicotine is positively reinforcing and aversive at the same dosage range and at the same time as has been shown for amphetamine in rats [18]. Also, it is not known what the nature of the interaction(s) between reward and aversion is after nicotine administration. Some have observed that tolerance to the positive reinforcing effects of drugs has not been sufficiently demonstrated experimentally [3]. Thus, the hypothesis may be forwarded that drug dependencies may arise after repeated administration if tolerance develops to the aversive or negative reinforcing effects of drugs but not the positive reinforcing effects. Since we have demonstrated the preexposure effect for nicotine-induced taste aversion which we believe is a form of tolerance, a relationship between nicotine-induced conditioned taste aversion and the etiology of nicotine dependence is suggested. We propose that the loss of novelty via the development of tolerance to nicotine-induced aversion, or the unmasking of the inherent positive reinforcing effects of nicotine as a result of the diminution of the aversive properties of nicotine after repeated exposure, may singly, or together, contribute to the dependence-inducing properties of nicotine.

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