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Conditioned Saccharin Aversions in Rats as a Result of Cutaneous Nicotine or Intraperitoneal Nicotine Administered in Divided Doses

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ETSCORN, F., G. A. MOORE, E. P. SCOTT, L. S. HAGEN, T. M. CATON, D. L. SANDERS AND K. K. DIVINE. *Conditioned saccharin aversions in rats as a result of cutaneous nicotine or intraperitoneal nicotine administered in divided doses.* PHARMACOL BIOCHEM BEHAV **28**(4) 495–502, 1987.—Nicotine base was used in a conditioned taste aversion (CTA) paradigm to avert male Sprague-Dawley rats to saccharin solution (0.1%, w/v). Experiments investigated different dose routes of nicotine administration and duration of action as determinants in nicotine-induced CTA. In Experiment 1 nicotine was injected intraperitoneally (IP) at doses of 0.5, 1.0, or 3.0 mg/kg 30 min after drinking saccharin solution. Using a two-bottle choice test, no CTA was observed, although all nicotine animals showed obvious symptoms of malaise including seizures in the highest dose group. Experiment 2 showed dose-related CTA when nicotine (10.0, 30.0, or 50.0 mg/kg) was cutaneously applied 30 min following saccharin drinking. Experiment 2B showed that the aversions were due to associative rather than nonassociative factors such as sensitization or enhanced neophobia. In Experiment 3, the following group treatments were begun 30 min after saccharin drinking to distribute identical total nicotine doses over an extended period of time: One IP injection of 2.0 mg/kg nicotine (in a saline vehicle) and four injections of saline solution, three injections of 0.67 mg/kg nicotine and two injections of saline, five injections of 0.40 mg/kg nicotine, or five injections of saline. All injections were spaced 30 min apart. Compared with saline-injected controls, CTA occurred in the rats receiving either three or five injections of nicotine but the group receiving one injection did not differ from the control group. There was no difference in CTA between the groups receiving three or five injections.

Conditioned taste aversion Nicotine Cutaneous dosing Drug duration

CONDITIONED taste aversion (CTA) is said to occur when an animal avoids a once palatable fluid that has previously been paired with delayed drug injection, rotational stimulation, or X-irradiation [3]. Nicotine has recently attracted the attention of CTA researchers for several reasons. Nicotine is self-administered by more people than any other drug, it is a very toxic convulsant (40–60 mg may be lethal in a human), it is very addictive due to pharmacological as well as behavioral factors [27], and it is a potent stimulant of the chemoreceptor triggering zone of the area postrema of the medulla, the locus of the vomiting center [21,32].

In two small-sample studies with each using one acquisition trial, mice showed CTA to sucrose solution (20%, w/v) when it was followed by either an intraperitoneal (IP) injection of nicotine base (2.0 mg/kg in a saline vehicle) or confinement for 4 minutes in an enclosure containing concentrated tobacco smoke [15]. In both studies, the interstimulus interval was 15 min and two-bottle choice tests were used to test for CTA.

Studies using rats have also shown nicotine-induced CTA. For example, Kumar, Pratt and Stolerman [31] demonstrated CTA using nicotine bitartrate (0.008, 0.08, and 0.8 mg/kg) injected subcutaneously (SC) in the animals' flank immediately after having drunk either sodium chloride (0.9%, w/v) or sodium saccharin solution (0.1%, w/v). More than one acquisition trial was required for CTA in all groups except the highest dose group. They tested for CTA using a sensitive technique which combined one-stimulus and two-stimulus tests [7]. In another study, Iwamoto and Williamson [26] paired saccharin solution (0.1%, w/v) with delayed (60 min) SC injections (at an unspecified location) of either 0.05, 0.16, or 0.50 mg/kg of nicotine base in a 0.9% saline solution vehicle. After one acquisition trial, CTA was shown using two-bottle choice tests in the 0.50 mg/kg group but not in the other dose groups.

The purpose of the present series of studies was to determine if the route of nicotine administration is a factor in the acquisition of nicotine-induced CTA. For example, the SC route generally produces a slower onset of drug action with a longer duration when compared to IP with its fairly rapid rate of absorption due to the large absorbing surface provided by the organs of the peritoneal cavity [18]. Moreover, different locations of SC dosing (nape of neck versus flank) could result in different amounts or concentra-

tions of drug reaching the brain due to such factors as blood dilution and/or routing the drug first pass through the liver where the majority of nicotine is metabolized. Finally, we wanted to investigate the cutaneous route of drug administration which is a novel drug route for producing CTA learning.

EXPERIMENT 1

METHOD

Subjects

The subjects were 32 naive male TEX:(SD)AM rats (100–125 g) obtained from Timco Breeding Laboratories of Houston, TX. The animals were individually housed in galvanized steel cages (Wahmann, No. LC75a, 20.5 cm W × 18 cm H × 24.5 cm D) suspended over sterilized hardwood bedding ("Sani-Chips"). Each cage was equipped with a stainless steel bottle holder/food hopper (Wahmann No. LC303S). The hoppers contained ad lib Wayne Lab Blox for the duration of the study. Lab temperature was maintained at 26°C with overhead fluorescent lights cycled on at 0700 hr and off at 1900 hr. White noise was on constantly.

Apparatus

Calibrated drinking bottles [16] were constructed as follows: Stainless steel sipper tubes (Wahmann No. LC312, 6.4 cm long) were machined to an opening of 0.125", buffed smooth, and fitted into No. 3 rubber laboratory stoppers. The stoppers with tubes were then fitted into 30 cc calibrated (to 1 cc) plastic syringes which had been sawed through at the "0 cc" mark. Finally a No. 1 rubber stopper was fitted over each tube and pushed flush with the No. 3 stopper. The No. 1 stopper prevented a tube from protruding into a cage more than 2.0 cm. The drinking bottles were affixed to the cages using modified Wahmann bottle holder/food hoppers which provided constant cage insertion angles for a pair of drinking bottles as well as angling the sipper tubes toward one another. When a pair of drinking bottles were inserted into a cage (at 30° below horizontal), the spout tips were approximately 1 cm apart. Different sets of drinking bottles and plastic washing/storage containers were used for flavored and unflavored water. The bottles were soaked overnight in covered containers and repeatedly rinsed before and after each use.

Procedure

On arrival from the supplier, all rats were placed in individual cages and allowed 5 days of ad lib tap water before starting the experiment. At 1400 hr on the first day of the study all rats were deprived of water until the same time on the next day when each animal was introduced to 8 days of baseline drinking conducted as follows: Using 20 sec intervals, a pair of drinking bottles (one filled with tap water, the other empty) was placed in the bottle holder of each cage using a counterbalanced sequence across days for the cage position of the full bottles (LRRLRLLR, relative to *E*). After 15 min access, the drinking bottles were removed (on the 20 sec schedule), and the amounts consumed were determined and recorded for each animal.

On conditioning day (Day 9) at 0900 hr all animals were weighed in order to compute drug doses. They were then assigned at random to four treatment groups (see Table 1). At 1400 hr on the same day all rats were given 15 min access to two drinking bottles filled with saccharin solution (0.1% w/v, purified sodium saccharin in tap water, Fisher Scientific, No. S-3). At the end of the drinking period the bottles were removed, and the amounts consumed were determined and recorded. Subsequent to a 30 min delay following bottle removal, the animals were injected IP with either 0.9% saline, 0.5, 1.0, or 3.0 mg/kg of nicotine base in a 0.9% saline vehicle. The injections were given on an equivolume basis with a 140 g rat receiving 0.50 ml. New 25 gauge, 0.5" needles were used for each rat. Nicotine base in 5 ml bottles was obtained from Sigma Chemical Co. (No. N-3876) and stored under refrigeration. It was allowed to reach room temperature before use. The nicotine was not pH adjusted. Physiological saline was injected at a volume equal to the mean (0.5 ml, SEM=0.1) of all nicotine injections. A 20 sec injection interval was followed by having two experimenters removing rats from their home cages and restraining the animals for their injections, one experimenter drawing up the nicotine and administering the injections, one experimenter monitoring time, and one experimenter verifying dosage, drug, and animal number. Following injections the animals were observed for 90 min.

Days 10 and 11 were recovery days in which the animals were returned to baseline drinking conditions.

On Days 12 and 13 all animals were tested for aversions by giving them 15 min choice tests using two drinking bottles: One filled with saccharin solution, the other filled with unflavored water (saccharin was on the left side of all cages for the first test day and reversed for the second).

TABLE 1

SACCHARIN PREFERENCE SCORES AS A FUNCTION OF INJECTED NICOTINE OR SALINE

Groups*	Collapsed Saccharin Preference Scores Test Days 1–2
Saline	81 (±3.9 SEM)
0.5 mg/kg	77 (±4.8)
1.0 mg/kg	74 (±6.3)
3.0 mg/kg	72 (±5.5)

Note: Preference scores were collapsed by averaging the two scores for each animal (±standard error of the mean).
*n=8.

RESULTS AND DISCUSSION

Prior to statistical analyses each animal's choice test data was converted to a saccharin preference score by dividing the amount of saccharin solution consumed by the total amount of fluid consumed and multiplying by 100. A score of 100 would therefore indicate that the animal drank only saccharin solution while 0 would indicate that only plain water was consumed.

The two scores for each animal were collapsed by averaging to balance the potential effects of any position habits. A one-way ANOVA was computed on the collapsed preference scores across the four groups with no reliable differences found, $F(3,28)<1$. The groups likewise showed no difference when comparing amounts of plain water consumed on the last baseline day, the amounts of saccharin solution consumed on conditioning day, or the amounts of plain water consumed on the two recovery days, $F(3,28)<1$, in each case. On conditioning day, animal weights averaged

142 g (SEM=2.0) and ANOVA indicated no significant difference between groups, $F(3,28)<1$.

Although no CTA was demonstrated in this study (see Table 1), the post-injection behavior of the animals receiving nicotine suggested that they were indeed sick. The 1.0 and 3.0 mg/kg animals became ataxic with fine motor tremors within 2 min post-injection, and the 3.0 mg/kg animals convulsed within 5 min post-injection. Prostration in both groups lasted for up to 45 min. Other symptoms in all dose groups included ptosis, diarrhea, occasional retching, circling, wet dog shakes (WDS), and labored breathing. By 60–90 min, the severity of all observable symptoms had subsided in even the 3.0 mg/kg group. Other toxic convulsants at subconvulsive doses have likewise failed to induce CTA even though pronounced symptoms of malaise were clearly evident. Ahlers and Best [1] and Millner and Palfai [34] using metrazol reported no CTA and Berger [6] and Vogel [38] likewise failed to demonstrate CTA using strychnine.

The absence of CTA in the 3.0 mg/kg animals (which all convulsed) could have been due to a retrograde amnesia memory deficit. Metrazol-induced convulsions have been effective for blocking apomorphine-induced saccharin aversions when the convulsions were elicited either within or after the taste-illness interval [1]. Similar effects have been demonstrated using electroconvulsive shock [29,30]. The question remains as to why the 0.5 and 1 mg/kg groups showed no CTA with one conditioning trial although they showed obvious and severe symptoms of malaise.

The delay following the termination of saccharin drinking before commencing injections could have been shortened from 30 min, as used in the present study, to no delay; however, to have done so could have introduced a confound biasing the study in favor of showing CTA. As saccharin was used for the taste cue in the present study, the potential existed that lingering aftertastes could have "bridged" a very short delay (or no delay) separating the termination of saccharin drinking from the nicotine injections. Also previous research has indicated that the difference between no delay and a 30 min delay would be minimal at best in terms of the strength of an aversion [19]. Indeed the hallmark of CTA is the effectiveness of conditioning with very long delays separating drinking of the flavored fluid and the ensuing drug, radiation, or rotational treatment.

Although nicotine is highly alkaline (pH 10.2), which could have produced irritation of the peritoneum (we did not pH adjust our nicotine in order to compare the present studies with our previous research), no writhing or squealing was observed which is usually indicative of such irritation [18].

Goodman and Gilman [21] indicate that, "Nicotine is readily absorbed not only from the oral and gastrointestinal mucosa and from the respiratory tract but also from the skin. Indeed, severe poisoning has resulted from percutaneous absorption" (p. 568). Such findings suggest that if CTA could be established via cutaneous administration of nicotine, then duration of action may be a significant factor in producing one trial nicotine-induced CTA. The cutaneous route generally offers a delayed peak action and prolonged duration of action as a result of slower absorption compared to the IP route. Earlier work using a small sample of rats and a within-subjects design showed that dose-related CTA to saccharin solution could be produced using cutaneous nicotine after a 30 min delay in doses ranging from 4 to 40 mg/kg [17]. More recent research has involved the cutaneous application of nicotine to humans as a potential substitute therapy to aid in tobacco cessation [36,37]. The purpose of Experiment 2 was to determine if cutaneously absorbed nicotine could produce one trial CTA in rats at subconvulsive doses.

EXPERIMENT 2

METHOD

Subjects

The subjects were 32 naive male rats (same strain, weight range, and supplier as in Experiment 1).

Apparatus

The apparatus was identical to that used in Experiment 1.

Procedure

Baseline drinking measures were obtained for all animals for a total of 8 days. On conditioning day (Day 9) at 0900 hours all animals were weighed in order to compute drug doses. At 1400 hours on the same day all rats were given 15 min access to two drinking bottles filled with saccharin solution (0.1%, w/v). Following 15 min of drinking the bottles were removed and a 30 minute delay was imposed; after which, dosing commenced. Based on random assignment, four groups of eight rats were administered nicotine base or normal saline solution (0.9%) directly to their skin using an adjustable micropipette. The doses of nicotine base as determined by pilot studies were either 10.0, 30.0, or 50.0 mg/kg. Our pilot work consisted of single-subject CTA using the same concentration of saccharin solution and delay as in the present studies but with doses ranging from 5 to 150 mg/kg. The average dose volume for all groups receiving nicotine in the present study was 3.4 microliters and this value was selected for the volume of saline administered to each control animal. The site of application on the unshaved animals was approximately 2.5 cm posterior from a point midway between the ears. Dosing was performed by restraining each rat in a cloth towel, exposing the neck region, brushing the hair rostrally at the site of application, and pipetting the respective agent on the skin. This site was chosen because it is a difficult area for the rat to groom and cutaneous delivery of nicotine to a human in this lab found that the drug produces a slight "tingling" sensation at the point of application. If this "tingling" occurred in our rats, it could cause the animals to attempt to lick or groom the area possibly causing oral ingestion of nicotine. In our studies we have observed no grooming attempts or other behaviors to suggest that the nicotine produces any acute skin irritation. Observations of gross behavior continued for three hours after drug dosing. Following two recovery days (as in Experiment 1), the animals were tested for six days using 15 min, two-bottle (plain water and saccharin water) choice tests with the saccharin bottles placed in the cages across days according to a RLLRRL counterbalance.

RESULTS AND DISCUSION

The choice-test data were collapsed by averaging successive pairs of test day preference scores for each animal in order to balance the effects of position habits.

A one-way ANOVA with replications indicated a significant dose effect, $F(3,28)=1445$, $p<0.0001$, trials effect, $F(2,52)=32.2$, $p<0.0001$, and dose by trials interaction, $F(6,52)=4.4$, $p<0.01$. Scheffe's test indicated that for Test Days 1 and 2 (collapsed) significant ($p<0.05$) differences oc-

TABLE 2

MEAN SACCHARIN PREFERENCE SCORES AS A FUNCTION OF CUTANEOUS NICOTINE OR SALINE

	Collapsed Saccharin Preference Scores		
Groups*	Test Days 1–2	Test Days 3–4	Test Days 5–6
Saline	80 (±2.9)	82 (±1.8)	81 (±3.0 SEM)
10 mg/kg	66 (±3.2)	76 (±3.0)	79 (±2.4)
30 mg/kg	39 (±6.0)	61 (±6.8)	71 (±5.3)
50 mg/kg	27 (±4.9)	50 (±8.6)	58 (±7.6)

Note: Preference scores were collapsed by averaging successive pairs of scores for each animal (±standard error of the mean).
*n=8.

curred between the saline group and the 30.0 and 50.0 mg groups as well as between the 10.0 mg group and the 30.0 and 50.0 mg groups. Analysis of Test Days 3 and 4 indicated reliable differences between the saline group and 50.0 mg group and between the 10.0 mg group and 50.0 mg group. Statistical analyses for Test Days 5 and 6 were identical to Test Days 3 and 4.

One-way ANOVAs were computed across the four groups for the amounts of plain water consumed on the last day of baseline drinking, for the amounts of saccharin solution consumed on conditioning day, as well as for the amounts of plain water consumed on Recovery Days 1 and 2 with no differences found, $F(3,28)<1$, in each case. On conditioning day, animal weights averaged 137 g (SEM=1.28) and ANOVA indicated no difference between groups, $F(3,28)<1$.

As can be seen in Table 2, dose-related CTA was demonstrated using 10.0, 30.0 and 50.0 mg/kg of cutaneously applied nicotine base. The onset of distress as measured by ataxia and fine motor tremors occurred within two minutes of drug administration which, in terms of latency, was virtually identical to nicotine delivered IP; however, compared to the IP nicotine the duration of prostration from cutaneous administration was considerably longer with a duration of at least 3 hours. No seizures were observed in any of the animals. Ptosis was observed in all dose groups except for the 10.0 mg/kg rats, and all nicotine animals showed an occasional retch. Several of the rats in the two high-dose groups also showed WDS. Pilot studies using same sex and same size Fisher rats given cutaneous nicotine (10, 30, 50 mg/kg) produced WDS in all animals as well as elevated activity levels compared to Sprague-Dawley rats.

EXPERIMENT 2B

When investigating a novel method of drug administration or the use of a new drug in an attempt to produce CTA, controls may be necessary to determine if nonassociative effects are producing the observed changes in preference for a flavored fluid. Such nonassociative effects have been termed sensitization [35] or enhanced neophobia [11]. "Sensitization occurs if presentation of the punishment without prior emission of the response reduces the subsequent probability of the response" ([35], p. 16). For determining sensitization effects in CTA, unflavored water is paired with delayed treatment such as drug injection, X-radiation, or rotational stimulation. Novel saccharin solution is then offered 24 or 48 hr later. If the animals reduce their consumption of the novel saccharin solution compared to animals not subjected to one of the delayed treatments, then sensitization is said to have occurred. The following study was therefore undertaken to determine if enhanced neophobia or sensitization could account for the findings of Experiment 2 instead of associative learning processes.

METHOD

Subjects

The subjects were 30 naive male rats (same strain, weight range, and supplier as in the previous studies).

Apparatus

The apparatus was identical to that used in Experiments 1 and 2.

Procedure

The procedure, times, and sequencing for this three-group study were essentially identical to those used in Experiment 2: 2 weeks of laboratory habituation with ad lib water, 8 days of Baseline Drinking, 1 Conditioning Day, 2 Recovery Days and 4 Test Days. All drinking sessions consisted of 15 min access per animal per day. On conditioning day all animals were weighed at 0900 hours and treatments began at 1400 hours. Conditioning day for the three groups of ten animals consisted of the following treatments: Group 1 animals received 15 min access to a 0.1% saccharin solution in both drinking bottles and following a 30 min delay they were given a 30 mg/kg dose of cutaneous nicotine. Group 2 was treated identically as Group 1 with the exception that instead of nicotine dosing, Group 2 received cutaneous saline (0.9%). Group 3 received 15 min access to unflavored water in both drinking bottles followed 30 min later by 30 mg/kg of cutaneous nicotine. The animals were then given two recovery days which constituted a return to baseline drinking conditions. The next four days were test days with each animal having simultaneous access to two drinking bottles: One filled with saccharin solution and the other filled with unflavored tap water. The cage position of the drinking bottle containing saccharin solution for each animal followed a RLLR counterbalance for the four test days.

RESULTS AND DISCUSSION

Saccharin preference scores were computed, and successive pairs of scores for each animal were collapsed by averaging to balance position habits.

A one-way ANOVA with replications indicated a significant dose effect, $F(2,54)=14.47$, $p<0.0001$, and a significant trials effect, $F(1,54)=6.40$, $p<0.05$. The dose by trials interaction was not significant, $F(2,54)<1$. Scheffe's test indicated that for Test Days 1 and 2 (collapsed) a significant difference ($p<0.05$) existed between the Saccharin/Saline group and the Saccharin Solution/Nicotine group. No other comparisons were statistically reliable. The same statistical outcome ($p<0.05$) was obtained for Test Days 3 and 4 (collapsed).

The animals' weights on conditioning day averaged 211 g (SEM=4.28), and there was no significant difference in body weights between groups, $F(2,27)<1$, ANOVA. The Plain Water/Nicotine group (sensitization controls) drank an average of 17.6 ml (SEM=0.85) of water on conditioning day. Saccharin Solution/Saline and Saccharin Solution/Nicotine groups drank an average of 18.9 ml (SEM=0.64) and 19.1 ml (SEM=0.92), respectively. Likewise the groups did not dif-

TABLE 2B

MEAN SACCHARIN PREFERENCE SCORES AS A FUNCTION OF CUTANEOUS NICOTINE OR SALINE

Groups[a]	Collapsed Saccharin Preference Scores: Test Days 1–2	Test Days 3–4
Saccharin Solution/ Saline	82 (±5.0)	84 (±4.2 SEM)
Saccharin Solution/ Nicotine	37 (±8.7)*	57 (±9.5)*
Unflavored Water/ Nicotine†	61 (±7.8)	81 (±4.1)

Note: Preference scores were collapsed by averaging successive pairs of scores for each animal (±standard error of the mean).

*Indicats a significant ($p<0.05$) difference from their respective controls (Saccharin Solution/Saline).

†Sensitation control group.

[a]n=10.

fer in their drinking of plain water on the last baseline day or in plain water consumed on each of the two recovery days, $F(2,27)<1$ in each case, ANOVA.

As can be seen from the preference scores in Table 2B, CTA occurred in the group receiving saccharin solution followed by cutaneous nicotine when compared to the group drinking saccharin followed by cutaneous saline solution. The sensitization control group (unflavored water followed by cutaneous nicotine) showed a nonsignificant trend suggestive of nonassociative effects; however, this trend is nonexistent by the second pair of collapsed preference scores. Others have likewise noted that enhanced neophobia or sensitization effects are short-lived [11,14].

Drugs with relatively short half-lives have produced conflicting results when used as unconditional stimuli in CTA learning. For example, cocaine, which has a biological half-life ($t_{1/2}$) in humans of approximately 20–30 minutes, has in one instance produced CTA learning in rats [22], but failed in other attempts [9,10]. When positive results were obtained with cocaine [22] the CTAs as noted by the authors were weaker on several measures when compared to effective doses of other drugs. These findings could be due to such effects as lipid solubility of the particular drug, rapidity of drug onset, or duration of drug action. Researchers are undecided as to whether duration of drug action is a potent variable in CTA acquisition [7, 9, 10, 12, 13, 23, 24]. Experiment 3 was undertaken to determine if duration of action is a factor for nicotine-induced CTA. The biological half-life of IV (intravenous) nicotine in humans is approximately 120 min [4] and in the rat 60 min [33], making it an appropriate drug for such a determination. Accordingly, IP nicotine injections providing identical total doses (2 mg/kg) were systematically distributed (fractionated) over a 2-hr period in order to extend the duration of drug action.

EXPERIMENT 3

METHOD

Subjects

The subjects were 28 naive male rats of the same strain, weight range, and supplier as in the previous experiments.

Apparatus

The apparatus was identical to that of the previous experiments.

Procedure

Following 8 days of laboratory habituation with ad lib water, baseline drinking measures were obtained for a total of 6 days. On conditioning day (Day 7) at 0900 hours all animals were weighed in order to compute drug doses. At 1400 hours on the same day all rats were given 15 min access to two drinking bottles containing saccharin solution. After drinking, the bottles were removed and the amounts consumed were determined and recorded. A 30 min delay was then imposed; after which, dosing began. According to random assignment, four groups of rats (see Table 3) received the following treatments: Five injections of normal saline; one injection of nicotine base (2.0 mg/kg) followed by four injections of normal saline; three injections of nicotine base (0.67 mg/kg per injection) followed by two injections of saline; or five injections of nicotine base (0.40 mg/kg per injection). The total dose of nicotine for all experimental animals was 2.0 mg/kg, and all injections (saline or nicotine) were given at 30 minute intervals. As in Experiment 1, the nicotine was not pH adjusted. Injections were administered according to a quadrant schedule whereby the first injection was placed in the upper right quadrant of the abdomen (relative to the animal) and rotated clockwise with succeeding injections. The fifth injection for all rats was in the original quadrant. The saline injections were 0.5 ml and the nicotine injections were adjusted with saline solution to produce 0.5 ml volumes. Following injections, the animals were observed hourly until symptoms of malaise abated.

Days 8 and 9 were recovery days as in the previous experiments.

Days 10–13 were test days using two-bottle choice tests with the saccharin bottles placed in all cages according to a RLLR counterbalance across days.

RESULTS AND DISCUSSION

Saccharin preference scores were computed, and successive pairs of scores for each animal were collapsed by averaging to balance position habits.

A one-way ANOVA with replications indicated a significant dose effect, $F(3,48)=11.30$, $p<0.0001$, and a significant trials effect, $F(1,48)=23.27$, $p<0.0001$. The dose by trials interaction was not significant, $F(3,48)<1$. Scheffe's test indicated that for Test Days 1 and 2 (collapsed) reliable differences ($p<0.05$) occurred between the saline group and the groups receiving either three or five nicotine injections; likewise, there were reliable differences between the group receiving one nicotine injection and the groups receiving either three or five nicotine injections. There were no significant comparisons obtained for the collapsed data for Test Days 3 and 4.

The animals' weights on conditioning day averaged 132 g (SEM=1.49) and there was no significant difference in body weights between groups, $F(3,24)<1$, ANOVA. Also the groups did not differ with respect to plain water consumption on the last baseline day, saccharin solution consumed on conditioning day, or plain water consumed on each of the two recovery days, $F(3,24)<1$ in each case, ANOVA.

As can be seen in Table 3, CTA occurred as a result of administering the same total dose (2.0 mg/kg) of nicotine

TABLE 3

MEAN SACCHARIN PREFERENCE SCORES AS A FUNCTION OF EXTENDING THE DURATION OF ACTION OF INJECTED NICOTINE

Groups*	Collapsed Saccharin Preference Scores	
	Test Days 1–2	Test Days 3–4
5 Saline Injections	78 (±4.0)	90 (±3.0 SEM)
1 Nicotine/4 Saline Injections	69 (±6.7)	82 (±6.8)
3 Nicotine/2 Saline Injections	43 (±3.5)	68 (±6.9)
5 Nicotine Injections	47 (±5.5)	73 (±6.6)

Note: Preference scores were collapsed by averaging successive pairs of scores for each animal (±standard error of the mean). Animals receiving 1 nicotine injection (2 mg/kg) also received 4 saline injections, those receiving 3 nicotine injections (0.67 mg/kg per injection) received 2 saline injections, and those receiving 5 nicotine injections (0.20 mg/kg per injection) received no saline injections. Nicotine animals received a total dose of 2 mg/kg and injections for all groups were spaced 30 min apart.

*n=7.

over an extended period of time. Aversions occurred in the groups receiving either three or five nicotine injections compared to the saline-injected controls. The group experiencing one nicotine injection did not differ from the saline group. Finding no CTA after one acquisition trial of saccharin drinking followed by a 2.0 mg/kg IP injection of nicotine essentially supports the results for the 1.0 and 3.0 mg/kg nicotine groups in Experiment 1. The CTA in all groups had extinguished by the final pair of test days suggesting that one-trial, nicotine-induced CTA is not as resistant to extinction as CTA produced by such drugs as lithium chloride or cyclophosphamide. Finally the data suggest that extending the duration of nicotine-induced toxicosis past a certain period produces no further increase in the strength of the CTA. A possible explanation for this finding is that in humans nicotine is capable of producing an acute form of tolerance called tachyphylaxis [25]. Whether such tolerance occurs in rats to the pharmacological effects of nicotine necessary to produce CTA is not known.

GENERAL DISCUSSION

Rats given a single CTA acquisition trial consisting of drinking saccharin solution followed 30 min later by IP injections of nicotine (0.5, 1.0 and 3.0 mg/kg) did not differ in choice-test saccharin preferences from saline-injected controls (Experiment 1). A single acquisition trial using cutaneous nicotine (10.0, 30.0 and 50.0 mg/kg) produced dose-related CTA to saccharin solution (Experiment 2). In Experiment 2B, no long-term enhanced neophobia or sensitization was evidenced suggesting that nonassociative factors could not account for the findings of Experiment 2. Finally, when the total IP dose of nicotine (2.0 mg/kg) was administered in divided doses at 30 min intervals for up to 2 hr, CTA was observed in the groups receiving either three doses of 0.67 mg/kg per injection or five doses of 0.40 mg/kg per injection. The group receiving a single 2.0 mg/kg dose evidenced no CTA compared to the saline-injected controls (Experiment 3). Even though duration was manipulated in Experiment 3, the intensity of the drug effect may have varied as a result of the individual dose differences (2.0, 0.67 or 0.40 mg/kg per injection) albeit the total dose administered across groups was identical.

Experiment 1 was a replication of an unpublished pilot study using larger male animals (mean of 302 g) and smaller treatment groups (5 per cell) with identical methods and procedures. Nicotine doses were 0.5, 1.0, 3.0, and 5.0 mg/kg. The 5.0 mg/kg dose produced profound seizures in all rats, and for this reason, the upper dose in Experiment 1 was limited to 3.0 mg/kg. While 3.0 mg/kg produced seizures in all of the animals, no seizures were induced by 1.0 mg/kg. In the previous pilot study as well as in Experiment 1, no CTA was demonstrated. Other things being equal, it should be expected that higher doses of any drug would result in a higher probability of CTA than smaller doses of the same drug. Experiments 1 and 2 obviously differed in this respect; however, the upper limit of IP nicotine in Experiment 1 (3.0 mg/kg) produced seizures in all animals and exploring higher doses would have yielded confounded results due to the possibility of retrograde amnesia blocking any CTA.

Experiment 2 has been replicated twice using doses ranging from 3.0 to 110 mg/kg and identical saccharin concentrations and delays with CTA observed for all doses except 3.0 mg/kg. All of our cutaneous nicotine work following the present series of studies have used animals with an area of approximately one square inch shaved at the intended site of application with electrical animal clippers. In animals with intact fur, we have noted that some of the nicotine is "wicked" away from the skin by capillary action of the fur. As the nicotine on the skin is absorbed, the drug remaining on the fur is then drawn back to the skin where it is absorbed. Apparently some of the nicotine either evaporates, is deactivated by light and/or oxygen, or is absorbed by the hair since doses of approximately 50 mg/kg are quite toxic (but subconvulsive) in animals with shaved skin. We have not observed convulsions in animals with intact fur even at a dose of 110 mg/kg. CTA pilot work with the same procedure as in the present cutaneous studies but with a patch of fur removed on the back of the neck with a cream depilatory ("Nair") apparently affects skin permeability to such an extent that previously tolerable doses of cutaneous nicotine became highly toxic (seizures, death). We have enhanced the absorption of cutaneous nicotine using 1 microliter of dimethylsulfoxide (DMSO) applied immediately before nicotine dosing; however, the increase in drug uptake produced extreme toxicity in animals at doses that had been tolerated in same size and same sex rats. We are currently exploring pharmacological means of retarding the cutaneous absorption of nicotine in rats.

A striking feature of our nicotine-induced CTAs is the rapidity with which extinction occurs especially in light of the obvious and profound symptoms of nicotine malaise during conditioning. A typical finding using such treatments as lithium chloride, cyclophosphamide, or X-irradiation is that CTA learning is quite resistant to extinction especially when two-bottle choice tests are used. Humans generally have a negative experience with their first use of tobacco; yet, the vast majority of novice users quickly escalate their consumption suggesting that the negative consequences produce litte if any avoidance learning. The distinct (and novel) flavor of tobacco coupled with the toxicity of nicotine would seem to be an ideal situation for producing CTA to the taste of tobacco; however, it is general knowledge that such is not the case.

The question of why such a presumably adaptive response as CTA fails to occur under conditions of extreme toxicity from IP nicotine, but does occur with cutaneously applied nicotine or with IP nicotine given in divided doses over time, suggests that duration of action may be a variable in nicotine-induced CTA. Many factors contribute to what is generally termed "drug effects" such as latency, duration, time to peak activity, biological/behavioral activity (e.g., depressant, stimulant, convulsant), active/inactive metabolites, and intensity of effects. Drugs administered IP will first be translocated to the liver via hepatic portal circulation and in humans 80–90% of nicotine deactivation occurs in the liver [21]. The possibility exists that with only one acquisition trial and with the nicotine doses used, the duration of illness from the IP route is just not long enough to produce CTA. This speculation is somewhat supported by the findings of Experiment 3. Increasing the doses of IP nicotine beyond those reported in this paper is essentially limited by the extreme systemic toxicity produced by this drug. The subcutaneous route via the back of the animal's neck [5] as well as the cutaneous route at the back of the neck both allow entry into systemic circulation and transport to the brain before passage through the liver, thus possibly producing a stronger initial effect with perhaps a longer duration of action. Finally, the nicotine which survives first pass through the liver from any route of administration may enter the stomach via the bloodstream to be "trapped" at a pH of approximately 1.0. Current work in this lab using gas chromatography and mass spectroanalysis is concerned with determining blood levels of nicotine as a function of time following various dose routes.

The results of Experiment 2 demonstrate that cutaneously applied nicotine in doses of 30–50 mg/kg is capable of producing blood levels in the rat sufficient to produce CTA. As the permeability of human skin and rat skin is somewhat similar [25], the present findings are suggestive of a technique and potential therapy for use in tobacco cessation in humans, i.e., transdermal application of nicotine via an occlusive patch (U.S. Patent No. 4,597,961) in doses sufficient to suppress craving and withdrawals. Others are similarly exploring the therapeutic potential of this technique [36,37]. The growing evidence supporting the negative health consequences of tobacco use has prompted the study of pharmacological methods for aiding people in eliminating the dependence by administering nicotine in a form other than in a tobacco product. The rationale for these techniques is the assumption that nicotine is the agent responsible for maintaining the addiction [2] and if nicotine can be administered in a fashion other than via smoking, then a possible substitution therapy could be devised which in addition would be less physically damaging than smoking. By reducing or eliminating smoking as the means of administering nicotine, tars (carcinogens), carbon monoxide (cardiac damage), and powerful secondary reinforcers maintaining the habit would be eliminated. Consequently, nicotine tablets [28], unsmoked vaporized nicotine [20], and gum [8] have been developed with varying degrees of effectiveness. The gum however has been determined effective enough to warrant its recent marketing under the trade name, Nicorette.

Cutaneous administration of lipid soluble drugs or drugs capable of being transported across the skin via transporting agents may offer behavioral researchers a useful tool for repeated drug dosing (for inducing tolerance and/or dependence) which would be far less stressful than needle puncture or gastric intubation. Finally the cutaneous technique offers the potential of varying the rate of uptake and duration of action via vehicles which either enhance or retard absorption.

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