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SOME ACTIONS OF HISTAMINE AND 5-HYDROXYTRYPTAMINE ON ISOLATED CHICKEN OESOPHAGUS

BY

A. L. BARTLET AND T. HASSAN

From the Department of Veterinary Pharmacology, Royal (Dick) School of Veterinary Studies, Edinburgh

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A large dose of histamine causes the isolated rectal caecum of the fowl to contract and then specifically blocks its own action (Barsoum & Gaddum, 1935 ; Cleugh, Gaddum, Holton & Leach, 1961), the isolated chicken heart becomes insensitive to the inhibitory action of histamine (Bartlet, 1963) and atropine antagonizes the vasodepressor action of histamine in this species (Bunag & Walaszek, 1961). These observations suggest that histamine may have an indirect action in the chicken. In the present experiments with the chick oesophagus, histamine had a muscarine-like action (Dale, 1914) which was antagonized by cocaine, suggesting the release of acetylcholine from a neural structure. Histamine also had a direct action on the external muscle and muscularis mucosae, which consist of plain muscle (Calhoun, 1933, 1954), but this action was only clearly shown after separation of the externa from the mucosa. 5-Hydroxytryptamine (5-HT) had a direct action on the plain muscle of the chick oesophagus, and had no demonstrable neural action. Some of the experiments have been described before (Bartlet & Hassan, 1966).

METHODS

Isolated tissues

Chicks aged less than 2 weeks were killed by an air embolus and the whole oesophagus was rapidly removed and placed in Krebs solution. The pre- and post-crop segments were separated from the crop and washed through with Krebs solution. The term " oesophagus " in the text, tables and figures refers to pre- or post-crop segments. A preparation of separated muscularis mucosae was made by drawing a piece of oesophagus onto a glass rod and peeling away the external muscle. Separated external muscle was prepared by everting the oesophagus, drawing it onto a glass rod and stripping the mucosa. The terms " external muscle " and " muscularis mucosae " in the text, tables and figures refer to the separated layers. A nerve-muscle preparation was made by cutting the right vagus and descending oesophageal nerves at the level of the pharynx, separating them from the oesophagus along 1 cm of their length and removing them with the pre-crop oesophagus.

The isolated tissues had open ends and were set up for the recording of longitudinal contractions in a 40 ml. organ-bath filled with Krebs solution, gassed with 5% carbon dioxide in oxygen and maintained at 35° C. The nerves were drawn through an electrode similar to that described by Burn & Rand (1960). The preparations were attached to an isotonic frontal writing lever which exerted

a force of 2 g cm and magnified the contractions thirteen times, except for preparations contracting to nervous stimulation where the magnification was four. The composition of the bathing solution was that described by Krebs & Henseleit (1932), with the Ca^{++} halved.

The preparations were exposed to an agonist for 0.5–1 min in every 10 min, and were stimulated through the nerves for 5–30 sec in every 5 min or for 1.5 min in every 10 min with square pulses width 10 msec, frequency 20/sec and voltage adjusted to produce a submaximal or a maximal contraction. When the responses to an agonist or nervous stimulation were steady, an antagonist was added to the Krebs solution. The effect of antagonists was expressed in terms of the dose ratio (Gaddum, Hameed, Hathway & Stephens, 1955), which is the ratio of equi-active doses of agonist in the presence and absence of antagonist. The effects of antagonists on pre- and post-crop segments of the oesophagus were similar, and measurements made with these preparations have been combined.

Choice of local anaesthetic

Local anaesthetics were tested on the oesophagus contracting to vagal stimulation and on the muscularis mucosae contracting to histamine. A local anaesthetic which blocked the vagus nerve and which did not combine with histamine receptors was required. Exposure of the oesophagus to amethocaine 1 μg/ml. for 1 hr reduced the height of the contraction to vagal stimulation by about 50%, and exposure to amethocaine 2.5 μg/ml. for 1 hr nearly abolished the response. Amethocaine 2.5 μg/ml., however, antagonized histamine acting on the muscularis mucosae. Lignocaine 100 μg/ml. produced a spasm of the oesophagus and muscularis mucosae although it did not abolish the contraction to vagal stimulation. Cocaine 50 μg/ml. abolished the response of the oesophagus to vagal stimulation and potentiated histamine acting on the muscularis mucosae. Cocaine was therefore a satisfactory local anaesthetic for the experiments described under RESULTS.

Drugs

The drugs used were acetylcholine chloride, amethocaine hydrochloride, atropine sulphate, cocaine hydrochloride, eserine salicylate, hexamethonium bromide, histamine acid phosphate, 5-hydroxytryptamine creatinine sulphate, hyoscine hydrobromide, lignocaine hydrochloride, methysergide bimaleate, mepyramine maleate, and tubocurarine chloride. Quantities of drugs in the text, tables and figures refer to these salts.

RESULTS

Histamine

Oesophagus

Histamine produced a contraction of the oesophagus after a delay of about 10 sec. Preparations from young chicks gave steady contractions to histamine 0.1 μg/ml. but those from older birds were much less sensitive to histamine and gave contractions which gradually became smaller. In experiments with histamine the oesophagus of a chick aged less than 2 weeks was therefore used.

Mepyramine was a specific antagonist of histamine acting on the chick oesophagus (Table 1). Mepyramine 1 μg/ml., however, did not antagonize the response of the oesophagus to submaximal vagal stimulation (two experiments).

Atropine antagonized both histamine and acetylcholine (Fig. 1). The calculated regression lines for the antagonism of histamine and acetylcholine were parallel. Atropine was, however, a more potent antagonist of acetylcholine than of histamine. The concentrations of atropine which produced a ten-fold antagonism of acetylcholine and histamine were 1 and 100 ng/ml., respectively. High concentrations of atropine had a spasmogenic

action; atropine 100 μg/ml. produced a rise in the tone of the oesophagus and atropine 1 mg/ml. produced a contraction.

Atropine has an action on the isolated cat superior cervical ganglion which cannot be attributed to an antimuscarine action, and because hyoscine has no such action (Brown, 1966) it seemed advisable to repeat the foregoing experiments substituting hyoscine for atropine. Hyoscine antagonized acetylcholine and histamine acting on the oesophagus. The antagonism of both drugs was prompt and surmountable and in the experiment shown in Fig. 2, the dose ratios for acetylcholine and histamine in the presence of hyoscine 1 ng/ml. were 35.5 and 3.5, respectively. Experiments with different concentrations of hyoscine showed that the antagonism of histamine increased with the concentration of antagonist (Table 1).

TABLE 1

ANTAGONISM OF ACETYLCHOLINE, HISTAMINE AND 5-HT ON THE CHICK OESOPHAGUS

Dose ratios were measured when the antagonism became steady, except those for 5-HT in the presence of methysergide which were measured after a 70 min exposure: Dose ratios are given as means with standard errors, except where only one or two observations were made; the number of observations is given in parentheses

Antagonist	Concentration (μg/ml.)	Dose ratio		
		Acetylcholine	Histamine	5-HT
Hexamethonium	20	1·0 (2)	1·0 (2)	1·0 (2)
d-Tubocurarine	250	1·0 (2)		1·0 (2)
Hyoscine	0·001	83·0±30·6 (3)	1·9, 3·5	
	0·1		5·6, 6·7	
	1		56·4, 160·0	
Atropine	0·0001	2·8± 0·5 (5)	1·7± 0·25 (5)	
	0·001	9·9± 2·3 (8)	2·3± 0·45 (5)	1·0 (3)
	0·01	46·0±21·2 (7)	4·1± 0·27 (5)	
	0·1	56·0±11·3 (7)	8·6± 4·8 (6)	1·0 (3)
	1	131·0±30·0 (7)	19·0± 4·9 (5)	
	10	256·0±74·0 (5)	72·0± 15·3 (5)	1·6± 0·2 (6)
Mepyramine	0·01	1·0 (2)	9·0± 1·4 (6)	1·0
	0·1		23·0± 4·0 (4)	
Methysergide	0·001	1·0 (2)	1·5, 1·5	878 ±884 (3)

In six experiments, eserine 5 ng/ml. potentiated the action of histamine but not that of 5-HT (Fig. 3). The contraction to histamine increased progressively in the presence of eserine.

Cocaine 50 μg/ml. reduced the height of the contraction produced by histamine but did not abolish it. In six experiments the mean dose ratio for histamine in the presence of cocaine 50 μg/ml. was 54 with a standard error of 34. Exposure of the preparation to cocaine 50 μg/ml. for 20–30 min always abolished the response to submaximal or supramaximal vagal stimulation and potentiated that to acetylcholine.

External muscle

Histamine produced steady contractions of preparations of external muscle when these were made from chicks aged less than 2 weeks. Mepyramine antagonized the action

of histamine on the external muscle but the dose ratio was only one-half that on the intact oesophagus ($P<0.01$) (Table 2). Hyoscine antagonized acetylcholine acting on the external muscle, however, as much as it did on the oesophagus.

The action of histamine on the external muscle was partially antagonized by hyoscine; in three experiments the dose ratio for histamine in the presence of hyoscine 1 μg/ml. was

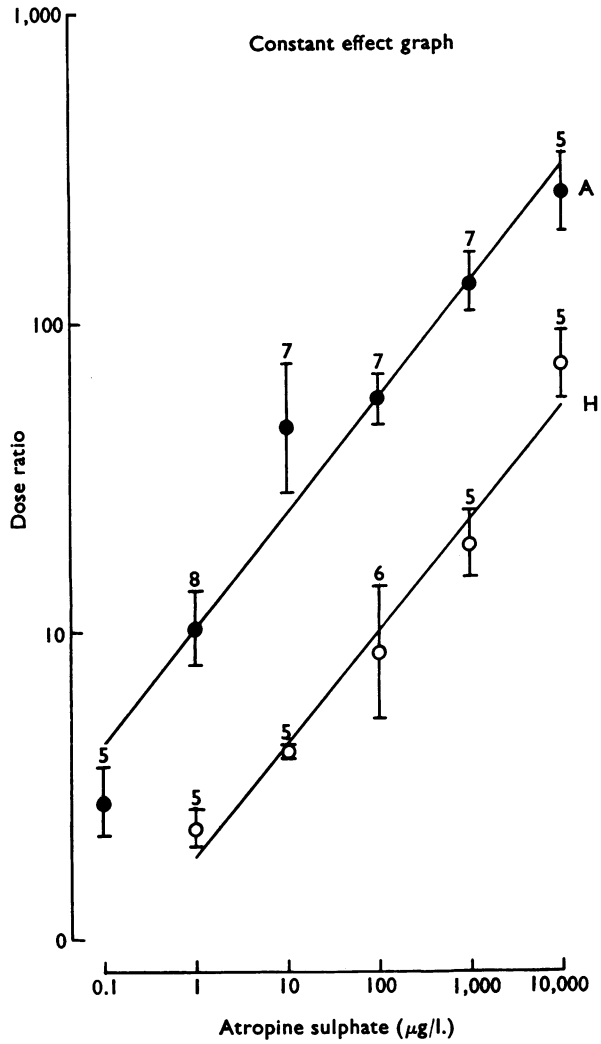

Fig. 1. Constant effect graph of the antagonism of histamine and acetylcholine by atropine on the oesophagus. Ordinate, dose ratio on a log scale; abscissa, concentration of atropine sulphate on the log scale. Mean dose ratios for histamine are shown as open circles (H) and for acetylcholine as closed circles (A). The vertical bars and numerals depict the standard errors and numbers of observations, respectively. The calculated regression lines are parallel and significant at the 1% level.

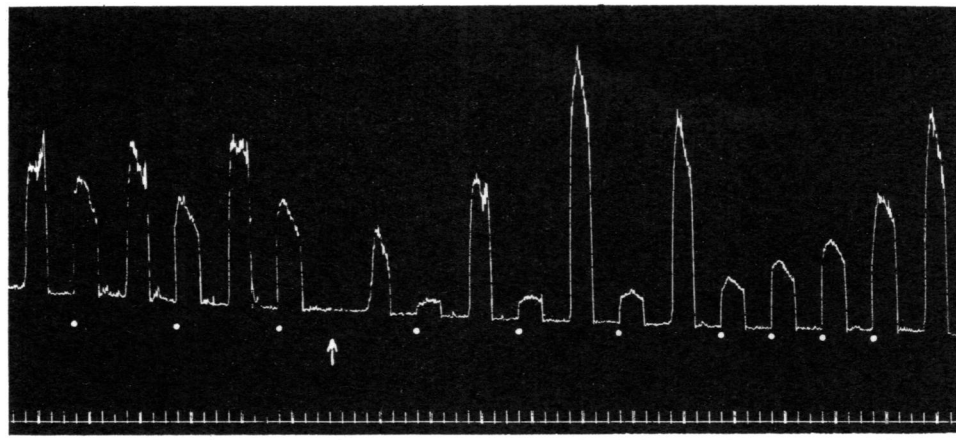

Fig. 2. Post-crop oesophagus. Numerals refer to organ bath concentrations, μg/ml., of acetylcholine (ACh) and histamine (H). From the arrow to the end of the tracing hyoscine 1 ng/ml. was present. Hyoscine antagonized acetylcholine and histamine. Time, 1 min.

1.6 ± 0.23. In one experiment the antagonism did not increase when the concentration of hyoscine was raised from 1 to 100 μg/ml.

Muscularis mucosae

The muscularis mucosae from chicks aged less than 2 weeks gave steady contractions to histamine, but that from older birds did not. On the muscularis mucosae the antagonism of histamine by mepyramine was similar to that on the external muscle and the dose ratio was less than that on the oesophagus (Table 2). The antagonism of acetylcholine by hyoscine on the muscularis mucosae was similar to that on the other oesophageal preparations.

Hyoscine 1 μg/ml. did not antagonize histamine acting on the muscularis mucosae (five experiments). In four experiments cocaine 50 μg/ml. potentiated the action of histamine on the muscularis mucosae.

5-Hydroxytryptamine

The oesophagus contracted to 5-HT 1–10 ng/ml. and methysergide specifically antagonized this action. The action of methysergide 1 ng/ml. developed slowly and did not reach a state of equilibrium (Table 1). When the action of methysergide was fully developed, the 5-HT contraction was abolished. The action of 5-HT was not antagonized by atropine, hexamethonium, tubocurarine or mepyramine (Table 1). Cocaine potentiated the action of 5-HT.

The action of 5-HT on the external muscle and muscularis mucosae was similar to that on the intact oesophagus.

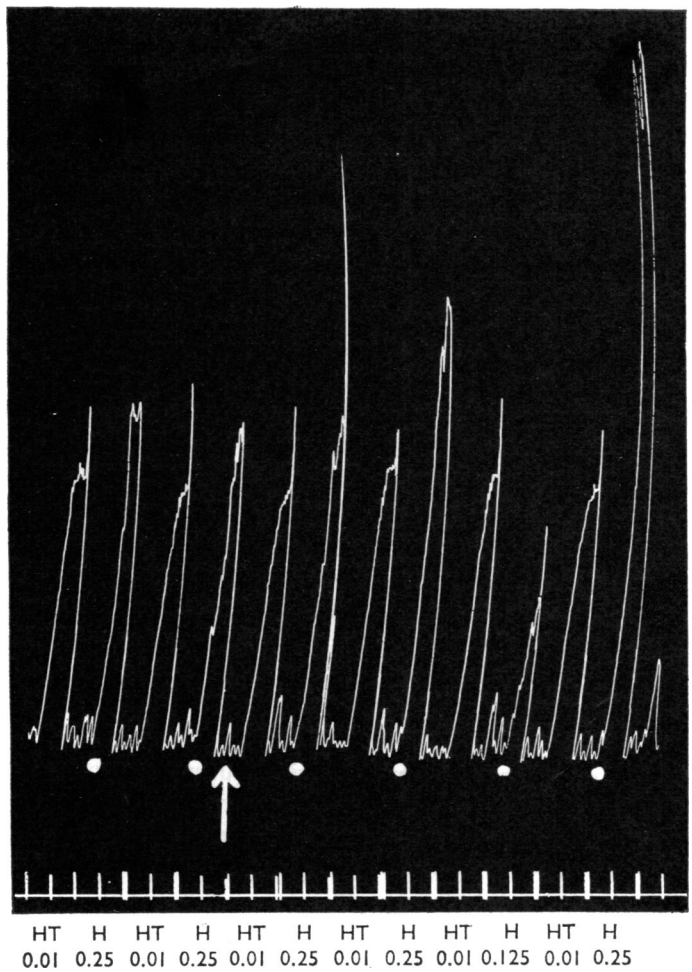

Fig. 3. Post-crop oesophagus. Numerals refer to organ bath concentrations, μg/ml., of 5-HT and histamine (H). From the arrow to the end of the tracing eserine 5 ng/ml. was present. Eserine produced a progressive potentiation of the effect of histamine. Time, 1 min.

TABLE 2

EFFECT OF SEPARATING THE EXTERNA FROM THE MUCOSA ON THE ACTIONS OF MEPYRAMINE AND HYOSCINE

Dose ratios are given as means with standard errors and number of observations in parentheses. Separation of the oesophageal layers reduced the action of mepyramine ($P<0{\cdot}01$), but not that of hyoscine

	Dose ratio	
Preparation	Histamine in the presence of mepyramine 10 ng/ml.	Acetylcholine in the presence of hyoscine 1 ng/ml.
Oesophagus	9·0±1·4 (6)	7·5±1·8 (6)
External muscle	4·4±0·9 (4)	5·4±0·6 (5)
Muscularis mucosae	4·3±0·3 (5)	9·2±2·3 (5)

DISCUSSION

The contraction of the oesophagus to histamine was antagonized by atropine or hyoscine and was potentiated by eserine, which suggests that histamine had a muscarine-like action. Cocaine, at a concentration sufficient to block the response to vagal stimulation, reduced the height of the contraction produced by histamine. This suggests that histamine stimulated a neural structure because cocaine did not antagonize acetylcholine acting on the oesophagus. Histamine was not antagonized by hyoscine or cocaine on the muscularis mucosae, and was only partially antagonized by hyoscine on the external muscle. Perhaps neural structures were damaged in the separation of the externa from the mucosa, and the muscarine-like action of histamine was thus impaired. The action of histamine which was not antagonized by hyoscine or cocaine was not clearly shown on the intact oesophagus where the neural action of histamine was predominant. Mepyramine did not antagonize the response of the oesophagus to vagal stimulation, although it blocked the neural action of histamine; this suggests that the neural histamine receptors were associated with an afferent pathway or an efferent pathway that was not activated during vagal stimulation.

Although histamine produced steady contractions of oesophageal preparations from young chicks, the response of preparations from older birds became progressively smaller. Tachyphylaxis to the hyoscine-resistant action of histamine on the muscularis mucosae occurred, suggesting that the viability of the histamine receptor was reduced with age. Mepyramine antagonized histamine acting on the oesophagus more readily than it did on the separated oesophageal layers. This may be because of a difference of functional state between the preparations rather than a difference of receptor.

The action of 5-HT was blocked by methysergide, but was not potentiated by eserine, or antagonized by atropine, hyoscine, cocaine or mepyramine. This suggests that 5-HT stimulated a specific receptor in the plain muscle of the oesophagus.

SUMMARY

1. Histamine produced a slow contraction of the fowl oesophagus, but the response could only be reproduced steadily on preparations made from young chicks.

2. The action of histamine on the oesophagus was antagonized by mepyramine, atropine, hyoscine and cocaine and was potentiated by eserine.

3. On the separated external muscle hyoscine blocked about 35% of the contraction to histamine. On the separated muscularis mucosae the contraction to histamine was not antagonized by hyoscine or cocaine.

4. Mepyramine antagonized histamine acting on the separated oesophageal layers less than on the intact oesophagus, and did not antagonize the response of the oesophagus to vagal stimulation.

5. 5-Hydroxytryptamine produced a contraction of the oesophagus and separated oesophageal layers which was only and specifically blocked by methysergide.

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