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INTERACTION BETWEEN HISTAMINE AND DICHLOROISOPROTERENOL, HEXAMETHONIUM, PEMPIDINE, AND DIPHENHYDRAMINE, IN NORMAL AND RESERPINE-TREATED HEART PREPARATIONS

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Histamine stimulated the isolated auricles and heart of the guinea-pig. The effect was best seen in auricles which had been previously depressed by treatment with reserpine. Ganglionic blocking drugs (hexamethonium and pempidine), applied to auricles which had been previously treated with reserpine, abolished the diphasic effect of nicotine, but did not alter the response to histamine. Dichloroisoproterenol did not modify the stimulant action of histamine in isolated auricles, either before or after treatment with reserpine; nor did it alter the response of the isolated heart. Diphenhydramine reduced or blocked the stimulant action of histamine in auricles which had been previously treated with reserpine. The results support the hypothesis that histamine stimulates the myocardium by a direct action on specific receptors.

Histamine is known to stimulate the isolated heart of the rabbit and cat (Dale and Laidlaw, 1910; Gunn, 1926; Staub and Grossmann, 1930), of the guinea-pig (Went and Lissak, 1935; Penna, Illanes, Ubilla, and Mujca, 1959), and of the rat and frog (Went, Varga, Szucs, and Feher, 1952). It also stimulates isolated auricles of the rabbit (Dews and Graham, 1946) and initiates contractions in isolated strips of ox ventricle (Iwao, 1938).

The stimulant action of histamine on cardiac tissue may be direct on myocardial receptors or indirect through the local release of catecholamines (Truex, 1950; Kottegoda, 1953). Evidence suggesting a direct action was recently obtained by Giotti and Jngianna (1959) and Pepeu, Mannaioni, and Giotti (1959) in experiments with isolated auricles which had been previously stopped by treatment with reserpine. Histamine, adrenaline, and noradrenaline restored the activity of the auricles but nicotine and ephedrine did not, presumably because the auricles had been partially depleted of catecholamines (Giotti, 1954; Pepeu, Masi, and Giotti, 1959).

The introduction of 1-(3,4 dichlorophenyl)-2 isopropyl aminoethanol hydrochloride (dichloroisoproterenol) (Powell and Slater, 1958), a specific antagonist of the catecholamines on the heart (Moran and Perkins, 1958), provided an opportunity to re-investigate the problem of the site of action of histamine on the heart.

METHODS

Isolated guinea-pig auricles were suspended in 50 ml. oxygenated Tyrode solution at 29°, according to the method of Giotti (1954). The auricles were connected to an isotonic lever with magnification of 5; the load on the auricles was 1.5 g. Contractions were recorded on a smoked drum.

The isolated guinea-pig heart was prepared by the method of Langendorff (1895) and Porter (1898) and was suspended in a modified Langendorff apparatus. The perfusion pressure was kept constant at 40 cm. water. The *p*H of the Ringer-Locke solution was 8.2 and of the coronary effluent 7.5. The temperature was adjusted to 38°. The apex of the ventricles was connected to an isotonic lever, which wrote on a smoked drum. The heart rate was taken from a dipolar surface electrogram. The coronary outflow was followed with the recording system of Giotti and Beani (1957).

The drugs used were: (−)-adrenaline bitartrate and (−)-noradrenaline bitartrate (Recordati); nicotine as supplied by the Italian State Monopoly of Tobacco, and crystallized by us as the bitartrate;

TABLE I

EFFECT OF DICHLOROISOPROTERENOL ON THE RESPONSE OF ISOLATED GUINEA-PIG AURICLES TO STIMULANT DRUGS

The experiments were carried out on the same preparations. Dichloroisoproterenol (10 μg./ml.) was allowed to act for 30 min. Each value is the mean±S.D. of 6 experiments.

Treatment of Auricles	Drug	Dose μg./ml.	Contraction Maximum Height in mm.		Rate per min.	
			Before Dose	After Dose	Before Dose	After Dose
Nil	Adrenaline	1	37·3±6	49·6±11	87±5	98±5
Dichloroisoproterenol ..	,,	1	14·5±6	17·6±2	83±5	83±5
Nil	Noradrenaline	1	37·5±6	49·8±11	86±4	96±2
Dichloroisoproterenol ..	,,	1	15·1±5	16·1±4	83±4	83±4
Nil	Histamine	2	37·7±6	47·2±8	89±4	95±3
Dichloroisoproterenol ..	,,	2	13·5±7	30·6±7	82±4	88±5

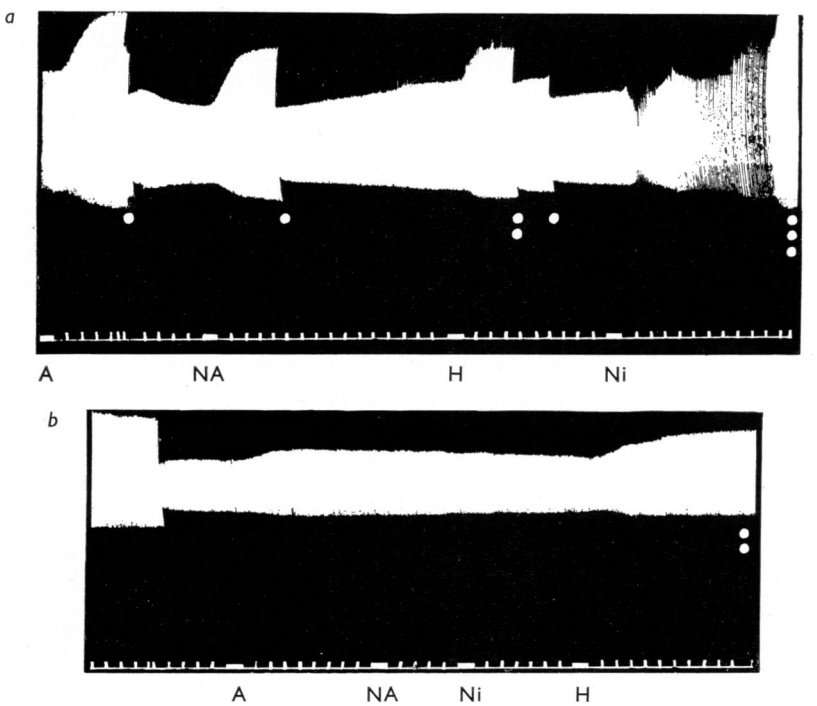

FIG. 1.—Dichloroisoproterenol 1×10^{-4} prevents the effects of the catecholamines and nicotine in guinea-pig isolated auricles; the effect of histamine is still present. (*a*) The effect of adrenaline 1×10^{-7} (*A*), noradrenaline 1×10^{-7} (*NA*), nicotine 5×10^{-5} (*Ni*), and histamine 1×10^{-6} (*H*). (*b*) The effect of the same substances 60 min. after treatment with dichloroisoproterenol. Upper tracing: height of contraction. Lower tracing: time, 10 sec. The washings are indicated by the white dots.

TABLE II

EFFECT OF RESERPINE AND DICHLOROISOPROTERENOL ON THE RESPONSE OF ISOLATED GUINEA-PIG AURICLES TO STIMULANT DRUGS

Reserpine (5 μg./ml.) was allowed to act for 5 hr.; dichloroisoproterenol (1 μg./ml.) for 10 min. before applying the stimulant drug. Experiments were carried out on the same preparations. Each value is the mean±S.D. of 6 experiments.

Treatment of Auricles	Drug	Dose μg./ml.	Contraction Maximum Height in mm.		Rate per min.	
			Before Dose	After Dose	Before Dose	After Dose
Reserpine	Adrenaline	1	2·4±2	39·8±6	19·2±19	75·8±16
,, +dichloroisoproterenol	,,	1	0·8±1	5±5	6·6±8	11·4±13
Reserpine	Histamine	2	3·2±4	22·4±9	14·4±15	70·2±11
,, +dichloroisoproterenol	,,	2	0·6±1	20·4±9	4·8±7	50·2±3

histamine dihydrochloride (Roche); reserpine was kindly supplied by Ciba Ltd., and used as the phosphate; pempidine (1,2,2,6,6,-pentamethylpiperidine) gratefully acknowedged as a gift from Dr. A. Banchetti, Istituto Gentili, Pisa; Benadryl (diphenhydramine) Parke Davis; dichloroisoproterenol kindly supplied by Dr. N. J. Giarman and Dr. G. Pepeu, of Yale University.

The drugs were dissolved in the appropriate physiological solution and concentrations (w/v) are expressed as the salt. Dose is quoted as the concentration of drug in the bath.

The stimulant drug was applied to the auricles in successive doses. When the response was maximal, the bath fluid was changed three times. The antagonist was then allowed to act on the auricles for a period of 30 to 60 min. before retesting the effect of the stimulant drugs. Reserpine, however, was left in contact with the auricles for 4 to 6 hr.

RESULTS

Effect of Histamine, Adrenaline, Noradrenaline, and Nicotine on Isolated Guinea-pig Auricles Before and After Treatment with Dichloroiso-

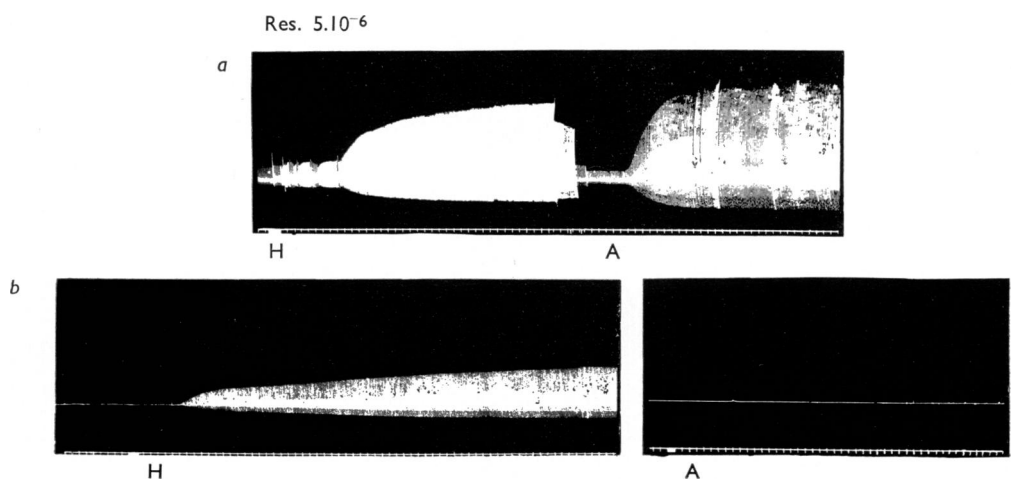

FIG. 2.—(*a*) Effects of histamine 1×10^{-6} and adrenaline 1×10^{-7} on guinea-pig auricles which had been previously treated with reserpine. (*b*) Effects of the same substances after treatment with dichloroisoproterenol 10^{-6} in addition to the previous treatment with reserpine. Upper tracing: height of contraction. Lower tracing: time, 10 sec.

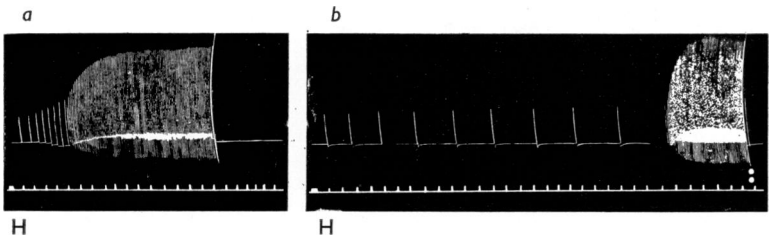

FIG. 3.—(*a*) Response of guinea-pig auricles, arrested by reserpine 5×10^{-6}, to histamine 1×10^{-6} (*H*). (*b*) Treatment with ganglionic-blocking agents (hexamethonium 1×10^{-4}) for 30 min. does not affect the histamine stimulation. Upper tracing: height of contraction. Lower tracing: time, 20 sec. The washings are indicated by the white dots.

proterenol (Table I, Fig. 1). — Dichloroisoproterenol (10 μg./ml.) abolished the stimulant effect of adrenaline (1 μg./ml.) and of noradrenaline (1 μg./ml.) on the rate, and greatly reduced the effect on the contraction, of the auricles. It also abolished the diphasic action (inhibition followed by stimulation) of nicotine (50 μg./ml.). Dichloroisoproterenol, however, did not abolish the response to histamine: the difference (17 mm.) between the mean for the contraction before the dose and the mean after the dose was significant ($P<0.05$).

Effect of Histamine and Adrenaline Before and After Dichloroisoproterenol on Isolated Guinea-pig Auricles Previously Treated with Reserpine (Table II, Fig. 2).—When dichloroisoproterenol (1 μg./ml.) was applied to auricles which had been previously treated with reserpine, the rate slowed and the contraction became smaller; in some experiments the auricles stopped. Under these conditions, the auricles

TABLE III

EFFECT OF ADRENALINE, NORADRENALINE, AND HISTAMINE ON THE ISOLATED HEART OF THE GUINEA-PIG, BEFORE AND AFTER TREATMENT WITH DICHLOROISOPROTERENOL

Experiments were carried out on the same preparations; dichloroisoproterenol was added to the perfusion fluid 30 min. before testing adrenaline, noradrenaline, and histamine. The values for the contraction are expressed as percentage increase of the height of the kymographic record. Each value is the mean±S.D. of 6 experiments.

Stimulant Drug	Dose μg.	Percentage Increase in Contraction			
		Dichloroisoproterenol			
		Dose (μg./ml.): 0	1	5	10
Adrenaline	0·1	135±30	0		
		115±27		0	
		103±20			0
Noradrenaline ..	0·1	129±6	0		
		94±35		0	
		108±16			0
Histamine	0·1	25±13	20±15		
		18±7		26±23	
		35±4			6±11
	0·5	46±14	57±16		
		41±14		67±22	
		77±40			71±42
	1·0	145±51	91±25		
		103±36		121±23	
		127±31			137±79

responded only slightly, or not at all, to adrenaline, but still retained their sensitivity to histamine. Thus, the mean values for the effect of adrenaline on the contraction (5.0 ± 5 mm.) and on the rate (11.4 ± 12 per min.), after the combined treatment, differed significantly ($P<0.05$) from the corresponding mean values (39.8 ± 6 mm. and 75.8 ± 16 per min.) obtained after treatment with reserpine alone. Dichloroisoproterenol (1 μg./ml.) did not reduce the effect of histamine on the contraction in the same auricles, but appeared slightly to depress its stimulant effect on the rate.

Effects of Histamine Before and After Diphenhydramine and Pempidine on Isolated Guinea-pig Auricles Previously Treated with Reserpine (Figs. 3 and 4).—When ganglionic-blocking agents (hexamethonium 100 μg./ml.; pempidine 0.1 μg./ml.) were allowed to act for 30 min. on auricles which had been previously treated with reserpine, the response to histamine was not affected. When they were allowed to act for 90 min., the stimulant action of histamine on the auricles was no longer obtained. Diphenhydramine (5 μg./ml.), applied to auricles which had been previously treated with reserpine (5 μg./ml. for 5 hr.), reduced or abolished the stimulant effect of histamine on the rate and force of contraction, but did not affect the response of the auricles to adrenaline.

Effects of Histamine, Adrenaline, Noradrenaline, and Nicotine on the Isolated Heart of the Guinea-pig, Before and After Treatment with Dichloroisoproterenol (Table III, Fig. 5).—Dichloroisoproterenol acting in a concentration of 1 μg./ml. was without effect on the heart. Concentrations of 5 to 10 μg./ml. reduced the contraction to $59.8\% \pm 11$, and the rate to $95.3\% \pm 4$, of the original values.

The stimulant effects of adrenaline and noradrenaline and the diphasic effect of nicotine all disappeared in hearts treated with dichloroisoproterenol in concentrations of 1 to 10 μg./ml. Within this range of concentrations, dichloroisoproterenol did not depress the response to small doses of histamine.

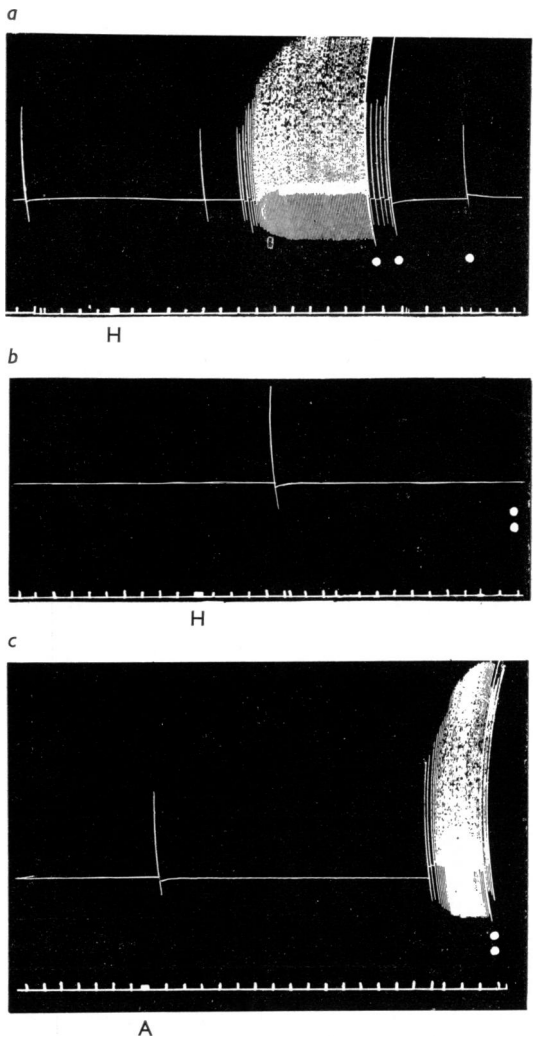

FIG. 4.—Diphenhydramine 5×10^{-6} prevents the response to histamine of guinea-pig auricles previously treated with reserpine. (*a*) Response to histamine 1×10^{-6} (*H*) on guinea-pig auricles previously treated with reserpine 5×10^{-6}. (*b*) Histamine 1×10^{-6} (*H*) 1 hr. after treatment with diphenhydramine 5×10^{-6}. (*c*) Adrenaline 2×10^{-7} (*A*) 90 min. after the treatment with diphenhydramine 5×10^{-6}. Upper tracing: height of contraction. Lower tracing: time, 20 sec. The washings are indicated by the white dots.

DISCUSSION

The results obtained in the present experiments suggest that histamine stimulates isolated cardiac tissue directly, and not through the release of catecholamines. They show that the stimulant action of histamine persisted in auricles which were first treated with reserpine, and then rendered insensitive to adrenaline and noradrenaline by suitable doses of dichloroisoproterenol. Went *et al.* (1952) reported that ergotamine abolished the stimulant effect of histamine on the isolated mammalian heart, and

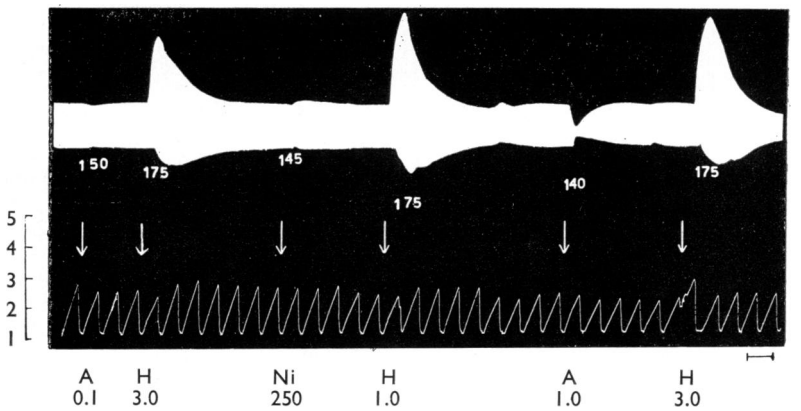

FIG. 5.—Dichloroisoproterenol 1×10^{-6} inhibits the effect of the adrenaline (*A*) and nicotine (*Ni*) in isolated guinea-pig heart, while histamine (*H*) stimulation is still present. Upper tracing: height of contraction. Lower tracing: flow of the perfusion fluid. The numbers indicate heart rate per min. Time, 2 min.

took this as indirect evidence for the release of adrenaline. However, not only is the anti-adrenaline action of ergotamine and related substances on the isolated mammalian heart open to some doubt (Nickerson, 1949), but the results of the present work fail to support the conclusion that histamine acts through the release of adrenaline.

It is unlikely that histamine exerted an effect on ganglia since its action was unaffected by ganglionic blocking agents which were applied in concentrations that abolished the diphasic action of nicotine. The action of ganglionic blocking drugs in abolishing the effect of histamine, after prolonged contact with the preparations, has been discussed elsewhere (Pepeu, Mannaioni, and Giotti, 1958, 1959). The fact that anti-histaminic drugs antagonized the stimulant effect of histamine without modifying the action of adrenaline and noradrenaline may be taken as further evidence for the existence of specific receptors for histamine in cardiac muscle. These findings, however, do not exclude the possibility that histamine acts through the release of stimulating substances which are not antagonized by dichloroisoproterenol. Went *et al.* (1954) have reported that when isolated perfused hearts are stimulated with histamine, substances which inhibit plain muscle appear in the perfusate. These substances have not yet been identified. The appearance of catecholamines in the perfusate during stimulation by histamine would suggest, in the light of the present findings, that their formation or release was not the cause, but the result, of the direct stimulant action of histamine on the heart.

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