Effects of Intravenous Administration of Slow-Reacting Substance of Anaphylaxis, Histamine, Bradykinin, and Prostaglandin $F_{2\alpha}$ on Pulmonary Mechanics in the Guinea Pig

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A B S T R A C T   The effects of intravenous administration of a purified preparation of slow-reacting substance of anaphylaxis (SRS-A), histamine, bradykinin, and prostaglandin $F_{2\alpha}$ (PGF$_{2\alpha}$) on the mechanics of respiration were assessed in the unanesthetized guinea pig. Geometrically increasing doses of SRS-A resulted in graded decreases in average pulmonary compliance, with only modest increases in average pulmonary resistance. A dose with apparent maximal effects, 3,000 U/kg, resulted in a decrease of $49\pm7\%$ of compliance below control values, with an increase in resistance of $24\pm8\%$ above control. Intravenous administration of geometrically increasing amounts of histamine, bradykinin, and prostaglandin $F_{2\alpha}$ also resulted in decreased compliance; but in each case this was accompanied by a marked increase in respiratory resistance. A decrease of compliance of approximately 50%, induced by intravenous histamine, bradykinin, or PGF$_{2\alpha}$, was accompanied by an increase of 60–140% in resistance. Thus, intravenously administered SRS-A alters pulmonary mechanics with a more peripheral effect than any of the other agents tested.

INTRODUCTION

The pathophysiologic changes of anaphylaxis are attributed to the action of chemical mediators released by sensitized cells upon interaction with antigen (1). Among the mediators identified as being released in vitro by the primary antigen-antibody reaction in the guinea pig lung are histamine (2), slow-reacting substance of anaphylaxis (SRS-A)[1] (2), and an eosinophil chemotactic factor of anaphylaxis (3), while rabbit aorta-contracting substance, prostaglandins $F_{2\alpha}$ and E_2 (PGF$_{2\alpha}$ and PGE$_2$) (4), and a kinin-forming enzyme (5) appear to be formed secondarily (6, 7). Since anaphylaxis in the guinea pig is characterized by death within minutes from respiratory insufficiency (8), the effects of a number of these mediators on respiration have been studied. Histamine (9), bradykinin (10), PGF$_{2\alpha}$ (11), and SRS (12) have all been shown to produce changes in guinea pig tracheal overflow preparations. The interpretation of these studies is limited by the use of an anesthetic which has profound effects on the response of the guinea pig to bronchoactive agents (13) and by the tracheal overflow method which only provides information on total pulmonary impedance. Accordingly, the effects of each of these mediators on the mechanics of respiration were reexamined in the unanesthetized animal by using the method of Amdur and Mead (14), which measures pulmonary compliance and resistance separately and therefore allows interpretation in terms of site of action.

METHODS

Male Hartley strain guinea pigs, weighing between 295 and 410 g, were lightly anesthetized for less than 10 min with ether and a small (0.38-mm ID, 0.76-mm OD) polyvinyl chloride catheter inserted through a skin window into a peripheral vein. The catheter was threaded centrally, approximately 5–7 cm, and secured into place with a silk tie. Proper placement was assured by aspiration of venous blood and confirmed at postmortem examination.

[1] *Abbreviations used in this paper:* PGE$_2$, prostaglandin E$_2$; PGF$_{2\alpha}$, prostaglandin F$_{2\alpha}$; SRS-A, slow-reacting substance of anaphylaxis.

Animals were prepared for measurements of pleural pressure by percutaneous placement of a fluid-filled catheter in the pleural space (14). The animals were then placed in a sloping front head-out body plethysmograph for measurement of tidal volume. From simultaneous recordings of tidal volume, pleural pressure, and the time rate of change of tidal volume, calculations were made of average pulmonary resistance and compliance (14). Since resistance and compliance varied considerably with body weight, all values were normalized by expressing compliance per kilogram and resistance multiplied by body weight in kilograms.

Crude SRS-A was obtained by intraperitoneal antigen injection of rats prepared 2 h before by administration of 1.0 ml of hyperimmune rat anti-egg albumin (15). Crude control material was prepared in the same manner, except that normal rat serum, rather than hyperimmune serum, was used to prepare the peritoneal cavity for antigen challenge. The crude materials were purified by base hydrolysis, nonionic polymeric (Amberlite XAD-2, Rohm and Haas Co., Philadelphia, Pa.) chromatography, and differential absorption silicic-acid chromatography (15). Material prepared in this fashion did not contain detectable amounts of histamine, PGF$_{2\alpha}$, or bradykinin. After purification, all samples were assayed for SRS-A activity on the guinea pig ileum in the presence of mepyramine maleate; 1 U of SRS-A was that amount of material which yielded a contraction of the guinea pig ileum similar in magnitude to that of 5 ng of histamine (16). Histamine diphosphate (Eli Lilly and Co., Indianapolis, Ind.), bradykinin (New England Nuclear, Boston, Mass.), and PGF$_{2\alpha}$ (Dr. John Pike, Upjohn Co., Kalamazoo, Mich.) were obtained as noted. SRS-A was dissolved in 1.0 ml Tyrode's solution (17). All other agents were dissolved in Hanks' balanced salt solution (Microbiological Associates, Inc., Bethesda, Md.). Each was administered by intravenous infusion over 10 s in a volume from 0.1 to 1.0 ml with a plastic syringe for SRS-A and a siliconized glass syringe with a Harvard apparatus infusion pump for the others (Harvard Apparatus Co., Millis, Mass.). Infusion of 3.0 ml of either buffer in 10 s did not alter respiratory frequency, pulmonary resistance, or pulmonary compliance.

Control measurements were made 1½–2 h after completing the set up of each experimental animal and before infusion of each agent. As noted by previous investigators (13, 14), control measurements were stable for periods of time up to 10–12 h. Data for time-course relationships represent the mean of three respiratory cycles near the time shown on the graphs. Values for dose-response relationships represent the mean of three respiratory cycles after administration of each agent at the time specified. Histamine, bradykinin, and PGF$_{2\alpha}$ were given at random, allowing sufficient time for resistance and compliance to return to control values; at least 30-min intervals were used for histamine and bradykinin and 90-min intervals for PGF$_{2\alpha}$. Separate animals were employed for each study of SRS-A.

RESULTS

The time-courses of pulmonary changes after intravenous infusion of SRS-A (3,000 U/kg) and control material are given in Fig. 1 ($n = 6$ animals in each group). During the first 45–50 s after infusion, reliable measurements of tidal volume could not be made because of excessive movement of the animals. 60 s after completion of the infusion, compliance had decreased 49±7%
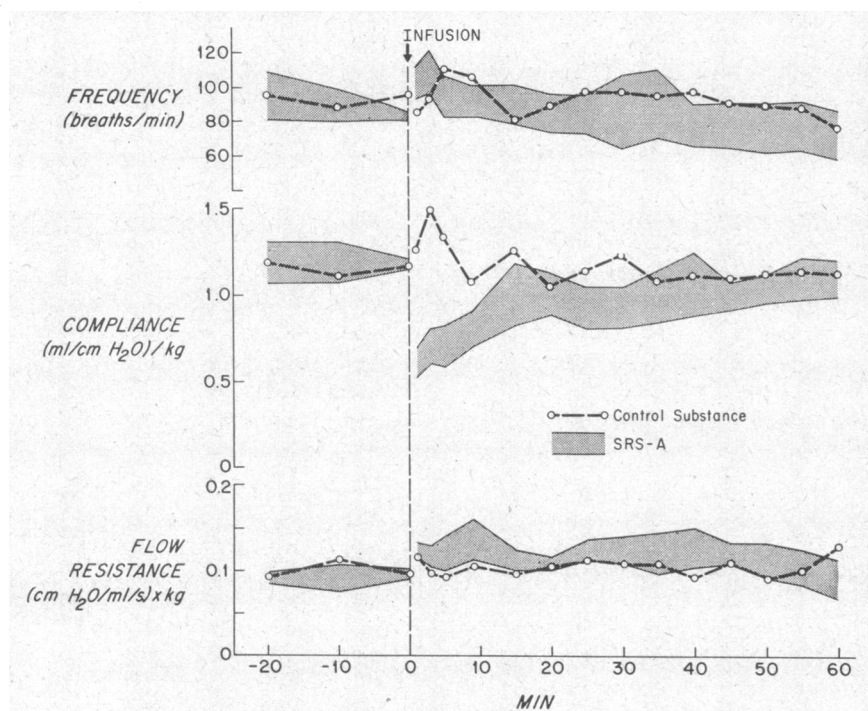

FIGURE 1 Effects of 3,000 U/kg SRS-A (shaded bar represents mean ±1 SEM) and control material (open circles) on the mechanics of respiration in the unanesthetized guinea pig. $n = 6$ animals in each group.

(1 SEM, $n = 6$ animals), while resistance had increased $24\pm8\%$ from control. The respiratory effects were still apparent 5 min after the infusion, but could no longer be detected at 15 min.

Dose-response relationships for the maximal observed effects of SRS-A (60 s after completion of the infusion) over a 200-fold range are given in Fig. 2. 50 U/kg had a slight effect, if any, on pulmonary mechanics, while 500 U/kg had marked effects; and both 3,000 and 10,000 U/kg yielded a maximum response. At this maximum observed response, compliance was decreased significantly from control ($P < 0.001$, Student's t test for paired variates), while resistance and frequency were not significantly changed ($P < 0.3$). Control material had no significant effect on pulmonary resistance, compliance, or respiratory frequency (Fig. 1).

After intravenous administration of 500 U/kg SRS-A, the animals were quiet enough at 30 s to permit reliable measurements of resistance and compliance. Compliance was $48\pm4\%$ below control, while resistance increased by $10\pm13\%$ above control. 60 s after completion of the infusion, compliance was $40\pm8\%$ below control, and resistance was increased $11\pm12\%$ above control. Compliance was still below control values 3 min after the infusion, but had returned to control levels by 9 min.

After 10,000 U/kg SRS-A there was a biphasic response (Fig. 3). The initial effect was similar to that seen after 3,000 U/kg. The secondary effect became apparent 4–5 min after infusion, was maximal at 15–20 min, and compliance was still significantly below control values ($P < 0.05$, Student's t test for paired variates) at 60 min.

Intravenous administration of histamine resulted in a marked increase in resistance associated with a marked decrease in compliance. The onset of the histamine effect was immediate and dissipated by 45 s (Fig. 4). Since the marked increase in respiratory frequency which occurred with histamine infusion might affect measured resistance and compliance, dose-response relationships for intravenous histamine are presented 10–12 s after completion of the histamine infusion, a time when frequency had returned to control levels. As shown in Fig. 5, 0.3 and 1.0 μg/kg had little or no effect on the mechanics of respiration, while doses of 3.0 and 9.0 μg/kg produced a marked alteration. A dose of 3.0 μg/kg decreased pulmonary compliance $30\pm10\%$, while resistance increased $80\pm18\%$. After 9.0 μg/kg, compliance decreased by $55\pm10\%$, and resistance increased $140\pm40\%$ from control values. Larger doses of histamine resulted in death or marked variation in respiratory patterns that made analysis of resistance and compliance unreliable.

Intravenous bradykinin also resulted in a decreased compliance and an increased resistance. The onset of
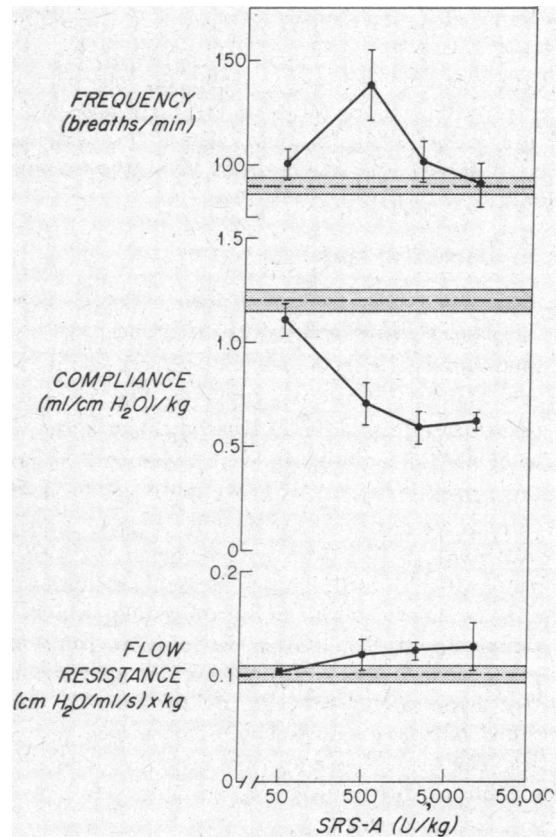

FIGURE 2 Dose-response relationship for intravenous SRS-A on the mechanics of respiration 1 min after completion of the infusion. Vertical bars represent 1 SEM. Shaded horizontal bars represent the average control value ±1 SEM. $n = 6$ animals.

the bradykinin action was rapid, with the maximal effect occurring 10–20 s after completion of the infusion (Fig. 4). The alterations produced by bradykinin decreased rapidly at first and then more slowly and were no longer detectable at 8 min. Dose-response relationships for the maximal effects of bradykinin are given in Fig. 6. 3.0 μg/kg of bradykinin increased resistance $83\pm20\%$, while compliance decreased $42\pm6\%$, and similar changes occurred with 30 μg/kg bradykinin. Both doses elicited an increased respiratory frequency of $30\pm9\%$. Tachyphylaxis to bradykinin was not observed after five consecutive doses of 10 μg/kg given at 30-min intervals.

PGF$_{2\alpha}$ had a biphasic effect on the mechanics of respiration. 300 μg/kg of PGF$_{2\alpha}$ produced an initial increase in resistance and a decrease in compliance with return of these parameters toward control values in the subsequent 3–5 min. The resistance again began to increase and compliance to decrease, with the maximal secondary effect occurring at 7 min and dissipating by

11 min. The secondary effect was greater in magnitude and lasted for 20–25 min after 3,000 µg/kg PGF₂ₐ (Fig. 3). Dose-response relationships and the maximal observed percent changes for both the early and late effects are given in Fig. 7 and Table I. Tachyphylaxis was not observed after five doses of 300 µg/kg at 60-min intervals.

DISCUSSION

The mediators studied differed in their action on pulmonary mechanics in the unanesthetized guinea pig in site and time-course of effects and with respect to the presence and nature of a biphasic response. The time-course of respiratory effects differed from agent to agent. The effects of histamine (Fig. 4) were ephemeral, as noted by other investigators (13), being undetectable at 45 s. In contrast, the effects of bradykinin (Fig. 4) were not maximal until 10–20 s after completion of the infusion. Thereafter there was an initial marked diminution followed by a slower return to control values, which was not complete until 8 min after the infusion. Infusion of SRS-A (3,000 U/kg) was followed by approximately 60 s of hyperkinetic behavior by the animals, which prevented reliable measurements of resistance and compliance. The first reliable measurements, made 60 s after completion of the infusion (Fig. 1), were also the

maximum observed effects of the agent; these decreased slowly and were no longer detectable at 15 min.

In contrast to these monophasic effects, 300 or 3,000 µg/kg PGF₂ₐ and 10,000 U/kg SRS-A had a biphasic effect on pulmonary mechanics. The onset of the secondary effects of both agents occurred at a time when compliance was returning to normal and when resistance was at control levels (Fig. 3). In duration, the SRS-A response was longer, with compliance remaining significantly below control values for 60 min after the infusion, while with PGF₂ₐ, resistance and compliance returned to control values at 30 min. The secondary effect observed after administration of these agents could be due to a metabolite of the mediator, which is more slowly cleared from the circulation, to a substance released by the initial infusion which ameliorated the mediator effects (18), or to a substance released by the initial infusion with an independent action of its own.

Over the range of doses studied, no plateau of the dose-response curve to histamine (Fig. 5) or PGF₂ₐ (Fig. 7) was noted. If sufficient amounts of these agents were given, death resulted from asphyxiation. In contrast, there was an apparent maximum magnitude of response observed with SRS-A (Fig. 2). An apparent maximum was also observed after bradykinin (Fig. 6). Accompanying the response to bradykinin was an in-
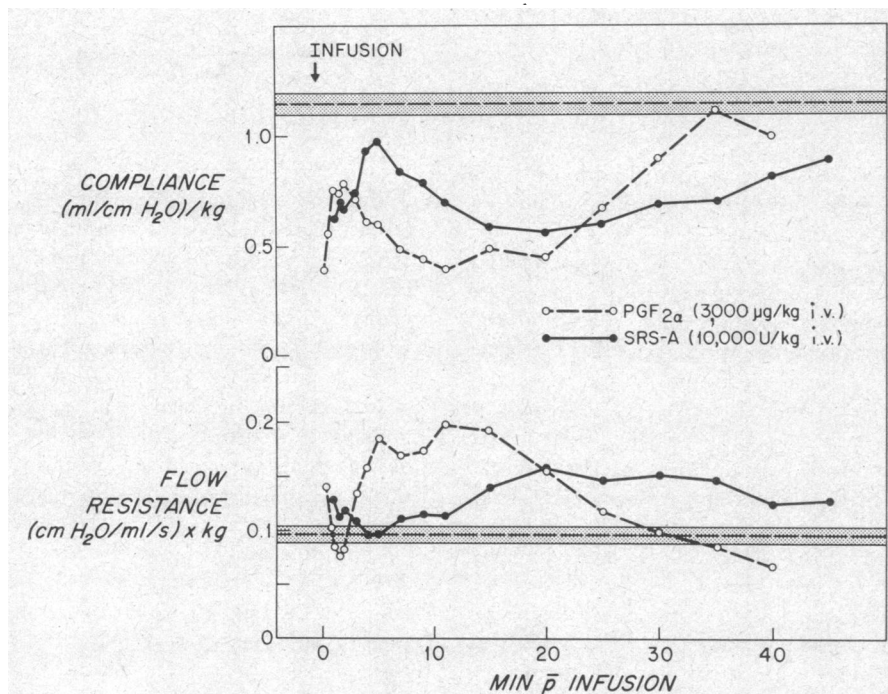

FIGURE 3 Comparative effects of intravenous SRS-A, 10,000 U/kg, and PGF₂ₐ, 3,000 µg/kg, on the mechanics of respiration. Shaded horizontal bar represents the average control value ±1 SEM.

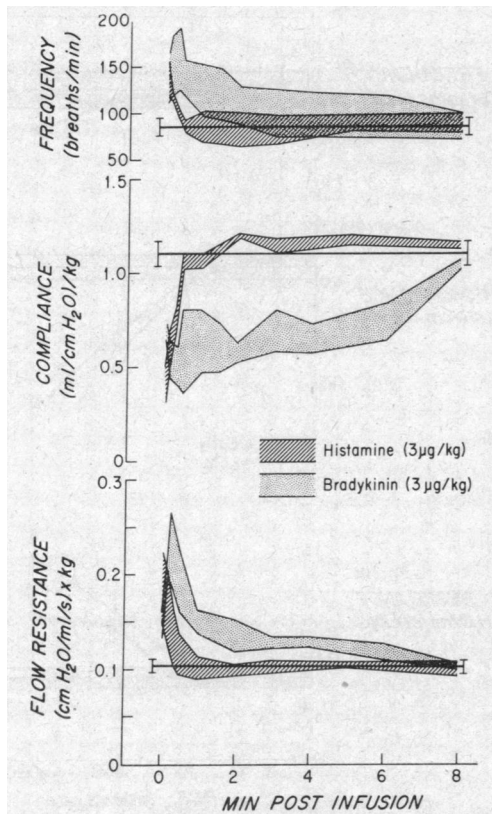FIGURE 4 Comparative time course of the respiratory effects of histamine, 3.0 μg/kg, and bradykinin, 3.0 μg/kg. Shaded areas represent the average experimental values ±1 SEM. Vertical side bars represent average predrug control ±1 SEM. $n = 3$ animals.

in resistance locally, might not be detected in terms of total resistance. Changes in the smaller airways, as measured by dynamic compliance, could reflect involvement of either alveolar ducts, bronchioles, or both. Alterations at the level of the alveolar ducts, which contain an abundance of smooth muscle in the guinea pig (21), would result in a decreased compliance by directly altering the distensibility of the parenchyma, while nonuniform constriction of the bronchioles would reduce dynamic compliance by decreasing the amount of parenchyma participating in tidal breathing. These anatomical inferences from physiologic data are not exact, and there may be situations in which changes in upper airway tone could alter measured compliance and vice versa. For example, an increase in airway resistance could alter measured airway compliance by increasing lung volume, i.e., compliance falls as volume increases. On the other hand, it is possible that decreased tissue compliance, accompanied by an increased elastic recoil, could result in a decreased resistance on the basis

crease in respiratory frequency. It is possible that the changes in respiratory frequency could account for the observed decrease in compliance, but this effect cannot account for the observed increase in resistance, which might be expected to fall with increasing respiratory frequency. Further, in a modified tracheal overflow preparation ventilated at constant frequency, intravenous bradykinin produced increased overflow (19); thus, it is likely that bradykinin had an effect on the airways directly.

Further distinctions among the mediators can be made by comparison of the site of action as suggested by their relative effects on resistance and compliance. In the unanesthetized guinea pig preparation, changes in small airways (alveolar ducts and bronchioles) are reflected by alterations in compliance, while changes in central airways are reflected by resistance. The small peripheral airways, because of their large total cross section relative to the central airways, contribute only a small fraction of total pulmonary resistance (20). Therefore, even a 50% reduction in their total cross section, resulting in approximately a fourfold increase
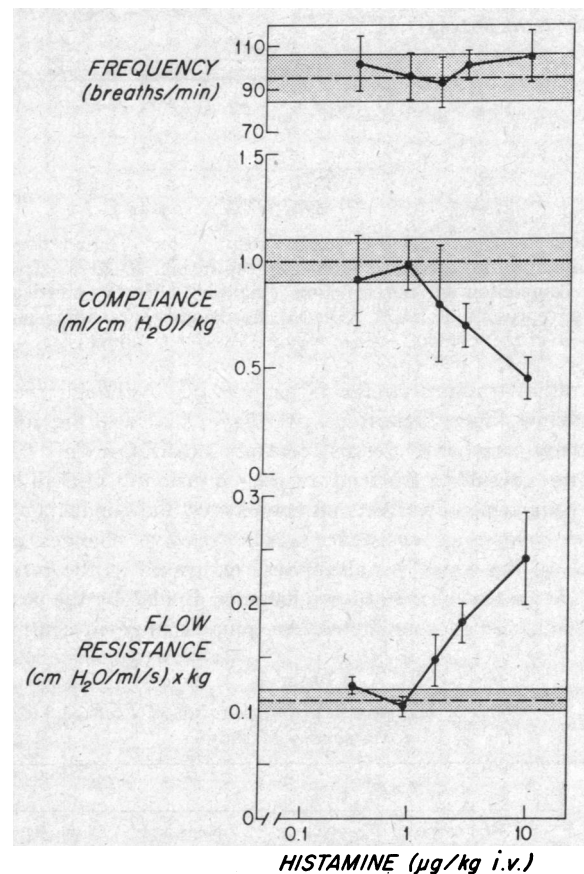

FIGURE 5 Dose-response relationship for intravenous histamine on the mechanics of respiration 10–12 s after completion of the infusion. Vertical bars represent 1 SEM. Shaded horizontal bars represent the average control value ±1 SEM. $n = 6$ animals.
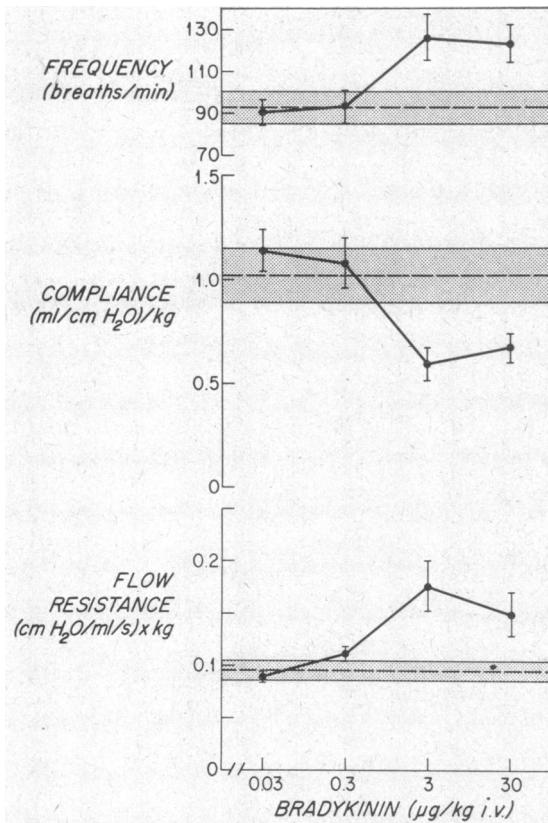
FIGURE 6 Dose-response relationship for intravenous bradykinin on the mechanics of respiration 10–20 s after the completion of the infusion (maximal effect). Vertical bars represent 1 SEM. Shaded horizontal bars represent the average control value ±1 SEM. $n = 6$ animals.
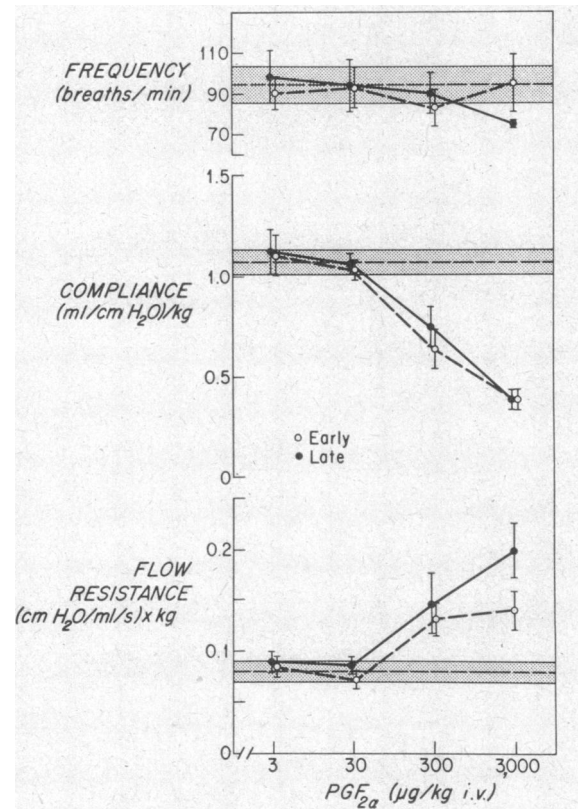

FIGURE 7 Dose-response relationship for intravenous $PGF_{2\alpha}$ on the mechanics of respiration. Interrupted line represents the mean change 20 s after completion of the infusion of the agent, while the solid line represents the mean maximal observed change 3–30 min after completion of the infusion. Vertical bars represent 1 SEM. Shaded SEM. $n = 6$ animals.

of radial traction on the large airways. Although recognizing these limitations, we have analyzed the apparent peripheral versus central localization of the changes resulting from intravenous infusion of mediators by comparing the effects on compliance and conductance (the inverse of resistance). The relative changes of each of these two variables were expressed as the ratio of the percent change of conductance divided by the percent change in compliance. An equal change in central

TABLE I

Effects of Intravenous Administration of $PGF_{2\alpha}$ on Pulmonary Mechanics

Dose μg/kg	Early*		Late*	
	Resistance	Compliance	Resistance	Compliance
	%		%	
300	+47±21	−28±10	+85±26	−28±10
3,000	+92±24	−63±5	+191±30	−65±17

* Percent changes from predrug control values. Values represent the average±SEM. $n = 6$ animals.

and peripheral airways would be represented by a ratio of 1, while agents with predominately large airway effects will exhibit a ratio greater than 1, and those with mostly small airway effects a ratio of less than 1. As shown in Fig. 8, histamine, bradykinin, and the early effects of $PGF_{2\alpha}$ were almost equally central and peripheral, while the late effect of $PGF_{2\alpha}$ appeared to be more central. In contrast, SRS-A had a low ratio of change in conductance to change in compliance, 0.38±0.21, suggesting that its effect was mainly on the terminal airways. For the secondary effect of SRS-A, observed only after the highest dose of the agent studied, the conductance to compliance ratio was still only 0.54±0.10.

Four theories have been used to account for the action of humoral agents on the lung. It has been proposed that they act by direct stimulation of respiratory smooth muscle (19), via a reflex arc (22, 23), by release of a second mediator (24), or by altering the sensitivity of one of the above effector mechanisms to humoral or nervous stimuli. Appreciation of which of these mecha-
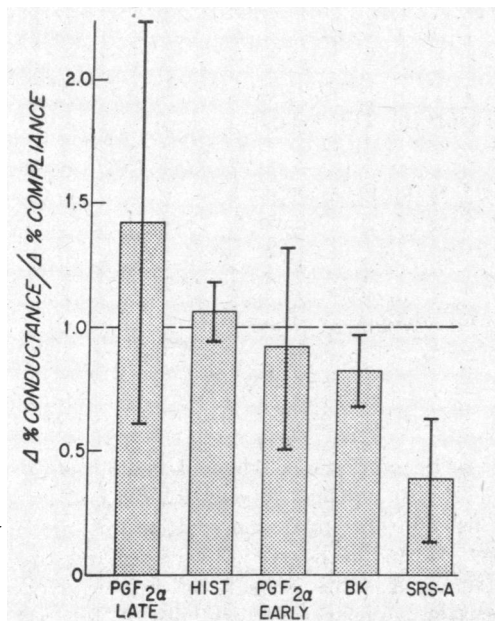
FIGURE 8 Ratios of percent change in conductance divided by percent change in compliance for the changes observed after intravenous infusion of mediators. Ratios greater than 1 suggest predominately large airway effects, while ratios less than 1 suggest predominantly small airway effects. Vertical bars represent 1 SEM. HIST, histamine; BK, bradykinin.

nisms is responsible for the observed action of a particular mediator will require further analysis by study of the effects of end organ antagonists and depletion of pools of secondary mediators and modulators.

ACKNOWLEDGMENTS

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