PHARMACOLOGIC PROPERTIES OF CYCLIZINE HYDROCHLORIDE (MAREZINE)

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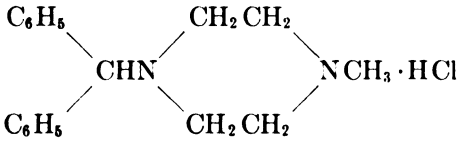
The use of cyclizine hydrochloride (Marezine[1]) (compound 47-83) for the prevention of seasickness has been described by Chinn *et al.* (1952, 1953). Its use in airsickness has also been described by Chinn, Gammon and Frantz (1953). Gutner *et al.* (1954), using microcaloric and galvanic stimulation methods, found that the drug notably decreased labyrinthine sensitivity in human subjects. Dent *et al.* (1954) have found that cyclizine alleviates post operative nausea and vomiting. The same investigators also found that the drug partially antagonized vomiting induced by the administration of apomorphine to dogs. Marcus (1954) also obtained relief of post operative nausea and vomiting with the aid of cyclizine. Cyclizine is a water-soluble, bitter, white crystalline solid with the formula:



Its synthesis was reported by Baltzly *et al.* (1949). Its antihistaminic action was discovered by Castillo *et al.* (1949). Roth (1953) stated that the compound is not adrenolytic, that it is mildly antihypertensive and that it is strongly antihistaminic. Mitchell *et al.* (1952) reported cyclizine possessed fungistatic properties but somewhat less than its congener, chlorcyclizine. The following paper presents additional details of the pharmacology of cyclizine.

METHODS. Cats and dogs were anesthetized with sodium pentobarbital i.p. In cats this was supplemented with chloralose, i.v. as required. Blood pressure was recorded with a mercury or bellows manometer from the carotid or femoral artery.

For testing autonomic reflexes, the vagus and cervical sympathetic nerves of the cat were stimulated for 3 to 5 seconds with square wave impulses. In some experiments the vagus was cut and both central and peripheral ends stimulated separately. Contractions of the nictitating membrane were recorded using a light lever with 6-fold magnification.

The spontaneous movements of the ileum of the anesthetized cat were recorded from a water-filled balloon under 30 cm. water pressure attached to a water manometer. The balloon (10 x 80 mm.) was inserted in a small ventral slit in the ileum about half way between the stomach and colon and was tied in place with a purse-string suture. Handling of the intestine was kept to a minimum. Under fairly deep anesthesia (Plane 2; slit-like pupils), spontaneous rhythm of the intestine was usually present within 5 to 10 minutes after closing the abdominal incision.

[1] Supplied as Marezine hydrochloride brand cyclizine hydrochloride by Burroughs Wellcome and Co. (U. S. A.) Inc.

Where drugs or electrical stimulations were given before and after cyclizine, at least two satisfactory control readings were obtained before cyclizine was injected. Testing was resumed within 5 to 10 minutes after administration of cyclizine.

Isolated segments of guinea pig ileum and rabbit duodenum were suspended in Tyrode's solution at 37.5°C. Antihistaminic properties of cyclizine were tested *in vitro* on isolated segments of guinea pig ileum. Varying concentrations of cyclizine were given to determine the dose necessary to produce 50 per cent inhibition of the spasm produced by histamine diphosphate.

The method of Loew *et al.*, as modified by Siegmund, was used for demonstrating *in vivo* block of histamine by cyclizine. Guinea pigs were exposed to an atmosphere of nebulized histamine at constant pressure. Details of the procedure used have been reported by Castillo *et al.* (1949).

Local anesthetic action was tested by intradermal injection in guinea pigs. Six shaved areas on the back of each guinea pig were delineated with a circle of india ink ½ inch in diameter. In the center of each circle 0.1 ml. of the compound to be tested was injected intradermally. At intervals of 2 minutes the site of injection was stimulated with weak shocks of 1 second duration from an inductorium. Squealing or jumping of the pig or twitches of large areas of the skin in response to shocks were considered as indicating no anesthesia. Duration of anesthesia was obtained.

Neuromuscular block was tested using the cat gastrocnemius-sciatic nerve preparation. The technique was the same as that previously described by Ellis *et al.* (1953). Cats were anesthetized with sodium pentobarbital and prepared to record contractions of the gastrocnemius on stimulation of the sciatic nerve. The nerve was stimulated at 6 second intervals with single square wave impulses. Supramaximal stimulations were used.

Acute toxicity experiments were performed on male albino mice, strain CF 1, weighing from 17 to 21 grams. After injection the mice were observed for one week. LD_{50}'s were calculated by probit analysis.

Chronic toxicity studies were conducted on CF male albino rats. The rats weighed 48 gm. (standard deviation = 4.67) at the start of the experiment. Cyclizine was incorporated into the ground fox chow diet at 4 different concentrations. Each concentration of cyclizine was fed to a group of 10 rats and all groups received food and water *ad lib*. The study was continued for 12 weeks.

Unbuffered solutions of cyclizine dihydrochloride were used for all tests except acute toxicity in mice for which both mono and dihydrochloride salts were used.

Other compounds used were synthetic *l*-epinephrine base, *l*-norepinephrine bitartrate, acetylcholine bromide, histamine diphosphate and serotonin creatine sulfate.[2]

Atropine sulfate was used as a standard for comparison with cyclizine whenever possible.

RESULTS. *Action on blood pressure:* Cyclizine was tested in anesthetized cats and dogs for effects on blood pressure (table 1). Doses up to 1 mgm./kgm. had no effect on blood pressure. At higher doses the blood pressure fell sharply and returned to the control level in less than three minutes. Even at large doses (8 mgm./kgm.) the effect on the blood pressure was very transient. One possible explanation for the blood pressure falls on intravenous administration was thought to be the effect of the pH of unbuffered solutions of cyclizine dihydrochloride. A solution containing 10 mgm./ml. has a pH of 2.6. However, injection of solutions of cyclizine buffered to pH 7.5 gave falls in blood pressure equal to those produced by unbuffered solutions.

Action on ileum of cat: Cyclizine relaxed both the tone and motility of the ileum of the anesthetized cat. This effect of cyclizine was present at doses below

[2] Supplied through the courtesy of Dr. R. K. Richards of the Abbott Laboratories.

TABLE 1

Blood pressure effects

Dose	No. of Cats	Average Maximum Fall	Range	Duration	Range
mgm./kgm.		*mm.* Hg	*mm.* Hg	*minutes*	*minutes*
0.25	1	0	—	—	—
0.5	2	0	—	—	—
1.0	5	0	—	—	—
2.0	6	38	16-60	2	0.5-2
4.0	2	50	30-70	2	0.5-3
5.0	4	63	50-78	2	1.0-2
8.0	8	62	40-90	2	1.0-3

TABLE 2

Duration of effect on balloon recording in ileum

Dose	No. of Cats	Average Duration	Range
mgm./kgm.		*minutes*	*minutes*
0.25	1	0	—
0.5	2	10	0-20
1.0	3	23	20-30
2.0	3	60	45-75
4.0	2	>60	60->120
5.0	1	>120	—
8.0	1	>120	—

those necessary to produce falls in blood pressure. The effect on the ileum lasted from ten minutes to more than two hours depending on the dose. The minimum dose which inhibited intestinal tone and motility was 0.5 mgm./kgm. (table 2).

There was no mydriasis at any of these doses (0.25 to 8 mgm./kgm.). In this, cyclizine differs markedly from atropine. The effect of 2 mgm./kgm. of cyclizine on the ileum is comparable to about 0.05 mgm./kgm. of atropine sulfate. However, at this dose, atropine produces marked mydriasis in anesthetized cats.

Action on the isolated intestine: Cyclizine is only weakly effective against acetylcholine-induced spasm of the isolated guinea pig ileum. Concentrations from 0.05 microgm. to 0.2 microgm./ml. which reduce tonus and decrease rhythmic contractions were tested. By calculating the amount necessary to produce 50 per cent block of acetylcholine spasm, cyclizine was found to possess about one per cent of the activity of atropine sulfate in this respect.

The tonus of isolated rabbit duodenum segments was reduced by concentrations of 0.25 microgm./ml. In this cyclizine is about one-tenth as active as atropine. Increased concentrations also decrease the magnitude of the spontaneous contractions of the intestine. Concentrations of cyclizine of 10 microgm./ml. produce complete loss of spontaneous contractions.

Action on vagus responses of the cat: The fall in blood pressure on stimulation of the preganglionic (cervical) fibers of the vagus was blocked by cyclizine. The

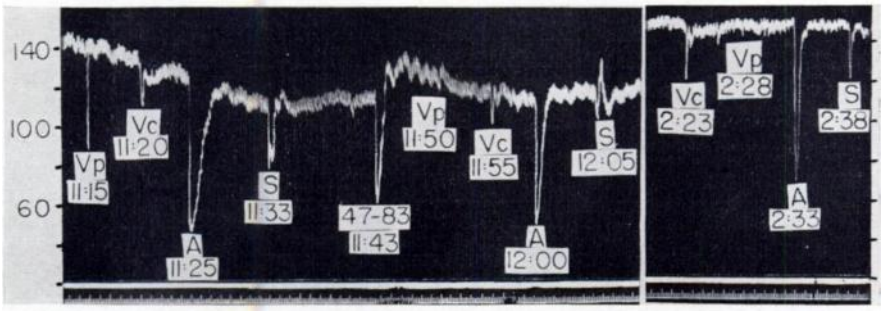


FIG. 1. Vp, stimulation of peripheral end of cut vagus; Vc, stimulation of central end of cut vagus; S, serotonin, 2 microgm./kgm.; A, acetylcholine, 4 microgm./kgm.; 47-83, cyclizine, 8 mgm./kgm. All injections were intravenous. Cat anesthetized with pentobarbital and chloralose. Blood pressure recorded from the left carotid artery. Break in kymograph record indicates elapse of two hours.

TABLE 3

Action of cyclizine on the vagus response and on injected histamine and serotonin

Dose	Per Cent Block of Vagus	Duration	Per Cent Block of Histamine	Duration	Per Cent Block of Serotonin	Duration
mgm./kgm.		*hrs.*		*hrs.*		*hrs.*
0.5	—	—	100	$1\frac{1}{2}$	—	—
0.5	60	$\frac{3}{4}$	50	$1\frac{1}{2}$	—	—
1.0	—	—	50	$>1\frac{1}{2}$	—	—
1.0	100	2	100	2	—	—
2.0	100	>2	—	—	—	—
2.0	—	—	50	$>1\frac{1}{2}$	—	—
2.0	100	$>2\frac{1}{2}$	—	—	—	—
2.0	60	2	—	—	—	—
2.0	100	>3	—	—	—	—
2.0	60	>4	—	—	—	—
5.0	100	$>2\frac{1}{2}$	—	—	—	—
8.0	100	>4	—	—	90	$1\frac{1}{2}$
8.0	100	$>3\frac{1}{2}$	—	—	100	1
8.0	100	>3	—	—	—	—
8.0	100	$>3\frac{1}{2}$	100	4	—	—
8.0	100	>2	—	—	—	—

degree of block was measured by the reduction in the response of the blood pressure to electrical stimulation. A dose of 2 mgm./kgm. blocked the vagus response for over two hours (figure 1). Marked block was obtained with doses as low as 0.5 mgm./kgm. (table 3).

Stimulation of the central end of the cut vagus produced variable blood pressure responses; both rises and falls were obtained. Neither response appeared to be modified by doses of cyclizine up to 8 mgm./kgm. (figure 1).

Action of cyclizine on injected acetylcholine, histamine and serotonin in the cat: Although cyclizine was very effective in blocking the response to vagus stimula-

TABLE 4

Effect of cyclizine on blood pressure response to acetylcholine, epinephrine and norepinephrine

Dose	Acetylcholine Response		Epinephrine Response		Norepinephrine Response	
	Before	After	Before	After	Before	After
mgm./kgm.	*mm.* Hg	*mm.* Hg	*mm.* Hg	*mm.* Hg	*mm.* Hg	*mm.* Hg
4	35	32	44	46	—	—
4	34	28	48	52	—	—
5	—	—	38	38	114	122
8	26	31	38	38	78	80
8	72	60	—	—	—	—
10	—	—	66	56	—	—

tion, there was no significant effect on the blood pressure response to injected acetylcholine (figure 1). In this cyclizine differs markedly from atropine. Doses of acetylcholine bromide from 2 to 4 microgm./kgm. were injected intravenously. These doses were sufficient to cause 25 to 80 mm. falls in blood pressure lasting 1 to 5 minutes. The variations in the response to acetylcholine before and after cyclizine are not greater than the expected variations in responses to acetylcholine (table 4).

The falls in blood pressure produced by 2 to 4 microgm./kgm. of histamine diphosphate were blocked by doses as low as 0.5 mgm./kgm. of cyclizine. The block lasted several hours with gradual return to normal response (table 3).

In the cat, low doses of serotonin creatine sulfate (1 or 2 microgm./kgm.) usually produce falls in blood pressure (figure 1). These falls were completely blocked by 8 mgm./kgm. of cyclizine (table 3).

Antihistamine action in guinea pig: On the isolated guinea pig ileum a concentration of 0.04 microgm./ml. of cyclizine caused 50 per cent inhibition of 0.2 microgm./ml. of histamine.

Cyclizine has been reported to be one-fourth as active as chlorcyclizine in blocking histamine-induced spasm of the tracheal chain preparation (Castillo *et al.*, 1949).

In vivo, cyclizine is as effective as diphenhydramine in preventing or reducing the severity of bronchoconstriction following exposure of guinea pigs to an atmosphere of nebulized histamine. Cyclizine and diphenhydramine were given orally at a dose of 10 mgm./kgm. The results (table 5) indicate that cyclizine is equal to diphenhydramine as an antihistamine *in vivo*.

TABLE 5

Per cent protected from histamine shock

Compound	No. of Guinea Pigs	Hours after Dosing			
		1 hr.	2½ hrs.	4 hrs.	5 hrs.
Cyclizine	17	59.4	46.5	44.9	28.4
Benadryl	8	50.0	50.0	12.5	12.5

TABLE 6

Effect of cyclizine on sympathetic responses in individual cats

Dose	Nictitating Membrane, Contraction		Heart Rate		Tracheal Occlusion, Rise in Blood Pressure	
	Before	After	Before	After	Before	After
mgm./kgm.	*mm.*	*mm.*			*mm.* Hg	*mm.* Hg
0.5	—	—	—	—	10	14
1.0	—	—	104	104	—	—
2.0	—	—	167	170	8	8
8.0	22	21	176	184	—	—
12.0	31	31	—	—	—	—

Local anesthetic action: No local anesthesia to mild shocks from an inductorium was obtained by intradermal injection of 0.1 ml. of a 0.5 per cent solution of cyclizine in 4 guinea pigs. A 1 per cent solution produced local anesthesia lasting about 25 minutes. In this respect, cyclizine is about as active as diphenhydramine and procaine.

Effect on sympathetic system and injected epinephrine in the cat: The contraction of the nictitating membrane in response to preganglionic stimulation of the cervical sympathetic was unaltered by injection of doses up to 12 mgm./kgm. of cyclizine (table 6). It was also found that the rise in blood pressure following injection of tetramethylammonium bromide (TMA) was not blocked. The rise in one cat after TMA was 20 mm. Hg, and after cyclizine (2 mgm./kgm.) the same dose of TMA 10 minutes later produced a 24 mm. rise.

The pressor responses to injected epinephrine and norepinephrine (2 to 4 microgm./kgm.) are not significantly changed by doses of 4 to 8 mgm./kgm. of cyclizine (table 4).

As with atropine, the heart rate in anesthetized cats increases slightly after injections of cyclizine (table 6).

The blood pressure response to brief (20–30 sec.) tracheal occlusion is mediated centrally and through the sympathetic nervous system. This reflex is blocked by hexamethonium (Paton and Zaimis, 1952). However, there is no change in the rise in blood pressure from tracheal occlusion after doses of cyclizine from 0.5 to 2 mgm./kgm. (table 6).

Neuromuscular block: At intravenous doses from 2 to 10 mgm./kgm. there was no neuromuscular block of skeletal muscle as measured by the cat gastrocnemius-sciatic nerve preparation.

Acute toxicity: Male albino mice were treated orally and intraperitoneally with cyclizine mono and dihydrochloride. A 0.4 per cent solution was used for the intraperitoneal injections and one per cent for the oral. The LD_{50} of cyclizine given orally is about twice the LD_{50} on intraperitoneal injection (table 7), indicating good absorption of the drug.

The toxic symptoms were nervousness and tremors, followed by convulsions with intermittent periods of prostration. The animals died in convulsions with respiratory failure occurring before heart failure. Deaths occurred from 22 to 65

TABLE 7

Acute toxicity of cyclizine, intraperitoneal LD_{50} determinations in duplicate

Salt	Route of Administration	LD_{50}
		mgm./kgm.
di HCl	i.p.	69
di HCl	i.p.	72
mono HCl	i.p.	69.6
mono HCl	i.p.	82
mono HCl	p.o.	165

TABLE 8

Effect of prolonged administration on growth of rats

Level of Drug in Diet	No. of Rats	Actual Drug Consumption	Final Body Weight	± s.d.	Gain in Weight in 12 Weeks	Deaths
gm./kgm. diet		*mgm./kgm. body wt./day*	*gm.*		*gm.*	
2.0	10	142	168.2	34.2	119.8	1
1.0	10	59	231.8	96.8	183.3	—
0.5	10	26	253.0	41.7	205.0	1
0.25	10	15	246.6	46.2	198.5	—
0	10	0	259.8	37	212.6	—

minutes after i.p. injection and from 15 to 80 minutes after oral administration. The symptoms disappeared in 24 hours in surviving mice.

Chronic toxicity: Chronic toxicity studies on cyclizine were conducted on male rats. The compound was incorporated into the diet at concentrations from 0.25 to 1 gm./kgm. of diet. After 12 weeks the rats were sacrificed for histological studies. The actual drug consumption and average growth are recorded in table 8.

At the highest dose (142 mgm./kgm. consumed/day), the gain in weight over the 12 week period is significantly lower than the controls ($P = < .01$ using Students t test). At lower levels of drug consumption there is no significant difference in weight of treated and control groups.

Red, white and differential counts, and hemoglobin determinations were made on 4 rats from each group at the end of the 12 week period. The results for each 4 rats are averaged in tables 9 and 10.

TABLE 9

Blood counts

Dose Level	Hemoglobin	Erythrocytes	Leucocytes
mgm./kgm. body weight	*gm./100 ml.*	*millions/cmm.*	*thousands/cmm.*
142	16.2	10.3	13.7
59	14.8	6.9	17.8
26	15.7	6.9	16.2
15	15.4	9.5	17.3
0	15.4	9.0	14.9

TABLE 10
Differential counts

Dose Level	Seg. Neutr.	Juv.	Total Neutr.	Lymph.	Mono.	Baso.	Eosin.
mgm./kgm. body wt./day							
142	19.50	5.50	25.00	72.50	1.50	0.25	0.50
59	16.75	8.50	25.25	72.75	2.25	0.00	0.50
26	16.50	9.00	25.50	71.00	2.00	0.50	1.00
15	17.25	8.75	26.00	71.00	2.25	0.00	0.75
0	16.75	8.75	25.50	71.50	2.50	0.00	0.50

The 50 rats were autopsied and the following tissues removed; thymus, pituitary, thyroid, heart, lung, liver, spleen, kidney, adrenal, gastrointestinal tract, testes and striated muscle. The gross autopsy disclosed that all the rats at the highest dose level had enlarged and spotted livers. Two rats from the next dose level (59 mgm./kgm. body weight/day) and two rats receiving 26 mgm./kgm. body weight/day, had light colored livers. The appearance of all the other organs in all groups was normal. Histological sections of all the tissues showed no pathological findings except at the highest dose. The lungs of all rats receiving 142 mgm./kgm. body weight/day showed pulmonary edema. All other tissues were normal.

DISCUSSION. Burn (1950) has commented on the fact that anti-acetylcholine, anti-histamine and local anesthetic properties are often displayed by the same compound. Such compounds may also block the hypotensive response to serotonin. Page and McCubbin (1953) have reported diminution of the depressor action of serotonin in cats by Phenergan, Benadryl and Thenfadil. Cyclizine possesses the above properties with the notable exception that it is not an anti-acetylcholine compound. The action of acetylcholine on the blood pressure and on the isolated ileum is not blocked, nor does cyclizine produce neuromuscular block. However, cyclizine is vagolytic and this may account for the block of serotonin. Comroe *et al.* (1953) have shown that after procedures which block the vagus (*e.g.* vagotomy or injection of atropine) hypotension is not produced consistently by serotonin.

With cyclizine, the parasympatholytic properties are limited, as far as has been determined, to structures innervated by the vagus. Thus cyclizine caused relaxation of the tone and motility of the ileum and blocked the blood pressure response to electrical stimulation of the vagus but did not produce mydriasis.

The site of block of the vagus by cyclizine is presumably central to the site of action of acetylcholine, since injected acetylcholine is still effective after cyclizine. Possibly acetylcholine is not liberated in response to electrical stimulation in the presence of cyclizine. For example, Jaco and Wood (1944) have demonstrated such a block of production of acetylcholine in the presence of the local anesthetic, procaine.

The observed block of the cardiovascular vagus response might be considered to be ganglionic. In this case one would also expect block of sympathetic ganglia

by cyclizine. In doses up to 8 mgm./kgm. no evidence of sympathetic block was found.

In the failure to block injected acetylcholine at doses which block electrical stimulation of the vagus, cyclizine resembles chlorpromazine. Courvoisier *et al.* (1952) have demonstrated that chlorpromazine is much more effective in relaxing the intact intestine and blocking the response to vagus stimulation than in blocking the depressor action of injected acetylcholine in the dog. They have also demonstrated that chlorpromazine produces complete block and reversal of injected epinephrine. However, the marked adrenolytic properties of chlorpromazine are not possessed by cyclizine.

SUMMARY

In anesthetized cats cyclizine blocks the vagus response, relaxes the tone and rhythmic contractions of the ileum and blocks injected histamine at low doses (0.5 mgm./kgm.).

Doses up to 8 mgm./kgm. have little or no effect on the pupil, or on injected acetylcholine, epinephrine or norepinephrine but block responses to serotonin.

Cyclizine is effective in reducing the mortality in guinea pigs exposed to nebulized histamine.

The local anesthetic effects of cyclizine are similar to procaine when compared by the guinea pig wheal method.

Cyclizine does not appear to block the sympathetic system and does not produce neuromuscular block.

It is suggested that cyclizine may specifically block the vagus nerve peripherally. That this block may also be central is indicated by the antiemetic properties of the compound.

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