Comparative *In Vitro* Evaluation of Cryogenine, Cyproheptadine, and Diphenhydramine as Antagonists of Furtrethonium, Histamine, and Serotonin

RALPH W. TROTTIER, Jr.* and MARVIN H. MALONE*

Abstract □ Cryogenine was a noncompetitive inhibitor of furtrethonium in isolated rat jejunum, while both diphenhydramine and cyproheptadine displayed mixed competitive-noncompetitive activity. All three compounds were relatively specific competitive antagonists of histamine in isolated guinea pig uterus, although each was capable of noncompetitive activity at high bath concentrations. Cryogenine and cyproheptadine had noncompetitive antihistaminic activity on guinea pig terminal ileum, while diphenhydramine exhibited mixed competitive-noncompetitive activity. Diphenhydramine appeared to be a fairly specific competitive antagonist of serotonin in rat uterus, while cryogenine was relatively noncompetitive. Cyproheptadine displayed mixed competitive-noncompetitive antiserotonin activity in this same tissue.

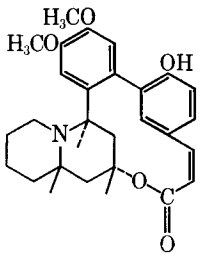
Keyphrases □ Cryogenine, cyproheptadine, diphenhydramine—*in vitro* activity comparison □ Antimuscarinic activity, comparative—cryogenine, cyproheptadine, diphenhydramine □ Antihistaminic activity, comparative—cryogenine, cyproheptadine, diphenhydramine □ Antiserotonin activity, comparative—cryogenine, cyproheptadine, diphenhydramine

Cryogenine (vertine), an alkaloid isolated from *Heimia salicifolia* Link and Otto by Blomster *et al.* (1), has been evaluated for anti-inflammatory activity by Kaplan *et al.* (2). Cryogenine appeared to be equipotent to phenylbutazone against both acute carrageenin-induced edema and chronic adjuvant-induced (*Mycobacterium butyricum*) inflammation. Previous kinetic studies of cryogenine had shown this alkaloid to be a noncompetitive inhibitor of furtrethonium in isolated rat jejunum and a competitive-noncompetitive inhibitor of acetylcholine in the isolated frog rectus abdominis (3). In light of these studies and other pharmacodynamic investigations (4, 5), it seemed desirable to determine the relative antimuscarinic, antihistaminic, and antiserotonin activity of cryogenine along with selected reference agents.

EXPERIMENTAL

Solubilization of cryogenine[1] was effected by dissolving the base in glacial acetic acid and then adding sufficient distilled water to make a 10-mg./ml. stock solution in 4% acetic acid. This stock was diluted progressively with water to yield the indicated incubation concentrations. Diphenhydramine[2] and cyproheptadine[3] were chosen as reference antagonists.

Drug-receptor interactions were studied and evaluated according to the kinetic methods of Ariëns (6), van Rossum (7) *et al.* Furtrethonium[4] was used as the reference muscarine-like agonist on



cryogenine

isolated rat jejunum suspended in aerated (95% oxygen and 5% carbon dioxide) Tyrode's perfusate (37°). Histamine diphosphate[5] was the reference agonist on both isolated guinea pig terminal ileum (aereated Tyrode's, 37°) and on isolated guinea pig uterus (aereated deJalon, Bayo, and deJalon's perfusate, 30–31°). Serotonin creatinine sulfate[6] was used as an agonist on isolated rat uterus (aereated deJalon, Bayo, and deJalon's, 30–31°). All donor animals were fasted for 24 hr. prior to sacrifice, and those to be used for isolated uterus experiments were subcutaneously preinjected 24–48 hr. prior to tissue extraction with 1 mg./kg. of diethylstilbestrol ethyloleate in peanut oil (8). After being placed in the 50-ml. perfusion bath, each isolated tissue was attached to a lightly loaded isotonic frontal writing lever arranged for kymographic tracing and then allowed to equilibrate for 60 min. with frequent washings of fresh, warmed perfusate. Cumulative concentration-response curves for the agonist were then made until at least two consecutive, reproducible concentration-response curves had been obtained. A 12–15-min. wash period was established between each curve utilizing five 50-ml. washings of fresh perfusate. Antagonistic effects were determined by allowing the antagonist to incubate with the tissue for 10 min. prior to documenting the cumulative log-concentration curve for the agonist in its presence.

RESULTS AND DISCUSSION

Figure 1 represents the effects of diphenhydramine on the cumulative log-concentration curves of furtrethonium in isolated rat jejunum. Possible competitive antagonism is illustrated by the parallel shift of the concentration-response curves at the lower incubation concentrations of diphenhydramine. Definite noncompetitive antagonism is seen at higher antagonist concentrations (1×10^{-5} *M*) as evidenced by a 50% depression of the curve maximum, although some specificity for the muscarinic receptor is still seen. The dashed line here and in the other figures represents the tissue recovery pattern after the highest concentration of the antagonist had been tested and after the tissue had been thoroughly washed with fresh perfusate. Cryogenine in the same system (Fig. 2) demonstrated pure noncompetitive antagonism at all concentrations, hence cryogenine does not occupy muscarinic receptors even though it has the ability to antagonize furtrethonium. A shallow, parallel displacement of the agonist concentration-response curve to the right with only low concentrations of the antagonist (Fig. 1) may indicate the presence of an agonist receptor reserve (6) rather than a true component of competitive antagonism.

Diphenhydramine when tested in guinea pig ileum against hista-

[1] Obtained from Dr. A. E. Schwarting, Div. of Pharmacognosy, Pharmacy Research Institute, The University of Connecticut, Storrs, Conn.

[2] Diphenhydramine HCl (control no. DA527) Parke, Davis & Co., Detroit, Mich.

[3] Cyproheptadine HCl (lot no. 18938), Merck Institute for Therapeutic Research, West Point, Pa.

[4] Furtrethonium iodide (lot no. C8-170A), Smith Kline & French Laboratories, Philadelphia, Pa.

[5] Nutritional Biochemicals Corp., Cleveland, Ohio (lot no. 2121) or California Corp. for Biochemical Research, Los Angeles, Calif. (lot no. 3783).

[6] Nutritional Biochemicals Corp., Cleveland, Ohio (lot no. 1573).

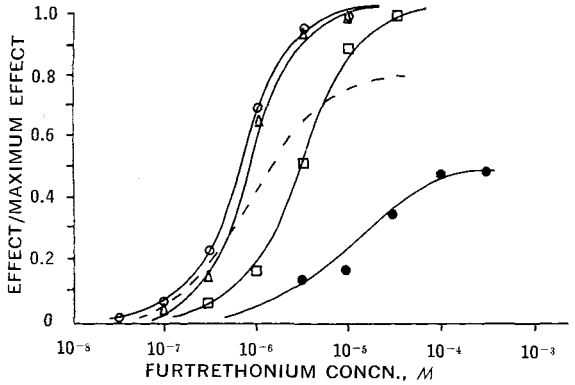


Figure 1—*Cumulative log-concentration curves of furtrethonium on rat jejunum in the presence of diphenhydramine. Key:* ○, *control;* △, 1×10^{-7} M *incubation concentration of diphenhydramine;* □, 1×10^{-6} M *diphenhydramine;* ●, 1×10^{-5} M.

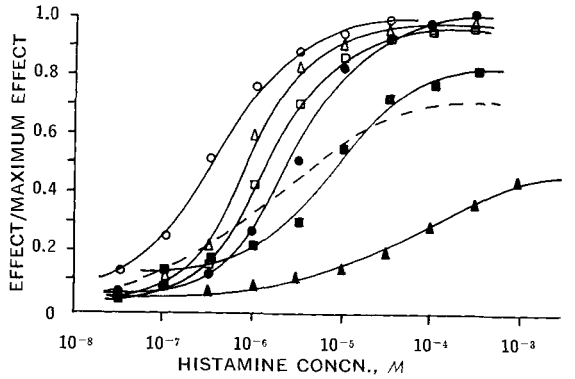


Figure 3—*Cumulative log-concentration curves of histamine on guinea pig ileum in the presence of diphenhydramine. Key:* ○, *control;* △, 1×10^{-9} M *incubation concentration of diphenhydramine;* □, 1×10^{-8} M *diphenhydramine;* ●, 1×10^{-7} M; ■, 1×10^{-6} M; ▲, 1×10^{-5} M.

mine displayed mixed competitive-noncompetitive antagonism (Fig. 3); however, when tested in isolated uterus (Fig. 4), diphenhydramine was dominantly competitive with a noncompetitive, partly specific activity at high concentrations. In the uterus preparation, complete tissue recovery was effected after the highest incubation concentration—this is generally a characteristic of reversible competitive antagonists.

Cryogenine displayed a classic noncompetitive antihistaminic action in guinea pig ileum (Fig. 5); but in the isolated uterus preparation, the qualitative action of cryogenine (Fig. 6) resembles that of diphenhydramine in the same tissue even to the extent that full tissue recovery could be achieved. In this tissue cryogenine may be said to react with a histaminic receptor. This qualitative difference in results between the two tissues may mean that the histaminic receptors in these tissues are structurally different, or that in the ileum cryogenine has a greater affinity for a receptor other than the histaminic.

In 1961, Rocha e Silva (9) proposed that a histidyl residue in a polypeptide chain might serve as a histaminic receptor site. After constructing Hirchfelder-La Pine molecular models of histamine and histidyl, it was seen that histamine could bind with the histidyl residue through hydrogen bonding between the imidazole nitrogen (histidyl) and the amine group (histamine) with a second anchorage point effected through hydrogen bonding between the carboxy oxygen of the adjacent amino acid moiety and the 1-nitrogen of the imidazole ring of histamine. A molecular model of cryogenine indicates two reactive groups; the quinidazole nitrogen and the phenolic hydroxyl bear the same spatial complimentarity (about 6 Å apart) as do the proposed reactive groups in the interactions of the histidyl-histamine. Since cryogenine could attach through hydrogen bonding to the proposed receptor, this might explain its possible competitive antagonism toward histamine in the uterus. This receptor surface would have to be readily available. since the cryogenine molecule is bulky and hindrance from surrounding chemical groups in the biophase could prevent receptor interaction. This in turn may account for the noncompetitive antihistaminic activity of cryogenine in the ileum. A molecular model study of diphenhydramine and histidyl relationships indicated that the carboxy group of the adjacent amino acid residue does not seem destined to react with any group of the diphenhydramine molecule. However, with rotation of the carboxy-amino amide linkage a weak hydrogen bond could form between the amide nitrogen of the histidyl residue and the ether oxygen of diphenhydramine. A second anchorage point probably involves a hydrogen bond between the imidazole nitrogen of histidine and the tertiary amino group of diphenhydramine. Additional receptor parts could be involved in hydrophobic bonding with the benzene rings of diphenhydramine.

In addition, cryogenine was tested for its antihistaminic activity using a modified version of the Castillo and DeBeer isolated guinea pig tracheal chain technique (aerated Krebs modification of Van Dyke-Hastings perfusate, 37°) (10, 11). Histamine was presented as a single challenge (1×10^{-4} or 1×10^{-5} *M* depending upon the sensitivity of the tissue). From the data, affinity (pD'_2) values were calculated to be 6.20 and 3.88 for diphenhydramine and cryogenine, respectively.

The antihistaminic activity of cryogenine and diphenhydramine was then compared with that of cyproheptadine, an effective serotonin antagonist (12). The cyproheptadine results indicated an antagonism qualitatively similar to that of cryogenine and diphenhydramine in guinea pig uterus. Parallel displacement of the concentration-response curves to the right was evident with all incubated concentrations ($6.97–27.89 \times 10^{-10}$ *M*), yet there was a tendency for a progressive lowering of the curve maxima as incubation concentrations were increased (−55% for 27.89×10^{-10} *M*).

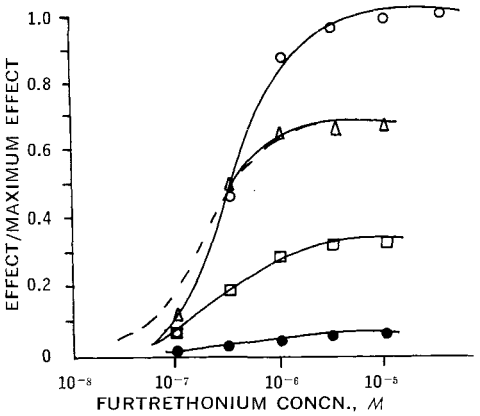


Figure 2—*Cumulative log-concentration curves of furtrethonium on rat jejunum in the presence of cryogenine. Key:* ○, *control;* △, 4.6×10^{-6} M *incubation concentration of cryogenine;* □, 14.5×10^{-6} M *cryogenine;* ●, 4.6×10^{-5} M.

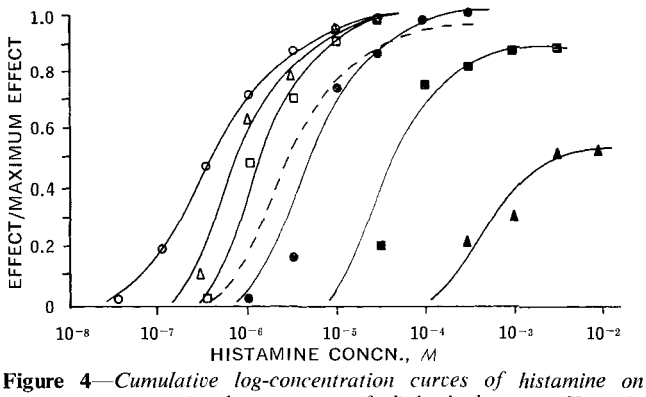


Figure 4—*Cumulative log-concentration curves of histamine on guinea pig uterus in the presence of diphenhydramine. Key:* ○, *control;* △, 1×10^{-9} M *incubation concentration of diphenhydramine;* □, 1×10^{-8} M *diphenhydramine;* ●, 1×10^{-7} M; ■, 1×10^{-6} M; ▲, 1×10^{-5} M.

Table I—Calculated *In Vitro* Constants

Agonist	Affinity, pD_2	System	Antagonist[a]	Affinity pA_2	Affinity pD'_2	Intrinsic Activity
Furtrethonium	6.16	Rat jejunum	Diphenhydramine	6.27	5.00	0(−1)
			Cyproheptadine	7.19	6.24	0(−1)
			Cryogenine	—	5.15	−1
Histamine	6.24	Guinea pig ileum	Diphenhydramine	8.46	5.18	0(−1)
			Cyproheptadine	—	8.97	−1
			Cryogenine	—	4.27	−1
Histamine	6.16	Guinea pig uterus	Diphenhydramine	8.49	4.97	0(−1)
			Cyproheptadine	10.18	8.92	0(−1)
			Cryogenine	5.02	3.60	0(−1)
Serotonin	7.07	Rat uterus	Diphenhydramine	8.46	5.44	0
			Cyproheptadine	10.02	8.41	0(−1)
			Cryogenine	6.09	4.39	−1

[a] Ten minutes incubation time.

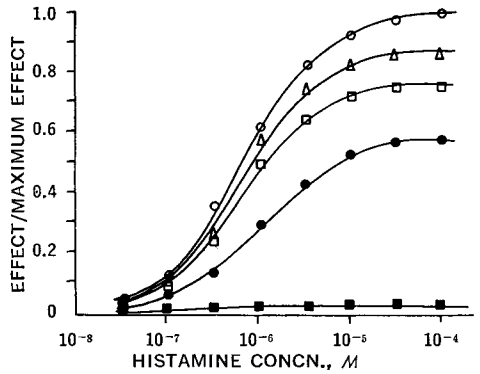


Figure 5—*Cumulative log-concentration curves of histamine on guinea pig ileum in the presence of cryogenine. Key:* ○, *control;* △, *4.6* × *10*$^{-6}$ M *incubation concentration of cryogenine;* □, *21.3* × *10*$^{-6}$ M *cryogenine;* ●, *4.6* × *10*$^{-5}$ M.

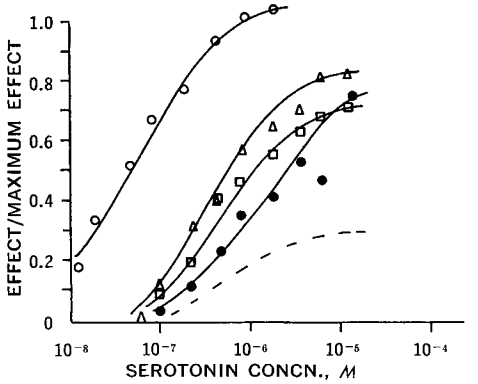


Figure 7—*Cumulative log-concentration curves of serotonin on rat uterus in the presence of cryogenine. Key:* ○, *control;* △, *4.6* × *10*$^{-6}$ M *incubation concentration of cryogenine;* □, *14.5* × *10*$^{-6}$ M *cryogenine;* ●, *4.6* × *10*$^{-5}$ M.

Cyproheptadine was clearly a noncompetitive histamine antagonist in guinea pig ileum.

The antifurtrethonium activity of cyproheptadine in isolated rat jejunum qualitatively resembled that of diphenhydramine in that tissue. Calculated affinity values (7) are summarized in Table I for all experiments.

Diphenhydramine appeared to be a more pure competitive inhibitor of serotonin than cyproheptadine in rat uterus, although clearly much less potent. This was shown by parallel displacement of the response curves for concentrations of 1 × 10^{-8} to 1 × 10^{-6} *M* of diphenhydramine with only a slight reduction of the curve maximum (−17%) noted with the incubation concentration of 1 × 10^{-6} *M*. Figure 7 represents the cumulative log-concentration curves of serotonin in the presence of cryogenine. While there was a trend for the curves to be shifted to the right along the log-concentration axis, there was a consistent dampening of the curve maximum with each incubation. When 8.18 × 10^{-5} *M* of cryogenine was incubated, no contractions could be produced by serotonin even though the cumulative bath concentration was raised to 1 × 10^{-4} *M*. The tissue recovery pattern after this incubation was drastically depressed (dashed line, Fig. 7). Cryogenine thus appears to be a much less potent and less specific serotonin antagonist than either cyproheptadine or diphenhydramine.

In general, these *in vitro* tests indicated that cryogenine possesses less specificity (pA_2) for the respective tissue receptors than do diphenhydramine and cyproheptadine as well as less potency to act as an antagonist of furtrethonium, histamine, and serotonin (pA_2 and pD'_2 values). Cryogenine appears to have some true specificity for the histaminic receptor of the guinea pig uterus.

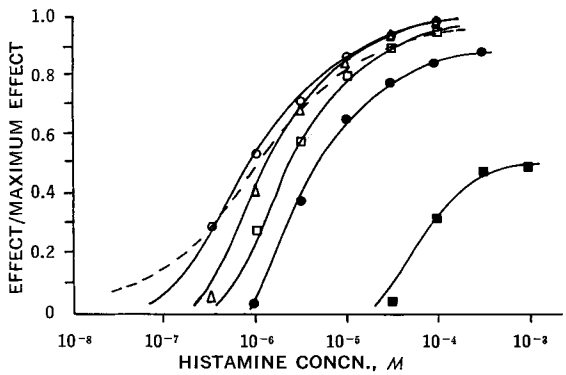


Figure 6—*Cumulative log-concentration curves of histamine on guinea pig uterus in the presence of cryogenine. Key:* ○, *control;* △, *4.6* × *10*$^{-6}$ M *incubation concentration of cryogenine;* □, *1.4* × *10*$^{-5}$ M *cryogenine;* ●, *4.6* × *10*$^{-5}$ M; ■, *14.5* × *10*$^{-5}$ M.

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ACKNOWLEDGMENTS AND ADDRESSES

Received April 24, 1969, from the *Division of Pharmacology, Pharmacy Research Institute, The University of Connecticut, Storrs, CT 06268*

Accepted for publication June 27, 1969.

Presented to the Pharmacology and Biochemistry Section, APhA Academy of Pharmaceutical Sciences, Montreal meeting, May 1969.

This work was supported by grant AM 11861-02 from the National Institute of Arthritis and Metabolic Diseases, United States Public Health Service.

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Interpretation of Percent Dissolved–Time Plots Derived from *In Vitro* Testing of Conventional Tablets and Capsules

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Abstract □ It is shown that under sink conditions a percent dissolved value at time t may simply be equivalent to the percent surface area generated to time t. If this is so, then percent dissolved-time data may best be described by a distribution function and the parameters of the distribution employed to describe the data. Simulated percent dissolved-time data, generated by means of the logarithmic normal distribution function, are shown to yield apparent first-order plots. Hence, if the new concept is correct, apparent first-order kinetics, derived from *in vitro* dissolution tests on conventional tablets and capsules, may be an artifact in some cases. In the special case when surface area of drug available for dissolution decreases exponentially with time after some lag time, t_0, then first-order kinetics appear applicable to the dissolution data. Relationships between many of the constants in formerly derived dissolution rate equations and some equations derived in this report are shown. Dimensions of the constants are clarified. The new method of dissolution rate data examination is capable of providing characterizing parameters of greater potential utility than conventional treatments heretofore used.

Keyphrases □ Tablets, capsules—percent dissolved-time plots interpreted □ First-order dissolution rate equation—sink conditions □ Surface area effects—dissolution rates □ Lag time—dissolution rates □ Distribution parameter, relation—dissolution data

Although there is extensive literature on dissolution rate theory it is appropriate here to review some of the equations in order to show relationships between some of the constants and establish their dimensions.

Quantitative Studies

Conditions of Constant Surface Area—Noyes and Whitney (1) quantitatively studied dissolution by rotating cylinders of benzoic acid and lead chloride in water, then analyzing the solution at intervals of time. In their experiments the surface area of chemical available for dissolution remained essentially constant. They showed that dissolution obeyed the equation

$$dC/dt = k\,(C_s - C) \qquad \text{(Eq. 1)}$$

where C is the concentration of solute at time t, C_s is the equilibrium solubility of the solute at the experimental temperature, and k is a first-order rate constant with dimension 1/time. In later experiments (2, 3) the surface area of solute available for dissolution, S, was incorporated into the equation to give

$$dC/dt = k_1 S\,(C_s - C) \qquad \text{(Eq. 2)}$$

where k_1 is a constant with dimensions length²/time. It should be noted that $k = k_1 S$ whence $k_1 = k/S$. Brunner (4) used Fick's law of diffusion to establish a relationship between the constants k and k_1 in the above equations and other variables. These relationships were:

$$k = DS/Vh \qquad \text{(Eq. 3)}$$

$$k_1 = D/Vh \qquad \text{(Eq. 4)}$$

where D is the diffusion coefficient of the solute in the dissolution medium, V is the volume of the dissolution medium, and h is the thickness of the diffusion layer. Equation 1 of Noyes and Whitney was written by Hixson and Crowell (5) as

$$dW/dt = KS\,(C_s - C) \qquad \text{(Eq. 5)}$$

where W is the amount of solute in solution at time t, dW/dt is the rate of appearance of solute in the solution at time t, and K is a constant with dimensions length/time. Equation 5 is obtained from from Eq. 2 by multiplying both sides by V and letting $K = k_1 V$. By comparing terms we find:

$$K = D/h \qquad \text{(Eq. 6)}$$

Equation 5 may be written as

$$dW/dt = KS/V\,(VC_s - W) = k\,(VC_s - W) \qquad \text{(Eq. 7)}$$

If a constant surface dosage form is studied under nonsink and nonreactive conditions then Eq. 7 should apply and the equation may be integrated to give

$$W = VC_s\,(1 - e^{-kt}) \qquad \text{(Eq. 8)}$$

Rearrangement of Eq. 8 and the taking of logarithms of both sides of the rearranged equation leads to:

$$\log\,(VC_s - W) = \log VC_s - \frac{k}{2.303}\,t \qquad \text{(Eq. 9)}$$

It should be noted that under conditions of constant surface area, nonsink, and nonreactive conditions, the asymptote is VC_s and not W^0 (the initial amount of drug in the dosage form) or W^∞ (the amount of drug ultimately dissolved at time infinity).