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Saccharin Aversions in Hamsters as a Result of Nicotine Injections[1]

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ETSCORN, F , G A MOORE, L S HAGEN, T M CATON AND D L SANDERS *Saccharin aversions in hamsters as a result of nicotine injections* PHARMACOL BIOCHEM BEHAV **24**(3) 567–570, 1986 —Golden Syrian hamsters (males, N=70) showed dose-related conditioned taste aversion (CTA) when saccharin drinking was followed by delayed nicotine injections Baseline consisted of measuring amounts consumed after 20 minutes of daily access to tap water Measures were taken for five days The hamsters were then conditioned by offering them saccharin solution (0 1%, w/v) for 20 minutes, afterwhich a 30 minute delay was imposed Subsequent to the delay, groups of 10 animals were treated as follows nicotine injection (1 0, 3 0, or 9 0 mg/kg, IP), saline injection, lithium chloride injection (2% body weight of a 0 15 M solution), sham injection, or left in their cages as handling/stress controls Following two recovery days with plain water available for 20 minutes, all animals were tested for CTA by offering them saccharin solution Dose-related CTA was demonstrated in the nicotine animals as measured by a decrease in saccharin consumption compared to drinking measures obtained from animals injected with saline Lithium chloride produced the same degree of CTA as 9 mg/kg of nicotine, and the aversions had extinguished in all groups by the third test day

Hamsters Nicotine Flavor aversion

AN animal can be made to avoid a once-palatable fluid if that fluid has been conditionally paired with delayed drug injection, rotational stimulation, or X-irradiation [1] Such learning has been termed conditioned taste aversion (CTA)

Nicotine, which is used more frequently than any other psychoactive drug, is highly toxic with 40–60 mg being potentially fatal in humans [8] Nicotine is also a powerful stimulant of the chemoreceptor triggering zone (CTZ) in the area postrema of the medulla which is the locus of the vomiting center In addition, the drug has a relatively short half-life in the rat of from 0 92 to 1 10 hr following IV administration [13] In humans the same value ranges from 20 to 30 min

In a small-sample study, Swiss-Webster mice showed CTA to a 20% (w/v) sucrose solution when it was followed by either a delayed intraperitoneal (IP) injection of nicotine base (2 mg/kg in a saline vehicle) or when forced to breathe concentrated tobacco smoke [5]

Studies using rats have also shown nicotine-induced CTA Kumar, Pratt and Stolerman [12] showed CTA using nicotine bitartrate (0 008, 0 08, and 0 8 mg/kg) injected subcutaneously (SC) in the animal's flank immediately after having drunk either sodium chloride solution (0 9%) or sodium saccharin (0 1%) solution More than one acquisition trial was required for CTA in all groups except the highest dose group Their procedure was based on the technique used by Booth, D'Mello, Pilcher and Stolerman [2] which combined one-stimulus and two-stimulus tests Iwamoto and Williamson [10] paired saccharin solution (0 1% w/v) with delayed (60 min) SC injections 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, reliable CTA was shown using two-bottle choice tests in the 0 50 mg/kg group but not in the other dose groups

Ksir [11] showed that hamsters will voluntarily eat commercial chewing tobacco without having to be food deprived When offered four grams of tobacco daily, the animals ate up to 2 6% of their body weight In some cases, they ingested the equivalent of 30 mg of nicotine per day when tobacco was simultaneously available along with nicotine in their drinking water The animals do eat the tobacco, it is not merely stored in their cheek pouches While the conditions would seem ideal for CTA learning (novel flavor, short delay of drug onset due to the lipid solubility of nicotine, and toxicity of the drug), the animals actually increased their consumption of both chewing tobacco and nicotine-laced drinking water over days Such increases suggest that negative consequences from nicotine in this species are either minimal or absent, at least when the drug is orally ingested

An understanding of how the hamster is able to consume such large daily amounts of a potent nauseant and emetic (in species that can vomit) while showing no evidence of aversion to the substance could be important for CTA research in general Moreover, basic information regarding nicotine and hamsters may be useful in establishing a hamster model of tobacco chewing or snuff "dipping," two increasingly popular methods of tobacco use in humans (Smoking is prohibited in New Mexico schools, but chewing or more often snuff "dipping" is generally ignored A recent and

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TABLE 1
MEAN AMOUNTS OF SACCHARIN SOLUTION CONSUMED

Treatments	Conditioning Day	Test Day 1	Test Day 2	Test Day 3
Saline	2 7 (0 23)	3 1 (0 24)	3 8 (0 24)	3 8 (0 28)
Nicotine 1 mg/kg	2 8 (0 19)	2 2 (0 21)	3 3 (0 15)	3 5 (0 16)
Nicotine 3 mg/kg	2 5 (0 19)	1 8 (0 21)	3 1 (0 23)	3 4 (0 24)
Nicotine 9 mg/kg	2 5 (0 14)	1 4 (0 12)	2 7 (0 15)	3 1 (0 22)
LiCl	2 6 (0 24)	1 7 (0 22)	2 8 (0 30)	2 9 (0 28)

(Milliliters—SEM)

growing trend among middle and senior high students is to swallow the excessive saliva and tobacco juice since they are not allowed to expectorate) The present study was undertaken to determine if a different route of nicotine administration (intraperitoneal injection) would produce CTA in the hamster If the hamster does show CTA from IP but not from oral nicotine, then the route of administration with resultant differences in absorption and duration of action could be a signficant factor in nicotine-based CTA in this species Finally, as previous studies [4,15] have shown CTA in hamsters based on lithium chloride (LiCl), we included a group receiving this drug to compare with any aversions produced by nicotine

METHOD

Animals

The subjects were 70 naive male golden Syrian hamsters *(Mesocricetus auratus)* purchased from Harlan Sprague-Dawley of Indianapolis, IN Each animal was assigned to an opaque polypropylene cage (17 8×29 2×13 cm) covered with a galvanized wire-bar top having a recessed food hopper and drinking bottle holder Each cage was supplied with ad lib Wayne Lab Blox and a 250 ml glass water bottle fitted with a rubber stopper, stainless steel sipper tube, and filled with fresh tap water The cages contained enough sterilized hardwood bedding ("Sani-Chips") to cover the bottom 2–3 cm Bedding was changed and cages washed weekly Lab temperature was maintained at 25°C with overhead fluorescent lights cycled on at 0700 hr and off at 1900 hr White noise was on constantly

Apparatus

Calibrated (0 2 ml) drinking tubes were fashioned from 10 ml disposable plastic syringes and fitted with 6 35 cm stainless steel spouts, and No 0 rubber laboratory stoppers [6]

Procedure

On arrival the hamsters were given 8 days of ad lib tap water from their glass water bottles prior to the study to allow for laboratory habituation

On Day 1 of the study at 1400 hr all hamsters were deprived of water until the same time on the next day when each animal was introduced to a baseline drinking session conducted as follows Every 10 sec a calibrated drinking tube containing fresh tap water was inserted into the bottle holder/stopper protector provided by the cage top This procedure continued sequentially until all animals had access to water In order to prevent an animal from dislodging or otherwise disturbing its drinking tube, a rubber band was placed around the width of the cage (approximately 8 cm from the end of the cage) with the drinking tube resting on the rubber band This arrangement elevated the entire tube approximately 2 5 cm from the cage top The spout tip protruded into the cage 4 5 cm at an angle of approximately 45° from horizontal After 20 min of access to tap water the drinking tubes were removed (on the 10 sec schedule), and the amounts consumed were determined and recorded for each animal Daily baseline drinking continued in this manner until five measures were taken (Days 2–6)

On Days 2 and 4 at 0900 hr, each hamster was removed from its home cage, handled for approximately 2 min, and then returned to its cage

Beginning at 0900 hr on the last day of baseline drinking (Day 6), all animals were weighed to the nearest gram in order to compute drug doses The hamsters were then randomly assigned to seven treatment groups consisting of 10 animals per group nicotine injection (1 0, 3 0, or 9 0 mg/kg), saline injection, lithium chloride injection (2% body weight of a 0 15 M solution), sham injection, or left in their home cages as handling/stress controls (see Table 1) All injections were delivered IP

Day 7 (Conditioning Day) consisted of offering all hamsters at 1400 hr saccharin solution (0 1% w/v, No S-3, Fisher Purified sodium saccharin in tap water) in their drinking tubes for 20 min, afterwhich the tubes were removed and the amounts consumed were determined and recorded A 30 min delay began with the end of the 20 min drinking period Following the delay each hamster (except the handling/stress control group) received its appropriate injection using for each animal a new 3/8 inch, 25 gauge needle affixed to a 1 cc plastic syringe Nicotine free base was obtained from Sigma Chemical Co (No N 3876 in 5 ml bottles) and lithium chloride from Fisher Scientific (Purified, No L-120) The nicotine base was diluted using normal (0 9%) saline into three concentrations to enable equivolume injections of the three doses of nicotine This resulted in a 50 gram animal receiving a dose volume of 0 5 ml Normal saline (0 5 ml/50 g of body weight of a 0 9% solution) was used for control injections Each sham animal was removed from its cage, sham injected, and returned to its cage Injections took 20–30 sec per animal The animals injected with nicotine were observed for any indication of tremor or seizure activity

Days 8 and 9 were Recovery Days with the animals receiving 20 min access to plain tap water from the calibrated drinking tubes

Days 10, 11, and 12 were Test Days with each animal

having a single bottle of saccharin solution to drink for 20 min No other liquids were offered

The drinking tubes received multiple soakings and rinsings in fresh tap water prior to and following all drinking sessions The tubes were filled via submersion by hand using fresh disposable vinyl gloves

RESULTS

Intraperitoneal injections of 1 0, 3 0, or 9 0 mg/kg of nicotine 30 min after having consumed a novel saccharin solution produced CTA in a dose-related manner (see Table 1)

As the three control groups (saline injected, sham injected, and cage controls) did not differ with respect to saccharin consumption on conditioning day or any of the test days (one-way analyses of variance, $F(2,27)<1$, in each case), only the saline injected group was used for comparisons with the three nicotine groups or the LiCl group

A repeated-measures analysis of variance (ANOVA) was performed on the amounts of saccharin consumed by the saline, 1 mg/kg, 3 mg/kg, and 9 mg/kg groups across the three test days resulting in a reliable dose effect, $F(3,72)=6\ 2$, $p<0\ 01$, trials effect, $F(2,72)=151$, $p<0\ 001$, and dose × trials interaction, $F(6,72)=3\ 9$, $p<0\ 01$ Pairwise comparisons were made using Scheffe's test (Test Day 1 The saline group differed reliably ($p<0\ 05$) from the three nicotine groups, Test Day 2 The saline group differed from the 9 mg/kg group, Test Day 3 no reliable comparisons)

A repeated-measures ANOVA was computed using the amounts of saccharin consumed by the saline and LiCl groups across the three test days resulting in a reliable drug effect, $F(1,18)=8\ 9$, $p<0\ 01$, and trials effect, $F(2,36)=44$, $p<0\ 001$ There was no interaction, $F(2,36)=3\ 1$, not significant Multiple t-tests indicated significant differences between the saline and LiCl groups on test days 1 and 2, $t(18)=4\ 28$, $2\ 51$, respectively, $p<0\ 05$

The animals in the saline, LiCl, 1 mg/kg, 3 mg/kg, and 9 mg/kg groups were equivalent with respect to Body weights as determined on the last day of baseline drinking, $F(4,45)<1$, ANOVA, amounts of plain water consumed on the last baseline day, $F(4,45)=1\ 06$, n s , amounts of saccharin solution consumed on conditioning day, $F(4,45)<1$, and water consumption on the two recovery days, $F(4,45)=1\ 98$ and $0\ 77$, respectively

The amount of plain water consumed on the last baseline day by the animals in the saline, LiCl, 1 mg/kg, 3 mg/kg, and 9 mg/kg groups averaged 3 4 ml (SEM=0 11) Body weights as determined on the last day of baseline drinking averaged 52 1 g (SEM=0 83)

DISCUSSION

The experiment demonstrates that hamsters can learn taste aversions to saccharin solution in a dose-related manner as a result of delayed IP nicotine injections CTA in the high dose nicotine group (9 mg/kg) was comparable to the CTA produced by LiCl (see Table 1) For nicotine to be such a powerful toxin and stimulant of the chemoreceptor triggering zone of the medulla, the aversions in the 9 mg/kg group had extinguished by the third test day Moreover, CTA in the animals injected with LiCl, a drug capable of producing strong CTA in hamsters [4,15], had likewise extinguished by the third test day, suggesting that nicotine is indeed a potent agent for CTA at least when compared to LiCl One-bottle, forced-choice tests as used in the present study do tend to produce faster extinction of CTA than two-bottle choice tests [3,9] In addition, the hamsters received only one acquisition trial

Ksir [11] had nicotine-tolerant hamsters consuming up to 30 mg of nicotine over a 24 hour period when given simultaneous access to a solution of 0 90 mg nicotine base per ml of water and commercial chewing tobacco ("Beech-Nut") Although his animals voluntarily consumed much higher total doses of nicotine (available for a longer time) than our animals received with their single dosing, route differences could have affected the actual blood levels of drug For example, gastrointestinal absorption routes a drug via the hepatic portal system directly to the liver where substantial amounts could be deactivated before entering general circulation In humans, 80–90% of nicotine metabolism occurs in the liver [8] Actual blood levels of nicotine following gastrointestinal absorption could be significantly below the dose ingested In addition, "alkaloid trapping" as a result of pH differences between body compartments could also reduce the amount of drug delivered to general circulation [7] Ksir [11], in addressing these problems, did note that significant blood levels were achieved following tobacco eating in his hamsters (43 9 ng nicotine/ml plasma) It could be hypothesized, based on the lack of CTA by Ksir's animals, that blood levels of nicotine on a short-term basis could be higher following IP doses in the range used in the present study than blood levels achieved from eating large amounts of tobacco or from drinking nicotine solution (IP dosing likewise results in first pass through the liver and the nicotine which escapes the first pass effect may enter the stomach via the bloodstream to be "trapped" at a pH of approximately 1) Finally, blood levels would rise much faster with IP injection compared to gastric absorption and rapid rises in blood levels of drugs or toxins (particularly alcohol) are most effective for stimulating the vomiting center [14]

Comparing the delivery route used by Ksir [11] with the route used in the present study suggests another possible reason for our results The highly alkaline nicotine (pH 10 2) could produce sufficient irritation of the peritoneum to act alone or in an additive fashion with any toxicosis from the nicotine to influence CTA, however, we observed no writhing or squealing which is usually indicative of such irritation Drugs which have been used to produce such irritation (as a screening test for analgesics) include IP acetic acid (0 6%) or phenylquinone [7] We have no evidence that these drugs have been used in an attempt to produce CTA or if "irritation" per se is capable of producing CTA learning

No seizures or fine motor tremors were observed in our hamsters even with the 9 mg/kg dose of nicotine, however, in pilot studies we have produced seizures with 12 mg/kg in hamsters of the same size and sex

Finally, we confirm that naive hamsters will unhesitantly eat chewing tobacco ("Beech-Nut") with latencies to consume approximately 0 5 gram of under 10 sec on the first and second day offered We have been unable to produce tobacco eating in rats even with 48 hr of food deprivation

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