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Conditioned Gustatory Avoidance Induced by Three Cholinergic Agents[1]

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PRESTON, K. L. AND C. R. SCHUSTER. *Conditioned gustatory avoidance induced by three cholinergic agents.* PHARMAC. BIOCHEM. BEHAV. 15(5)827–828, 1981.—Conditioned gustatory avoidance of sweetened condensed milk was induced in rats by drugs which differentially affect the cholinergic nervous system. After baseline water intake during a 15 minute daily session was established, sweetened condensed milk was presented in place of water, followed 15 minutes later by an IP injection of saline, physostigmine (0.5 mg/kg), atropine (25 mg/kg) or mecamylamine (25 mg/kg). Three presentations of milk were given, alternating daily with a water presentation. Atropine, a muscarinic blocker, and mecamylamine, a nicotinic blocker, both produced a strong avoidance. Physostigmine, an indirect agonist, produced a less pronounced avoidance at the dose employed. This study indicates that cholinergic agonists and antagonists are capable of inducing conditioned gustatory avoidance.

Conditioned gustatory avoidance Atropine Mecamylamine Physostigmine

THE phenomenon of conditioned gustatory avoidance (CGA) has been demonstrated utilizing a wide variety of agents. Most notably, methamphetamine [6,9] and lithium chloride [7,9] have proven to be very effective drugs, which, when administered following the consumption of a novel food or fluid, caused a reduced ingestion of that substance upon its subsequent presentation.

To date, little work has been done with cholinergic agents as avoidance inducing agents. The administration of scopolamine (a muscarinic blocker) following the consumption of sweetened condensed milk has been shown to increase latency to drink upon the subsequent presentation of the milk [1]. Methylatropine, when administered in a similar paradigm, also caused an increase in lick latency [8]. Atropine pretreatment blocked the formation of an avoidance response to saccharin induced by the administration of lithium chloride while methylatropine pretreatment did not [3]. However, there have only been a few studies [4, 5, 10] using the CGA paradigm in which cholinergic agents are used to induce gustatory avoidance and which use intake as the direct measure used to assess the avoidance. In the present study, single injections of physostigmine (an indirect nicotinic and muscarinic agonist), atropine (a muscarinic antagonist), or mecamylamine (a nicotinic antagonist) were given to rats following the consumption of a novel milk solution. All three agents were shown to produce avoidance.

METHOD

Subjects and Apparatus

Twenty-four male Sprague-Dawley rats weighing between 375 and 500 g were housed individually with ad lib access to food (Teklad Co., Winfield, OH). Colony room lights were automatically turned on at 0600 and off at 1900. The temperature was maintained at 21±1°C. All fluid was presented in modified, calibrated 50 ml syringes attached to the front of each cage.

Training

Rats were water deprived for 24 hours after which water was presented for a 15 minute session at 1000 for 7 daily sessions. Rats were weighed each day of the experiment 30 minutes prior to the presentation of water.

Testing

Rats were randomly divided into 4 groups of 6 rats each. On the eighth and tenth days, all rats received bottles of sweetened condensed milk (Borden C.; diluted 2 parts water to 1 part milk) at room temperature for 15 minutes in place of the water. Fifteen minutes after the milk was removed, each group of rats was given intraperitoneal injections of atropine SO_4 (25 mg/kg), mecamylamine HCl (25 mg/kg), physostig-

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mine SO_4 (0.5 mg/kg), or physiological saline. These doses were based on pilot studies and chosen to enhance the likelihood of producing avoidance. On days 9 and 11 water was presented without injections. On day 12, milk was again presented as on day 8, however, no further injections were given.

Atropine SO_4 (Aldrich) and mecamylamine HCl (MSD) were each given in a concentration of 25 mg/ml in physiological saline. Physostigmine SO_4 (Sigma) was given in a concentration of 0.5 mg/ml in physiological saline. Control subjects received 1 ml/kg of physiological saline (Travinol). All doses are expressed as the salt.

Statistics

Statistical analysis was performed using the Mann-Whitney U test [2]. Significance was accepted at the 0.05 level. The amount of fluid consumed was converted to ml/kg to control for weight variations between rats.

RESULTS

The amount of water consumed on day 7 averaged 48±2 ml/kg and was not significantly different across the 4 groups of rats. Comparison of water intake by rats in each of the 3 drug groups with that of the saline (control) group on days 9 and 11 also showed no significant differences (Fig. 1).

The amount of milk consumed during the first exposure was lower than the amount of water consumed under baseline conditions for all 4 groups (27±3 ml/kg milk vs 48±2 ml/kg water; Fig. 1). Those rats receiving saline 15 minutes after milk removal demonstrated an increased milk consumption during the second (50±4 ml/kg) and third (44±7 ml/kg) exposures on days 10 and 12 (Fig. 1A). The milk intake of each of the 3 drug treated groups was significantly lower than that of the control group on the second and third milk exposures (days 10 and 12). The atropine treated group drank 9±2 ml/kg and 2±2 ml/kg on days 10 and 12, respectively, U(6,6)=0; the mecamylamine treated group drank 12±3 ml/kg and 3±1 ml/kg on days 10 and 12, U (6,6)=0, and physostigmine treated group drank 26±6 ml/kg of milk on days 10 and 12, U(6,6)=5(Fig. 1B,C,D).

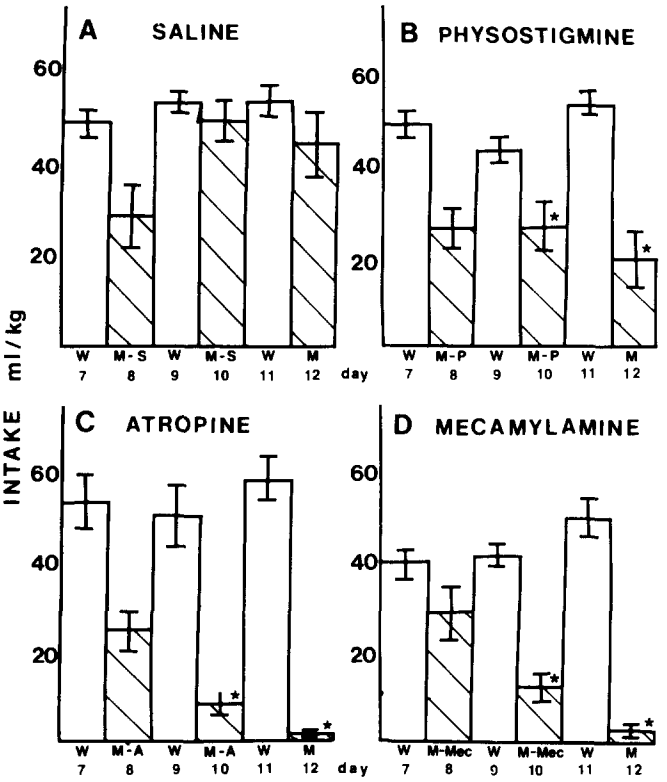

FIG. 1. Fluid intake in ml/kg on days 7 to 12. Letters below each histogram indicate which fluid was presented (W=water; M=milk) and which drug (days 8 and 10 only) was injected 15 min after fluid removal (S=saline 1 ml/kg; P=physostigmine 0.5 mg/kg; A=atropine 25 mg/kg; Mec=mecamylamine 25 mg/kg). *Indicates significance at $p<0.05$. Mann-Whitney U test.

DISCUSSION

Previous studies have shown that drugs affecting the cholinergic system can induce gustatory avoidance [1, 4, 5, 8, 10]. Several of these studies, however, have utilized indirect measures of the avoidance, such as latency from the presentation of the fluid to the first lick. The present study used as a direct measure (fluid intake in ml) and thereby confirmed and extended the avoidance inducing properties of atropine. In addition, two previously untested cholinergic agents, physostigmine and mecamylamine, were found to be capable of inducing gustatory avoidance.

Atropine and mecamylamine, at the doses chosen, proved to be effective in producing avoidance. In addition, observation of the rats showed that these treatments caused agitation (including biting of the drinking tube and vocalizations) upon the subsequent presentations of the milk.

Physostigmine was less effective in reducing milk intake and in causing agitation. Since only one dose of each agent was tested, it is not possible to make comparisons of their potency or efficacy in producing avoidance. It remains to be determined whether the lesser effect of physostigmine is a function of dose or a difference in efficacy of this agent in producing gustatory avoidance.

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