Pharmacology Biochemistry & Behavior, Vol. 27, pp. 81–85. © Pergamon Journals Ltd., 1987. Printed in the U.S.A. 0091-3057/87 $3.00 + .00

Conditioned Taste Aversion and Cholinergic Drugs: Pharmacological Antagonism[1]

JAMES A. ROMANO AND JAMES M. KING[2]

US Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD 21010-5425

Received 30 June 1986

ROMANO, J. A. AND J. M. KING. *Conditioned taste aversion and cholinergic drugs: Pharmacological antagonism.* PHARMACOL BIOCHEM BEHAV 27(1) 81–85, 1987.—The effectiveness of drugs as unconditioned stimuli (UCSs) in the conditioned taste aversion (CTA) procedure may be influenced by specific pharmacological antagonism. The present studies examined the UCS effects of two carbamates, physostigmine salicylate (PS) and pyridostigmine bromide (PB), and three anticholinergic compounds, atropine methyl nitrate (AMN), atropine sulfate (AS), and benactyzine hydrochloride (BH). Individual drugs, as well as combinations of the carbamates and the anticholinergics, were studied in a two-bottle procedure in rats. The lowest effective doses for eliciting significant CTAs were PS, 0.32 mg/kg; PB, 1.00 mg/kg; AMN, 0.04 mg/kg; AS, 0.07 mg/kg and BH, 0.90 mg/kg, IP. Combinations of PS with either AMN or BH were mutually antagonistic as UCSs, whereas PS with AS was not. PB with AMN, but not with AS, also showed antagonism in the procedure. The present results suggest that the CTA procedure is well-suited for direct examination of cholinergic drug effects and may also be used to explore interactions of different classes of cholinergic drugs.

Anticholinesterase Anticholinergic Conditioned taste aversion Antagonism Physostigmine salicylate Pyridostigmine bromide Atropine methyl nitrate Atropine sulfate Benactyzine hydrochloride

THE conditioned taste aversion (CTA) procedure has been proposed as a possible element in a broad battery of screens for behavioral toxicity. The bases for this proposed usage are the facts that (1) most known toxins do reliably condition CTAs, (2) CTAs generally corroborate known toxicity, and (3) the procedure is moderately sensitive and cost effective [19]. In this procedure, an animal is exposed to a novel stimulus, such as saccharin, which serves as a conditioned stimulus (CS). A toxicosis is then induced in the animal through use of an unconditioned stimulus (UCS) which may be a drug, a chemical or ionizing radiation [2,7]. Often a rat will develop an enduring aversion to the CS taste after a single association with the UCS-induced toxicosis [23]. The essentiality of this illness to the formation of CTAs has been questioned [4,6]. From the list of toxins which have been demonstrated to produce CTAs, it appears that although illness may not be essential for formation of CTAs, it may well be sufficient. Thus, one may use the CTA as a gross index of the animal's ability to discern a drugged state [6].

Although some studies have been carried out using cholinergic agents in radiation-induced taste aversions [3], until recently few studies have been conducted employing cholinergic compounds as UCSs [5]. CTAs have been reported following administration of the anticholinergics scopolamine [10], atropine sulfate (AS) [18], and its quaternary analogue, atropine methyl nitrate (AMN) [18]. These studies, however, did not examine dose-effect relationships for these anticholinergic drugs. Physostigmine (PS), an anticholinesterase, was found to produce a weak CTA at a single dose of 0.50 mg/kg in the one-bottle procedure [18]. We have previously reported CTAs to the toxic organophosphorus compounds sarin [11], soman [21] and tabun [22] at doses ranging from 0.60 to 0.75 LD50, which were occasionally accompanied by overt signs of cholinergic intoxication. Similarly, MacPhail [15], using a two-bottle procedure, found no CTA to the anticholinesterase carbaryl but reported a CTA to another, Baygon, only at the highest dose employed, i.e., one accompanied by noticeable signs of peripheral cholinomimetic stimulation. MacPhail [15] concluded that the CTA procedure may not be a sensitive measure of anticholinesterase intoxication. In general, however, the sensitivity of rats to induction of CTAs by low doses of cholinergic drugs remains to be determined.

The CTA procedure may also be used to determine the

[1]The experiments reported here were conducted according to the Guide for Care and Use of Laboratory Animals (1978), as prepared by the Committee on Care and Use of Laboratory Animals, National Research Council, DHEW Publication No. (NIH) 80-23. The opinions or assertions contained herein are the private views of the authors, and are not to be construed as reflecting the views of the Department of the Army or the Department of Defense.

[2]Present address: US Army Human Engineering Laboratory, APG, MD 21005-5001.

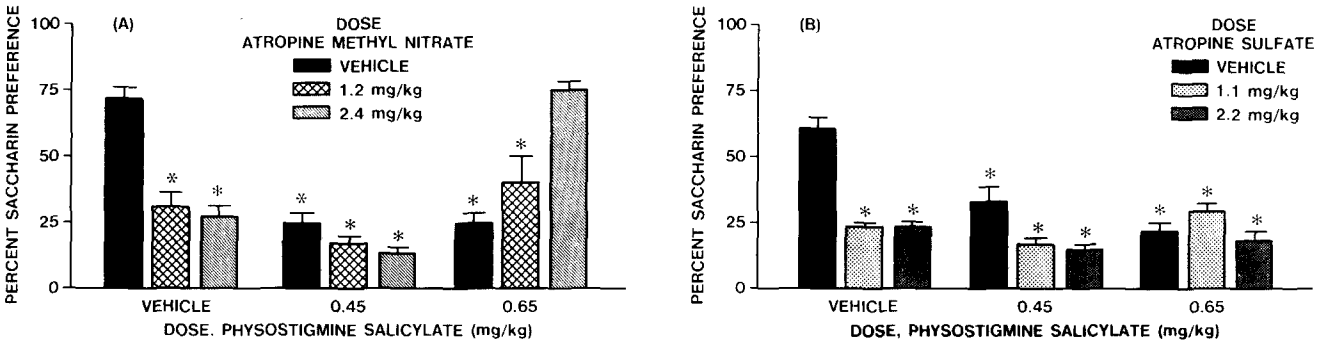

FIG. 1. (A) Effects of pairing PS (veh, 0.45, 0.65 mg/kg) and AMN (veh, 1.2, 2.4 mg/kg) on percent saccharin preference. Ten rats were tested in the vehicle-vehicle group. Five rats were in all other groups. (B) Effects of pairing PS (veh, 0.45, 0.65 mg/kg) and AS (veh, 1.1, 2.2 mg/kg) on percent saccharin preference. The vehicle-vehicle and the vehicle-1.1 mg/kg AS groups contained 10 animals; the 0.65 PS-vehicle group contained four animals; all other groups contained five animals. The vertical bars express the mean (±S.E.M.) percent saccharin preference for each experimental group. The asterisks indicate significance compared with vehicle-vehicle control ($p<0.05$). Drugs were given IP.

pharmacological specificity of the UCS. Pharmacologic specificity in the CTA procedure has been demonstrated by a number of studies of agonist-antagonist pairs [8, 12, 13, 25]. To a degree, anatomic specificity has also been demonstrated [1,20]. However, these studies did not employ cholinergic agents. Thus, the purposes of the present study were to demonstrate (1) the utility of the conditioned taste aversion procedure in evaluating the discriminative stimulus properties of cholinergic drugs, particularly at low doses, and (2) blockade of the behavioral changes produced by cholinergic drugs through the use of pharmacologic antagonists.

METHOD

Subjects

Three hundred and ninety-one male albino rats (AMRI: (SD X WI)BR), weighing between 250 and 370 grams were used in this study. They were housed individually in plastic cages (25×46×20 cm) in temperature-controlled animal quarters, and maintained on a 12-hour light-dark cycle, with artificial light provided between 0600 and 1800 hours. The rats were allowed at least 4 days to become acclimated to the animal quarters and to daily handling prior to experimental use. Laboratory rat chow was available ad lib throughout the studies. Group sizes are given in the figure legends.

Drugs

PS, pyridostigmine bromide (PB), AMN, AS and benactyzine hydrochloride (BH) were employed as UCSs. Drug doses were for the salt. Doses of PS used ranged from 0.20 to 0.65 mg/kg, PB from 0.40 to 2.00 mg/kg, AMN from 0.04 to 2.40 mg/kg, AS from 0.07 to 2.20 mg/kg, and BH from 0.29 to 9.0 mg/kg. The dosages varied by 0.16 log increments for PS, 0.20 for PB, 0.30 for AMN and AS, and 0.50 for BH. The vehicle for all drugs was water for injection, USP, with 0.5% methylparaben and 0.05% propylparaben (w/v) added for stabilization and with pH adjusted to 2.8 using 0.1 N hydrochloric acid. All solutions were prepared so that injection volumes were proportional to 1.0 ml/kg. The drug solutions were prepared in lots and stored under refrigeration between drug tests. The vehicle was used for control injections and all drugs were given intraperitoneally.

TABLE 1

EFFECTS OF CARABAMATE, ANTIMUSCARINIC, AND CHOLINOLYTIC DRUGS ON FORMATION OF CONDITIONED TASTE AVERSIONS IN RATS

Drug	Lowest Dose Yielding Significant CTA ($p<0.05$)
Physostigmine Salicylate (PS)	0.32 mg/kg
Pyridostigmine Bromide (PB)	1.00 mg/kg
Atropine Methyl Nitrate (AMN)	0.04 mg/kg
Atropine Sulfate (AS)	0.07 mg/kg
Benactyzine Hydrochloride (BH)	0.90 mg/kg

CTA Procedure

After acclimation, animals were trained in the home cage for four days to drink on a 30 min access per day drinking schedule. On day 5, they were offered only a distinctively sweet-flavored (0.2% w/v saccharin) solution, the CS, during this drinking period. This exposure was followed shortly by an injection of the drugs or vehicle, whichever was to serve as the UCS. The animals were maintained on the 30 min daily water consumption schedule for three additional days. On the fourth day following the pairing of drugs and flavored solution, animals were offered a two-bottle choice between the sweetened saccharin solution and tap water during their 30 min drinking period. Test day consumption for each animal was expressed as percent saccharin preference: (saccharin solution consumed−(saccharin solution consumed + water consumed) × 100).

Design and Statistical Analysis

A series of dose-ranging studies with the individual drugs was conducted to determine minimally effective dosages for eliciting the CTA. Following these studies, a series of antagonism experiments were conducted. PS and PB were each paired with AMN and AS, respectively (Experiments I–IV). Finally, PS was studied in conjunction with BH (Experiment V). In Experiments I and II, AMN (vehicle, 1.2 and 2.4

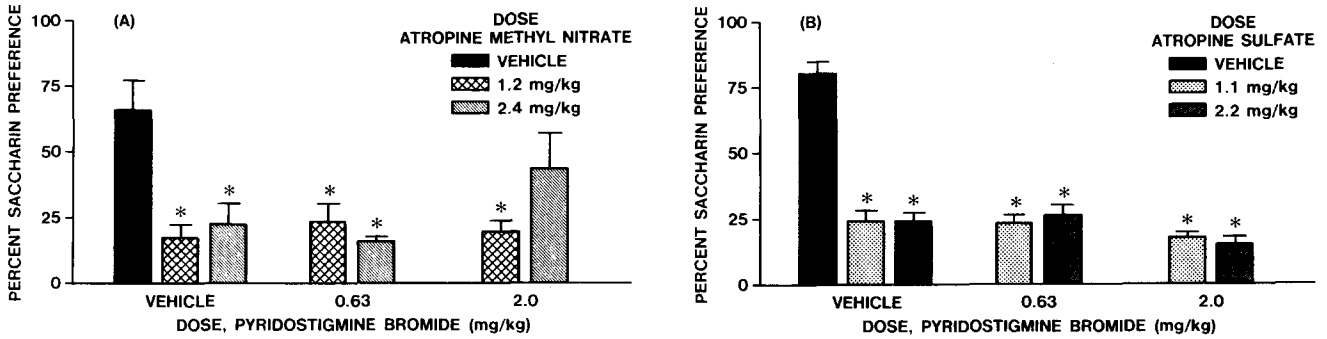

FIG. 2. (A) Effects of pairing PB (veh, 0.63, 2.0 mg/kg) and AMN (veh, 1.2, 2.4 mg/kg) on percent saccharin preference. Nine rats were tested in the vehicle-vehicle group. The vehicle-1.2 mg/kg AMN and the vehicle-2.4 mg/kg AMN groups contained 10 animals. Five rats were in all other groups. (B) Effects of pairing PB (veh, 0.63, 2.0 mg/kg) and AS (veh, 1.1, 2.2 mg/kg) on percent saccharin preference. Ten rats were tested in the vehicle-vehicle, vehicle-1.1 mg/kg AS, and the vehicle-2.2 mg/kg AS groups. All other groups contained five rats. The vertical bars express the mean (±S.E.M.) percent saccharin preference for each experimental group. The asterisks indicate significance compared with vehicle-vehicle control ($p<0.05$). Drugs were given IP.

mg/kg) and AS (vehicle, 1.1 and 2.2 mg/kg) were paired with PS (vehicle, 0.45 and 0.65 mg/kg) in 3×3 between-groups factorial designs. Following the two-way between groups ANOVA, Newman-Keuls analysis was used to compare groups to the vehicle-vehicle control and to each other. For Experiments III and IV, AMN (vehicle, 1.2 and 2.4 mg/kg) and AS (vehicle, 1.1 and 2.2 mg/kg) were paired with PB (veh, 0.63 and 2.0 mg/kg) in one-way between-groups designs. Following the one-way ANOVA, Dunnett's test compared each group to the vehicle-vehicle control. In Experiment V, PS (veh and 0.65 mg/kg) was paired with BH (veh, 0.29, 0.90, 2.90, and 9.00 mg/kg) in a 2×5 between-groups factorial design. Again, following the two-way between groups ANOVA, the Newman-Keuls analysis was used to compare groups to the vehicle-vehicle control and to each other [27]. In Experiments I–V, the anticholinergics AMN, AS, and BH were injected immediately following the 30 min drinking period, while PS and PB were administered five min later. Pilot studies had indicated that this was the most efficacious timing of drug presentation.

In addition, analysis of variance was calculated using test day total fluid intake to determine if any drug or drug combination significantly influenced the amount of fluid consumed.

RESULTS

Individually, all drugs produced dose-related CTAs without affecting total test day fluid intake. Use of the Newman-Keuls procedure allowed for the determination of the lowest drug dosage which would yield significant differences from the vehicle control (see Table 1 for summary). The dose-effect cuves for AS and AMN were steep and parallel, whereas the dose-effect curves for BH, PS, and PB were relatively shallow and nearly parallel (data not shown here).

During the course of these experiments two animals at the vehicle-0.65 mg/kg PS group, one vehicle control animal, and one animal receiving 2.9 mg/kg BH-0.65 PS were lost. These deaths may not have been directly attributable to toxic drug effects, although 0.65 mg/kg PS was often accompanied by signs of cholinergic hyperstimulation.

Experiments I–V

The effects of pairing PS and AMN as UCSs are shown in Fig. 1A. Results of the analysis of variance indicated that flavor aversions were produced by PS, $F(2,41)=16.0$, $p<0.001$, and AMN, $F(2,41)=49.0$, $p<0.001$. Furthermore, there was a significant interaction between these drugs, $F(4,41)=34.0$, $p<0.001$. That is, the results of the Newman-Keuls analysis indicated that the vehicle control and the 2.4 mg/kg AMN-0.65 mg/kg PS dose groups did not differ from each other, although they were significantly different from all other treatment combinations. In addition, the 1.2 mg/kg AMN-0.65 mg/kg PS treatment combination, although resulting in greater flavor aversions than either of the two groups mentioned above, had significantly weaker flavor aversions than all but one of the remaining drug treatment combinations. Thus, the significant interaction indicates that PS and AMN were mutually antagonistic. However, the only statistically significant antagonism, as shown by Newman-Keuls analysis, was found in the 2.4 mg/kg AMN-0.65 mg/kg PS group.

The results of the PS and AS UCS pairings are presented in Fig. 1B. Results of the analysis of variance indicated that CTAs were produced by PS, $F(2,45)=3.71$, $p<0.05$, and AS, $F(2,45)=5.5$, $p<0.01$. No significant interaction was observed. All groups demonstrated significant CTAs when compared to the vehicle control condition. No other significant differences were detected among treatment combinations. The lack of a significant interaction indicates that PS and AS were not mutually antagonistic.

For Experiment III, in which PB was paired with AMN, significant differences were found among groups $F(6,41)=7.51$, $p<0.01$, with all groups being significantly different from control, with the exception of the 2.4 AMN-2.0 PB group. The lack of a statistically significant difference for the latter drug combination suggests that PB and AMN are mutually antagonistic, at least at one dose level (see Fig. 2A). In Experiment IV, pairing PB with AS resulted in significant differences among groups, $F(6,43)=69.99$, $p<0.01$. Application of the Newman-Keuls test demonstrated that all groups were significantly different from the vehicle-vehicle control (see Fig. 2B).

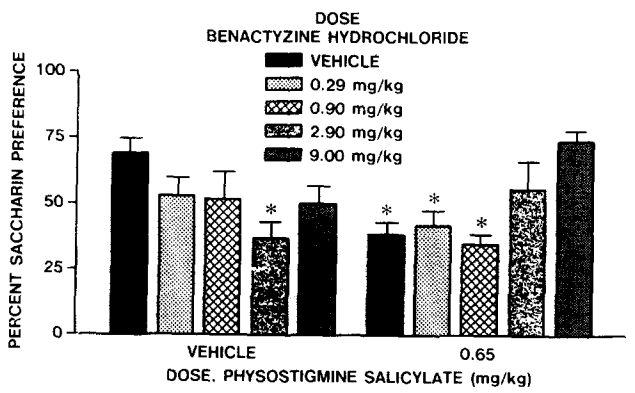

FIG. 3. Effects of pairing PS (veh, 0.65 mg/kg) and BH (veh, 0.29, 0.90, 2.9, 9.0 mg/kg) on percent saccharin preference. Ten rats were tested in the vehicle-vehicle, vehicle-0.29 mg/kg BH, vehicle-0.90 mg/kg BH, vehicle-2.9 mg/kg BH, vehicle-9.0 mg/kg BH, and the 0.90 mg/kg BH-PS groups. The vehicle-PS, 0.29 BH-PS, and the 2.9 BH-PS groups contained nine rats. Seven rats were tested in the 2.9 mg/kg BH-PS group. The vertical bars express the mean (±S.E.M.) percent saccharin preference for each experimental group. The asterisks indicate significance compared with vehicle-vehicle control ($p<0.05$). Drugs were given IP.

In Experiment V, BH, $F(4,84)=4.34$, $p<0.01$, but not the PS factor, collapsed across BH doses, $F(1,84)=1.0$, $p>0.05$, produced a significant CTA as demonstrated by reduced saccharin preference. In this experiment, a significant BH×PS interaction, $F(4,84)=9.92$, $p<0.01$, was demonstrated (see Fig. 3). Note that PS (0.65 mg/kg), when paired with vehicle, did produce a significant CTA (with a saccharin preference of 0.48). The latter result suggests a mutual antagonism between the two test compounds.

It is important to note that there was no effect on total test day fluid intake for any of the drug combinations used in these experiments. Furthermore, no signs of cholinergic intoxication were observed in any of the drug-combination studies (i.e., Experiments I through V). However, doses of the carbamates PS and PB which were sufficient to produce CTAs were occasionally accompanied by tremor and slight salivation.

DISCUSSION

Each of the five drugs produced a dose-related CTA to a normally preferred flavor. Cholinergic antagonists proved particularly potent and efficacious in the present two-bottle choice procedure with significant CTAs at dosages as low as 0.04, 0.07, and 0.90 mg/kg for AMN, AS, and BH, respectively. The use of this procedure resulted in lower effective doses than hitherto reported [18,23] and attests to its sensitivity in detecting the behavioral effects of the anticholinergic drugs. For example, in the rat BH was shown to affect schedule-controlled behavior or shuttle avoidance only at doses of 4.4 mg/kg or greater in our laboratory (unpublished observations). Conversely, the indirect cholinergic agonists PS and PB were shown to produce significant CTAs at doses of 0.32 or 1.00 mg/kg and above which, as stated above, were accompanied by some signs of cholinergic intoxication. Perhaps the CTA procedure is not as readily influenced by "sign-free") doses of the latter compounds as by the cholinergic antagonists (i.e., doses not otherwise accompanied by obvious signs of cholinergic stimulation). However, PB produced CTAs at doses which did not disrupt response rates in a rat operant procedure in this laboratory [16].

PS and AMN proved to be mutually antagonistic, at least at one dose combination, as were PS and BH. However, this was not true for PS and AS. Conversely, AS but not AMN has been shown to block PS-induced tail flick analgesia [17]. The reasons for the lack of interaction between AS, which has both central and peripheral actions, and the carbamates in our present CTA experiments are not clear at this time. It is well known that AMN is more potent than AS in its gastrointestinal effects [14]. Perhaps larger dosages of AS would have given results similar to those obtained with AMN. Alternatively, AMN, but not AS, has potent ganglionic blocking activity, being significantly more effective in that respect than tetraethylammonium [9]. This property may be shared with BH, and this common ganglionic blocking property may also explain the interactions with the carbamates PS and PB in the CTA procedure. Actions of cholinergic drugs at the area postrema (AP) may also be important. In an animal not capable of emesis, viz, the rat, the AP remains significant in formation of CTAs. Lesions of this area prevent formation of drug-induced CTAs including CTAs induced by the anticholinergic drug scopolamine methylnitrate [20]. The AP is peripherally accessible as the blood-brain barrier is comparatively weak at this point. The present experiments were not designed to evaluate the role of AP vs. peripheral gastrointestinal factors in the formation of CTAs by cholinergic drugs. Nevertheless, these experiments indicate the feasibility of demonstrating antagonism with the CTA procedure. Thus, the present findings suggest that the CTA procedure may demonstrate blockade of the behavioral changes produced by cholinergic drugs through the use of pharmacologic antagonists. The locus of the pharmacological antagonism remains a matter of speculation.

As the present findings demonstrate, significant pharmacological antagonism can be obtained even when both UCSs produce substantial CTAs in their own right. Therefore, the CTA procedure appears useful in (1) detecting the aversive stimulus properties of cholinergic drugs, and (2) exploring the specificity of CTAs produced by cholinergic drugs through use of pharmacologic antagonists.

ACKNOWLEDGEMENTS

The authors wish to express their thanks to Renee Alvarez and Larry Moore for assisting with the experiments, to Dr. John McDonough for providing valuable editorial advice, and to Ms. Melanie Murrow for editorial and administrative assistance.

REFERENCES

1. Amit, Z., D. E. Levitan, Z. W. Brown and F. Rogan. Possible involvement of central factors in the mediation of conditioned taste aversion. *Neuropharmacology* **16:** 121–124, 1977.
2. Braveman, N. S. Visually guided avoidance of poisonous foods in mammals. In: *Learning Mechanisms in Food Selection*, edited by L. M. Barker, M. R. Best and M. Domjan. Waco: Baylor University Press, 1977.
3. Cairnie, A. B. and K. E. Leach. Dexamethasone: A potent blocker for radiation-induced taste aversion in rats. *Pharmacol Biochem Behav* **17:** 305–311, 1982.
4. Cappell, H. and A. E. LeBlanc. Gustatory avoidance conditioning by drugs of abuse: Relationships to general issues in research on drug dependence. In: *Food and Aversion Learning*, edited by N. W. Milgram, L. Krames and T. W. Alloway. New York: Plenum Press, 1976.
5. Deutsch, R. Effects of atropine on conditioned taste aversion. *Pharmacol Biochem Behav* **8:** 685–694, 1978.
6. Gamzu, E., G. Vincent and E. Boff. A pharmacological perspective of drugs used in establishing conditioned taste aversions. *Ann NY Acad Sci* **443:** 231–249, 1985.
7. Garcia, J. and W. G. Hankins. On the origin of food aversion paradigms. In: *Learning Mechanisms in Food Selection*, edited by L. M. Barker, M. R. Best and M. Domjan. Waco: Baylor University Press, 1977.
8. Goudie, A. J., E. W. Thornton and J. Wheatley. Attenuation by alpha-methyl-para-tyrosine of amphetamine-induced conditioned taste aversion in rats. *Psychopharmacologia* **45:** 119–123, 1975.
9. Innes, I. R. and M. Nickerson. Atropine, scopolamine, and related antimuscarinic drugs. In: *The Pharmacological Basis of Therapeutics*, edited by L. S. Goodman, A. Gilman and G. B. Koelle. New York: MacMillan Publishing Co., Inc., 1975.
10. Kral, P. A. Effects of scopolamine injection during CS-UCS interval on conditioning by naloxone. *Psychol Rep* **28:** 690, 1971.
11. Landauer, M. R. and J. A. Romano. Acute behavioral toxicity of the organophosphate sarin in rats. *Neurobehav Toxicol Teratol* **6:** 239–243, 1984.
12. Landauer, M. R., S. T. Philips, R. L. Balster and L. S. Harris. Effects of cannabinoids on cyclophosphamide-induced taste aversion in mice. *Fed Proc* **43:** 952, 1984.
13. LeBlanc, A. E. and H. Cappell. Antagonism of morphine-induced aversive conditioning by naloxone. *Pharmacol Biochem Behav* **3:** 185–188, 1975.
14. Lumb, W. V. and E. W. Jones. *Veterinary Anaesthesia*. Philadelphia: Lea and Febiger, 1973.
15. MacPhail, R. C. Studies on the flavor aversions induced by n-substituted carbamates and by Alkyl-tin compounds. *Toxicologist* **1:** 44, 1981.
16. Modrow, H. E. and J. H. McDonough. Effects of soman and pyridostigmine on variable interval responding. *Neurobehav Toxicol Teratol* **7:** 528–529, 1985.
17. Pedigo, N. W., W. L. Dewey and L. S. Harris. Determination and characterization of the antinociceptive activity of intraventricularly administered acetylcholine in mice. *J Pharmacol Exp Ther* **193:** 845–852, 1975.
18. Preston, K. L. and C. R. Shuster. Conditioned gustatory avoidance induced by cholinergic agents. *Pharmacol Biochem Behav* **15:** 827–828, 1981.
19. Riley, A. L. and D. L. Tuck. Conditioned taste aversions: A behavioral index of toxicity. *Ann NY Acad Sci* **443:** 272–292, 1985.
20. Ritter, S., J. J. McGlone and K. W. Kelly. Absence of lithium-induced taste aversion after area postrema lesions. *Brain Res* **201:** 501–506, 1980.
21. Romano, J. A., J. M. King and D. M. Penetar. A comparison of physostigmine and soman using taste aversion and nociception. *Neurobehav Toxicol Teratol* **7:** 243–249, 1985.
22. Romano, J. A. and M. R. Landauer. Effects of the organophosphorus compound, O-ethyl-N-dimethyl-phosphoramidocyanidate (Tabun), on flavor aversions, locomotor activity, and rotarod performance in rats. *Fundam Appl Toxicol* **6:** 62–68, 1986.
23. Rondeau, D. B., F. B. Jolicoeur, A. D. Merkel and M. J. Wayner. Drugs and taste aversion. *Neurosci Biobehav Rev* **5:** 279–294, 1981.
24. Stolerman, I. P. and G. D. D'Mello. Oral self-administration and the relevance of conditioned taste aversions. In: *Advances in Behavioral Pharmacology*, edited by T. Thompson and D. B. Dews. New York: Academic Press, Inc., 1981.
25. van Der Kooy, D. and A. G. Phillips. Temporal analysis of naloxone attenuation of morphine-induced taste aversion. *Pharmacol Biochem Behav* **6:** 637–641, 1977.
26. Vogel, J. R. and D. E. Clody. Habituation and conditioned taste aversion. *Psychon Sci* **28:** 275–276, 1972.
27. Winer, B. J. *Statistical Principles in Experimental Design*, 2nd edition. New York: McGraw-Hill, 1971.