Effects of Chlorpheniramine and Pyrilamine on the Atrial Actions of Acetylcholine, Tyramine, and Ephedrine

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Abstract □ The experiments described herein were performed on isolated, spontaneously beating rabbit atria. The muscarinic actions of acetylcholine in the presence of physostigmine were antagonized by pyrilamine, chlorpheniramine and procaine which demonstrates that these drugs have an "antimuscarinic" action. The nicotinic (positive inotropic and chronotropic) actions of acetylcholine in the presence of atropine were also antagonized by pyrilamine and chlorpheniramine. Cocaine, pyrilamine, and chlorpheniramine inhibited the atrial stimulation by tyramine and ephedrine. The fact that the antimuscarinic and antinicotinic effects of pyrilamine and chlorpheniramine show similar slopes may indicate that similar receptors are involved. Cocaine and pyrilamine also have parallel slopes indicating that pyrilamine has a cocaine-like effect which is not related to local anesthesia.

Keyphrases □ Atrial depression—acetylcholine, physostigmine produced □ Chlorpheniramine, pyrilamine, procaine effect—induced atrial depression □ Tyramine, ephedrine atrial stimulation—chlorpheniramine, pyrilamine, cocaine effect □ Antimuscarinic activity—chlorpheniramine, pyrilamine, procaine □ Antinicotinic activity—chlorpheniramine, pyrilamine, cocaine

This investigation was undertaken to compare the effects of two antihistamines, namely, pyrilamine maleate and chlorpheniramine maleate on the muscarinic (direct) and nicotinic (indirect) actions of acetylcholine and on the indirectly acting sympathomimetic amines, tyramine and ephedrine. Isaac and Goth (1) and Johnson and Kahn (2) have suggested that antihistamines have a cocaine-like effect since, like cocaine, they potentiate the effects of norepinephrine and reduce significantly the effects of tyramine. The cocaine-like property of these antihistamines was investigated since previous work from this laboratory has shown that cocaine alone of a series of local anesthetics studied antagonized the positive inotropic and chronotropic responses to tyramine on the isolated rabbit atria. All other local anesthetics and cocaine inhibited the stimulatory responses to nicotinic doses of acetylcholine (3). The results obtained from the antihistamine studies were compared with the responses elicited by procaine (against acetylcholine) and cocaine (against tyramine and ephedrine) to ascertain if any similarities or differences exist between these antihistamines and local anesthetics in their effects on the above mentioned agonists.

EXPERIMENTAL

Apparatus and Method—All of the following experiments have been carried out on spontaneously beating, isolated rabbit atria. The atria were excised from male albino rabbits and suspended in a 40-ml. organ bath, filled with oxygenated Locke solution and maintained at a constant temperature of 30 ± 1°. The force of contractions was recorded on a smoked drum kymograph with a heart lever (Starling), and the rate with a impulse counter (Thorp), set at 10-sec. intervals (3).

To obtain accurate measurements of atrial force, the following procedure was instituted. While the atria were equilibrating, the heart lever was weighted with 1 g. and a 1-g. tension line was inscribed on the smoked paper. The weight was removed, the atrium reattached, and the tension adjusted so that during the momentary pause between beats the level of the inscribing lever coincided with the 1-g. tension line. A writing pen was placed to inscribe a line, coinciding with 1-g. tension to aid in measuring the force of atrial contraction since a part of the recording, above the line, is due to spring recoil. The recording below the 1 g. tension line was used to measure atrial force.

Using methods described in Snedecor (4), mean values and standard errors of the means were obtained. Student's *t*-test was utilized to determine statistical significance. A computer (Olivetti-Underwood programma 101) was programmed for regression calculations to determine the slopes and coordinates for the regression line points of the dose-response curves.

A probability of 5% ($p = 0.05$) was considered to be the minimum for an event not to have occurred by chance alone.

All doses reported in this communication are expressed in terms of the respective salts.

RESULTS

Effect of Chlorpheniramine, Pyrilamine, and Procaine on Atrial Depression Produced by Acetylcholine in the Presence of Physostigmine—Acetylcholine bromide (0.025 mcg./ml.) followed, 4 to 5 min. later, by physostigmine salicylate (2.5 mcg./ml.) produced atrial standstill. No spontaneous resumption of atrial contractions occurred for periods of up to 20 min. A single exchange of the bath fluid with drug-free Locke solution resulted in a restoration of contractions within 2 to 5 min. If, instead of washing, increasing doses of procaine were added to the bath fluid, atrial contractions resumed rapidly and returned essentially to control levels (Table I).

Table I—Effect of Acetylcholine (ACH., 0.025 mcg./ml.) Followed by Physostigmine (PHYSO., 2.5 mcg./ml.) on the Force and Rate of Contractions of Isolated Rabbit Atria and its Modification by Procaine (PROC.)

PROC. Dose mcg./ml.	(*n*)	Parameter Measured	Depression[a] of Controls, %	Depression[b] Remaining after PROC., %	Time to Onset of Return of Beats, sec. ± *SE*
0	(6)	Force	100	28.5[c]	127 ± 31
		Rate	100	36.5[c]	
3	(6)	Force	100	58.3	166 ± 45
		Rate	100	53.4	
8	(6)	Force	100	9.0	58 ± 21
		Rate	100	23.6	
15	(6)	Force	100	0	37 ± 10
		Rate	100	20.8	
30	(4)	Force	100	0	39 ± 12
		Rate	100	8.7	

[a] Depression produced by the addition of ACH. (0.025 mcg./ml.) and PHYSO. (2.5 mcg./ml.). [b] 100 indicates no effect of PROC. on the depression and 0 complete reversal. [c] Responses following a single exchange of the bath fluid.

Table II—Effect of Acetylcholine (ACH., 0.025 mcg./ml.) Followed by Physostigmine (PHYSO., 2.5 mcg./ml.) on the Force and Rate of Contractions of Isolated Rabbit Atria and its Modification by Pyrilamine (PM.)

PM. Dose, mcg./ml.	(*n*)	Parameter Measured	Depression[a] of Controls, %	Depression[b] Remaining After PM., %	Time to Onset of Return of Beats, sec. ± *SE*
0	(6)	Force	100	0[c]	279 ± 74
		Rate	100	61.6[c]	
12	(5)	Force	100	79	478 ± 97
		Rate	100	78.6	
16	(6)	Force	100	55	332 ± 63
		Rate	100	63	
24	(6)	Force	100	42.3	220 ± 33
		Rate	100	55.9	

[a] Depression produced by the additon of ACH. (0.025 mcg./ml.) and PHYSO. (2.5 mcg./ml.). [b] 100 indicates no effect of PM. on the depression and 0 complete reversal. [c] Responses following a single exchange of the bath fluid.

Table III—Effect of Acetylcholine (ACH., 0.025 mcg./ml.) Followed by Physostigmine (PHYSO., 2.5 mcg./ml.) on the Force and Rate of Contractions of Isolated Rabbit Atria and its Modification by Chlorpheniramine (CT.)

CT. Dose, mcg./ml.	(*n*)	Parameter Measured	Depression[a] of Controls, %	Depression[b] Remaining after CT., %	Time to Onset of Return of Beats, sec. ± *SE*
0	(7)	Force	100	13.5[c]	210 ± 35
		Rate	100	62.8[c]	
5	(7)	Force	100	71.4	379 ± 63
		Rate	100	79.4	
10	(7)	Force	100	35.8	166 ± 27
		Rate	100	61.6	
30	(6)	Force	100	37.5	84 ± 25
		Rate	100	53.3	

[a] Depression produced by the addition of ACH. (0.025 mcg./ml.) and PHYSO. (2.5 mcg./ml.). [b] 100 indicates no effect of CT. on the depression and 0 complete reversal. [c] Responses following a single exchange of the bath fluid.

The addition of increasing doses of pyrilamine and chlorpheniramine likewise antagonized the atrial depression produced by acetylcholine-physostigmine (Tables II and III). Atrial contractions did not return as rapidly as with procaine.

Table IV—Analysis of Dose-Response Curves to Pyrilamine, Chlorpheniramine, and Procaine in the Presence of Acetylcholine (0.025 mcg./ml.) and Physostigmine (2.5 mcg./ml.)

Drug	Parameter Measured	Slope ± *SE*	*X*-Intercept[a] ± *SE*
Procaine	Force	86.7 ± 21.6[b]	3.009 ± 0.2
Procaine	Rate	43.2 ± 8.3[b]	2.428 ± 0.3
Pyrilamine	Force	102.6 ± 39.6	3.885 ± 0.1
Pyrilamine	Rate	74.3 ± 21.7[b]	3.797 ± 0.1
Chlorpheniramine	Force	39.4 ± 32.8	3.282 ± 0.7
Chlorpheniramine	Rate	32.2 ± 11.1[b]	3.080 ± 0.4

[a] Log ng./ml. [b] Significantly different from zero (p equals or less than 0.05).

Table V—Effect of Pyrilamine (PM.) on the Responses of Isolated Rabbit Atria to Acetylcholine (ACH., 100 mcg./ml.) in the Presence of Atropine (ATR. 3 mcg./ml.)

PM. Dose, mcg./ml.	(*n*)	Parameter Measured	Effect of[a] PM. Depression (% of Control)	Effect of[b] ACH. and ATR. Excitation (% of Control)	Inhibition of ACH. and ATR. after PM. %	Time to Onset of ACH. Responses, sec. ± *SE*
0	(6)	Force	—	75	—	18 ± 4
		Rate	—	28.1	—	20 ± 0
0.25	(6)	Force	5.26	75	20	22 ± 4
		Rate	0.8	28.1	35.4	20 ± 3
0.5	(6)	Force	5.26	75	26.6	20 ± 2
		Rate	1.72	28.1	62	27 ± 3
1	(6)	Force	5.55	75	60	29 ± 3
		Rate	5.9	28.1	88.4	23 ± 4
2.5	(6)	Force	0	75	86.8	28 ± 4
		Rate	13	28.1	100	35 ± 6

[a] Depression caused by PM. in the absence of acetylcholine-atropine. [b] Stimulation caused by acetylcholine-atropine in the absence of PM.

Tables I, II, and III summarize the antagonistic effects of chlorpheniramine, pyrilamine, and procaine on acetylcholine-physostigmine induced atrial depression.

Table IV shows the values for the slopes and *X*-intercepts of the dose-response curves for the effects of the acetylcholine-physostigmine antagonists. The slopes of these dose-response curves using atrial rate as the measured parameter are not significantly different from each other. The values for the *X*-intercepts demonstrate that procaine is the most potent of the antagonists followed by chlorpheniramine and pyrilamine.

The dose-response curves in which force is the measured parameter show that procaine is the only antagonist which has a dose-response curve with a statistically significant slope. Even though the slopes of the dose-response curves to the antihistamines are not significantly different from zero, an antagonism to acetylcholine-physostigmine by these compounds is apparent.

Table VI—Effect of Chlorpheniramine (CT.) on the Responses of Isolated Rabbit Atria to Acetylcholine (ACH., 100 mcg./ml.) in the Presence of Atropine (ATR., 3 mcg./ml.)

CT. Dose, mcg./ml.	(*n*)	Parameter Measured	Effect of[a] CT. Depression (% of Control)	Effect of[b] ACH. and ATR. Excitation (% of Control)	Inhibition of ACH. and ATR. after CT. %	Time to Onset of ACH. Responses, sec. ± *SE*
0	(6)	Force	—	27.8	—	21 ± 5
		Rate	—	39.4	—	21 ± 1
1	(6)	Force	0	27.8	0	24 ± 7
		Rate	1.87	39.4	27.9	30 ± 14
2.5	(6)	Force	0	27.8	+20[c]	32 ± 7
		Rate	5.5	39.4	53.5	51 ± 27
5	(6)	Force	0	27.8	40	52 ± 16
		Rate	4.7	39.4	100	45 ± 7
10	(6)	Force	0	27.8	80	78 ± 34
		Rate	14.8	39.4	100	58 ± 11

[a] Depression caused by CT. in the absence of acetylcholine-atropine. [b] Stimulation caused by acetylcholine-atropine in the absence of CT. [c] + denotes a stimulatory response to acetylcholine.

Table VII—Analysis of Dose-Response Curves to Pyrilamine and Chlorpheniramine in the Presence of Acetylcholine (100 mcg./ml.) and Atropine (3 mcg./ml.)

Drug	Parameter Measured	Slope ± *SE*	*X*-Intercept[a] ± *SE*
Pyrilamine	Force	85.4 ± 11.2[b]	2.367 ± 0.1
Pyrilamine	Rate	88.1 ± 2.9[b]	1.995 ± 0.0
Chlorpheniramine	Force	133.7 ± 13[c]	3.501 ± 0.03
Chlorpheniramine	Rate	79.8 ± 18.1[b]	2.725 ± 0.2

[a] Log ng./ml. [b] Significantly different from zero (p equals or less than 0.05). [c] Estimated standard error.

Effect of Pyrilamine and Chlorpheniramine on the Indirect Effects of Acetylcholine—Positive inotropic and chronotropic responses were obtained when acetylcholine (100 mcg./ml.) was added 3 min. after atropine sulfate (3 mcg./ml.).

Increasing doses of pyrilamine and chlorpheniramine were added in a randomized series 7 min. before atropine in order to determine the effects of these drugs in modifying the indirect (nicotinic) actions of acetylcholine on the rabbit atria. In this experimental design, the atria were exposed to the antihistamine for 10 min. prior to the addition of acetylcholine.

Tables V and VI summarize the effects of the antihistamines on the nicotinic responses to acetylcholine.

The antihistamines antagonized the responses of the atria to acetylcholine and the highest doses of chlorpheniramine and pyrilamine, in all cases, unmasked a muscarinic action of the quaternary ammonium compound.

Table VII shows the values for the slopes and *X*-intercepts of the dose-response curves on the effects of pyrilamine and chlorpheniramine as antagonists to nicotinic acetylcholine. When atrial rate was the measured parameter, the slopes of the dose-response curves were not significantly different from each other.

The *X*-intercept values show that pyrilamine is more potent than chlorpheniramine as an antagonist of the nicotinic effects of acetylcholine.

Using force as the response, pyrilamine produced a dose-response curve which has a statistically significant slope. Chlorpheniramine exhibited an antagonism to the production of force but no significant dose-response curve was apparent within the dose range used.

Effect of Pyrilamine, Chlorpheniramine, and Cocaine on the Stimulation of Rabbit Atria Produced by Tyramine and Ephedrine—Various doses of chlorpheniramine, pyrilamine, and cocaine were compared in their actions on the responses of isolated rabbit atria to the stimulating effects of tyramine and ephedrine.

A series of control studies were performed to determine the responses to six consecutive doses of tyramine (5 mcg./ml.), given every 30 min., and of ephedrine (10 mcg./ml.), given at 45-min. intervals to ascertain whether or not any change in the positive inotropic and chronotropic responses would ensue. No significant change in the respose to these drugs was observed.

Table VIII—Effect of Cocaine (COC.) on the Responses of Isolated Rabbit Atria to Tyramine (TYR., 5 mcg./ml.)

COC. Dose, mcg./ml.	(*n*)	Parameter Measured	Effect of[a] TYR. Excitation (% of Control)	Effect of[b] COC. Depression (% of Control)	Inhibition of TYR. after COC. %	Time to Onset of TYR. Responses, sec. ± *SE*
0	(6)	Force	42.8	—	—	24 ± 2
		Rate	56.8	—	—	24 ± 3
1.25	(6)	Force	42.8	0	0	34 ± 3
		Rate	56.8	0	37.9	52 ± 5
2.5	(6)	Force	42.8	0	16.7	58 ± 5
		Rate	56.8	4.17	65.5	77 ± 7
5	(6)	Force	42.8	7.15	66.6	88 ± 21
		Rate	56.8	3.12	77.5	140 ± 21

[a] Excitation caused by tyramine in the absence of cocaine. [b] Depression caused by cocaine in the absence of tyramine.

Table IX—Effect of Pyrilamine (PM.) on the Responses of Isolated Rabbit Atria to Tyramine (TYR., 5 mcg./ml.)

PM. Dose mcg./ml.	(*n*)	Parameter Measured	Effect of[a] TYR. Excitation (% of Control)	Effect of[b] PM. Depression (% of Control)	Inhibition of TYR. after PM. %	Time to Onset of TYR. Responses, sec. ± *SE*
0	(7)	Force	100	—	—	22 ± 3
		Rate	47.4	—	—	26 ± 2
1	(7)	Force	100	0	50	24 ± 4
		Rate	47.4	10.1	25	31 ± 3
2.5	(7)	Force	100	0	56.2	32 ± 5
		Rate	47.4	18	3.57	34 ± 2
5	(6)	Force	100	0	43.8	38 ± 5
		Rate	47.4	28.2	21.4	48 ± 5
10	(7)	Force	100	6.7	37.4	42 ± 4
		Rate	47.4	28.2	42.8	56 ± 6

[a] Excitation cause by tyramine in the absence of pyrilamine. [b] Depression caused by pyrilamine in the absence of tyramine.

Table X—Effect of Chlorpheniramine (CT.) on the Responses of Isolated Rabbit Atria to Tyramine (TYR., 5 mcg./ml.)

CT. Dose, mcg./ml.	(*n*)	Parameter Measured	Effect of[a] TYR. Excitation (% of Control)	Effect of[b] CT. Depression (% of Control)	Inhibition of TYR. after CT., %	Time to Onset of TYR. Responses, sec. ± *SE*
0	(7)	Force	100	—	—	22 ± 2
		Rate	33.8	—	—	29 ± 4
1	(7)	Force	100	5	33.4	29 ± 3
		Rate	33.8	6.4	+9.3[c]	40 ± 5
2.5	(7)	Force	100	5.25	51.9	26 ± 3
		Rate	33.8	7.3	+9.3	36 ± 8
5	(7)	Force	100	5.55	59.2	38 ± 8
		Rate	33.8	10.7	0	57 ± 13
10	(7)	Force	100	0	66.6	54 ± 21
		Rate	33.8	8.6	25.3	63 ± 5

[a] Excitation caused by tyramine in the absence of chlorpheniramine. [b] Depression caused by chlorpheniramine in the absence of tyramine. [c] + denotes a stimulatory response to tyramine.

Doses of the antihistamines or cocaine were added to the organ bath 10 min. prior to the addition of either tyramine or ephedrine. Tables VIII, IX, X, XIII, XIII and XIV summarize the results of these experiments.

Table XI shows the values for the slopes and *X*-intercepts of the dose-response curves to pyrilamine, chlorpheniramine, and cocaine in response to tyramine. The table shows that the slopes of the rate

Table XI—Analysis of Dose-Response Curves to Cocaine, Pyrilamine, and Chlorpheniramine in the Presence of Tyramine (5 mcg./ml.)

Drug	Parameter Measured	Slope ± *SE*	*X*-Intercept[a] ± *SE*
Cocaine	Force	111.4 ± 30.6[b]	3.166 ± 0.09
Cocaine	Rate	66.2 ± 14[b]	2.526 ± 0.2
Chlorpheniramine	Force	32.8 ± 4.1[b]	1.966 ± 0.2
Chlorpheniramine	Rate	84.6 ± 8.5[c]	3.612 ± 0.1
Pyrilamine	Force	31.3 ± 5.7[b]	5.117 ± 0.3
Pyrilamine	Rate	65.2 ± 2.8[b]	3.353 ± 0.0

[a] Log ng./ml. [b] Significantly different from zero (p equals or less than 0.05). [c] Estimated standard error.

Table XII—Effect of Cocaine (COC.) on the Responses of Isolated Rabbit Atria to Ephedrine (EPH., 10 mcg./ml.)

COC. Dose, mcg./ml.	(*n*)	Parameter Measured	Effect of[a] EPH. Excitation (% of Control)	Effect of[b] COC. Depression (% of Control)	Inhibition of EPH. after COC. %	Time to Onset of EPH. Responses, sec. ± *SE*
0	(9)	Force	22.2	—	—	62 ± 20
		Rate	33.7	—	—	49 ± 5
0.5	(9)	Force	22.2	0	0	82 ± 26
		Rate	33.7	3.2	21.2	78 ± 13
1.25	(9)	Force	22.2	4.8	25	97 ± 24
		Rate	33.7	4.1	42.4	114 ± 20
2.5	(7)	Force	22.2	4.35	25	131 ± 48
		Rate	33.7	5.6	57.5	146 ± 32
5	(6)	Force	22.2	8.7	50	191 ± 68
		Rate	33.7	7.85	51.5	168 ± 37

[a] Excitation caused by ephedrine in the absence of cocaine. [b] Depression caused by cocaine in the absence of ephedrine.

dose-response curves for cocaine and pyrilamine are not significantly different from each other. A comparison of the *X*-intercepts show that cocaine is more potent than pyrilamine. No linear dose-response curve for chlorpheniramine could be shown even though it antagonized the effects of tyramine on atrial rate responses.

Table XV shows the values for the slopes and *X*-intercepts of the dose-response curves of the antihistamines and cocaine in response to ephedrine. The table shows that the slopes of the rate dose-response curves for pyrilamine and cocaine are not significantly different from each other. A comparison of the *X*-intercepts shows that cocaine is more potent than pyrilamine in antagonizing ephedrine. No statistically significant dose-response curve for chlorpheniramine could be demonstrated although some antagonism was apparent.

Comparisons regarding the effects of the antagonists (pyrilamine and chlorpheniramine) on the force responses of the atria to tyramine and ephedrine could not be made since the concentrations of the antihistamines were apparently too low to show linear dose-response curves. Cocaine was the only antagonist for which a linear dose-response curve concerning atrial force was obtained. Although the antihistamines did not produce a significant dose-response curve, inhibitory effects on the production of force by the agonists was noted (Tables XIII and XIV).

Table XIII—Effect of Pyrilamine (PM.) on the Responses of Isolated Rabbit Atria to Ephedrine (EPH., 10 mcg./ml.)

PM. Dose, mcg./ml.	(*n*)	Parameter Measured	Effect of[a] EPH. Excitation (% of control)	Effect of[b] PM. Depression (% of control)	Inhibition of EPH. after PM., %	Time to Onset of EPH. Responses, sec. ± *SE*
0	(6)	Force	25	—	—	21 ± 4
		Rate	34	—	—	42 ± 5
1	(6)	Force	25	6.25	+50[c]	29 ± 2
		Rate	34	8.2	3.34	70 ± 6
2.5	(6)	Force	25	0	+25[c]	44 ± 7
		Rate	34	13.8	13.6	110 ± 18
5	(6)	Force	25	6.25	0	48 ± 7
		Rate	34	11	43.3	147 ± 40
10	(6)	Force	25	12.5	50	57 ± 9
		Rate	34	18.5	60	190 ± 48

[a] Excitation caused by ephedrine in the absence of pyrilamine. [b] Depression caused by pyrilamine in the absence of ephedrine. [c] + denotes a stimulatory response to ephedrine.

Table XIV—Effect of Chlorpheniramine (CT.) on the Responses of Isolated Rabbit Atria to Ephedrine (EPH., 10 mcg./ml.)

CT. Dose, mcg./ml.	(*n*)	Parameter Measured	Effect of[a] EPH Excitation (% of Control)	Effect of[b] CT. Depression (% of Control)	Inhibition of EPH. after CT. %	Time to Onset of EPH. Responses, sec. ± *SE*
0	(6)	Force	31.2	—	—	40 ± 1
		Rate	17	—	—	58 ± 9
1	(6)	Force	31.2	6.7	40	49 ± 7
		Rate	17	2.3	31.8	90 ± 24
2.5	(5)	Force	31.2	5.55	60	44 ± 5
		Rate	17	7.2	45.4	147 ± 23
5	(6)	Force	31.2	0	40	63 ± 10
		Rate	17	10.4	36.4	172 ± 35
10	(6)	Force	31.2	0	60	70 ± 8
		Rate	17	21.3	45.4	167 ± 42

[a] Excitation caused by ephedrine in the absence of chlorpheniramine. [b] Depression caused by chlorpheniramine in the absence of ephedrine.

DISCUSSION AND CONCLUSIONS

An investigation has been made of two antihistamines, pyrilamine, and chlorpheniramine, as to their ability to reverse atrial arrest induced by acetylcholine in the presence of physostigmine. Using a series of doses of these antihistamines, graded dose-response curves were obtained against acetylcholine-physostigmine which demonstrated the antimuscarinic action of these drugs. The linearity of the dose-response curves indicates that with increasing doses of the antihistamines, the degree of reversal of atrial arrest increases.

The observation that chlorpheniramine is more potent than pyrilamine (dose ratio: 6 to 1) in antagonizing acetylcholine is not surprising, since many authors (5–9) ascribed a weak or negligible anticholinergic component to pyrilamine in contrast to chlorpheniramine to which other investigators (9, 10) attributed a moderate anticholinergic action.

In view of the above information and the fact that pyrilamine and chlorpheniramine possess greater local anesthetic activities than does procaine (9), a statement can be made concerning the antimuscarinic activity of the antihistamines and procaine. Since pyrilamine has been shown to have a greater local anesthetic activity than chlorpheniramine (9), and since both compounds are stronger local anesthetics than procaine, a local anesthetic component of action can certainly be dismissed as the mechanism for acetylcholine antagonism because procaine was the most potent and strongest of the antagonists used in this study.

Many authors have demonstrated atrial stimulation resulting from the administration of a nicotinic dose of acetylcholine in the presence of atropine (3, 11–13). This action was shown by many authors to depend upon the release of catecholamines from isolated, intact hearts and atria (3, 12–14, 26).

Table XV—Analysis of Dose-Response Curves to Cocaine, Pyrilamine, and Chlorpheniramine in the Presence of Ephedrine (10 mcg./ml.)

Drug	Parameter Measured	Slope ± *SE*	*X*-Intercept[a] ± *SE*
Cocaine	Force	52.3 ± 2.9[b]	2.291 ± 0.0
Cocaine	Rate	45.8 ± 9.8[b]	2.723 ± 0.1
Chlorpheniramine	Force	0.0 ± 38.4	—
Chlorpheniramine	Rate	10.2 ± 8.6	1.926 ± 1.4
Pyrilamine	Force	71.3 ± 3.8[b]	3.715 ± 0.0
Pyrilamine	Rate	59.7 ± 9.9[b]	3.047 ± 0.1

[a] Log ng./ml. [b] Significantly different from zero (p equals or less than 0.05).

It has been demonstrated that various concentrations of pyrilamine and chlorpheniramine, as well as all conventional local anesthetics (3), can partially or completely block the positive inotropic and chronotropic responses to acetylcholine in the presence of atropine.

In comparing the dose-response curves obtained, it becomes apparent that there is a direct relationship between the dose of antihistamines and the degree of inhibition of the acetylcholine response.

By a comparison of the slopes and *X*-intercepts of the dose-response curves of the antihistamines (Table VII), it can be shown that the slopes of the rate dose-response curves are not significantly different from each other which may indicate a similar mechanism of antagonism to the indirect effects of acetylcholine. *X*-intercept values show that pyrilamine is more potent than chlorpheniramine in antagonizing this indirect action of acetylcholine, in contrast to its antimuscarinic efficacy.

Upon an analysis of the rate dose-response curves for acetylcholine-physostigmine and acetylcholine-atropine in the presence of the antihistamines, one notices that these slopes are not significantly different from each other. This may indicate that the acetylcholine receptors that have a role in both the direct and indirect responses are very similar in structure.

Various authors have demonstrated that some antihistamines: pyrilamine, chlorpheniramine *etc.*, can modify the tissue responses to tyramine (2, 15).

These studies have also demonstrated that pyrilamine and chlorpheniramine can antagonize the actions not only of tyramine but also of ephedrine despite the difference in the proportions of direct and indirect effects of these two sympathomimetic amines, classified by Trendelenburg *et al.* (16).

A significant rate dose-response curve was obtained for pyrilamine against tyramine and ephedrine but the dose-response curves for chlorpheniramine against these indirectly acting sympathomimetic amines was not significant even though atrial rate increases were antagonized. These results may indicate that pyrilamine and chlorpheniramine have different actions on tyramine and ephedrine or that tyramine and ephedrine differ in their mechanism of action in the rabbit atrium. However, the first part of the last statement is made with some reservations because we were unable to use higher concentrations of chlorpheniramine since atrial depression was becoming apparent.

The slopes of the dose-response curves for cocaine against tyramine and ephedrine are similar to that of pyrilamine which may indicate a similar mechanism of action. A comparison of the *X*-intercepts of cocaine and pyrilamine shows that cocaine is more potent as a tyramine and ephedrine antagonist than is pyrilamine.

An analysis of the slopes of the rate dose-response curves for pyrilamine against nicotinic acetylcholine and the indirectly acting sympathomimetic amines shows that the slopes are significantly different from each other. This may indicate that norepinephrine release by acetylcholine and the indirectly acting amines (anti-tyramine and -ephedrine slopes were shown not to be significantly different when they were compared) may occur by different mechanisms.

It also seems possible for certain antihistamines to antagonize the actions of tyramine and ephedrine by the mechanism postulated by Iversen (17) for cocaine. Such a proposal has already been made by Johnson and Kahn (2) for the inhibition of tyramine by the antihistamines; this corroborates the theory of Furchgott *et al.* (18) and was defended by many authors including Iversen (19) who relates the antagonism of the effects of tyramine to blockade of reuptake by the active transport system at the sympathetic neuronal membrane thereby decreasing norepinephrine release simultaneously.

Table XVI illustrates the similarities and differences encountered with antihistamines, local anesthetics and atropine concerning their actions on acetylcholine, tyramine and ephedrine. The table shows that the effects of nicotinic doses of acetylcholine in the presence of atropine can be antagonized by both local anesthetics and antihistamines while the effects of tyramine and ephedrine can only be antagonized by cocaine and various antihistamines. The property common to both types of antagonists is the local anesthetic component. It would therefore seem reasonable to hypothesize that those drugs possessing the strongest local anesthetic action should be the most effective antagonists of the nicotinic effects of acetylcholine. Naranjo and de Naranjo (9) ranked the relative local anesthetic potencies of both local anesthetics and antihistamines in the following descending order: dibucaine, tetracaine, pyrilamine, chlorpheniramine, lidocaine, and procaine. Results from this laboratory show that pyrilamine is more potent than chlorpheniramine in antagonizing nicotinic acetylcholine while Koppanyi and MacFarlane (3) have shown that dibucaine, tetracaine, cocaine, lidocaine, and procaine in order of descending potency antagonized the actions of nicotinic acetylcholine. Therefore there appears to be a close correlation between local anesthetic potency and the antagonism of the indirect effects of acetylcholine.

Table XVI—Effects of Antihistamines, Local Anesthetics, and Atropine on the Actions of Indirectly Acting Sympathomimetic Amines and Acetylcholine

Modulating Agents	Direct Action of Acetyl-Choline	Indirect Action of Acetylcholine	Action of Tyramine and Ephedrine
Synthetic local anesthetics	↓[a] (3, 24)	↓ (3)	0[b] (3, 23)
Cocaine	↓ (3)	↓ (3)	↓ (20, 23, 24)
Antihistamines	↓ (9, 22, 24)	↓ (24)	↓ (2, 24)
Atropine	↓ (25)	↑[c] (3, 24)	0 (20, 21)

[a] ↓ = decrease. [b] 0 = no effect. [c] ↑ = increase.

The ultimate explanation for the interactions between the previously mentioned antagonists and agonists hinges upon the problem of the mode of release of norepinephrine from storage sites by nerve stimulation, by indirectly acting sympathomimetic amines and by acetylcholine in the presence of atropine.

REFERENCES

(1) L. Isaac and A. Goth, *Life Sci.*, **4,** 1899(1965).

(2) G. Johnson and J. Kahn, *J. Pharmacol. Exptl. Therap.*, **152,** 458(1966).

(3) T. Koppanyi and M. MacFarlane, *Ann. N. Y. Acad. Sci.*, **144,** 543(1967).

(4) G. Snedecor, "Statistical Methods," Iowa State College Press, Ames, Iowa, 1956.

(5) T. Sherrod, H. Schloemer, and E. Loew, *Federation Proc.*, **5,** 202(1946).

(6) T. Sherrod, E. Loew, and H. Schloemer, *J. Pharmacol. Exptl. Therap.*, **89,** 247(1947).

(7) E. Loew, *Physiol. Rev.*, **27,** 542(1947).

(8) E. Loew, *Ann. N. Y. Acad. Sci.*, **50,** 1142(1950).

(9) P. Naranjo and E. de Naranjo, *Arch. Intern. Pharmacodyn.*, **113,** 313(1958).

(10) A. Labelle and R. Tislow, *J. Pharmacol. Exptl. Therap.*, **113,** 73(1955).

(11) S. Kottegoda, *Brit. J. Pharmacol.*, **8,** 83(1953).

(12) M. Torchiana and E. Angelakos, *Arch. Intern. Physiol.*, **71,** 762(1963).

(13) T. Koppanyi and M. MacFarlane, *Life Sci.*, **3,** 1135(1964).

(14) E. Angelakos and E. Bloomquist, *Arch. Intern. Physiol.*, **73,** 397(1965).

(15) R. Buchholz, F. Haka, D. Plester, *Arch. Exptl. Pathol. Pharmakol.*, **212,** 189(1951).

(16) U. Trendelenburg, A. Muskus, W. Fleming, and B. de la Siera, *J. Pharmacol.*, **138,** 170(1962).

(17) L. Iversen, *J. Pharm. Pharmacol.*, **18,** 481(1966).

(18) R. Furchgott, S. Kirpekar, M. Rieker, and A. Schwas, *J. Pharmacol. Exptl. Therap.*, **142,** 39(1963).

(19) L. Iversen, "Uptake and Storage of Norepinephrine in Sympathetic Nerves," Cambridge U. Press, 1967, chap. 8, pp. 147–198.

(20) C. Cannon-Alfred, T. Koppanyi, F. Cowan, and G. D. Maengwyn-Davies, *Med. Pharmacol. Exptl.*, **16,** 135(1967).

(21) F. Cowan, T. Koppanyi, and G. D. Maengwyn-Davies, *Arch. Intern. Pharmacodyn.*, **160,** 424(1966).

(22) N. Dutta, *Brit. J. Pharmacol.*, **4,** 281(1949).

(23) M. Tainter and D. Chang, *J. Pharmacol.*, **30,** 193(1927).

(24) *Results* section of this communication.

(25) L. Goodman and A. Gilman, "The Pharmacological Basis of Therapeutics," Macmillan Co., 3rd ed., 1965, pp. 523–524.

(26) J. Richardson and E. Woods, *Proc. Soc. Exptl. Biol. Med.*, **100**, 149(1959).

ACKNOWLEDGMENTS AND ADDRESSES

Received May 12, 1969 from the *Department of Pharmacology, Schools of Medicine and Dentistry, Georgetown University, Washington, DC 20007*

Accepted for publication July 17, 1969.

This work was supported in part by a Hoffmann-LaRoche Fellowship.

Abstracted in part from a thesis presented by Robert E. Osterberg to the Graduate School, Georgetown University, Department of Pharmacology, in partial fulfillment of Master of Science degree requirements.

The authors wish to acknowledge the invaluable suggestions and criticisms of Drs. L. Isaac, T. E. Lynes, G. D. Maengwyn-Davies, and F. G. Standaert.

Mutual Inhibitory Effect of (−)-Mandelic Acid and Certain Sulfonamides on the Kinetics of Their Urinary Excretion in Humans

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Abstract □ Pseudo-first-order rate constants for the overall elimination of (−)-mandelic acid both in the absence (*K*) and in the presence (*Kas*) of sulfadiazine, sulfamethazine, or sulfamerazine were determined in three human subjects. Since the extent of metabolism of (−)-mandelic acid was not significantly altered in the presence of these sulfonamides, the ratio of the rate constants, *K*/*Kas*, has been calculated as a measure of the inhibitory effect of each sulfonamide on the urinary excretion of (−)-mandelic acid. For the dosage levels of the inhibitors employed, the range of such ratios found in all three subjects is 1.29–1.55 due to sulfadiazine, 1.33–1.62 due to sulfamethazine, and 1.33–1.76 due to sulfamerazine. Similar inhibitory effects were observed on the urinary excretion of sulfadiazine in the presence of (−)-mandelic acid in two subjects. Therefore, it is concluded that these compounds probably share the same renal tubular transport system(s) for their secretion in humans.

Keyphrases □ (−)-Mandelic acid urinary excretion—sulfonamide effect □ Sulfonamide urinary excretion—(−)-mandelic acid effect □ Urinary excretion, sulfonamides, (−)-mandelic acid—mutual inhibition □ GLC—analysis □ UV spectrophotometry—analysis

Since the time Marshall *et al.* (1–3) produced evidence for the renal tubular secretion of phenol red, numerous studies have been conducted to show that other substances are also secreted by the renal tubules. In addition, considerable efforts are being made to establish the mechanism of renal tubular secretion. Weiner and Mudge (4–6) have reviewed the tubular mechanisms for the excretion of organic acids and bases while Despopoulos (7) has reviewed the renal transport of organic ions. Current theory suggests the presence of separate mechanisms for renal tubular secretion of acids and bases in humans (4). Extensive lists of drugs, which are secreted by the renal tubules, are cited by Weiner and Mudge (4) and Despopoulos (7).

Nagwekar and Kostenbauder (8) have demonstrated that the excretion and metabolism of both optical isomers of mandelic acid follow pseudo-first-order kinetics in humans and that there is no significant difference in the rate constants for excretion of these isomers. They showed that the principal metabolites of mandelic acid are benzoylformic acid and benzoic acid and that mandelic acid is completely recovered in the urine. The rate of metabolism of (+)-mandelic acid was found to be twice the rate of metabolism of (−)-mandelic acid. A gas-chromatographic method was used to quantitatively determine intact mandelic acid and its metabolites excreted in the urine.

Nagwekar and Kostenbauder (8) also studied in humans the effect of probenecid on the urinary excretion of mandelic acid and since probenecid decreased the rate of urinary excretion of both isomers of mandelic acid, they concluded that mandelic acid is probably involved in active renal tubular transport.

Although Despopoulos and Callahan (9) could show no effect of probenecid on the rate of transport of sulfadiazine, sulfamerazine, and sulfamethazine in rabbit kidney slice studies, Crosley *et al.* (10) demonstrated, in humans, an increase in the plasma levels of both the intact and the acetylated forms of triple sulfonamides in the presence of probenecid. They attributed this to the inhibitory effect of probenecid on the excretion of these sulfonamide(s). Hansen *et al.* (11) also studied the effect of probenecid on the plasma concentration of the same triple sulfonamide mixture in humans, and reported an increase in the plasma concentration of intact sulfonamide in some of their studies.

These experimental observations suggest that both sulfonamides and the mandelic acids may be secreted by an active transport mechanism present in the kidney tubules, and that probenecid interferes with this active process. They also suggest that both sulfonamides and the mandelic acids are secreted by the same mechanism. If this is the case, an optical isomer of mandelic acid and a sulfonamide, when administered simultaneously