ANTISEROTONIN-ANTIHISTAMINIC PROPERTIES OF CYPROHEPTADINE

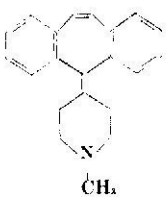
CLEMENT A. STONE, HERBERT C. WENGER, CARL T. LUDDEN, JOHN M. STAVORSKI AND CHARLES A. ROSS

Merck Institute for Therapeutic Research, West Point, Pennsylvania

Received for publication May 14, 1960

The demonstration that serotonin may be released alone or along with histamine in certain allergic or anaphylactoid situations in experimental animals (see Page, 1958) has given rise to studies of the antiserotonin actions of various antihistaminics. Among those which have been shown to possess such properties are various phenothiazine derivatives, such as chlorpromazine, promethazine and trimeprazine (Gyermek *et al.*, 1956; Parratt and West, 1958), thenalidine (Rothlin and Cerletti, 1955) and others (Rapport and Koelle, 1953; Doepfner and Cerletti, 1957). From these investigations, however, it is clear that none of the agents studied was particularly outstanding as an antagonist of serotonin. For the most part, the demonstration of activity was restricted to antagonism of serotonin effects on isolated tissues, such as the rat uterus or colon and the guinea pig ileum, or upon the bronchoconstriction induced by aerosolization of serotonin in guinea pigs. The latter two preparations, particularly, reveal antiserotonin effects of a large group of chemically and pharmacologically unrelated substances (Jaques *et al.*, 1956). In addition, in those instances wherein the antiserotonin actions of the antihistaminics have been compared to lysergic acid diethylamide (LSD) it was evident that most of the antihistaminics were considerably less effective than the latter, although the phenothiazine mentioned approached it (Doepfner and Cerletti, 1957; Schmid *et al.*, 1959). In view of these considerations it was somewhat surprising to find that the antihistaminic agent 1-methyl-4-(5-dibenzo[a,e]cycloheptatrienylidene)-piperidine hydrochloride (cyproheptadine)[1] was not only a potent antagonist of histamine but also approached, equalled or exceeded LSD with respect to antagonism of serotonin in a variety of experimental situations. The present report concerns itself with a description of the antihistaminic and antiserotonin actions of the new agent in comparison with certain of the more specific and potent reference agents within both pharmacologic classes.

The structural configuration of cyproheptadine is shown below:



The compound, in the form of the hydrochloride, is a crystalline solid melting at 214–216°C and is soluble in water to the extent of 5 mg/ml. From its structure it is evident that it resembles in certain respects the phenothiazine-type antihistaminics. Moreover, it bears some resemblance to LSD and other ergot derivatives in that they possess an N-substituted heterocyclic ring. On the other hand, there is no obvious structural resemblance to the many simpler indole-type serotonin antagonists which have received such wide attention (Gaddum *et al.*, 1955; Erspamer, 1955; Shaw and Woolley, 1956).

METHODS. *Antagonism of the pressor effect of serotonin in the dog.* Mongrel dogs, anesthetized with vinbarbital (50 mg/kg i.v.) were prepared for recording mean arterial blood pressure from the carotid artery. Since the vascular action of serotonin in the otherwise untreated anesthetized dog is so variable and unstable, all animals were treated with a long-acting ganglionic blocking agent [4,5,6,7 - tetrachloro - 2 - (2 - dimethylaminoethyl)-indole bismethochloride (chlorisondamine)], in a dose of 0.4 mg/kg i.v. This was injected 30 to 45 minutes prior to beginning the ex-

[1] Periactin is the registered trademark of Merck & Co., Inc., for its brand of cyproheptadine.

periment. The ability of various agents to antagonize the pressor effect of serotonin then was measured in the following manner. Pressor responses to intravenous injections of epinephrine (1.0 μg/kg i.v.), norepinephrine (0.5 μg/kg i.v.) and serotonin (25 μg/kg i.v.) were obtained in that order. Five- to seven-minute intervals separated each response. Two responses to each pressor agent were obtained prior to injecting the first dose of the antagonist. The antagonist was then injected and 5 minutes later the test responses were again determined in the order named. The antagonist was reinjected and test responses recorded as before. This process was continued until the antagonistic effect *versus* serotonin was nearly complete. The results were expressed as % inhibition of each of the test responses at each of the cumulative doses, as compared to the second control response. Only the pressor phase was considered in these calculations even though certain of the agents tended to "reverse" the response to epinephrine. The data obtained with norepinephrine will not be included because the results with that substance qualitatively paralleled those obtained with epinephrine. Since the primary interest was the antagonism of serotonin, it is to be noted that each test response to serotonin was obtained at about 15 to 20 minutes after the injection of each dose of antagonist. An experimental tracing illustrative of the procedure is found in figure 1.

Ten control experiments were performed in which the solvent used to effect solution of the antagonists was given alone. Five of these consisted of injecting 0.1-ml doses of dimethylformamide and five in which similar volumes of saline were employed. The dimethylformamide was used to effect solutions of BAS and hydroxindasol which are poorly soluble in saline; all other antagonists were dissolved in saline (see below). These ten experiments indicated that the responses to the various pressor agents were sufficiently uniform and stable for assay purposes extending through a 4-hour period. Tachyphylaxis to repeated injections of serotonin was not evident under the conditions employed.

It is felt that without the use of the ganglionic blocking agent the assessment of antiserotonin activity would have been impossible because of the instability of the serotonin responses (see Page, 1958). In addition, the ganglionic blocking agent produced a stable blood pressure floor which was essentially unaltered by any of the antagonists studied. Hence, changes in initial pressures just prior to test injections of the pressor substances were remarkably constant throughout the course of the assays.

The standard dose of 25 μg/kg of serotonin in 63 randomly selected "ganglion-blocked" dogs was found to produce an average increase in blood pressure of 95 (S.D. ± 26.3 mm Hg). This amount of serotonin was shown, in other experiments, to produce maximal responses, as determined from the dose-response relationship. On the other hand, the responses elicited by epinephrine and norepinephrine were submaximal.

Antagonism of the spasmogenic effect of serotonin. The general procedure employed by Gaddum and Hameed (1954) was employed. Rats were pretreated with stilbestrol (0.1 mg/kg i.m.) 24 hours prior to sacrifice. The isolated uterus was suspended in a 20-ml muscle chamber through which DeJalon's solution was perfused. Muscle contractions were recorded with an isotonic lever, magnifying the response 5 times. The perfusion solution was maintained at about 30°C. The standard spasmogenic dose of serotonin was 0.05 μg/ml, and this amount was kept in contact with the tissue for 45 seconds prior to its removal by washing. Contractions were elicited every 4 minutes, except when the antagonists were added to the chamber; then the interval between contractions was slightly greater than 4 minutes to allow 4 minutes' contact of the antagonist with the tissue. In general, 4 control contractions of approximately equal height were obtained prior to the antagonist. If the inhibition induced by the antagonist was rapidly reversible by washing, the same tissue was employed for further doses of the same antagonist after the responses had returned to pre-antagonist levels. If the antagonist produced slowly irreversible effects, the tissue was discarded after recording nine subsequent contractions.

Antagonism of edema induced by local injection of serotonin and egg white in rats. Rats were pretreated subcutaneously with various doses of cyproheptadine or other antagonists 30 minutes prior to the production of local edema by serotonin or egg white. The latter effect was induced in the one hind foot of each animal by injection of 5 μg of serotonin (base) or 0.05 ml of an 8% (v/v) solution of fresh egg white into the plantar surface. Saline in equal volume (0.05 ml) was injected into the other foot. Thirty minutes following the edema-producing agent, the animals were sacrificed and the hind feet were removed in a standard manner and weighed. The results were expressed as increase of weight, in %, of the serotonin- or egg white-injected foot as compared to the saline-treated foot.

The effect of cyproheptadine on serotonin-induced hind foot edema was also studied after oral administration. In this instance, 4 dose levels with 10 rats/level were employed and the compound was injected into 24-hour fasted rats 1 hour

prior to the injection of serotonin, as above. The hind feet were removed and weighed 30 minutes later as described.

The selection of the hind foot which was to be serotonin-treated was determined by appropriate randomization procedures. A similar procedure was used to determine the order of dosing. The experiments were conducted by the "blind" technique.

Antagonism of the bronchoconstrictor effect of histamine in guinea pigs. Guinea pigs were pretreated intraperitoneally with various doses of the test compound 30 minutes prior to exposure in an appropriate chamber for three minutes to an aerosol containing 0.1% histamine base. Protection was assessed during the exposure period by the presence or absence of loss of righting, the latter presumably due to bronchoconstriction and the resulting asphyxia. Since the end point was somewhat subjective, the experiments were conducted "blind." Ten or 12 guinea pigs were employed at three to five doses. ED50's were calculated by the Litchfield-Wilcoxon procedure (1949).

Saline-injected controls were always employed concurrently. In the 74 animals so employed, only 2 failed to demonstrate loss of righting. Insofar as this was essentially 100% response, a correction for insensitivity among control animals was not needed among treated groups.

Antagonism of histamine-induced vasodepressor responses in the dog. Mongrel dogs of either sex were anesthetized with vinbarbital (50 mg/kg i.v.), bilaterally vagotomized and prepared for recording mean arterial pressure from the carotid artery by means of a mercury manometer. Histamine, at 4 different dose levels, was injected and the responses to each dose recorded, with 5-minute intervals separating each response. Following these responses the first dose of the antagonist was injected and the responses to histamine were again obtained, beginning 10 minutes after the dose of antagonist. A second dose of antagonist was then administered and the test responses again obtained in the manner described.

The doses of histamine (base) employed were 0.5, 1.0, 2.0 and 4.0 μg/kg i.v. In alternate dogs, the order of histamine dosing was varied from the lowest to highest dose of histamine to one which began with the highest dose. Four control experiments in which saline was given instead of antagonist showed that within the time required to complete the experiment there was no consistent or unidirectional change in the responses to the various levels of histamine.

In the experiments described above a number of reference agents were employed for comparative purposes. In the serotonin antagonism studies, known antagonists were employed as well as a number of known antihistaminic agents which have been reported to possess antiserotonin properties. In addition, an indole derivative, [1 - (*p* - methoxybenzyl) - 2 - methyl - 5 - hydroxytryptamine hydrochloride (hydroxindasol)], whose antiserotonin properties have not previously been described was also studied. This compound is structurally and pharmacologically similar to a closely related analog, 1-benzyl-2-methyl-5-methoxytryptamine (BAS), first described by Woolley and Shaw (1956), and to the 5-hydroxy derivative of the latter (BAS-phenol, Shaw and Woolley, 1957).

Of the various materials employed in the studies described above, the following were employed as the hydrochloride or the dihydrochloride salts: cyproheptadine, hydroxindasol, BAS, thiopropazate (Dartal, Searle), trimeprazine (Temaril, Smith Kline & French), promethazine (Phenergan, Wyeth), chlorpromazine (Thorazine, Smith Kline & French), diphenhydramine (Benadryl, Parke, Davis), SY-21 (N-ethyl-N-(9-fluorenyl)-2-aminoethyl chloride hydrochloride, Parke, Davis), and thenylpyramine (Thenylene, Abbott). LSD (Delysid, Sandoz), thenalidine (Sandostene, Sandoz), pyrilamine (Neo-Antergan, Merck Sharp & Dohme), chlorpheniramine (Chlor-Trimeton, Schering) and prochlorperazine (Compazine, Smith Kline & French) were used in the form of the maleate salt. Epinephrine, norepinephrine and perphenazine (Trilafon, Schering) existed as the free base and in each case solution was effected by means of a few drops of dilute hydrochloric acid. Serotonin was in the form of creatinine sulfate salt. In all instances dosages of the above materials were calculated in terms of the base weight.

Any expression of variation, in terms of standard errors, was estimated by the approximate procedures outlined by Snedecor (1946).

RESULTS. *Antiserotonin actions.* Dog blood pressure: The nature of the antagonistic effect of cyproheptadine against the pressor action of serotonin in the "ganglion-blocked" dog is illustrated by the tracing in figure 1. As is evident, an initial dose of 10 μg/kg i.v. produced a small decrease in the response while larger doses produced an increasingly greater effect. There was little or no tendency to produce the type of "reversal" that may be readily observed with epinephrine responses in the presence of adrenergic blocking agents. The compound did not significantly modify the pressor action of

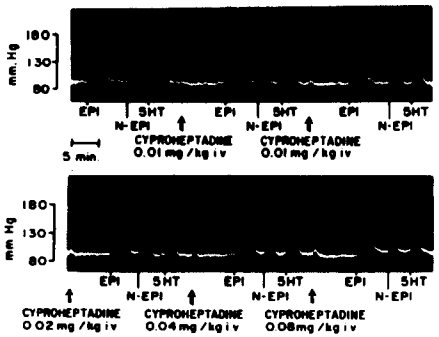


FIG. 1. Effect of cyproheptadine on the pressor responses to serotonin in the "ganglion-blocked" dog.

Note that successive doses of cyproheptadine produced blockade of the response to 25 μg/kg of serotonin (5HT) without materially influencing those to 1.0 μg/kg of epinephrine (epi) or to 0.5 μg/kg of norepinephrine (N-epi). The lower tracing is continuous with the upper. Chlorisondamine, 0.4 mg/kg i.v., was given 30 to 45 minutes prior to beginning the experiment.

epinephrine or norepinephrine in the dose range depicted in figure 1.

In the experiment in figure 1, it is evident that the inhibitory effect of cyproheptadine was related to the cumulative amount administered. In the 10 experiments so conducted, the graphically estimated dose required to inhibit the standard serotonin response by 50% (ED50) was 0.05 mg/kg (table 1). Comparison of similar values from the average dose-response curves obtained with certain of the indole-type serotonin antagonists, studied under identical circumstances, indicate that cyproheptadine approached the activity of LSD and was more active than either hydroxindasol or BAS (table 1). Comparison with LSD, however, was rendered difficult owing to the distinctly curvilinear dose-response curve which was obtained with the latter agent and which prevented a reasonable estimate of an ED50.

The data in table 1 also show the results obtained with thenalidine, trimeprazine, promethazine and chlorpromazine. As is evident none of these agents was as active as cyproheptadine or LSD and all except thenalidine blocked epinephrine pressor responses as well. Other compounds pharmacologically or structurally similar to cyproheptadine were also studied with respect to a capacity to inhibit serotonin and epinephrine pressor responses in the dog. Pyrilamine, chlorpheniramine and diphenhydramine in cumulative doses ranging from 1.0 to 8.0 mg/kg i.v. failed to antagonize serotonin (4 to 6 experiments with each agent). The phenothiazine derivatives perphenazine, thiopropazate and prochlorperazine, however, were capable of blocking serotonin responses and, like those phenothiazines listed in table 1, also blocked epinephrine. With these agents the active dose range varied from 0.2 to 4.0 mg/kg, cumulative i.v. (2 experiments with each).

As mentioned above, cyproheptadine produced a blockade of serotonin responses in doses which did not concomitantly influence those induced by epinephrine or norepinephrine. However, other experiments in which higher amounts of cyproheptadine were employed revealed that the compound did possess some adrenergic blocking activity. In an effort to determine the importance of the latter action, additional assays similar to those described above were performed using larger doses. A specific and potent adrenergic blocking agent, N-ethyl-N-(2-chloroethyl)-9-fluorenamine (SY-21), was employed for comparative purposes.

The experiments revealed that cyproheptadine reduced the pressor phase to epinephrine only after doses considerably above those required to antagonize serotonin. The data presented in figure 2 show that doses about 60 times those needed to block serotonin to an equal degree were required. SY-21, on the other hand, blocked epinephrine in lower doses than serotonin.

None of the other specific antagonists was similarly studied, but in other experiments in the anesthetized dog intravenous doses of 0.5 mg/kg of LSD or 10 mg/kg i.v. of hydroxindasol or BAS (4 to 6 experiments with each agent) have failed to reduce significantly epinephrine or norepinephrine pressor responses. These agents, therefore, are also relatively free of adrenergic blocking activity.

No information with respect to the effect of the various agents on vasodepressor responses to serotonin that have been reported to occur in the dog (see Page, 1958) was obtained in the present experiments. This was prevented by the fact that serotonin responses were almost always totally monophasic (pressor); any depressor phase was minor in magnitude and erratic in the "ganglion-blocked" dog.

Rat uterus: Cyproheptadine was found to be highly effective in blocking the spasmogenic

TABLE 1

Antagonism of the pressor effects of serotonin in the "ganglion-blocked" dog by cyproheptadine and reference agents

Compound	No. of Expt.	Cumulative Dose	Mean % Change*		ED50 mg/kg i.v.†	
			Serotonin	Epinephrine	Serotonin	Epinephrine
		mg/kg i.v.	*25 μg/kg i.v.*	*1 μg/kg i.v.*		
Cyproheptadine	10	0.01	−24 ± 3.4‡	−4		
		0.02	−36 ± 4.0	−5		
		0.04	−47 ± 3.9	−3	0.05	—
		0.08	−56 ± 4.4	−3		
		0.16	−64 ± 4.4	−4		
LSD	5	0.001	−6 ± 3.1	−4		
		0.002	−21 ± 6.7	−1		
		0.004	−36 ± 5.0	0	*ca.* 0.01	—
		0.008	−47 ± 7.3	+2		
		0.016	−52 ± 9.8	+4		
		0.032	−57 ± 10.6	+13		
Hydroxindasol	9	0.05	−19 ± 3.2	−2		
		0.10	−30 ± 5.1	+0.3	0.25	—
		0.20	−50 ± 6.6	−3		
		0.40	−77 ± 6.5	−0.3		
BAS	4	1.0	−5 ± 4.3	—		
		2.0	−27 ± 7.2	—	3.6	—
		4.0	−55 ± 7.5	—		
		8.0	−81 ± 5.1	—		
Thenalidine	6	0.25	−12 ± 4.9	+1		
		0.50	−21 ± 7.1	+1		
		1.0	−33 ± 9.8	+7	2.1	—
		2.0	−47 ± 13.7	+14		
		4.0	−67 ± 9.2	+24		
Chlorpromazine	5	0.10	−47 ± 5.2	−39 ± 2.7		
		0.20	−62 ± 6.9	−52 ± 3.6	0.12	0.22
		0.40	−71 ± 3.5	−55 ± 4.4		
Promethazine	8	0.125	−29 ± 11.3	−45 ± 8.6		
		0.25	−30 ± 4.9	−52 ± 5.2		
		0.50	−48 ± 6.6	−65 ± 4.0	0.50	0.20
		1.0	−66 ± 4.0	−70 ± 2.6		
		2.0	−78 ± 3.0	−72 ± 4.8		
Trimeprazine	6	0.25	−37 ± 7.4‡	−26 ± 3.4		
		0.50	−48 ± 6.9	−37 ± 3.4	0.54	1.5
		1.0	−57 ± 8.0	−47 ± 2.7		
		2.0	−63 ± 9.0	−52 ± 3.4		

* The mean % change in response to serotonin or epinephrine after the administration of the indicated cumulative doses as compared to the control response obtained just prior to injection of the first dose of test compound. A minus sign indicates a decrease in the response; a plus sign, an increase. All dogs were given chlorisondamine (0.4 mg/kg i.v.) 30 to 45 minutes prior to beginning of the experiments.

† Estimated graphically from the average dose response line.

‡ Standard errors.

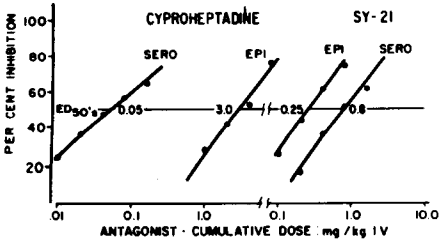


FIG. 2. Relative blocking effect of cyproheptadine on serotonin *versus* epinephrine pressor effects in the "ganglion-blocked" dog.

Note that cyproheptadine was 60 times more effective against serotonin than epinephrine, while the specific adrenergic blocking agent, SY-21 (N-ethyl-N-(2-chloroethyl)-9-fluorenamine), blocked epinephrine effects in lower doses than serotonin. The data with SY-21 are the means of 7 experiments. The data relating to antagonism of serotonin by cyproheptadine are from table 1, while those relating to epinephrine antagonism are the mean values derived in 4 additional assays.

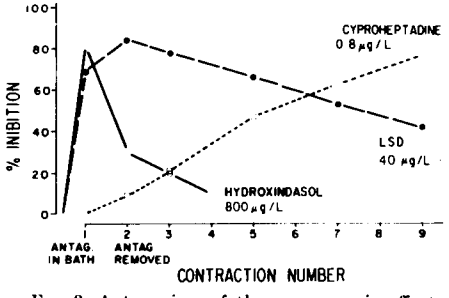


FIG. 3. Antagonism of the spasmogenic effects of serotonin on the isolated rat uterus.

The antagonist was present in the organ bath along with serotonin only during that contraction labeled 1. In subsequent responses, labeled 2 through 9, the antagonist has been removed and the tissue washed an increasingly greater number of times. Note that the antagonism exerted by cyproheptadine (5 experiments) develops slowly and when it is no longer present in the bath. The inhibitory effects of both LSD (6 experiments) and hydroxindasol (6 experiments) were more quickly obtained and more readily reversible.

effect of serotonin on the isolated rat uterus. An unusual characteristic of this inhibitory effect was the fact that little or no inhibition occurred when the antagonist was present, along with serotonin, in the organ bath. After its removal by washing the inhibitory activity gradually appeared and became more intensified with each subsequent response to serotonin.

This slowly developing activity was in contrast

TABLE 2

Antagonism of serotonin-induced contractions of the isolated rat uterus by various agents

Compound	No. Strips	Conc.	Maximal % Inhibition ± S.E.*	EC50†
		µg/l		µg/l
Cyproheptadine	5	0.2	23 ± 6.9	
	5	0.4	65 ± 13.4	0.34
	5	0.8	79 ± 11.5	
LSD	5	10	10 ± 2.7	
	5	20	44 ± 11.3	22
	6	40	84 ± 9.5	
Hydroxindasol	4	200	12 ± 1.2	
	6	400	19 ± 2.6	680
	6	800	63 ± 10.4	
BAS	6	2000	11 ± 2.7	
	5	4000	39 ± 7.5	4,400
	5	8000	78 ± 11.3	

* The maximal effect noted after the single exposure to the indicated concentration irrespective of whether or not the antagonist had been removed from the organ chamber by washing. See METHODS and RESULTS sections for additional details.

† That concentration required to produce 50% inhibition. It was estimated graphically.

to that observed with the other antagonists studied and the data in figure 3 illustrate the differences among the agents studied by similar procedures. It is seen that after a single exposure to cyproheptadine and its later removal, the slowly developing antagonism appeared. LSD exerted maximal inhibitory effects against the serotonin response just subsequent to its removal from the bath, while hydroxindasol produced its greatest inhibitory effect while present in the organ chamber. The inhibitory effects of hydroxindasol were immediately reversible, while those of LSD were slowly reversible upon repeated washing. BAS, not shown in figure 3, behaved qualitatively like LSD.

Using concentrations of cyproheptadine lower than those depicted in figure 3, again the slowly developing antagonism was observed, although the maximal effects were not as great. In table 2 are data relating to the maximal inhibitory action at the various concentrations studied, along with similar values obtained with the

other antagonists and the graphically estimated median effective concentration required to inhibit the response by 50%. From these latter values, it is evident that cyproheptadine was by far the most active agent under the conditions employed. As in the experiments made with respect to the antagonism of serotonin pressor effects (table 1) a relatively large degree of variation was encountered.

The slow onset and apparently completely irreversible nature of the inhibitory action of cyproheptadine against the serotonin response of the rat uterus suggested that the agent may interfere with the contractile mechanisms in a nonspecific manner. Six additional experiments in which acetylcholine (1 μg/ml) was employed alternately with serotonin to induce contractions of the isolated rat uterus showed, however, that the acetylcholine responses were not reduced, while those to serotonin were affected in the

TABLE 3

Inhibitory effect of various agents on edema induced by injection of serotonin into the hind feet of rats

Compound	No. of Rats/Dose	Dose*	Mean % Increase in Weight of Serotonin-Injected Feet†			ED50‡
			Control group	Treated group	% Inh.	
		mg/kg s.c.				*mg/kg s.c.*
Cyproheptadine	24	0.01	47 ± 2.7	36 ± 2.6	23	
		0.03		23 ± 2.3	51	0.03
		0.09		7 ± 0.9	85	
LSD	16	0.0067	47 ± 2.6	35 ± 2.6	26	
		0.02		23 ± 2.8	51	0.02
		0.06		10 ± 2.9	79	
Hydroxindasol	8	1.5	52 ± 4.8	32 ± 2.4	38	
		4.5		21 ± 2.2	60	2.8
		13.5		16 ± 0.7	69	
Pyrilamine	8	10	46 ± 4.1	37 ± 4.0	20	>10
Chlorpheniramine	8	10	46 ± 4.1	41 ± 4.7	11	>10
Thenalidine	8	4.5	52 ± 4.5	35 ± 2.5	33	
		9.0		23 ± 1.5	56	7.2
		18.0		17 ± 1.7	67	
Chlorpromazine	8	0.67	52 ± 3.8	35 ± 2.7	30	
		2.0		26 ± 2.0	50	2.0
		6.0		16 ± 1.2	68	
Promethazine	8	2.5	52 ± 3.8	36 ± 2.5	30	
		5.0		27 ± 2.2	49	5.0
		10.0		15 ± 2.0	70	
Trimeprazine	8	0.25	37 ± 3.6	28 ± 3.6	23	
		0.75		17 ± 2.0	53	0.94
		2.25		13 ± 3.8	64	

* Administered 30 minutes before the injection of serotonin and an additional 30 minutes later the degree of weight change determined as described in the text.

† The ± values are standard errors.

‡ Estimated graphically and represents that dose required to effect 50% inhibition of the increase in weight of the serotonin-injected feet.

manner described after the single exposure of 0.001 μg/ml. Such evidence indicates that the contractile mechanisms were intact and that some specificity was attached to the ability of cyproheptadine to antagonize the effect of serotonin on the isolated rat uterus.

Local edema in rats: The local injection of serotonin into the plantar surface of the hind feet of the rat has been demonstrated (Parratt and West, 1957, 1958) to produce edema, presumably due to an increase in capillary permeability. Experiments with cyproheptadine showed that this agent was highly effective in blocking this effect of serotonin (table 3). Subcutaneous doses of as little as 10 μg/kg reduced the effect while 90 μg/kg subcutaneously nearly obliterated the response. Comparative data with LSD indicate that cyproheptadine was nearly as active as the latter, while both agents were considerably more active than hydroxindasol (table 3).

Cyproheptadine, administered orally, was also

TABLE 4

Inhibitory effect of various agents on edema induced by injection of egg white into the hind feet of rats

Compound	No. of Rats/Dose	Dose*	Mean % Increase in Weight of Egg White-Injected Feet†			ED50‡
			Control group	Treated group	% Inh.	
		mg/kg s.c.				*mg/kg s.c.*
Cyproheptadine	8	0.04	34 ± 3.1	24 ± 2.1	29	
		0.12		15 ± 1.4	56	0.1
		0.36		8 ± 1.2	76	
LSD	8	0.02	34 ± 3.1	30 ± 4.7	12	
		0.06		24 ± 3.7	29	0.09
		0.18		10 ± 1.8	71	
Hydroxindasol	8	1.5	38 ± 3.6	32 ± 1.3	16	
		4.5		24 ± 1.3	37	8.6
		13.5		15 ± 1.3	61	
Pyrilamine	8	10	45 ± 2.3	26 ± 4.4	42	>10
Chlorpheniramine	8	10	45 ± 2.3	36 ± 1.6	20	>10
Thenalidine	8	2.5	38 ± 3.5	29 ± 2.9	25	
		7.5		18 ± 1.5	54	6.8
		22.5		7 ± 1.9	81	
Chlorpromazine	8	0.67	28 ± 3.5	18 ± 1.6	37	
		2.0		13 ± 1.1	54	1.4
		6.0		6 ± 1.1	82	
Promethazine	8	2.5	28 ± 3.5	19 ± 2.1	33	
		5.0		9 ± 1.0	66	3.7
		10.0		4 ± 0.7	84	
Trimeprazine	8	0.083	18 ± 2.6	14 ± 2.5	22	
		0.25		10 ± 1.9	42	0.38
		0.75		7 ± 1.2	62	

* Administered 30 minutes before the injection of egg white and an additional 30 minutes later the degree of weight change determined as described in the text.

† The ± values are standard errors.

‡ Estimated graphically and represents that dose required to effect 50% inhibition of the increase in weight of the egg white-injected feet.

effective in inhibiting the edema produced by the local injection of serotonin. Doses as little as 0.05 mg/kg produced a significant reduction (20%) whereas 1.35 mg/kg caused a 90% reduction in the response. The ED50, estimated as described in table 3 and based upon a 4-dose level assay, was 0.23 mg/kg. This value is about 8 times that determined after subcutaneous administration (table 3) and indicates that the agent is reasonably well absorbed from the gastrointestinal tract.

Since cyproheptadine possesses marked antihistaminic activity, it was of interest to determine whether other antihistaminics were capable of blocking the edema induced by serotonin. The specific antihistaminics, pyrilamine (10 mg/kg s.c.) and chlorpheniramine (10 mg/kg s.c.), studied under similar circumstances did not exert any inhibitory actions (table 3). On the other hand, thenalidine, chlorpromazine, trimeprazine and promethazine all exerted significant antagonism. It is to be noted that these agents were considerably less active than cyproheptadine and LSD even though they approached the activity of the latter with respect to blocking the pressor action of serotonin in the dog (table 1).

Egg white edema in rats: The above experiments indicate that cyproheptadine and other antagonists can inhibit the edema induced by the local injection of exogenous serotonin. Since the release of endogenous serotonin from tissue depots has been implicated in the edema which results from local injection of egg white (Rawley and Benditt, 1956; Parratt and West, 1957), it was of interest to study the effects of cyproheptadine on the edema so produced.

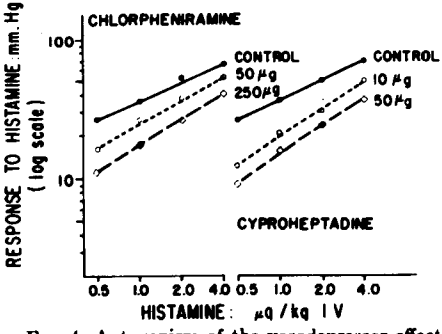


FIG. 4. Antagonism of the vasodepressor effect of histamine in the dog by cyproheptadine and chlorpheniramine.

The doses are in terms of cumulative dose in µg/kg, intravenously. The data are the means derived from 4 experiments made with each agent. See text for other details.

TABLE 5

Protective effect of cyproheptadine and reference agents against histamine-induced bronchoconstriction in guinea pigs

Compound	ED50: mg/kg i.p.* (95% C.L.)
Cyproheptadine	0.032 (0.021 to 0.048)
Chlorpheniramine	0.049 (0.025 to 0.096)
Trimeprazine	0.11 (0.048 to 0.25)
Pyrilamine	0.28 (0.17 to 0.45)
Thenylpyramine	0.96 (0.50 to 1.84)
Thenalidine	6.9 (3.1 to 15.3)
Diphenhydramine	5.8 (3.8 to 8.8)

* As determined 30 minutes after administration and based upon 3 to 5 dose levels with 10 to 12 animals per dose. The values were calculated after the Litchfield-Wilcoxon procedure (1949). See text for details of method.

The data in table 4 demonstrate that cyproheptadine was effective in blocking the edema induced by egg white. Comparatively, both LSD and cyproheptadine appeared to be equivalent in activity, and again both were much more active than hydroxindasol. Thenalidine and the various phenothiazine derivatives studied were capable of exerting antagonism but they were distinctly less active than cyproheptadine and LSD. Pyrilamine and chlorpheniramine exerted only minor inhibition at relatively large doses.

Antihistaminic actions. Dog blood pressure: Intravenous doses of as little as 10 µg/kg significantly reduced the vasodepressor effect of histamine in the anesthetized dog. The additional dose of 40 µg/kg (50 µg/kg, cumulative) produced a greater degree of inhibition (fig. 4). Chlorpheniramine, after intravenous doses of 50 and 200 µg/kg (250 µg/kg, cumulative) produced a degree of inhibition roughly equivalent to that which followed 10 and 40 µg/kg of cyproheptadine. Thus, the latter under the conditions employed was about 5 times more effective than the former.

Both cyproheptadine and chlorpheniramine were relatively more effective in blocking low doses of histamine than higher amounts. In figure 4, this is evident by the nonparallel histamine dose-effect lines in the presence of the antagonists as compared to those obtained in their absence.

Guinea pig bronchoconstriction: Cyproheptadine proved capable of preventing the appearance of symptoms referable to bronchoconstriction due to aerosolization of appropriate concentrations of histamine to guinea pigs. In this respect it resembled other antihistaminic agents in that effective doses protected against the typical hyperpnea, coughing, dyspnea, asphyxial convulsions, loss of righting, and death that occurs in unprotected guinea pigs. As the data in table 5 demonstrate, the intraperitoneal ED50 was 0.032 mg/kg and this amount was somewhat smaller than the similar value estimated for chlorpheniramine. The other antihistaminics studied for comparative purposes all required considerably higher doses. On this basis, therefore, cyproheptadine may be considered a highly effective antagonist of histamine in the order of potency of that obtained with chlorpheniramine.

Discussion. The antiserotonin actions of various antihistaminic agents have been the subject of several investigations (Doepfner and Cerletti, 1957; Schmid *et al.*, 1959; and Parratt and West, 1958). It is clear from these studies, as well as from those by Gyermek *et al.* (1956), that the most active antagonists were phenothiazine derivatives, or closely related structures, even though differing test procedures were employed by the various workers. The present investigation has confirmed such findings. Aside from cyproheptadine which was the most active of the antihistaminics studied, the phenothiazines were considerably more active than the more specific and classical antihistaminics examined. Of the latter category, which included chlorpheniramine, diphenhydramine and pyrilamine, only thenalidine exerted significant antiserotonin action. Comparatively, this agent was appreciably less active than chlorpromazine, promethazine and trimeprazine. From these observations, as well as those of the other investigators, cited above, it would appear that compounds with the phenothiazine nucleus possess antiserotonin activity of a relatively high order. It is of interest that, structurally, cyproheptadine bears a resemblance to the phenothiazines and, in addition, contains an N-substituted heterocyclic ring—a moiety also found in the highly active natural and synthetic ergot alkaloids, such as LSD (Cerletti and Doepfner, 1958). It is, perhaps, these structural components which confer upon cyproheptadine its high degree of antiserotonin activity.

The slowly developing antagonistic action of cyproheptadine against the serotonin-induced contractions of the isolated rat uterus after it has been removed from the organ bath resembles a similar action of LSD when it is exposed to that tissue constantly or for prolonged periods (Gaddum, 1958). In the present instance this action of cyproheptadine was probably related to irreversible fixation to receptors and this, in turn, produced the same result as does the constant exposure of more reversible antagonists. It is thus possible that the fifty-fold potency differential between cyproheptadine and LSD, as shown by the data in table 2, would likely be less marked if LSD were studied under constant or more prolonged contact conditions.

As Parratt and West (1958) have demonstrated, the ability of serotonin antagonists to reduce the edema that results from the local injection of egg white into the hind feet of rats is probably related to their ability to block the effect of endogenous serotonin released by an anaphylactoid effect of the egg white. However, it is of interest that cyproheptadine, LSD and hydroxindasol were more effective in blocking the edema induced by the serotonin than that resulting from the injection of egg white (compare ED50's, tables 3 and 4). This was observed in spite of the fact that the resulting edema was less severe with egg white than with serotonin. Chlorpromazine, promethazine, trimeprazine and thenalidine, on the other hand, were either equally effective in both circumstances or more active against egg white edema, a difference also evident in the data of Parratt and West (1958). These observations suggest that individual antagonists may differ in terms of mechanism of action in the two test situations.

The experiments in the dog indicate that of the agents effective in blocking serotonin-induced pressor responses only LSD, BAS, hydroxindasol, thenalidine and cyproheptadine were significantly free of an ability to block epinephrine responses concomitantly. All the phenothiazines studied and SY-21 blocked both responses nearly in parallel, although the latter may have been more active against epinephrine than serotonin (figure 2). The results suggest that the phenothiazines may owe their ability to block serotonin pressor responses to their adrenergic blocking activity.

This interpretation is supported by the observation that many adrenergic blocking agents are active antagonists to serotonin, not only in the dog but also in the isolated rat uterus (see Gaddum, 1958). Moreover, depletion of catecholamines from peripheral tissues of the dog by appropriate treatment with reserpine significantly reduced serotonin pressor activity (Stone, unpublished results), in a manner similar to that described for various sympathomimetic amines by Burn and Rand (1958). Thus, it is possible that serotonin acts, in part at least, indirectly; either by ganglionic stimulation (which is resistant to blockade by conventional ganglionic blocking agents) or by a more peripheral release of the sympathetic mediator. Such a mechanism would account for the antagonistic activity of adrenergic blocking drugs (including the phenothiazines) in blocking serotonin pressor responses in the dog, although a similar mechanism probably would not apply to the activity of such agents on the isolated rat uterus. On the basis of this explanation, cyproheptadine, LSD and the simpler indole antagonists would possess a more direct action in exerting their antagonistic effect in the dog.

From the data reported, it is clear that cyproheptadine cannot be considered a specific antihistaminic or antiserotonin agent. From other studies, the compound possesses a variety of other actions, including a capacity to antagonize nicotine- and electroshock-induced convulsions in mice, and to prevent tremorine-induced tremors in mice. In addition, the agent possesses weak peripheral anticholinergic actions, equivalent to about $\frac{1}{50}$ that of atropine (Ross and Stone, unpublished). Thus like LSD (Rothlin, 1957), cyproheptadine possesses a number of pharmacologic effects apparently unrelated to its antiserotonin or antihistaminic action. While such actions may have no practical significance they detract from the usefulness of such compounds in delineating the physiological and/or pathological role of serotonin.

SUMMARY

The antihistaminic-antiserotonin actions of 1-methyl-4-(5 - dibenzo[a,e] cycloheptatrienylidine)-piperidine hydrochloride (cyproheptadine) have been demonstrated in several different experimental situations. Antihistaminic activity was revealed by a capacity to antagonize the vasodepressor effects of histamine in the dog and by an ability to annul the signs of toxicity of aerosolized histamine in guinea pigs. The antiserotonin actions of cyproheptadine included an ability to block the vasopressor actions of serotonin in the anesthetized, ganglionic blocking agent treated dog, a capacity to block the spasmogenic effect of serotonin on the isolated rat uterus and an inhibitory effect of the swelling and edema produced by the local injection of serotonin in the hind feet of rats. The inhibitory action against similarly-induced egg white edema probably was related to the basic antiserotonin action of the agent.

The antihistaminic and/or antiserotonin actions of cyproheptadine were compared with similar properties of a variety of other agents, including lysergic acid diethylamide and other simple indole derivatives, chlorpromazine, chlorpheniramine, pyrilamine, thenalidine, promethazine, trimeprazine, thenylpyramine and diphenhydramine. Under the conditions described for the various comparisons, the antihistaminic action equalled or exceeded that of chlorpheniramine and the antiserotonin activity approached, equalled or exceeded that of lysergic acid diethylamide. Since both of these latter agents are among the most active members of their respective pharmacologic classes, it was evident that cyproheptadine possesses both actions to a relatively high degree.

ACKNOWLEDGMENTS. The authors are grateful to Drs. E. L. Engelhardt and J. M. Sprague of the Merck Sharp & Dohme Research Laboratories Division for supplies of cyproheptadine and to Drs. E. F. Rogers and L. H. Sarett of the same laboratories for supplies of hydroxindasol and BAS.

REFERENCES

BURN, J. H. AND RAND, M. J.: J. Physiol. **144**: 314, 1958.

CERLETTI, A. AND DOEPFNER, W.: THIS JOURNAL **122**: 124, 1958.

DOEPFNER, W. AND CERLETTI, A.: Int. Arch. Allergy, N. Y. **10**: 348, 1957.

ERSPAMER, V.: Science **121**: 369, 1955.

GADDUM, J. H.: in 5-Hydroxytryptamine, edited by G. P. Lewis, p. 245, Pergamon Press, London, 1958.

GADDUM, J. H. AND HAMEED, KHAN A.: Brit. J. Pharmacol. **9**: 240, 1954.

GADDUM, J. H., HAMEED, KHAN A., HATHWAY, D. E. AND STEPHENS, F. F.: Quart. J. exp. Physiol. **40**: 49, 1955.

GYERMEK, L., LAZAR, I. AND CZÁK, A. ZS.: Arch. int. Pharmacodyn. **107**: 62, 1956.

JAQUES, R., BEIN, H. J. AND MEIER, R.: Helv. physiol. acta **14**: 269, 1956.
LITCHFIELD, T. J., JR. AND WILCOXON, F.: THIS JOURNAL **96**: 99, 1949.
PAGE, I. H.: Physiol. Rev. **38**: 277, 1958.
PARRATT, J. R. AND WEST, G. B.: J. Physiol. **139**: 27, 1957.
PARRATT, J. R. AND WEST, G. B.: Brit. J. Pharmacol. **13**: 65, 1958.
RAPPORT, M. M. AND KOELLE, G. B.: Arch. int. Pharmacodyn. **93**: 464, 1953.
RAWLEY, D. A. AND BENDITT, E. P.: J. exp. Med. **103**: 399, 1956.
ROTHLIN, E.: J. Pharm., Lond. **9**: 569, 1957.
ROTHLIN, E. AND CERLETTI, A.: Ann. Allergy **13**: 80, 1955.
SCHMID, E., ZICHA, L., SCHEIFFARTH, F. AND BÜTTNER, O.: Ärztl. Forsch. **9**: 474, 1950.
SHAW, E. AND WOOLLEY, D. W.: THIS JOURNAL **116**: 164, 1956.
SHAW, E. AND WOOLLEY, D. W.: Proc. Soc. exp. Biol., N. Y. **96**: 439, 1957.
SNEDECOR, G. W.: Statistical Methods, p. 97, Iowa State College Press, Ames, Iowa, 1946.
WOOLLEY, D. W. AND SHAW, E.: Science **124**: 34, 1956.