

Role of amines in anaphylactic contraction of guinea pig isolated smooth muscle

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The possible role of acetylcholine (ACh), histamine (His), and 5-hydroxytryptamine (5-HT) in the contractile response to ovalbumin (Oval) of ileal and tracheal muscle from sensitized guinea pigs was examined. Antagonists to these agents (atropine, mepyramine and methysergide, 5-benzyloxygramine, or 5-HT tachyphylaxis, respectively) were employed singly or in combinations. Care was always taken to ensure specificity of blockade. Results indicate that about half of the peak magnitude of the oval-induced contraction is absent in the presence of a specific inhibitory concentration of mepyramine. This finding suggests that His plays a considerable role in the oval-induced contraction. Contrary to previous reports, similar evidence was not obtained of a role for ACh and 5-HT in the Oval-induced contraction. The source of the agonists released by Oval was also investigated. Pretreatment with compound 48/80 almost completely eliminated the Oval-induced contraction of ileal muscle while reducing the tracheal contraction about one-half. It would appear that the Oval-induced ileal contraction involves primarily agonists released from mast cells in the preparation whereas tracheal contraction is only partly dependent on mediators from mast cells.

Important advances in the elucidation of the mechanism of anaphylaxis were the demonstration by Schultz¹ of the anaphylactic response of smooth muscle in vitro and the work of Dale² on in vitro anaphylaxis. Since these early studies, a number of conflicting reports have appeared purporting to identify the factors involved in the so-called Schultz-Dale reaction. These alleged factors include (1) agonists released locally by an antigen-antibody reaction occurring at their sites of storage and (2) the direct stimulation of the smooth muscle cell by an antigen-antibody reaction occurring at its plasma membrane.³

Agonists suggested to have a role in the Schultz-Dale reaction include at least 3 simple amines: histamine (His),⁴ acetylcholine (ACh),⁵⁻⁷ and 5-hydroxytryptamine (5-HT).⁵ These amines have also been postulated to be involved in anaphylaxis in the intact animal.⁸⁻¹⁰ Presumably, His exists primarily in mast cells. In the guinea pig, 5-HT may not be stored in mast cells but rather is present in platelets and in the mucosal layer of the gastrointestinal tract.¹¹ ACh is the neurotransmitter released at autonomic ganglia and nerve endings of the parasympathetic division of the autonomic nervous system.

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Supported in part by National Science Foundation Grant No. GB-23353, and National Institutes of Health Grant No. HL 15580 and 11802.

Received for publication June 13, 1973.

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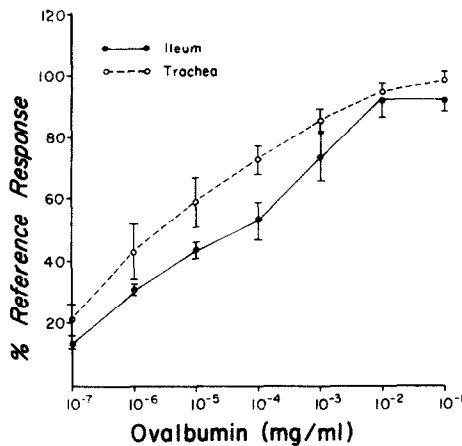


FIG. 1. Dose-response relationship for ovalbumin contraction of ileal and tracheal strips. 100% reference response was that elicited later by ovalbumin (1 milligram per milliliter) in the same tissue. Closed circles represent the means from 6 experiments with ileal muscles. Open circles represent the means from experiments with 4 tracheal segments. Brackets enclose the standard errors of the means. The magnitudes of the contractile responses to 10^{-2} , 10^{-1} , and 1 milligram per milliliter were not statistically different.

The studies implicating these amines have often involved the use of blocking agents presumed to be specific. It is now generally recognized that the specificity of such antagonists is often related to concentration. At high levels, many antagonists appear to act nonspecifically. This report describes experiments on the anaphylactic contraction of guinea pig isolated ileal and tracheal smooth muscle during which precautions were taken to ensure the specificity of the various blockers employed. In addition, the source of mediators released during anaphylaxis was studied by means of compound 48/80, a substance known to promote the degranulation of mast cells.¹²

METHODS

Male guinea pigs weighing 250 to 300 Gm. were sensitized by receiving 0.1 ml. 5 per cent Oval by intraperitoneal injection on alternate days for 5 days. They were maintained on standard laboratory guinea pig chow and tap water ad lib. After 3 to 4 weeks, the animals were sacrificed by a blow to the head. Segments of the ileum and trachea were removed.

Ileal segments 3 to 4 cm. in length were mounted in 15 ml. muscle baths. Care was taken to ensure that the lumen was freely accessible to the bathing medium. Trachea were cut into a series of attached rings after the manner of Timmerman and Scheffer.¹³ Two tracheal preparations were obtained per guinea pig and mounted in 15 ml. muscle baths. One was used as a control and the other as an experimental preparation. Ileal muscle shortening was detected and recorded by means of an inkwriting lever system and standard kymograph. The load on the muscle was one gram. Isometric contraction of the tracheal muscle was detected by a Grass force-displacement transducer and recorded on a Grass Polygraph Model 5. The resting load on the tracheal muscle was one gram.

The muscle preparations were continuously bathed in physiological salt solution containing: 125 mM NaCl, 2.7 mM KCl, 1.8 mM CaCl_2 , and 11 mM glucose. The solution was buffered with THAM (Trishydroxymethylaminomethane, Sigma-Trizma base buffer), 23.8 mM. The pH was adjusted to 7.4 with HCl. The medium was bubbled constantly with 100

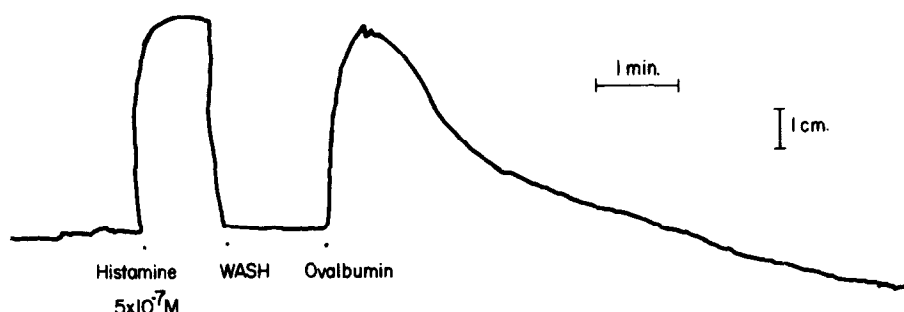


FIG. 2. Comparison of histamine- and ovalbumin-induced contractile responses of an isolated ileal strip. An ileal strip resting in normal physiological salt solution was exposed to 5×10^{-7} M histamine. After about 1 minute, the muscle strip was returned to normal physiological medium lacking histamine. One hour later, the muscle was exposed to a bathing solution containing ovalbumin (1 milligram per milliliter). The contractile activity was followed for about 5 minutes.

Table 1. Effect of mepyramine on peak contractile responses elicited by equipotent concentrations of Oval and His

Concentration of mepyramine (M)	Magnitude*† of peak contractile response to:	
	Oval (1 mg./ml.)	His (5×10^{-7} M)
0	101.1 $\pm$ 4.3 (39)	100 (39)
10^{-6}	73.0 $\pm$ 8.1 (5)	49.5 $\pm$ 3.1 (24)
10^{-8}	54.4 $\pm$ 9.6 (7)	14.1 $\pm$ 4.4 (8)
5×10^{-7}	40.7 $\pm$ 6.1 (27)	5.7 $\pm$ 2.0 (37)

*As mean $\pm$ S.E. (number of experiments).

†As % of the standard reference contraction induced by 5×10^{-7} M His.

per cent oxygen. Temperature of the medium bathing the ileal muscle was maintained at 31° to 32° C. Temperature of the solution bathing the tracheal strips was kept at 36° to 37° C.

Drugs were added to the bathing media from concentrated stock solutions prepared in distilled water. Oval was always employed at a final bath concentration of 1 mg. per milliliter. This concentration was chosen because it was found to be supramaximal in experiments assessing the cumulative dose-response relationship¹⁴ for Oval (Fig. 1). Thus its use presumably ensured the complete activation of the processes by which Oval elicits contraction. When blockers were used, they were added to the bathing medium 5 minutes before the agonist.

RESULTS

Experiments on isolated ileum

The concentration of Oval employed (1 mg./ml.) induced a substantial contractile response of the isolated whole ileal strip. The peak magnitude was virtually identical to that obtained with 5×10^{-7} M His in the same strip (Fig. 2). The His-induced response was always sustained for as long as the agonist was allowed to remain in the bathing solution. In the experiment depicted in Fig. 2, the contraction was terminated by draining out His-containing bathing solution after one minute of exposure and replacing it with fresh physiological bathing medium.

Table II. Contractile response elicited by equipotent concentrations of Oval, ACh, or 5-HT in the presence of ACh and 5-HT antagonism

Antagonist (M)	Magnitude*† of contractile response to:		
	Oval (1 mg./ml.)	ACh (10^{-6} M)	5-HT (10^{-6} M)
Atropine (10^{-6})	98.7 ± 8.7 (7)	1.9 ± 0.6 (18)	20.0 ± 7.3 (11)
Methysergide (10^{-5}) + 5-Benzylxygramine (10^{-5})	92.0 ± 2.8 (5)	—	17.6 ± 8.1 (5)
Prior exposure for 15 min. to 5-HT (10^{-6})	85.8 ± 8.2 (8)	—	0 (8)

*As mean ± S.E. (number of experiments).

†As % of the standard reference contraction induced by 5×10^{-7} M His, a concentration equipotent to 10^{-6} M 5-HT and Oval (1 mg./ml.) in physiological bathing medium.**Table III.** Contractile response elicited by equipotent concentrations of Oval, ACh, His or 5-HT in the presence of combinations of antagonists

Antagonist (M)	Magnitude*† of contractile response to:			
	Oval (1 mg./ml.)	ACh (10^{-6} M)	His (5×10^{-7} M)	5-HT (10^{-6} M)
Mepyramine (5×10^{-8}) + Atropine (10^{-6})	53.5 ± 12.2 (4)	0 (4)	0 (4)	—
Mepyramine (5×10^{-8}) + Atropine (10^{-6}) + 5-Benzylxygramine (10^{-5})	37.0 ± 6.5 (3)	0 (3)	0 (3)	0 (3)
Atropine (10^{-6}) + Methysergide (10^{-6})	121.0 ± 3.4 (6)	0 (22)	—	4.6 ± 1.3 (22)

*As mean ± S.E. (number of experiments).

†As % of the standard reference contraction induced by 5×10^{-7} M His, a concentration equipotent to 10^{-6} M ACh, 10^{-6} M 5-HT, and Oval (1 mg./ml.) in physiological bathing medium.

Once the response to Oval had occurred, the tissue was insensitive to Oval for at least the following 2 hours. This same concentration of Oval was ineffective when applied to isolated ileal strips from normal, nonsensitized guinea pigs. The His response could be repeated and was also obtained in isolated ileal strips from normal, nonsensitized animals.

The effects of various concentrations of the antihistamine, mepyramine, on the magnitude of the Oval (1 mg./ml.) and His (5×10^{-7} M)-induced contractions are illustrated in Table I. Increasing the level of mepyramine from 10^{-9} to 5×10^{-8} M reduced the magnitude of the contractile responses in both cases. However, the highest level (5×10^{-8} M) almost completely inhibited the His response while blocking the Oval-induced contraction only about 60 per cent. This concentration of mepyramine was without effect on the ACh and 5-HT-induced contractions. Raising the mepyramine level to 10^{-7} M, however, resulted in profound inhibition of the contractions due to Oval, His, 5-HT, and ACh, suggesting a nonspecific blockade.

Similar experiments were performed comparing the mechanical responses to Oval (1 mg./ml.) and equipotent concentrations of ACh (10^{-6} M) and 5-HT (10^{-6} M). The anticholinergic agent, atropine (10^{-6} M), was found to block the ACh response almost completely without affecting the His-induced contraction (Table II). This level of atropine was ineffective versus the Oval-induced

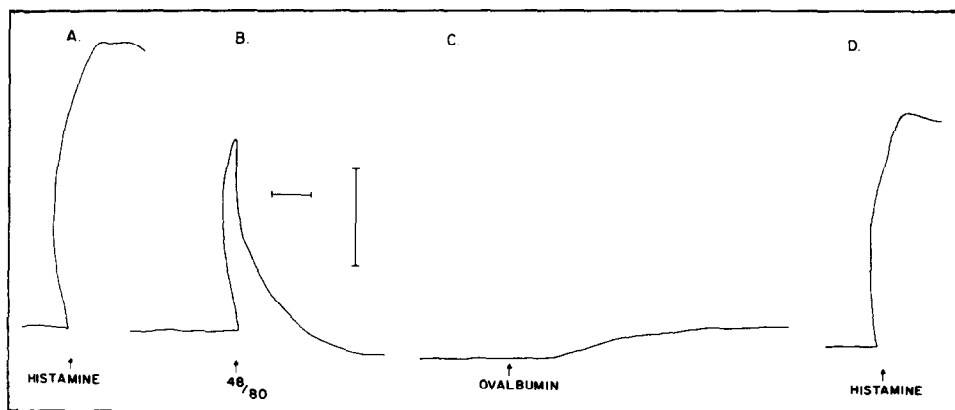


FIG. 3. Effect of prior exposure to compound 48/80 on ovalbumin and histamine-induced contraction of ileal segments. A typical kymograph tracing is illustrated. **A**, An ileal strip resting in physiological salt solution was exposed to 5×10^{-7} M histamine. After about 2 minutes, the strip was returned to physiological medium lacking histamine (not shown). **B**, One hour later, compound 48/80 (1 milligram per milliliter) was added to the bathing medium. Thirty minutes later, the bathing solution was replaced by physiological salt solution lacking compound 48/80 (not shown). **C**, After 5 minutes in physiological medium, the strip was exposed to ovalbumin (1 milligram per milliliter). **D**, Thirty minutes after the ovalbumin-induced response declined to base line, histamine (5×10^{-7} M) was introduced into the bathing medium. The horizontal bar represents one minute. The vertical bar represents 3 cm.

response but inhibited the 5-HT contraction about 80 per cent. Inhibition of 5-HT contraction by atropine has been reported before.¹⁵ It apparently results from the blockade of ACh released by 5-HT-induced nerve stimulation.

The 5-HT antagonists methysergide¹⁶ and 5-benzyloxygramine¹⁷ either separately or in combination greatly inhibited the 5-HT response without altering the His contraction. The Oval-induced response was also essentially unchanged (Table II). When the concentrations of these 5-HT antagonists were increased until they completely inhibited the 5-HT response, the contractions induced by His and ACh were also appreciably affected, indicating a non-specific blockade.

However, it was found that a 15 minute prior exposure of the muscle to 10^{-6} M 5-HT resulted in a subsequent loss of the 5-HT response for 15 to 30 minutes, probably owing to the development of a receptor-related specific tachyphylaxis since responses to His were little affected.¹⁵ The contractile response to Oval applied during this period of 5-HT tachyphylaxis was almost unchanged from the normal response (Table II).

Some experiments were carried out employing two or more antagonists. In these studies, the antagonists were added together 5 minutes before the agonists. The order of agonists added was: ACh, His, 5-HT, and Oval. On occasion, either His or 5-HT was omitted.

Mepyramine (5×10^{-8} M) plus atropine (10^{-6} M) completely blocked responses to ACh and His, while inhibiting the Oval response about one-half (Table

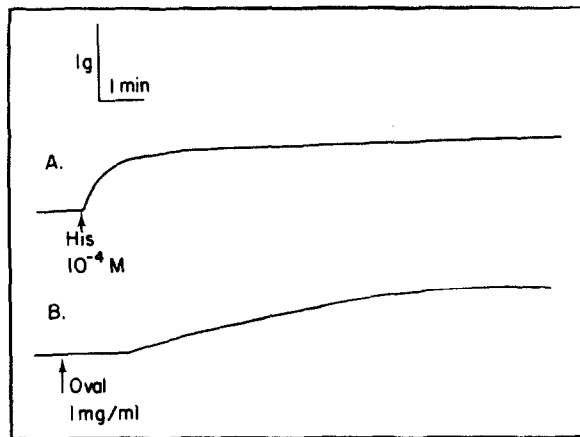


FIG. 4. Comparison of histamine- and ovalbumin-induced contractile responses of an isolated tracheal strip. **A.** A tracheal strip resting in physiological salt solution was exposed to 10^{-4} M histamine. After about 10 minutes, the strip was returned to physiological medium lacking histamine (not shown). **B.** One hour later, the muscle was exposed to a solution containing ovalbumin (1 milligram per milliliter). The contractile activity was followed for about 10 minutes.

III). The addition of 5-benzyloxygramine (10^{-5} M) to this antagonist mixture resulted in the further blockade of 5-HT while the Oval-induced contraction was still present to an appreciable degree.

Atropine (10^{-6} M) plus methysergide (10^{-6} M) blocked the contraction induced by ACh and greatly attenuated the response to 5-HT. The Oval-induced contraction was, if anything, enhanced by this antagonist combination.

Some insight into the source of agonists involved in the Oval-induced contraction was gained in 6 experiments using compound 48/80. This substance promotes the degranulation of mast cells and induces the release of histamine and other substances.^{18, 19} Ileal strips were tested with 5×10^{-7} M His to elicit a reference response. The agonist was then washed out and the tissues allowed to recover in physiological salt solution for one hour, whereupon the strips were exposed to compound 48/80 (1 mg./ml.) for 30 minutes. The introduction of compound 48/80 resulted in an immediate spikelike contraction followed by relaxation to or beyond the previous resting length. The muscle segments were next returned to physiological salt solution for 5 minutes and then Oval (1 mg./ml.) was added to the bathing medium. The resultant contraction was weak and developed slowly (Fig. 3) compared to that obtained in tissues not exposed to compound 48/80 (Fig. 2). Thirty minutes after the Oval-elicited contraction had subsided, His (5×10^{-7} M) still induced a substantial contractile response (Fig. 3) suggesting that the cellular effects of compound 48/80 did not seriously inhibit the stimulatory activity of this agent.

Experiments on isolated trachea

Oval produced a slowly developing contraction of the sensitized tracheal smooth muscle preparation (Fig. 4). Mechanical activity did not begin im-

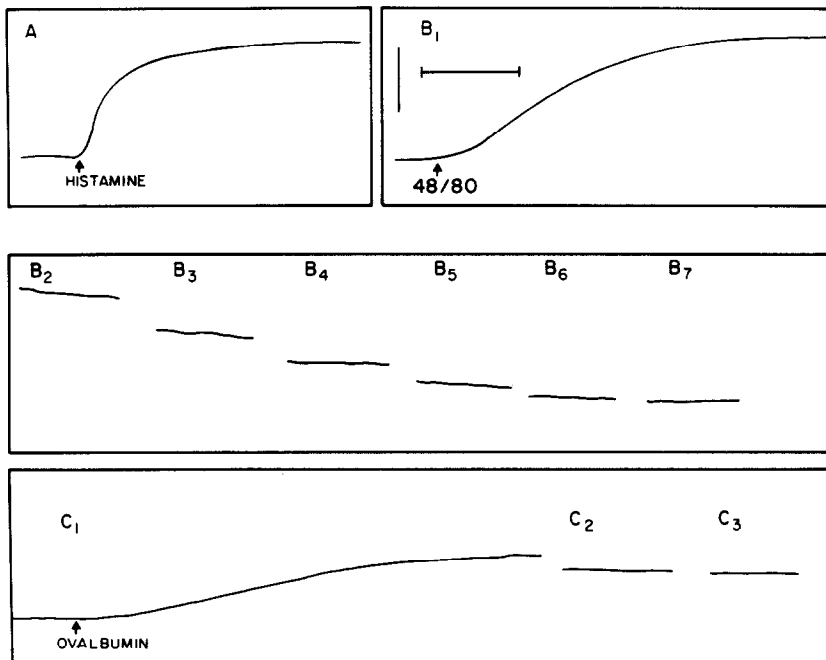


FIG. 5. Effect of compound 48/80 on ovalbumin-induced contraction of tracheal muscle. A typical physiograph record is shown. **A**, A tracheal strip bathed in physiological salt solution was exposed to 10^{-4} M histamine. After about 5 minutes, the muscle was returned to histamine-free physiological medium (not shown). **B₁**, After one hour, the strip was exposed to compound 48/80 (1 milligram per milliliter). The contractile response during the first 8 minutes is illustrated. **B₂-B₇**, Contractile responses at 12, 22, 32, 42, 52, and 62 minutes, respectively, after the addition of compound 48/80. The muscle segment was then transferred to physiological salt solution (not shown). **C₁**, After 5 minutes, ovalbumin (1 mg./ml.) was added to the bath. The contractile response during the first 10 minutes is shown. **C₂-C₃**, Contractile response 40 and 75 minutes, respectively, after the addition of ovalbumin. Horizontal bar = 0.5 Gm.

mediately after the application of the antigen. Onset was delayed about one minute. Peak tension was reached some 5 minutes later. The contraction could not be terminated early by removal of the Oval from the bathing medium. In contrast, responses to His (Fig. 4), ACh, or 5-HT began immediately upon the introduction of the agonist into the bathing solution. Peak tension was reached in less than 2 minutes. While tracheal preparations from nonsensitized animals also responded well to His, ACh, and 5-HT, they were insensitive to Oval.

The magnitude of the peak tension response to Oval (1 mg./ml.) was usually about one gram and was similar to that induced by 10^{-4} M His, 5×10^{-5} M ACh, and 10^{-4} M 5-HT. In contrast to the contractions elicited by these agents, the response to Oval (1 mg./ml.) could not be repeated.

In 6 experiments, mepyramine (5×10^{-7} M) was found to block completely the mechanical response to 10^{-4} M His while the Oval-induced contraction was still 59 ± 4 (S.E.M.) per cent of control. The Oval response in the presence of mepyramine was always slower to develop and sometimes more delayed in onset than control responses elicited in the absence of mepyramine. Contractile

responses to ACh (5×10^{-5} M) and 5-HT (10^{-4} M) still occurred in the presence of 5×10^{-7} M mepyramine.

Four experiments were performed to test the effect of ACh and 5-HT blockade. 5-HT block was obtained by inducing tachyphylaxis of the tissue to the drug. This was accomplished by exposing the tissue to 10^{-4} M 5-HT for 30 minutes. A second application of 5-HT now had no effect while the responses to ACh and His could still be obtained. Atropine (10^{-6} M) was employed to block the ACh response. In the presence of complete blockade of ACh and 5-HT by atropine and 5-HT tachyphylaxis, respectively, the Oval-induced contraction was still not significantly altered (99 ± 4 per cent) from that of the control.

Four tracheal strips were treated with compound 48/80 in a manner similar to that described previously for ileal segments (Fig. 5). After being exposed to 10^{-4} M His to elicit the reference contraction and allowed to recover in physiological salt solution, tracheal strips were placed in contact with compound 48/80 (1 mg./ml.). A large, long-lasting contractile response occurred that required 30 to 65 minutes to subside, after which the strips were returned to physiological medium and 5 minutes later exposed to Oval (1 mg./ml.). The resulting contraction was about half that expected (Fig. 4) but was quite prolonged, lasting 60 to 90 minutes.

DISCUSSION

A contractile response is elicited by Oval (1 mg./ml.) in sensitized guinea pig isolated ileum and trachea but not in tissues from nonsensitized animals. The peak magnitude reached is similar to that seen with 5×10^{-7} M His in ileum or 10^{-4} M His in trachea. Once obtained in a preparation, the Oval-induced contraction could not be repeated for at least 2 hours. This desensitization has been noted before and attributed to the temporary exhaustion of the processes coupling the antigen-antibody complex formation with the release of smooth muscle agonists.²⁰

The data indicate that about half the Oval-induced contractile response of both tracheal and ileal smooth muscle can be ascribed to the action of His. This agonist is apparently released into the vicinity of the smooth muscle cells following the antigen-antibody reaction. A role for His in the anaphylactic response has often been postulated.⁴

Unlike previous workers,⁵⁻⁷ we were unable to obtain convincing evidence of a role for ACh or 5-HT in this response. Several possible reasons might account for this discrepancy: (1) The earlier workers often made no attempt to compare equipotent concentrations of Oval, His, 5-HT, and ACh. (2) They usually demonstrated inhibition of the Oval-induced response only when combinations of blockers were used. (3) Little attempt was made to quantitate their results. (4) Little information was reported of any effort made to test for specificity of blockade. In the present study, equipotent concentrations of agonists were employed. Experiments were performed with single blockers as well as combinations of antagonists. All the contractile responses were compared quantitatively to a standard reference contraction in the same muscle strip. Moreover, in our studies we routinely tested for specificity of blockade.

The present findings agree with those of other recent studies describing the participation of His in the Schultz-Dale reaction. Cirstea and Suhaciu²¹ and Kurihara and Shibata²² detected His release during the anaphylactic response of guinea pig ileum. In addition, the latter investigators report finding no evidence of 5-HT release.

The residual contractile response to Oval in the presence of His blockade by mepyramine may have resulted from the release of other agonists or from the direct stimulatory effect of an antigen-antibody reaction on the muscle cell surface itself.³ While a number of agonists other than amines have been postulated to be released during systemic anaphylaxis, those that have attracted the most attention are bradykinin,²³ prostaglandins,²⁴ and a "slow-reacting substance of anaphylaxis" or SRS-A.²⁵ The latter is an acidic, lipidlike substance. Bradykinin is produced from a plasma substrate¹¹ and is not likely to be involved in anaphylactic responses of isolated tissues. On the other hand, SRS-A²⁶ and prostaglandins²⁷ are possible candidates for mediating the residual Oval-induced response obtained in the presence of mepyramine in both ileal and tracheal preparations.

Our results with ileal muscle do not support the postulate that the mepyramine-resistant residual contraction involves a direct action of the antigen on the smooth muscle cell itself. After treatment with compound 48/80, ileal strips responded very weakly to Oval although substantial contractions to His could still be elicited. Presumably, the mast cell serves as the principal source of agonists for Oval-induced contraction of ileal segments. This conclusion is in agreement with that of Dale and Zilletti²⁸ who used *n*-octylamine to disrupt the mast cells.

Tracheal strips still responded well and in a prolonged manner to Oval after compound 48/80 treatment. It may be that compound 48/80 is a less effective agent for releasing agonists from tracheal than from ileal mast cells, although the large, prolonged contraction associated with its introduction into the medium bathing the airway muscle (Fig. 5) would tend to argue against this. More likely, it would appear that a direct stimulatory effect of the antigen-antibody reaction on the tracheal muscle cell may have occurred. Alternatively, agonists from sources other than mast cells may also be involved in the Oval-induced tracheal contraction.

The adequacy of these guinea pig isolated preparations as animal models for human anaphylaxis is, of course, questionable. Reservations have been voiced frequently.¹¹ Nevertheless, the clinical evidence for the lack of efficacy of anticholinergic agents and the limited usefulness of antagonists of His and 5-HT²⁹ are in agreement with the failure of these inhibitors to block the anaphylactic contraction of isolated ileal and tracheal preparations.

We wish to thank Miss Elizabeth C. Thomason for her aid in preparing the manuscript, Sandoz Pharmaceuticals for the donation of methysergide, Dr. V. B. Haarstad for the gift of 5-benzyloxygramine, and Burroughs Wellcome & Co. for compound 48/80.

REFERENCES

- 1 Schultz, W. H.: Physiological studies in anaphylaxis. I. The reaction of smooth muscle of the guinea-pig sensitized with horse serum, *J. Pharmacol. Exp. Ther.* 1: 549, 1910.

- 2 Dale, H. H.: The anaphylactic reaction of plain muscle in the guinea-pig, *J. Pharmacol. Exp. Ther.* **4**: 167, 1913.
- 3 Alonso-De Florida, F., Del Castillo, J., Garcia, X., and Gijon, E.: Mechanism of the Schultz-Dale reaction in the denervated diaphragmatic muscle of the guinea-pig, *J. Gen. Physiol.* **51**: 677, 1968.
- 4 Dragstedt, C. A.: Anaphylaxis, *Physiol. Rev.* **21**: 563, 1941.
- 5 Geiger, W. B., and Alpers, H. S.: The mechanism of the Schultz-Dale reaction, *J. ALLERGY* **30**: 316, 1959.
- 6 Danielopolu, D., Rudeanu, A., Bruckner, I., and Simionescu, S.: Liberation d'acetylcholine dans le choc paraphylactique (anaphylactique), *C. R. Soc. Biol. (Paris)* **142**: 757, 1948.
- 7 Nakamura, K.: Nouvelle opinion sur la production der choc anaphylactique, specialement sur le role de l'acetylcholine dans l'anaphylaxie, *Jap. J. Exp. Med.* **19**: 31, 1941.
- 8 Eyre, P., Lewis, A. J., and Wells, P. W.: Acute systemic anaphylaxis in the calf, *Br. J. Pharmacol.* **47**: 504, 1973.
- 9 Csaba, B., and West, M.: Role of histamine, 5-hydroxytryptamine and bradykinin in the rat anaphylaxis, *Acta Physiol. Acad. Sci. Hung.* **39**: 369, 1971.
- 10 Gold, W. M., Kessler, G. F., and Yu, D. Y. C.: Role of vagus nerves in experimental asthma in allergic dogs, *J. Appl. Physiol.* **33**: 719, 1972.
- 11 Austen, K. F., and Humphrey, J. H.: In vitro studies of the mechanism of anaphylaxis, *Adv. Immunol.* **3**: 1, 1963.
- 12 Nösal, R., Slorach, S. A., and Uvnäs, B.: Quantitative correlation between degranulation and histamine release following exposure of rat mast cells to compound 48/80 in vitro, *Acta Physiol. Scand.* **80**: 215, 1970.
- 13 Timmerman, H., and Scheffer, N. G.: A new tracheal strip preparation for the evaluation of β -adrenergic activity, *J. Pharm. Pharmacol.* **20**: 78, 1968.
- 14 Van Rossum, J. M.: Cumulative dose-response curves. II. Technique for the making of dose-response curves in isolated organs and the evaluation of drug parameters, *Arch. Int. Pharmacodyn. Ther.* **143**: 299, 1963.
- 15 Erspamer, V.: Pharmacology of indolealkylamines, *Pharmacol. Rev.* **6**: 425, 1954.
- 16 Doepfner, W., and Cerletti, A.: Comparison of lysergic acid derivatives and antihistamines as inhibitors of the edema provoked in the rat's paw by serotonin, *Int. Arch. Allergy Appl. Immunol.* **12**: 89, 1958.
- 17 Gaddum, J. H., Hammed, K. A., Hathaway, D. E., and Stephens, F. F.: Quantitative studies of antagonists for 5-hydroxytryptamine, *Q. J. Exp. Physiol.* **40**: 49, 1955.
- 18 Paton, W. D. M.: Compound 48/80: A potent histamine liberator, *Br. J. Pharmacol.* **6**: 499, 1951.
- 19 Rothschild, A. M.: Mechanisms of histamine release by compound 48/80, *Br. J. Pharmacol.* **38**: 253, 1970.
- 20 Dale, M. M., and Okpako, D. T.: Recovery of anaphylactic sensitivity in the guinea-pig ileum after desensitization, *Immunology* **17**: 653, 1969.
- 21 Cîrstea, M., and Suhaciu, G.: Role of histamine, substance P and slow-reacting substance of anaphylaxis (SRS-A) in the Schultz-Dale reaction of the guinea-pig ileum, *Arch. Int. Physiol. Biochim.* **76**: 344, 1968.
- 22 Kurihara, N., and Shibata, K.: Mediators in anaphylaxis of guinea-pig small intestine, *Jap. J. Pharmacol.* **22**: 581, 1972.
- 23 Brocklehurst, W. E., and Lahiri, S. C.: The production of bradykinin in anaphylaxis, *J. Physiol. (Lond.)* **160**: 15P, 1962.
- 24 Piper, P., and Vane, J.: The release of prostaglandins from lung and other tissues, in Ramwell, P. W., and Shaw, J. E., editors: Prostaglandins, *Ann. N. Y. Acad. Sci.* **180**: 363, 1971.
- 25 Orange, R. P., and Clusten, K. F.: Slow reacting substance of anaphylaxis, *Adv. Immunol.* **10**: 105, 1969.
- 26 Berry, P. A., Collier, H. O. J., and Holgate, J. A.: Bronchoconstrictor action in vivo of slow-reacting substance in anaphylaxis (SRS-A) and its antagonism, *J. Physiol. (Lond.)* **165**: 41P, 1963.
- 27 Bergström, S., Carlson, L. A., and Weeks, J. R.: The prostaglandins: a family of biologically active lipids, *Pharmacol. Rev.* **20**: 1, 1968.
- 28 Dale, M. M., and Zilletti, L.: The anaphylactic reaction in the longitudinal muscle strip of guinea pig ileum, *Br. J. Pharmacol.* **37**: 518P, 1969.
- 29 Aviado, D. M.: Krantz and Carr's Pharmacologic principles of medical practice, ed. 8, Baltimore, The Williams & Wilkins Company, chap. 51, 1972.