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REDUCTION IN ANTIGEN-INDUCED RELEASE OF HISTAMINE AND SLOW REACTING SUBSTANCE OF ANAPHYLAXIS FROM GUINEA PIG LUNG WITH INCREASING AGE

JEROME H. FLEISCH, PATRICIA J. CALKINS and CAROL S. HOOKER

Division of Pharmacological Research, The Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46206, U.S.A.

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Abstract—Release of histamine and slow reacting substance of anaphylaxis (SRS-A) from passively sensitized guinea pig lung was found to be related to the age of the donor animal. Minced lungs from 1-week, 1-month and 2-month-old guinea pigs were passively sensitized *in vitro* to ovalbumin (OA). Subsequent challenge with OA released histamine and SRS-A. Lungs from 1- and 2-month-old animals released less mediators than lungs from 1-week-old animals. A reduced tissue content of histamine and SRS-A and a smaller per cent total release of these mediators from the older guinea pig lungs were responsible for this decline. Lungs from 9- and 53-day-old guinea pigs actively sensitized to OA and challenged 21 days later released similar amounts of histamine and SRS-A. Passively sensitized lung from 1-week-old animals released more histamine than the actively sensitized tissues. SRS-A released from actively sensitized lung was too variable for comparison. These experiments suggest passive sensitization *in vitro* of lung from 1-week-old guinea pigs may be a means of maximizing mediator release. More significantly, they indicate the importance of the animal's age when studying antigen-induced mediator release.

Elucidation of the mechanisms responsible for generation and release of anaphylactic mediators, such as histamine and slow reacting substance of anaphylaxis (SRS-A), from immunologically sensitized lung has contributed to our understanding of the asthmatic state [1]. Feigen and Conrad [2] and Orange and Austen [3] have recently noted the variability in antigen-induced histamine release from animal to animal. Generally, this poses only minor technical problems. However, unlike histamine, SRS-A must be detected by bioassay [4, 5], and high yields of this mediator upon antigen challenge greatly enhance reproducibility.

Previous studies from this and other laboratories found large variations in the response of arteries to isoproterenol. This variability was reduced when tissues from animals of the same age were selected for analysis. Subsequently, this was related, at least partially, to a decrease in beta receptor activity with increasing age [6, 7]. The present study was conducted to determine if animal age is a factor in antigen-induced mediator release from guinea pig lung. The results show that passively sensitized lungs from 7- to 10-day-old guinea pigs release more histamine and SRS-A upon antigen challenge than lungs from 30- and 60-day-old guinea pigs. This decline with age was due to a smaller tissue content of the mediators and to a reduced percentage of total release of histamine and SRS-A from the older guinea pig lungs.

MATERIALS AND METHODS

Male and female Hartley guinea pigs (William Cavies, Ferncreek, KY) of known weight and age were killed by decapitation. Lungs were excised and perfused through the pulmonary artery with Krebs bicarbonate solution of the following composition in m-moles/liter: KCl, 4.6; $CaCl_2\ 2H_2O$, 1.8; KH_2PO_4, 1.2; $MgSO_4 \cdot 7H_2O$, 1.2; NaCl, 118.2; $NaHCO_3$, 24.8; and dextrose, 10.0. Poorly perfused and bloody areas were discarded. Normal lung was minced with surgical scissors, washed a few times with Krebs solution, divided into 400-mg replicates and incubated for 1 hr at 37.5° in vials containing 2.5 ml of 1:33 dilution of serum obtained from actively sensitized guinea pigs (see below). After passive sensitization *in vitro*, the tissues were washed with Krebs solution containing 10^{-6} M indomethacin which has been previously shown to enhance SRS-A release [8]. Tissue samples were then reincubated at 37.5° in 2.5 ml of indomethacin–Krebs solution for 15 min. Antigen (10^{-4} g/ml of ovalbumin, final concn) was added and the incubation continued for an additional 15 min. The diffusates were decanted and centrifuged at 3000 g (Sorval RC 5) for 5 min. The resultant supernatant fractions were collected at 0° and assayed for SRS-A and histamine. Total histamine and SRS-A were determined by adding 2.5 ml of indomethacin–Krebs solution to the remaining tissues and immersing the vials in boiling water for 10 min. The amounts of histamine and SRS-A released into the incubation medium after boiling plus that released by antigen were considered the total tissue content. All incubations were carried out in duplicate and appropriate tissue controls were run.

Antiserum was prepared by actively sensitizing guinea pigs with 2 mg ovalbumin in 50% complete Freund's adjuvant i.p. on days 1 and 5. On day 21,

Table 1. Age-related decrease in mediator release from passively sensitized guinea pig lung*

Age	Animal wt (g)	Histamine (μg free base/ml)	SRS-A (relative units)†
7–10 days old (14)	166.3 ± 5.6‡§	2.02 ± 0.20‡§	32.5 ± 5.9‡§
30–33 days old (16)	255.4 ± 3.3§	1.05 ± 0.14	12.2 ± 2.5
60–63 days old (16)	426.4 ± 5.8	0.68 ± 0.15	8.4 ± 1.8

* Values represent means of the number of animals in parentheses ± S.E.
† Relative to a standard sample.
‡ Significantly different ($P < 0.05$) from 30- to 33-day-old animals.
§ Significantly different ($P < 0.05$) from 60- to 63-day-old animals.

the animals were bled and serum was collected. Lungs from similarly sensitized animals were used in the experiments described in Table 3.

SRS-A was bioassayed on guinea pig ileum in the presence of 10^{-6} M atropine and 10^{-6} M pyrilamine [4, 5]. Quantitation was based on the potency of the experimental samples relative to a standard sample. SRS-A was defined as that substance capable of contracting the guinea pig ileum in the presence of atropine and pyrilamine and antagonized by 10^{-6} M FPL 55712, a selective SRS-A-blocking agent [9]. The ileum was bathed in Tyrode's solution of the following composition in m-moles/liter: NaCl, 136.9; KCl, 2.7; $CaCl_2$ $2H_2O$, 0.9; $MgCl_2$ $6H_2O$, 1.1; NaH_2PO_4 H_2O, 0.4; $NaHCO_3$, 11.9; and dextrose, 5.6. Histamine was extracted from 1-ml portions of the samples using the procedures outlined by Tauber *et al.* [10] and Brocklehurst [11]. A modification of the fluorometric assay of Shore [12] was used to assay histamine [13]. Fluorescence was read at 360 nm excitation and 450 nm emission (Aminco–Bowman spectrophotofluorometer). Known concentrations of histamine were treated similarly to produce a standard curve for each experiment. Differences between means were determined by Student's *t*-test for unpaired data. Statistical significance was assumed when $P < 0.05$. The quantitation of mediators in lung tissue from different age animals should, ideally, be referenced to parameters such as dry weight and total proteins, in addition to wet weight. The wet weight/dry weight ratio and total proteins of five lungs from each group of animals were not different; therefore, results have been expressed on the basis of tissue wet weight. Dry weights were determined after subjecting the tissues to 45° for 48 hr. Total proteins were ascertained by the method of Lowry *et al.* [14].

The following drugs were used: ovalbumin grade 5, atropine sulfate, indomethacin, *o*-phthaldialdehyde (Sigma Chemical Co., St. Louis, Missouri); pyrilamine maleate (ICN Pharmaceuticals, Inc., Plainview, New York); FPL 55712 (gift of Fisons, Ltd., Leicester, U.K.) and complete Freund's adjuvant (Difco Laboratories, Detroit, Michigan).

RESULTS

Age-related decrease in antigen-induced release of histamine and SRS-A from guinea pig lung. Release of histamine and SRS-A from passively sensitized guinea pig lung by ovalbumin decreased as the animals aged (Table 1). Data obtained from male and female guinea pigs were similar and therefore pooled for analysis. Lungs from 1-week-old guinea pigs released twice as much histamine and nearly three times more SRS-A than lungs from 1-month-old animals and three times as much histamine and four times more SRS-A than lungs from 2-month-old animals.

We then asked whether a reduction in the concentration of histamine and SRS-A in lung tissue with age could account for the decreased release of mediators. This provided a partial explanation. Lungs from 2-month-old guinea pigs contained 44 per cent less histamine and about 70 per cent less SRS-A than 1-week-old guinea pig lungs (Fig. 1). A decrease in response to antigen challenge also contributed to the reduction in mediator release with aging. Ovalbumin released 52 per cent of the total histamine from 1-week-old guinea pig lungs and 38 per cent from 2-month-old guinea pig lungs (Table

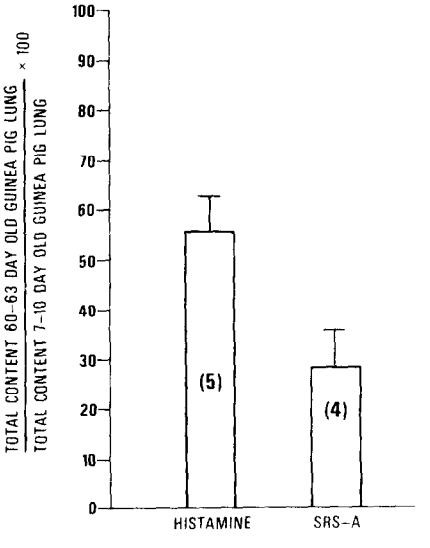

Fig. 1. Relationship between total levels of histamine and SRS-A in lungs from 2-month- and 1-week-old guinea pigs. The older lungs had 56 per cent of the histamine and 28 per cent as much SRS-A as the younger lungs. Total histamine and SRS-A were determined by adding 2.5 ml of indomethacin–Krebs solution to 400 mg of lung tissue after antigen challenge and immersing the vials in boiling water for 10 min. The amount of mediators released into the incubation medium after boiling plus that released by antigen was considered the total tissue content.

Table 2. Per cent of total histamine and total SRS-A released on antigen challenge from passively sensitized guinea pig lung*

Age	Histamine	SRS-A
7–10 days old	51.5 ± 3.8† (6)	79.6 ± 1.8† (5)
60–63 days old	38.2 ± 4.1 (5)	69.6 ± 2.4 (4)

* Values for histamine and SRS-A represent means of number of animals in parentheses ± S.E.

† Significantly different ($P < 0.05$) from 60- to 63-day-old animals.

2). The older lungs also released 10 per cent less of the total SRS-A (Table 2).

Antigen-induced release of histamine and SRS-A from actively sensitized guinea pig lungs. Nine-day-old guinea pigs were actively sensitized to ovalbumin. Histamine was released from their lungs by challenge with ovalbumin 21 days later when the animals were 1 month old. The amount of histamine released was less than that from 1-week- and 1-month-old passively sensitized lungs and similar to histamine released from 2-month-old passively sensitized lungs (Tables 1 and 3). SRS-A released from actively sensitized lungs was too variable to make a valid comparison between actively and passively sensitized tissue. Another group of guinea pigs, nearly 2 months old when actively sensitized, released the same amounts of histamine and SRS-A from their lungs in response to ovalbumin as did the younger actively sensitized animals (Table 3).

DISCUSSION

Antigen-induced release of the mediators of anaphylaxis from immunologically sensitized lung is variable [2, 3]. Previous experience with other types of pharmacologic differences [6] led us to question whether the amount of histamine and SRS-A released from passively sensitized guinea pig lung is related to animal age. Austen *et al.* [15] previously showed that immunological release of histamine from rat peritoneal mast cells decreased with increasing age of the cell donor. In agreement with this observation, we found the per cent of total histamine released from mixed peritoneal cells of the rat by concanavalin A, dextran, Compound 48/80, and the Ca^{2+} ionophor A23187 was less when the cells were obtained from older animals (Hooker and Fleisch, unpublished observations). Despite similarities in antigen-induced mediator release from different cell types, guinea pigs used in experiments on mediator release from lung are usually selected on the basis of weight and not by age. Thus, in the present study, we explored the relationship between a donor guinea pig's age and the quantity of histamine and SRS-A released from passively and actively sensitized lung upon antigen challenge.

Lungs from 1-week-, 1-month- and 2-month-old guinea pigs were passively sensitized to ovalbumin. Release of histamine and SRS-A in response to ovalbumin decreased as the animals aged. The major portion of this decline was attributable to a smaller total tissue content of these substances in the older animals; the 2-month-old guinea pig lungs contained 44 per cent less histamine and 70 per cent less SRS-A than lungs from 1-week-old animals. Total values represented that released upon antigen challenge plus residual mediators released upon immersing the tissues in boiling water. This is important in regard to SRS-A, since recent evidence indicates that some SRS-A is preformed but total tissue levels are substantially increased if measured after antigen challenge [16]. The reduction in histamine content of guinea pig lung with increasing age probably reflects either a decrease in the number of mast cells per gram of tissue or a reduction in the number of histamine-containing granules per mast cell. In addition, a small decrease in the per cent of total releasable mediators was found in lungs from 2-month-old animals (14% histamine, 10% SRS-A). This may indicate a slight reduction in the degree of antibody fixation to the mast cells of older lungs.

Table 3. Mediator release from actively sensitized guinea pig lung*

Group	Histamine (μg free base/ml)	SRS-A (relative units)†
A	0.55 ± 0.10 (4)	16.6 ± 7.2 (4)
B	0.66 ± 0.18 (4)	18.6 ± 8.0 (4)

* Values represent means ± S.E. Numbers in parentheses indicate the number of animals tested. The animals in group A were 9 days old when given the first sensitizing dose of ovalbumin; the animals in group B were 53 days old.

† Relative to a standard sample.

Active immunization takes 3 weeks before maximal tissue sensitization becomes evident. Therefore, the youngest actively sensitized guinea pigs (9 days old) were 1 month old when their lungs were challenged with ovalbumin. The procedure used for active sensitization was found previously to result in highly sensitized lung [17]. Tissues from these animals and those from 2-month-old actively sensitized guinea pigs released 25 per cent of the histamine released from 1-week-old passively sensitized lung. SRS-A was too variable for a reliable analysis.

The life span of the guinea pig is approximately 5–8 years. Even the oldest animals used in the present experiments were very young by comparison. Strictly speaking then, our observations are as much related to maturation as to aging *per se*. Nevertheless, they emphasize the importance of the experimental animal's age when evaluating antigen-induced mediator release. Most studies in the literature have used guinea pigs that weighed anywhere from 200 to 500 g. Table 1 shows that 250-g guinea pigs are already too old for maximal mediator release. Our experiments also suggest that passive sensitization of lung obtained from the same age guinea pigs will decrease the variability often seen in this type of experiment. Additionally, our results indicate that passive sensitization *in vitro* of 7- to 10-day-old guinea pig lung may be more desirable than active sensitization, especially for the maximal release of SRS-A upon antigen challenge.

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