The Inhibitory Action of Lidocaine in Anaphylaxis[1-3]

EARLE B. WEISS, WALTER A. HARGRAVES, and SRI GUHA VISWANATH

SUMMARY

The action of lidocaine, a local anesthetic, was investigated during anaphylaxis in guinea pigs after passive sensitization *in vitro* of lung tissue and trachealis muscle. Pretreatment of the trachealis muscle with 8.54 m*M* lidocaine resulted in the total inhibition of anaphylactic isometric tension. Full reversal of anaphylactic-induced contractures was rapidly achieved with concentrations of 4.27 m*M* lidocaine. Release of histamine from both lung tissue and trachealis muscle was inhibited by 73 to 82 per cent, respectively, over concentration ranges of 2.13 to 8.54 m*M* lidocaine. A bimodal effect on sensitized tissues was noted, with lidocaine causing a slight release of histamine in the trachealis muscle of 1.6 per cent at a concentration of 8.54 m*M*. Lidocaine did not impair the initial passive sensitization process, nor did it appear to elute antibody once it was cell bound. The dual inhibitory effect on mast-cell release of mediators and on muscle contraction by lidocaine may be related in part to common processes involving the binding or flux of calcium.

Introduction

The myostabilizing action of local anesthetics on agonist-induced contractures in striated and smooth muscles has been well described, including the airways of the guinea pig (1–3). Partial explanation for the mechanism of such action is the ability of these compounds to influence the flux or binding of calcium ions, which results in muscle relaxation (2, 4–6). Because the exocytic secretion of histamine and other mediators from the mast cell after interaction of antigen and antibody is similarly dependent on the influx of calcium after a change in the permeability of the mast-cell membrane, we evaluated the effect of lidocaine on the anaphylactic reaction in the guinea pig trachealis muscle and lung (7–9). This paper reports both the inhibition of smooth muscle contraction and the inhibition of mediator release from mast cells by lidocaine during the Schultz-Dale reaction after passive sensitization *in vitro*.

Materials and Methods

Adult male Hartley guinea pigs (Elm Hill Breeding Laboratory, Chelmsford, Mass.) weighing 450 to 600 g were killed by stunning and exsanguination, and the lungs were perfused at 37° C in Krebs-Henseleit solution until they were free of blood. The trachea was removed, was transferred to a Krebs-Henseleit solution at 37° C, was gassed with 95 per cent O_2 and 5 per cent CO_2, was dissected free of extraneous tissue, and was cut into rings. Two to 4 preparations were obtained from each animal. Passive, *in vitro* sensitization was accomplished by immersing the muscle at zero force into a 1:10 normal saline dilution of reconstituted rabbit antichicken egg-albumin antiserum (ICN Pharmaceuticals, Irvine, Ca.) in Krebs-Henseleit solution for 180 min at room temperature. For studies of histamine release, lung tissues were diced to approximately 3 mm by 3 mm and then were passively sensitized as described previously.

(Received in original form May 27, 1977 and in revised form January 9, 1978)

1 From the Division of Respiratory Diseases, St. Vincent Hospital, and the Department of Medicine, University of Massachusetts Medical School, Worcester, Mass. 01604.

2 Supported in part by a grant from the Foundation for Research in Bronchial Asthma and Related Diseases and the St. Vincent Hospital Research Foundation.

3 Requests for reprints should be addressed to Earle B. Weiss, M.D., Division of Respiratory Diseases, St. Vincent Hospital, 25 Winthrop St., Worcester, Mass. 01604.

Challenge with specific antigen to either trachea or lung was performed with 5 × recrystallized egg albumin at a final bath concentrations of 100 μg per ml (ICN Pharmaceutical, Irvine, Ca.), at periods specified subsequently. Control studies were conducted with Krebs-Henseleit solution alone. Before all studies, the sensitized trachealis muscle or lung tissue was washed free of excess antiserum (3 washes of 5.0 ml each, 2 min per wash) with Krebs-Henseleit solution. Immunologic specificity was shown as the failure of sensitized muscles to react to normal saline solution or ragweed pollen antigen; neither antigen nor antibody caused the development of tension in nonsensitized muscles.

Isometric tension. For measurements of trachealis tension, the cartilage was cut, and one end was fastened with No. 50 cotton thread to a fixed clamp; the other end was fastened with a thin piece of platinum wire to a Grass FT03C force displacement transducer amplified by a Hewlett-Packard 8805B amplifier to record isometric tension changes, in milligrams on a precalibrated Hewlett-Packard 7754A thermal tip polygraph; full scale was 5 g. The muscle was oriented parallel to the direction of the force displacement. The strips were suspended at 37° C under 2 g of initial tension in a 20-ml muscle chamber (Harvard) containing Krebs-Henseleit solution continuously aerated with 95 per cent O_2 and 5 per cent CO_2. Optimal length-tension relationships for this experiment were determined by exposing the muscle to increasing tensions of 0.25, 0.5, 1.0, 2.0, and 3.0 g against a standard histamine dose of 1.0 μg per ml, and verification of the selected 2-g maximums was conducted periodically. All tracheas were analyzed for force development, and those exhibiting baseline instability (±10 per cent), inconsistent rates, or erratic responses were discarded. All tracheal preparations were initially equilibrated for 60 to 90 min with the bath fluid changed 3 times. Before any new experiment, the bathing medium was flushed 3 times, and the muscles were re-equilibrated for at least 10 min. O_2 and CO_2 tensions and the pH of the bathing fluid were monitored for each experiment by assay in a 313 blood gas analyzer (Instrumentation Laboratories, Inc.). Partial pressure for CO_2 and O_2 ranged from 35 to 44 mm Hg and from 350 to 550 mm Hg, respectively, with an average pH of 7.42 ± 0.02, unless otherwise cited.

After equilibration, the tension responses to challenge with 100 μg of egg albumin per ml were recorded to the period of maximal change and to a constant plateau. For protection studies, sensitized muscles were incubated with and without lidocaine, in final concentrations of 500 (2.13 m*M*), 1,000 (4.27 m*M*), and 2,000 (8.54 m*M*) μg per ml. The drug was placed in the bath until baseline equilibration occurred (20 to 60 min) and before challenge with antigen. For reversal experiments, similar concentration ranges were used after the maximal anaphylactic plateau tension had been reached. At the termination of all studies, the length of the muscle at the 2.0-g tension was measured with a micrometer accurate to ±0.1 mm. Then the muscles were removed, were dissected free of cartilage, and were dried at 50° C to constant weight on a 5-place Mettler balance. Force development was expressed either in milligrams or in absolute units of tension (kg per cm^2) (10).

Histamine assay. The excised fragments (3 mm by 3 mm) of guinea pig lungs were equilibrated in Krebs-Henseleit solution aerated with 95 per cent O_2 and 5 per cent CO_2 at 37° C for 45 min. They were then weighed into 0.4 ± 0.001-g aliquots; 6 to 8 aliquots were obtained from each lung. This was followed by sensitization with rabbit antichicken egg albumin antiserum for 180 min at room temperature, as described previously. The tissues were then removed, were washed in Krebs-Henseleit solution at 37° C and were diced into 1-mm by 1-mm slices. These slices were incubated for 20 min in 10 ml of reaction mixtures, as follows: (*1*) control, sensitized tissue in Krebs-Henseleit solution alone, (*2*) anaphylactic challenge, exposed to egg albumin, (*3*) lidocaine only, or (*4*) exposure to lidocaine for 20 min followed by anaphylactic challenge. Bath samples (volume, 2.0 ml) for analysis of histamine release into the bath buffer were removed 5 min after anaphylactic challenge for all of the above experiments, the point of maximal tension observed in the anaphylactic trachea. This sample was added to 2.0 ml of 0.8 N $HClO_4$ yielding a final concentration of 0.4 N $HClO_4$. At the same time, the lung fragments were removed for tissue assay and were immersed in 2 ml of 0.4 N $HClO_4$, were homogenized in a ground glass homogenizer, were sonicated at 150 watts for 30 sec (Braunsonic 1510), and were centrifuged (3,000 rpm for 15 min at room temperature). A 1.5-ml aliquot of this supernatant was transferred to a 50-ml volumetric flask, and dilutions were made with 0.4 N $HClO_4$ and were assayed by the procedure of May and coworkers (11). Fluorescence at 450 mμ was measured on an Aminco-Bowman dual monochromatic spectrophotofluorometer from activation at a wavelength of 360 mμ and was normalized to a quinine sulfate standard. The concentration of histamine was determined from a standard histamine curve. Lidocaine, at the concentrations used in this study, 2.13 to 8.54 m*M* (0.5 to 2.0 mg per ml), had no significant effect on fluorescence or the histamine assay for the wavelengths cited.

The trachealis muscle was also assayed for histamine. The trachealis was cut into rings 2 mm in width, and 4 such rings were pooled for each experiment. The rings were sensitized as described previously in a 1.0-ml bath volume. After 3 rinses in Krebs-Henseleit solution, the muscles were segregated as follows: (*1*) control, Krebs-Henseleit solution alone, (*2*) anaphylactic challenge with egg albumin, (*3*) incubation with lidocaine for 20 min at concentrations of 2.13 m*M*, 3.2 m*M*, 4.27 m*M*, and 8.54 m*M*,

and (4) incubation with lidocaine for 20 min at these same concentrations before challenge with egg albumin. Effluents (1.0 ml) were removed after 5 min for histamine analysis. The tracheal tissues were also removed, the muscles were excised, their wet weights were recorded, they were homogenized in 2.0 ml of 0.4 N $HClO_4$, and were centrifuged as described for lung tissue. Thereafter, the assay was identical to that for lung tissue except that the trachealis homogenate was not diluted before assay. Paired assays of lung and trachea from the same animal were not necessarily conducted. Per cent of histamine release into the supernatant was calculated as follows: per cent release = [total amount of histamine in supernatant (μg) − histamine leak (lidocaine alone)]/[total amount of histamine in tissue (μg) + histamine leak (lidocaine alone) + total amount of histamine in supernatant] × 100. Per cent of histamine release for octylamine experiments from the tissue was calculated as follows: (initial control tissue histamine concentration − histamine tissue concentration after release)/initial histamine tissue concentration × 100; these concentrations were expressed as μg of histamine per g of wet tissue.

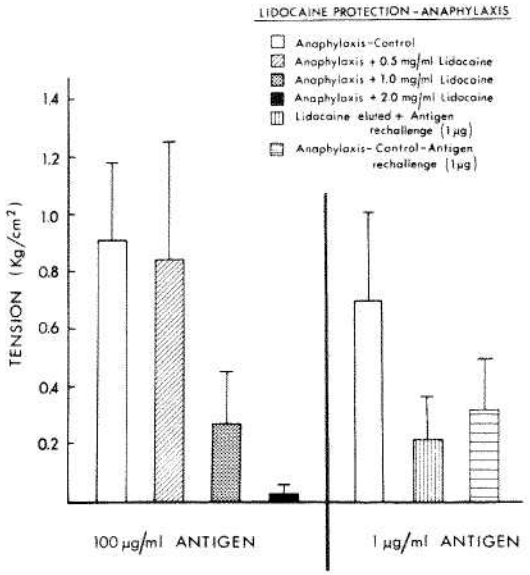

Fig. 1. Effect of pretreatment with lidocaine on anaphylactic tension. Lidocaine was administered to sensitized muscles for 20 to 60 min followed by challenge with 100 μg of antigenic egg albumin per ml. Values are expressed as mean tension ± SD. Complete suppression occurred with a final bath concentration of 8.54 m*M* lidocaine (2.0 mg per ml). In a separate experiment, removal of lidocaine by washing 4 times and rechallenge with 1.0 μg of antigen per ml produced tensions equal to those in the control tissue challenged with antigen alone (1.0 μg per ml) (n = 10) (columns 5 and 6) ($P < 0.3$). All values presented were calculated from baseline changes induced by lidocaine alone; these baseline decreases were 0.3 kg per cm^2 for 4.27 m*M* lidocaine (1.0 mg per ml), and 0.6 kg per cm^2 for 8.54 m*M* lidocaine (2.0 mg per ml).

The Krebs-Henseleit buffer was prepared as follows: NaCl, 118.1 m*M*; KCl, 4.7 m*M*; $NaHCO_3$, 24.8 m*M*, $CaCl_2$, 2.52 m*M*; $MgSO_4 \cdot 7H_2O$, 2.4 m*M*; KH_2PO_4, 1.10 m*M*; glucose, 10 m*M*, in distilled, deionized water. Lidocaine hydrochloric monohydrate (Astra Pharmaceutical Products, Inc., Framingham, Mass.) was prepared daily in distilled, deionized water in concentrations appropriate to produce the desired final bath concentrations, expressed as μg per ml as the free base, or m*M*; volume additions were 0.25 ml or less. Octylamine hydrochloride (Eastman Kodak, Rochester, N.Y.) was prepared in distilled, deionized water.

Statistical analysis. Tension responses were expressed in kg per cm^2, and force was expressed in mg. Differences between paired observations within a group or between the means of different groups were analyzed by "Student's" t test. All data were expressed as mean ± SD, unless otherwise specified.

Results

Immunogenic specificity and effect of lidocaine on anaphylactic tension. Nonsensitized lung tissue challenged with 100 μg of egg albumin per ml and sensitized lung tissue with 500 μg of ragweed pollen antigen per ml released zero μg of histamine per ml into the organ bath (n = 6, for each). Neither egg albumin antigen nor antibody alone caused tension development in nonsensitized muscles. The dose-dependent suppression of tension developed during anaphylaxis by the pretreatment with lidocaine is depicted in figure 1 (columns 1 through 4). Maximal protection (98 to 100 per cent) occurred with 8.54 m*M* lidocaine (2.0 mg per ml) present in the incubation medium before and during challenge with egg albumin (100 μg per ml).

When sensitized muscles were exposed to 1.0 μg of egg albumin per ml, control tensions of 0.7 ± 0.3 kg per cm^2 were observed; those muscles pretreated with 8.54 m*M* lidocaine yielded tensions of 0.03 kg per cm^2. Then, the bath solution from the control muscles and those pretreated with 8.54 m*M* lidocaine was removed, and the muscles were washed 4 times for 30 min with 20-ml Krebs-Henseleit buffer, eluting both antigen and lidocaine. Re-exposure of each pair of muscles to 1.0 μg of egg albumin per ml (n = 10) yielded subsequent equivalent mean anaphylactic tensions (figure 1, columns 5 and 6) ($P < 0.3$).

Application of lidocaine at the point of initial

maximal anaphylactic tension, followed by cumulative administration of doses at concentrations specified in figure 2, produced relaxation at each concentration for 15 to 20 min; complete relaxation occurred with 1.0 mg of lidocaine per ml. This was similar to the action the drug exhibited against agonist-induced contractions in nonsensitized trachea (1). With 8.54 m*M* lidocaine (2.0 mg per ml), tension decreased (within 5 to 10 min) to less than the initial resting baseline. Control muscles (those not exposed to lidocaine) did not begin to relax for at least 30 to 45 min after the maximal anaphylactic peak was reached, and even then did not return to baseline unless the antigen was washed free from the preparation. Pretreatment of the trachealis muscle with 2.0 μg per ml of diphenhydramine blunted the initial rapid tension component at 1 min attributable to histamine ($P < 0.01$), yet the more prolonged contracture, presumably due to slow-reacting substance of anaphylaxis (SRS-A), persisted (figure 3). Lidocaine was still effective in reversing tension under these circumstances. Therefore, 4.27 m*M* lidocaine can completely lyse anaphylactic tension due not only to histamine but probably also to SRS-A or any other released chemical mediators.

Histamine release. To evaluate the role of lidocaine in mediator release, histamine activity was measured in the muscle or lung bath effluent as well as in the respective tissues. As shown in figure 4 and table 1, during anaphylaxis, guinea pig lung liberated approximately 9.7 ± 1.1 per cent (mean ± SE, n = 22) of the total tissue histamine into the supernatant at 37° C after a 5-min exposure to 100 μg of egg albumin per ml. Lidocaine (8.54 m*M*) inhibited this release by approximately 82 per cent. Over the range of 2.13 to 8.54 m*M* lidocaine, some release of histamine by the drug itself was observed from control (sensitized) lung tissue, thereby decreasing the total net amount released. Sensitized trachealis muscle, in the absence of lidocaine, released approximately 13.1 ± 2.2 per cent (mean ± SE, n = 16) of the total tissue histamine into the supernatant after challenge with 100 μg of egg albumin per ml. Then, the effect of lidocaine on histamine release by trachealis muscle followed a pattern qualitatively similar to that in lung tissue with approximately 73 per cent maximal inhibition of histamine activity with 8.54 m*M* lidocaine (2.0 mg per ml). We found a similarly small amount of histamine liberation by sensitized trachealis muscle into the bath effluent after

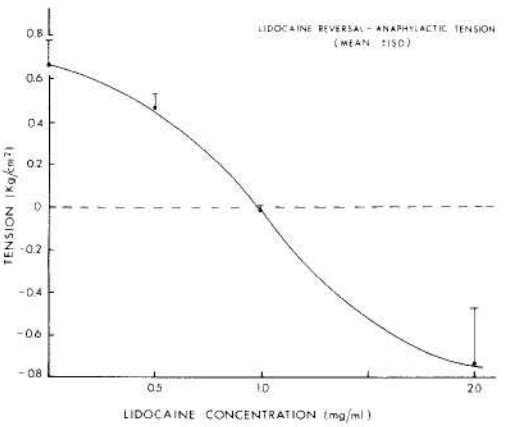

Fig. 2. Effect of lidocaine on reversal of anaphylactic tension. Lidocaine was added at the concentrations cited first at the point of maximal anaphylactic tension and then cumulatively at each concentration until a plateau for that dose was reached. Complete inhibition occurred with 4.27 m*M* lidocaine (1.0 mg per ml), within 15 min, with tension decreasing below baseline at 8.54 m*M* lidocaine (2.0 mg per ml). Mean ± SD.

exposure to lidocaine over the concentration range of 3.2 m*M* to 8.54 m*M*, as occurred with lung tissue; the maximal release was 1.6 ± 0.7 per cent (mean ± SE, n = 4) with 8.54 m*M* lidocaine (2.0 mg per ml). At 22° C, a substantial decrease in histamine activity was noted during anaphylaxis, with only 1.0 ± 0.7 per cent (mean ± SE, n = 6) of histamine recovered in the supernatant from the trachealis tissue. Similarly, the release of histamine by lidocaine in sensitized trachealis muscle was influenced by temperature, decreasing from 1.6 ± 0.7 per cent at 37° C to 0.59 ± 0.1 per cent at 22° C at the lidocaine concentration of 8.54 m*M* (2.0 mg per ml) (mean ± SE, n = 4).

Lung and trachealis tissues were also assayed for residual histamine content (table 1). The histamine content of sensitized control lung tissue was 25.6 ± 2.3 μg of histamine per g of wet weight. After challenge with egg albumin antigen this decreased to 21.3 ± 2.0 μg of histamine per g of wet weight, significantly less than that of the control tissue ($P < 0.02$, group and paired "Student's" t test). Exposure of the sensitized lung to lidocaine alone significantly increased its histamine content to 48.3 ± 2.5 μg per g of wet weight, as compared to that of sensitized lung alone or to that after anaphylaxis in the absence of lidocaine ($P < 0.001$); this response was found to be equivalent for all concentrations of lidocaine in the range of 3.2 m*M* to 8.54 m*M*. Pre-

treatment of sensitized lung fragments with lidocaine followed by exposure to antigen led to an equivalent value for the histamine content of lung tissue, 44.7 ± 1.7 μg per g of wet weight ($P < 0.8$), as compared to that of sensitized tissue pretreated with lidocaine and not receiving antigen, and significantly different from that of sensitized lung tissue alone ($P < 0.01$) (table 1). However, trachealis tissue showed no significant decrease in histamine content after anaphylaxis, with or without 8.54 m*M* lidocaine. Tracheal histamine contents in μg per g of wet weight (mean ± SE) were: (*1*) sensitized, 37.8 ± 6.7, n = 10; (*2*) after anaphylaxis, 26.5 ± 3.7, n =16; $0.1 > P > 0.05$ by group t test; (*3*) lidocaine followed by anaphylaxis, 34.0 ± 4.2, n = 13 (not significant as compared to sensitized trachealis).

In addition, lidocaine increased the histamine content of normal, nonsensitized lung tissue; histamine contents were 13.1 ± 1.2 μg per g of wet weight in control tissues, compared to 22.0 ± 2.6 μg per g of wet weight with 3.2 m*M* lidocaine present ($P > 0.01$) (table 1).

Passive sensitization. Incubation of lidocaine (at concentrations of 2.13 m*M*, 8.54 m*M*, and 17.1 m*M*) with rabbit antichicken egg albumin antiserum (1:10 dilution) for 3 hours did not prevent sensitization of the trachealis muscle *in vitro* at room temperature. After 4 washings in Krebs-Henseleit solution at 37° C, under isometric conditions, application of egg albumin produced an anaphylactic reaction equivalent in tension to that of control tissue in all instances. Two animals were studied for each dose of lidocaine (table 2A).

Elution studies. The affinity of the antiserum for the tissue preparation under the influence of lidocaine was studied by repeated washing. A previously sensitized muscle incubated with 8.54 m*M* lidocaine for 20 min and then washed 4 times with Krebs-Henseleit solution responded to albumin challenge with isometric tension equal to that of control tissue ($P < 0.9$). Therefore, lidocaine can be added and eluted without affecting the sensitized state (table 2B).

Octylamine. To compare the effect of a known organic mast cell releaser of histamine, the lung tissues were exposed to octylamine (0.1 to 1.0 mg per ml) and assayed as described for release of antigen. The maximal liberation of histamine

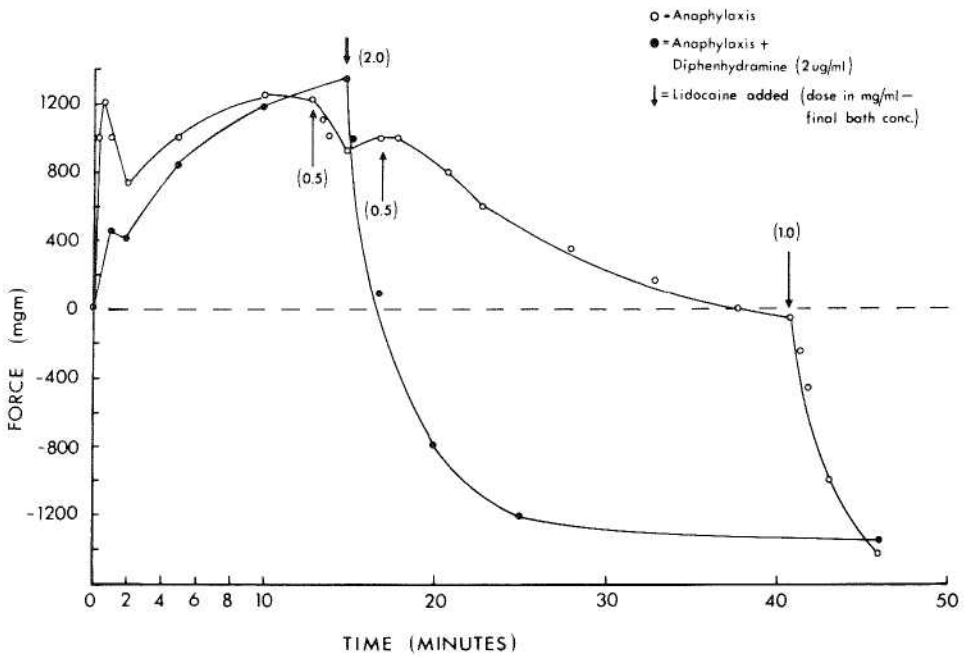

Fig. 3. Typical lidocaine reversal with and without 2.0 μg of diphenhydramine per ml present (redrawn from original recordings). With 4.27 m*M* lidocaine (1.0 mg per ml) anaphylactic force (in mg) reached baseline and decreased to below baseline at 8.54 m*M* lidocaine (2.0 mg per ml). Pretreatment with diphenhydramine blunted the initial tension at 1.0 min ($P < 0.01$). The more persistent maximal tension, which was not completely due to histamine, was reversed rapidly once 8.54 m*M* lidocaine (2.0 mg per ml final bath concentration) was added.

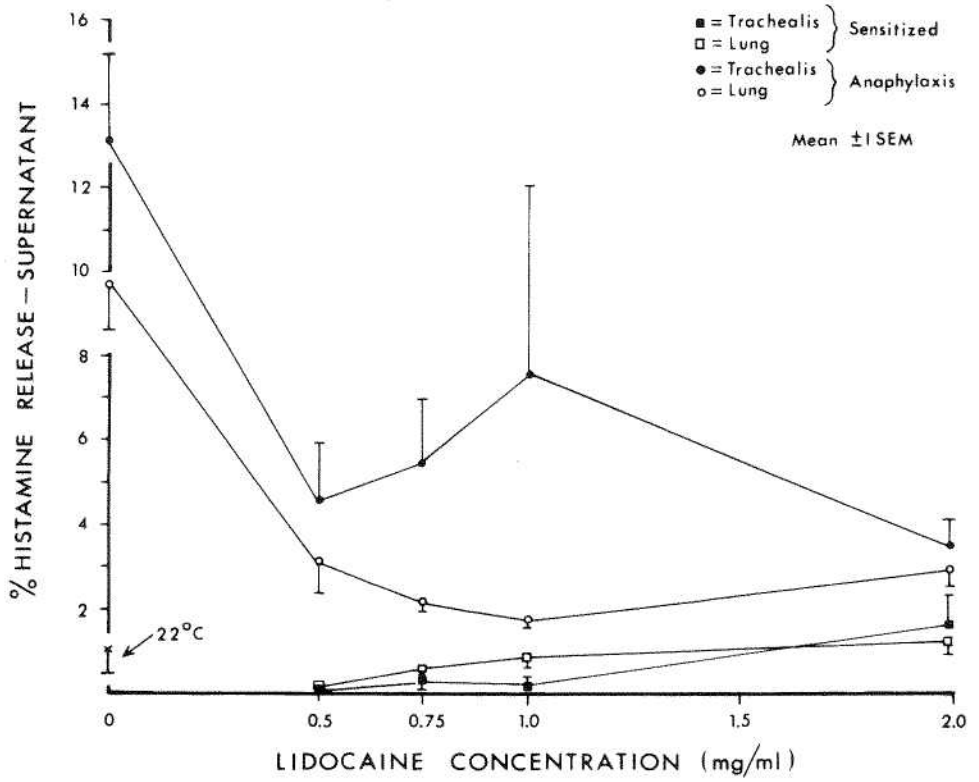

Fig. 4. Histamine released into the supernatant for sensitized and anaphylactic lung and trachealis tissues at various concentrations of lidocaine. Samples were analyzed after 5.0 min of anaphylaxis. Control lung tissue released 9.7 ± 1.1 per cent (mean ± SE, n = 22) and control trachealis muscle 13.1 ± 2.2 per cent (n = 16). Significant inhibition was observed to be maximal with 8.54 m*M* lidocaine (2.0 mg per ml). Note that lidocaine alone released histamine from sensitized tissues, approximately 1.6 per cent from trachealis tissue and 1.2 per cent from lung tissue at 8.54 m*M* lidocaine (2.0 mg per ml). At 22° C, only 1.0 per cent of the histamine was released from the control trachealis muscle. The number of assays for each data point for both anaphylactic and sensitized trachealis was 3, 3, 3, and 4 at 0.5 mg per ml, 0.75 mg per ml, 1.0 mg per ml, and 2.0 mg per ml of lidocaine, respectively; the number of assays was 8, 8, 4, and 9 for the same doses in both sensitized and anaphylactic lung tissue.

was found to be 77 per cent, based on assay of the total histamine content of tissue (table 3). Lidocaine at a concentration of 2.13 m*M* blunted this effect and caused a maximal release of 47.7 ± 3.6 per cent ($P < 0.01$) at the octylamine concentration of 1.0 mg per ml.

Discussion

The ability of local anesthetics to stabilize the smooth muscle of the airways to a wide variety of agonistic stimuli was extended in this present study to their action during anaphylaxis in guinea pigs (1–3). The initial observation was that of a protective, dose-dependent suppression of anaphylactic isometric tension by lidocaine with essentially complete inhibition at concentrations of 8.54 m*M* (2.0 mg per ml) *in vitro*. The subsequent experiments were designed to clarify this mechanism for both smooth muscle and mast cell components of the trachealis tissue.

The rapid reversal of anaphylactic tracheal contraction by the addition of lidocaine at the point of maximal tension was consistent with a myorelaxant effect directly upon the smooth muscle cell. The final effect on tension was dose-dependent, with complete inhibition at 4.27 m*M* lidocaine (1.0 mg per ml), and then relaxation at less than baseline with 8.54 m*M* lidocaine (2.0 mg per ml). This was maximal within 15 to 20 min of drug application. The ability of lidocaine to prevent and/or reverse development of tension during experimental anaphylaxis in guinea pigs is similar to its action in nonsensitized guinea pig trachealis muscle against a wide vari-

TABLE 1

EFFECT OF LIDOCAINE ON LUNG HISTAMINE CONTENT*

Treatment of Lung Tissue†	Histamine Content			Net Effluent Histamine Concentration		
	(no. samples)	(μg/g wet weight)	P**	(no. samples)	(net total μg/g wet weight)	P**
(A) Sensitized	19	25.6 ± 2.3	—	19	0.11 ± 0.01	—
(B) Sensitized + antigen	22	21.3 ± 2.0	S ($P < 0.02$)†† compared to (A)	22	2.00 ± 0.12	S ($P < 0.001$) compared to (A)
(C) Sensitized + lidocaine, 3.2 to 8.54 m*M*	8	48.3 ± 2.5	S ($P < 0.001$) compared to (A) NS ($P < 0.8$)†† compared to (D)	35	0.25 ± 0.04	S ($P < 0.001$) compared to (B) + (D)
(D) Sensitized + lidocaine, 3.2 to 8.54 m*M*, + antigen***	34	44.7 ± 1.7	S ($P < 0.01$) compared to (A) + (B)	34	0.31 ± 0.04	S ($P < 0.001$) compared to (B)
(E) Nonsensitized control	18	13.1 ± 1.2	—			
(F) Nonsensitized control + lidocaine, 3.2 m*M*	18	22.0 ± 2.6	S ($P < 0.01$) compared to (E)			

Definition of abbreviations: S = significant; NS = not significant.

* Results given as mean values ± SE.

† Experiments A–D were conducted on a single set of tissues, and E + F on a separate set; data for these 2 groups are not directly comparable.

** Group t test.

†† Paired t test.

*** In the sequence cited.

ety of stimuli including histamine, acetylcholine, depolarizing potassium, and direct-current electrical stimulation (1). However, the concentration of lidocaine required in anaphylaxis was much greater, perhaps because of the potency or cumulative action of released mediators. This may have also been reflected in our observation that after pretreatment with 2.13 m*M* lidocaine there was a minimal inhibition of anaphylactic tension (figure 1), yet histamine release was significantly inhibited (figure 4) at this concentration. Because lidocaine was capable of fully reversing anaphylactic tension in muscles pretreated with diphenhydramine, the stabilization of the trachealis muscle against other chemical mediators of anaphylaxis besides histamine (viz., SRS-A, kinins, etc.) could be inferred. Although unresolved at present, this inhibitory mechanism may occur by an action on membrane calcium flux or intracellular calcium binding, effective by preexposure to lidocaine or after anaphylactic tension has developed (2, 5, 12, 13).

The other major site of action for lidocaine in guinea pig anaphylaxis is the mast cell, because inhibition of mediator release could also account for some of our observations. This appeared important because histamine release after interaction of antigen and antibody is calcium-dependent, and local anesthetics are believed to act in part by inhibition of transmembrane flux or translocation of Ca^{++} by a binding effect at cell membrane surfaces (5, 14, 15). We observed a concentration-related inhibition of histamine release into the bath effluent, maximal at a lidocaine concentration of approximately 8.54 m*M* (2.0 mg per ml) (figure 4). Here, approximately 73 per cent and 82 per cent net inhibition of histamine release was found for trachealis and lung tissue, respectively. This degree of inhibition included the small amount of histamine released by lidocaine in both sensitized lung and sensitized trachealis tissue. Our studies did not clarify whether the latter process was secretory or cytotoxic. However, it decreased somewhat the complete inhibition by lidocaine of histamine release during anaphylaxis. The small quantity of histamine liberated could also cause some development of tension. However, this effect was eliminated by the concurrent, direct, smooth-muscle relaxant properties of lidocaine (1). The anaphylactic release of histamine was temperature-dependent, with a significant decrease at 22° C (versus 37° C). This also occurred with lidocaine for sensitized tissue alone. The temperature effect was consistent with previous reports (16).

The histamine content of tissue on the basis of wet weight was also analyzed to corroborate observations on effluent release (table 1). The histamine content that we observed in control lung tissue was similar to that reported elsewhere (17). In lung tissue, there is significant variability in the amount of releasable histamine among strains of guinea pigs (17), and histamine content may vary with animal age,

TABLE 2A

EFFECT OF LIDOCAINE ON PASSIVE SENSITIZATION

	Mean Tension (± SD) (*kg per cm²*)	P
Control anaphylaxis plus	0.914 ± 0.267	NS*
lidocaine, 2.13 m*M*	0.877 ± 0.231	NS
lidocaine, 8.54 m*M*	0.731 ± 0.100	NS
lidocaine, 17.1 m*M*	0.843 ± 0.168	NS

* Not significant.

as has been shown in the rat (18). We attempted to minimize this variation by using animals of the same strain and comparable age. To decrease individual animal variability further in the random-bred Hartley strain, aliquots of lung tissue from several animals were pooled, and paired experiments were conducted in all instances, with and without lidocaine. Essentially, after anaphylaxis in lung tissue, a decrease in histamine content of tissue occurred that was significantly inhibited by lidocaine. Because of experimental design, this was not an artifact due to inflammatory edema (18). Effluent histamine concentrations (table 1, figure 4) measured separately, but from the same lung assayed for histamine content of tissue corroborated the inhibition by lidocaine on anaphylactic histamine release of approximately 80 per cent. Lidocaine itself also increased the histamine content of the sensitized lung. The mechanism for this latter finding in sensitized tissue is not clear from our data, but it could contribute in part to the small quantity of histamine released by lidocaine itself in the effluent studies. The data for non-sensitized control lung tissue are not directly comparable to those from studies in sensitized animals because of experimental design. Yet a similar increase in lung histamine followed exposure to lidocaine. In this regard, increases in tissue histamine content have been reported after specific antigen exposure to sensitized leukocytes *in vitro* and *in vivo* (19). Assem and co-workers demonstrated that aside from immunogenic histamine release by antigen, there was a concurrent significant increase in total histamine and histamine-forming capacity (19). Other literature describes this effect after anaphylaxis, compound 48/80, and inflammatory stimuli (20–22). This stimulation of histamine-forming capacity can occur within 30 min *in vitro* (19). Although tissue histamine content after anaphylaxis was decreased, as compared to that of sensitized lung tissue, our observation of a stimulating effect by lidocaine appears consistent with the ability of guinea pig lung to increase histamine content within the time period of our experiment. Whether this is related to activation by lidocaine of histidine decarboxylase, as suggested by Assem and associates (19), Kahlson and co-workers (20) and Slorach and Uvnäs (21), or is due to other mechanisms is unclear at present. We can offer no explanation for the limited changes in histamine content of trachealis tissue during anaphylaxis or with lidocaine. Maximal experimental error of tissue weight and histamine assay is about 8 per cent. A deviation of this magnitude from observed experimental values could be one explanation for the non-significant differences in tracheal histamine content that we observed. However, small changes in histamine concentration of tracheal tissue would be sufficient to induce the observed increases in tension.

Besides the inhibition of histamine release from mast cells, a similar action was observed toward the potent organic releaser, octylamine.

TABLE 2B

EFFECT OF LIDOCAINE ELUTION ON ANAPHYLAXIS

	Mean Tension (± SD) (*kg per cm²*)	P
Control anaphylaxis	0.873 ± 0.263	–
Elution after exposure to 8.54 m*M* + octylamine	0.897 ± 0.332	NS* < 0.9

* Not significant.

TABLE 3

EFFECT OF OCTYLAMINE AND LIDOCAINE ON HISTAMINE RELEASE (AT 37° C)

	Released Histamine from Tissue* (% *mean* ± *SE*)	No.	"P"
Octylamine			
0.1 mg/ml	−1.5 ± 22.0	6	–
0.25 mg/ml	35.7 ± 7.5	14	–
0.5 mg/ml	59.9 ± 2.2	20	–
1.0 mg/ml	77.2 ± 1.7	12	–
Lidocaine, 2.13 m*M* + octylamine			
0.1 mg/ml	–	–	–
0.25 mg/ml	16.6 ± 6.3	6	< 0.01
0.5 mg/ml	31.2 ± 7.5	8	< 0.01
1.0 mg/ml	47.7 ± 3.6	4	< 0.01

*(Initial control-tissue histamine concentration − histamine tissue concentration after release)/(initial histamine tissue concentration) × 100.

Octylamine, which may be more active *in vitro* than compound 48/80, released histamine in concentration ranges cited for guinea pig lung, 0.1 to 1.0 mg per ml (16). The mechanism and extent of histamine liberation from mast cells may vary with the tissue, animal species, and releasing agent, although morphologic changes in mast cells of several animal sources have features in common during release of histamine. In contrast to antigen-antibody induced extrusion of mast cell granules, Mota (23) has shown that histamine release by octylamine in guinea pig mesentery is associated with a loss of the definition of the intracellular metachromatic granules, which is perhaps indicative of osmotic swelling of these granules. However, the precise step(s) in anaphylaxis in guinea pig lung compared to octylamine release of histamine activity influenced by lidocaine were not clarified by the present studies.

Austen and Lichtenstein (9) have reviewed the factors that control histamine release from sensitized lung fragments and sensitized human leukocytes. This process first involves an immunologic activation stage requiring antigen, but not calcium. Then a secretory stage of several complex biochemical steps follows, leading to mediator release, which is dependent on both influx of extracellular calcium and intracellular calcium pools (24). Ionophorous antimicrobial drugs, presumed to increase selectively cell-membrane permeability to calcium, have become useful in the study of histamine-release phenomena (25). Foreman and associates (25) demonstrated that ionophores X537A (Hoffman-LaRoche) and particularly A23187 (Eli Lilly) caused a calcium-dependent histamine release from mast cells and that antigen-antibody interaction led to flux of calcium-45 into the mast cells. The observation is important because colchicine, which inhibits normal microtubular function, or agents influencing cellular cyclic adenosine monophosphate do not affect mediator release induced by ionophores (26). For example, Foreman and Gomperts (27) reported that neither theophylline nor dibutyryl cyclic adenosine monophosphate could inhibit A23187 histamine release, whereas both were effective under antigen-induced conditions. In addition, mast cell secretory extrusion of granules after exposure to A23187 in the presence of calcium supports the concept of a calcium flux triggering histamine release by influencing exocytosis (28). Apparently, local anesthetics can inhibit this process. Johnson (29) described the protection of lidocaine against histamine release by ionophore A23187 and compound 48/80 and calcium-45 flux in rat mast cells; the maximal effect occurred in the concentration range of 15 to 150 μg per ml. At slightly greater concentrations of lidocaine, stimulation of calcium-45 flux and of histamine release was observed. Both observations bear similarity to our data for lidocaine in guinea pig lung and trachealis muscle; that is, a significant inhibition of histamine release during antigen-induced excitation and a small quantity of histamine released from sensitized tissues by the anesthetic itself. Further, the report by Kazimierczak and co-workers (30) indicates that tetracaine, lidocaine, procaine (in that order of potency) inhibited histamine release by compound 48/80 from isolated rat mast cells in a dose-dependent pattern. At a concentration of

20 m*M*, there was almost total inhibition of histamine release by lidocaine. More than 1.0 m*M* tetracaine also caused histamine release, as described previously for lidocaine. Calcium ions were shown to play a significant role in the 48/80 release phenomenon by exhibiting antagonism towards lidocaine; an increase in the concentration of calcium antagonized the inhibitory action of lidocaine on release of histamine (30).

Therefore, the diverse stimuli of octylamine, antigen-antibody interaction, compound 48/80, and calcium ionophores may have some common pathway, possibly calcium flux and mobilization, by which inhibition of local anesthetic is operative. This pathway appears distinct from the suppression of antigen-induced histamine release by sympathomimetic amines and methylxanthines by their stimulation of cyclic adenosine monophosphate (5, 31, 32). In summary, anaphylaxis in guinea pigs was significantly suppressed by lidocaine, by both inhibition of histamine release and concurrent pharmacologic antagonism of released mediators on the smooth muscle cells. Lidocaine did not appear to influence the initial sensitization process, nor did it cause elution of the antibody once it became cell bound. These observations in the guinea pig model may provide some explanations for the effects of lidocaine in certain patients with bronchial asthma (33).

References

1. Weiss, E. B., Anderson, W. H., and O'Brien, K. P.: The effect of local anesthetic, lidocaine, on guinea pig trachealis muscle *in vitro*, Am Rev Respir Dis, 1975, *112*, 393.
2. Feinstein, M. B., and Paimre, M.: Pharmacological action of local anesthetics on excitation-contraction coupling in striated and smooth muscle, Fed Proc, 1969, *28*, 1643.
3. Fleisch, J. H., and Titus, E.: Effect of local anesthetics on pharmacologic receptor systems of smooth muscle, J Pharmacol Exp Ther, 1973, *186*, 44.
4. Carvalho, A. P.: Calcium-binding properties of sarcoplasmic reticulum as influenced by ATP, caffeine, quinine, and local anesthetics, J Gen Physiol, 1968, *52*, 622.
5. Feinstein, M. B.: Inhibition of contraction and calcium exchangeability in rat uterus by local anesthetics, J Pharmacol Exp Ther, 1966, *152*, 516.
6. Abert, G., and Andersson, R.: Studies on mechanical actions of mepivacaine (carbocaine) and its optically active isomers on isolated smooth muscle: Role of Ca^{++} and cyclic AMP, Acta Pharmacol Toxicol (Kbh), 1972, *31*, 321.
7. Foreman, J. C., and Mongar, J. L.: The role of the alkaline earth ions in anaphylactic histamine secretion, J Physiol (Lond), 1972, *224*, 753.
8. Orr, T. S., Hall, D. E., and Allison, A. C.: Role of contractile microfilaments in the release of histamine from mast cells, Nature, 1972, *236*, 350.
9. Austen, K. F.: A review of immunological, biochemical and pharmacological factors in the release of chemical mediators from human lung, in *Asthma*, K. F. Austen and L. M. Lichtenstein, ed., Academic Press, New York, 1973, p. 111.
10. Hargraves, W. A., and Weiss, E. B.: The effect of histamine on the length-tension properties of guinea pig trachealis muscle, Respir Physiol, 1977, *29*, 211.
11. May, C. D., Lyman, M., Alberto, R., and Cheng, J.: Procedures for immunochemical study of histamine release from leukocytes with small volume of blood, J Allergy, 1970, *46*, 12.
12. Johnson, P. N., and Incsi, G.: The effect of methylxanthines and local anesthetics on fragmented sarcoplasmic reticulum, J Pharmacol Exp Ther, 1969, *169*, 308.
13. Bianchi, C. P., and Bolton, T. C.: Action of local anesthetics on coupling systems in muscle, J Pharmacol Exp Ther, 1967, *157*, 388.
14. Kaliner, M., and Austen, K. F.: Immunologic release of chemical mediators from human tissues, Ann Rev Pharmacol, 1975, *15*, 177.
15. Mongar, J. L., and Svec, P.: The effect of phospholipids on anaphylactic histamine release, Br J Pharmacol, 1972, *46*, 741.
16. Rothschild, A. M.: Histamine release by basic compounds, in *Handbook of Experimental Pharmacology*, M. Rocha e Silva, ed., Springer-Verlag, New York, 1966, p. 386.
17. Stone, S. H., Liacopoulos, P., and Halpern, N.: Histamine: Differences in amount available for release in lungs of guinea pigs susceptible and resistant to acute anaphylaxis, Science, 1964, *146*, 1061.
18. Muus, P., Ridgway, P., Douglas, J. S., and Bouhuys, A.: Histamine content of tracheal and lung tissue as a function of age in rats, Respir Physiol, 1974, *21*, 317.
19. Assem, E. S. K., Schild, H. O., and Vickers, M. R.: Stimulation of histamine-forming capacity by antigen in sensitized human leucocytes, Int Arch Allergy, 1972, *42*, 343.
20. Kahlson, G., Rosengren, E., and Thunberg, R.: Accelerated histamine formation in hypersensitivity reactions, Lancet, 1966, *1*, 782.
21. Slorach, S. A., and Uvnäs, B.: Amine formation by rat mast cells in vitro, Acta Physiol Scand, 1968, *73*, 457.
22. Schayer, R. W.: Evidence that induced histamine is an intrinsic regulator of the microcirculatory

system, Am J Physiol, 1961, *202*, 66.

23. Mota, I.: Effect of antigen and octylamine on mast cells and histamine content of sensitized guinea pig tissues, J Physiol (Lond), 1959, *147*, 425.
24. Lichtenstein, L. M.: The control of IgE-mediated histamine release: Implications for the study of asthma, in *Asthma*, K. F. Austen and L. M. Lichtenstein, ed., Academic Press, New York, 1973, p. 91.
25. Foreman, J. C., Mongar, J. L., and Gomperts, B. D.: Calcium ionophores and movement of calcium ions following the physiological stimulus to a secretory process, Nature (New Biol), 1973, *245*, 249.
26. Johnson, H. G., and Bach, M. K.: Prevention of calcium ionophore-induced release of histamine in rat cells by disodium cromoglycate, J Immunol, 1975, *114*, 14.
27. Foreman, J. C., and Gomperts, B. D.: The relationship between anaphylactic histamine secretion and the premeability of the mast cell membrane to calcium, Int Archs Allergy Appl Immunol, 1975, *49*, 179.
28. Cochrane, D. E., and Douglas, W. W.: Calcium-induced extrusion of secretory granules (exocytosis) in mast cells exposed to 48/80 or the ionophores A-23187 and X-537A, Proc Nat Acad Sci (Wash), 1974, *71*, 408.
29. Johnson, H. G.: Inhibition of histamine release and ionophore-induced calcium flux in rat cast cells by chloropromazine and lidocaine (abstract), J Allergy Clin Immunol, 1976, *57*, 239.
30. Kazimierczak, W., Peret, M., and Maslinski, C.: The action of local anaesthetics on histamine release, Biochem Pharmacol, 1976, *25*, 1747.
31. Assem, E. S. K.: Cyclic 3′,5′-adenosine monophosphate and the anaphylactic reaction, in *Effects of Drugs on Cellular Control Mechanisms*, B. R. Rabin and R. B. Freedman, ed., University Park Press, Baltimore, 1972, p. 259.
32. Assem, E. S. K., Pickup, P. M., and Schild, H. O.: The inhibition of allergic reactions by sympathomimetic amines and methylxanthines, Br J Pharmacol, 1970, *39*, 212.
33. Weiss, E. B., and Patwardhan, A. V.: The response to lidocaine in bronchial asthma, Chest, 1977, *72*, 429.