Reduced Anaphylactic Responsiveness of Strain 2 Guinea Pigs (43322)

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Abstract. **Strain 2 guinea pigs have been shown to have diminished anaphylactic responsiveness. In the present study, experiments were conducted comparing various characteristics of the anaphylaxis-resistant Strain 2 guinea pigs to those of an outbred anaphylaxis-prone Dunkin-Hartley strain. To bypass the possibility that differences in antibody titers accounted for the difference in anaphylactic reactivity, both strains of guinea pig were passively sensitized with the same amount of IgG antibody to ovalbumin. Measures of anaphylactic responsiveness to subsequent antigen challenge with ovalbumin included (i) systemically induced respiratory responses; (ii) isolated cardiac responses; and (iii) cutaneous responses. In all cases, using an amount of antibody sufficient to sensitize Dunkin-Hartley guinea pigs, the anaphylactic responses of the Strain 2 guinea pigs were either nonexistent or significantly less than those of the Dunkin-Hartley strain. To further determine which factors might be responsible for this difference, tissue histamine content, histamine releasability, and histamine responsiveness of the two strains were measured. The results of these studies indicated that the respiratory hyporesponsiveness of the Strain 2 guinea pigs may be due to a low pulmonary histamine content combined with reduced pulmonary responsiveness to histamine. However, since the cardiac histamine content and the responsiveness of the Strain 2 guinea pigs were not different from those of the Dunkin-Hartley strain, these factors cannot contribute to the reduced Strain 2 cardiac anaphylactic responsiveness. Compound 48/80 released equal quantities of histamine from the isolated hearts of the Strain 2 and the Dunkin-Hartley animals, but antigen challenge evoked histamine release only from the isolated Dunkin-Hartley hearts. We conclude that the cardiac anaphylactic hyporesponsiveness of the Strain 2 guinea pigs may be due to an inability of antigen to evoke release of anaphylactic mediators such as histamine.** [P.S.E.B.M. 1991, Vol 198]

Guinea pigs are often used as models for studying various allergic reactions since, in general, they develop dramatic immediate hypersensitivity or anaphylactic reactions. Much information about the mediators of anaphylaxis, their pulmonary, cardiac, and systemic effects, and the processes that evoke their release has been obtained from such guinea pig models (1–6). In 1925, Lewis and Loomis (7) reported that certain inbred National Institutes of Health strains of guinea pig (including the currently maintained Strain 2) did not develop the usual hypersensitivity to foreign proteins (in this case, sheep red blood cells or horse serum). Although this particular observation has received little attention since that time, it is clear that a better understanding of the factors responsible for this hyporesponsiveness may provide important clues about intraspecies immunomodulation. Therefore, in the present study, experiments were conducted comparing various characteristics of the anaphylaxis-resistant Strain 2 guinea pigs to those of an outbred anaphylaxis-prone strain (Dunkin-Hartley descendants).

Diminished hypersensitivity reactions may reflect reduced antibody production in response to foreign protein exposure. For example, Lundberg and colleagues (8, 9) have developed an anaphylaxis-resistant inbred strain of guinea pigs (8) characterized by low homocytotropic antibody production (9). To bypass the possibility that low antibody production contributed to the anaphylactic resistance of the Strain 2 guinea pig, the present experiments were conducted using guinea pigs that were passively sensitized with exogenous IgG

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antibody to ovalbumin to assure that adequate amounts of antibody were indeed present. Responses monitored included: pulmonary and cardiovascular components of the systemic anaphylactic response, anaphylactic reactions of isolated perfused hearts, and local cutaneous anaphylactic reactions. Reduced anaphylactic responsiveness may also result from other abnormalities, such as reduced quantities of mediators, reduced release of mediators, or abnormal tissue responses to these mediators. Since histamine is a primary mediator of the anaphylactic response in the guinea pig (10), experiments were conducted to address the issues of whether the differences in anaphylactic responsiveness between the strains were a result of differences in (i) the total amount of available histamine in the lung and heart tissue, (ii) the releasability of that histamine, and (iii) the pulmonary and cardiac responses to histamine.

Materials and Methods

Animals. Random-bred Dunkin-Hartley descendant, male guinea pigs (body wt of 300–650 g) were obtained from the Bio-lab Corporation, St. Paul, MN. Strain 2 male guinea pigs (body wt of 400–700 g) were obtained from Ribi Immunochem Research, Inc., Hamilton, MT.

Antibody Preparation. IgG antibody used for all experiments was obtained from pooled serum samples of the Dunkin-Hartley guinea pigs immunized with ovalbumin. As described previously (11), the IgG was affinity purified by passage of serum over a protein A-Sepharose column to separate IgG from other serum constituents. The IgG fraction was dialyzed against saline and stored in aliquots containing 1.0 mg/ml at −20°C for later use. Passive sensitization of the guinea pigs was achieved by intracardiac injection of the antibody (0.8 mg IgG/kg) under ether anesthesia 12–24 hr before the experiment.

Systemic Reactions. Pentobarbital-anesthetized (25 mg/kg ip) guinea pigs were mechanically respirated after succinylcholine chloride treatment (2.1 mg/kg iv), and pulmonary resistance, dynamic lung compliance, mean arterial pressure, and heart rate were measured as described previously (12). The adequacy of ventilation was confirmed by the measurement of blood gases. Tracheal airflow was measured with a Fleisch pneumotachograph and transpulmonary pressure measured via a needle inserted in the pleural cavity. Both tracheal airflow and transpulmonary pressure were fed into an on-line pulmonary mechanics computer (model 6; Buxco Electronics, Sharon, CT) that calculated pulmonary resistance and dynamic lung compliance by the method of Amdur and Mead (13). Animals were allowed to stabilize 15–20 min prior to experimental manipulations. For anaphylactic reactions, an antigen challenge of passively sensitized guinea pigs was initiated by an intravenous injection of ovalbumin (44 μg/kg or, in two of the Strain 2 guinea pigs, 460 μg/kg) and responses were observed for the subsequent 20-min period. For assessing the effect of histamine on measured variables, increasing doses of histamine were given to nonsensitized guinea pigs by bolus intravenous injections at 1-min intervals and maximum responses were determined.

Cardiac Reactions. Hearts were removed from pentobarbital-anesthetized (35 mg/kg ip), sensitized (or nonsensitized) guinea pigs, and perfused by Langendorff's method at a constant flow of ~9 ml/min/g (32°C) with modified Krebs-Henseleit solution (containing: 118.0 m*M* NaCl, 4.7 m*M* KCl, 25.0 m*M* $NaHCO_3$, 3.0 m*M* $CaCl_2$, 1.2 m*M* $MgSO_4$, 1.2 m*M* KH_2PO_4, 10.0 m*M* glucose, 0.5 m*M* disodium EDTA, 10 units/liter of insulin, and 1000 units/liter of heparin sodium), and bubbled with 95% O_2 and 5% CO_2. Perfusion pressure was measured from a side arm in the perfusion line and used to calculate coronary vascular resistance. A fluid-filled balloon attached to a pressure transducer placed in the left ventricle via the mitral valve was used to measure left ventricular pressures and heart rate. Oxygen content of venous effluent obtained anaerobically from a cannula placed in the right ventricular outflow track was used along with arterial samples to calculate oxygen consumption. For anaphylactic reactions, after a 45-min equilibration period, hearts from passively sensitized guinea pigs were challenged by intra-aortic bolus injection of ovalbumin (4.0 mg in 0.4 ml of saline), and reactions were observed and effluent samples were collected over the subsequent 10 min. Details of these procedures have been published previously (14, 15). For assessing the effect of histamine on measured variables, after a 45-min equilibration period, increasing concentrations of histamine were added to the perfusate in cumulative fashion at 5-min intervals and variables at the end of each infusion period were measured. For determining the "releasability" of the cardiac histamine, the isolated hearts of nonsensitized guinea pigs received a bolus intra-arterial injection (0.4 mg in 0.2 ml of saline) of the mast cell degranulator compound 48/80 (16), and coronary effluent was collected for the subsequent 5 min for histamine determination.

Cutaneous Reactions. Guinea pigs were anesthetized with ether, their backs were shaved, and 12 sites on each animal were injected intradermally with 0.1-ml dilutions of the IgG antibody to ovalbumin described above. After a latent period of 4 hr, the animals were challenged by cardiac puncture with 2 mg of ovalbumin and 25 mg/kg of Evans blue in normal saline. The diameter and intensity of blueing were evaluated 30 min after ovalbumin challenge on the undersurface of the skin and the minimum amount of IgG given a 5-mm bluing reaction was noted (11).

Histamine Determinations. Lung and cardiac tis-

sue were rinsed free of blood, homogenized in 0.4 *N* perchloric acid for 2 min, and centrifuged at 17,600*g* for 15 min, and the supernatants were stored at −20°C until assayed for histamine content (17). The coronary effluent from hearts challenged either with antigen or with compound 48/80 was collected on ice, acidified with 0.1 ml of 2 *N* perchloric acid/ml effluent, and stored at −20°C until assayed. The histamine content of the various samples was determined by the manual fluorometric method of Shore *et al.* (18) modified by Anton and Sayre (19) with a detection limit of 20 ng.

Data Analysis. Data are reported throughout as mean ± SE. Significant differences between groups were declared at $P < 0.05$ using unpaired Students' *t* test. For determining the differences in the responses to each concentration of exogenous histamine, the *t* test employed was Satterthwaites' approximation, which does not assume equal variances (20). In most cases, differences between the two groups were so great that the small Strain 2 sample size did not limit the conclusions of this study.

Results

Systemic Anaphylactic Responses. The systemic responses of anesthetized, passively sensitized guinea pigs to antigen challenge included significant increases in pulmonary airway resistance, decreases in dynamic airway compliance, transient increases followed by decreases in mean arterial pressure, and increases in heart rate. (Details of the time course of the pulmonary events have been published previously [21].) The peak values of these anaphylactic responses are indicated in Figure 1. Initial values for the Dunkin-Hartley ($n = 10$) and Strain 2 ($n = 3$) groups, respectively, are as follows: pulmonary airway resistance in cm H_2O/ml/sec, 0.30 ± 0.01 and 0.35 ± 0.01 ($P = 0.07$); dynamic airway compliance in ml/cm H_2O, 0.39 ± 0.04 and 0.39 ± 0.02 ($P = 0.99$); mean arterial pressure in mm Hg, 42 ± 4 and 30 ± 1 (P = 0.14); and heart rate in beats/min, 209 ± 8 and 214 ± 2 ($P = 0.56$). The data in Figure 1 clearly show that the respiratory components of the systemic anaphylactic response of the Strain 2 guinea pigs were significantly lower and the antigen-induced decrease in arterial pressure was less pronounced than comparable responses of the Dunkin-Hartley strain.

Cardiac Anaphylactic Responses. The responses of isolated hearts from passively sensitized guinea pigs to antigen challenge included significant increases in coronary vascular resistance, heart rate, left ventricular isovolumic systolic pressure (transient increase followed by depression), and myocardial oxygen consumption. (Details of the time course of these cardiac events have been published elsewhere [14, 15].) A summary of the cardiac peak responses to antigen challenge in the two

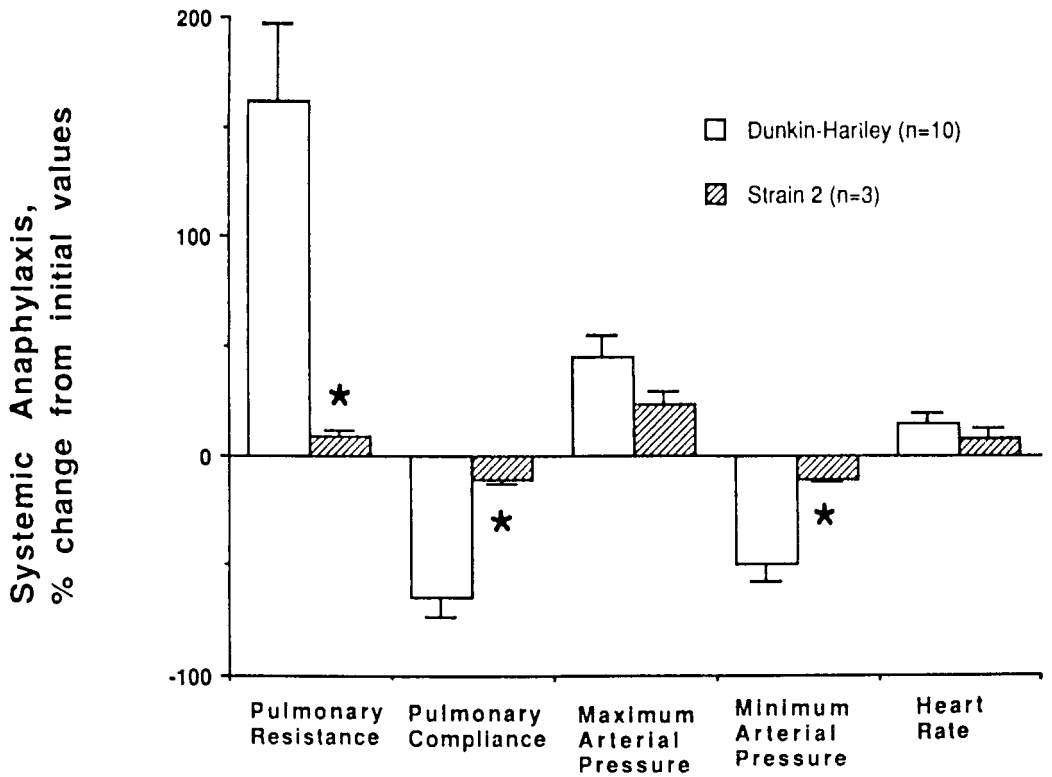

Figure 1. Peak systemic anaphylactic responses of anesthetized, passively sensitized guinea pigs to intravenous injection of antigen as percentage of change from initial values. Mean ± SE. *$P < 0.05$, as compared with the response achieved by the Dunkin-Hartley strain (unpaired *t* test).

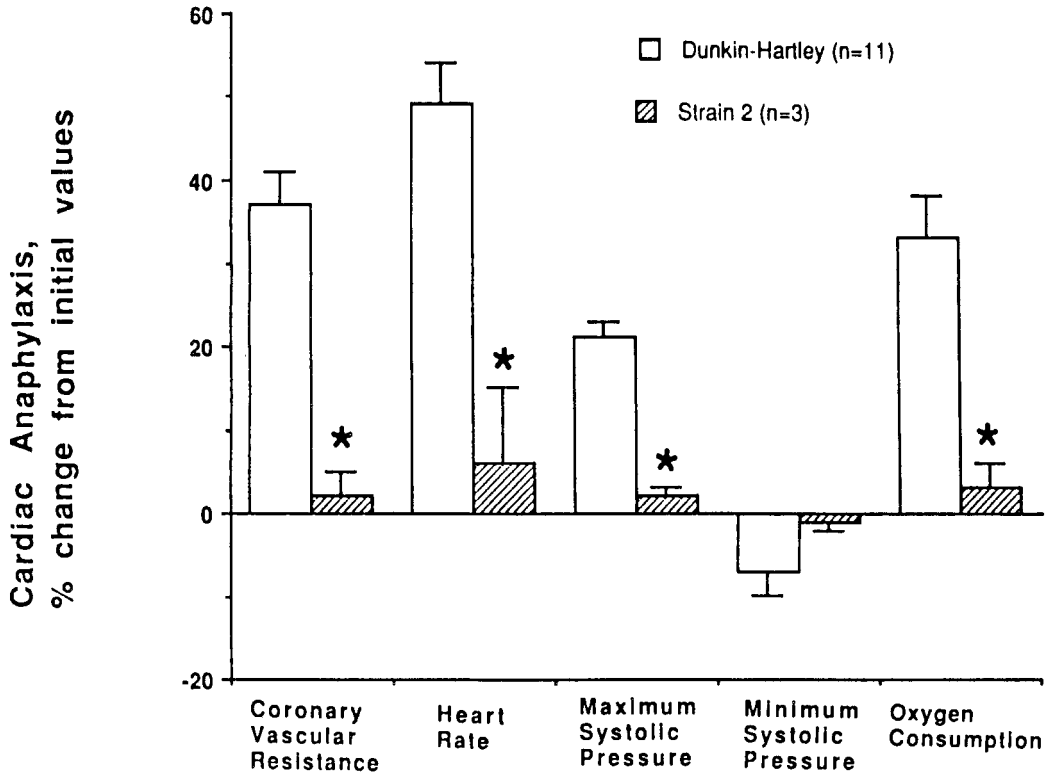

Figure 2. Peak cardiac anaphylactic responses of isolated, perfused, passively sensitized guinea pig hearts to antigen challenge as percentage of change from initial values. Mean ± SE. *$P < 0.05$, as compared with the response achieved by the Dunkin-Hartley strain (unpaired t test).

Table I. Release of Histamine from Isolated Hearts of Strain 2 and Dunkin-Hartley Strain Guinea Pigs[a]

	Compound 48/80-induced release (mg/g wet wt/5 min)	Antigen-induced release (mg/g wet wt/5 min)
Dunkin-Hartley	3.35 and 6.45 ($n = 2$)	1.36 ± 0.26 ($n = 8$)
Strain 2	4.11 and 6.22 ($n = 2$)	<0.01[b] ($n = 3$)

[a] Release of histamine from isolated hearts of the two strains of guinea pig during the 5 min following bolus injections of compound 48/80 or antigen.
[b] Values for histamine release in this group were below the detection level of the assay (0.01 mg/g/5 min). Using this value, $P < 0.01$ as compared with the value obtained in the Dunkin-Hartley group.

strains is shown in Figure 2. Initial values for the Dunkin-Hartley ($n = 11$) and Strain 2 ($n = 3$) groups, respectively, are as follows: coronary vascular resistance in mm Hg/ml/min/g, 5.06 ± 0.32 and 6.83 ± 0.33 ($P = 0.02$); heart rate in beats/min, 165 ± 7 and 163 ± 3 ($P = 0.90$); left ventricular systolic pressure in mm Hg, 98 ± 5 and 127 ± 2 ($P = 0.015$); and myocardial oxygen consumption in μl O_2/min/g, 97 ± 6 and 99 ± 5 ($P = 0.86$). The data of Figure 2 clearly show that the isolated hearts of the Strain 2 guinea pigs do not undergo significant cardiac anaphylactic reactions.

Cutaneous Anaphylactic Responses. The cutaneous anaphylactic reactions of the Strain 2 guinea pigs were also significantly reduced as compared with those of the Dunkin-Hartley strain. In four Dunkin-Hartley guinea pigs, 0.25 ng of IgG caused a 5-mm blueing reaction, whereas 10 times that amount (2.5 ng of IgG) was required to cause a 5-mm blueing reaction in two Strain 2 guinea pigs.

Pulmonary and Cardiac Histamine Content. The histamine content in the isolated lungs of two Strain 2 guinea pigs (13.4 and 12.4 μg/g wet wt) was less than that of four Dunkin-Hartley guinea pigs (39.4 ± 5.2 μg/g wet wt). However, the cardiac histamine content of two Strain 2 guinea pigs (6.52 and 4.68 mg/g wet wt) was not different from that of eight Dunkin-Hartley strain guinea pigs (5.67 ± 0.47 mg/g wet wt).

Histamine Release from Isolated Perfused Hearts. As shown in Table I, injection of the mast cell degranulator, compound 48/80, evoked substantial histamine release from the isolated perfused hearts of both groups, with no obvious difference between the groups.

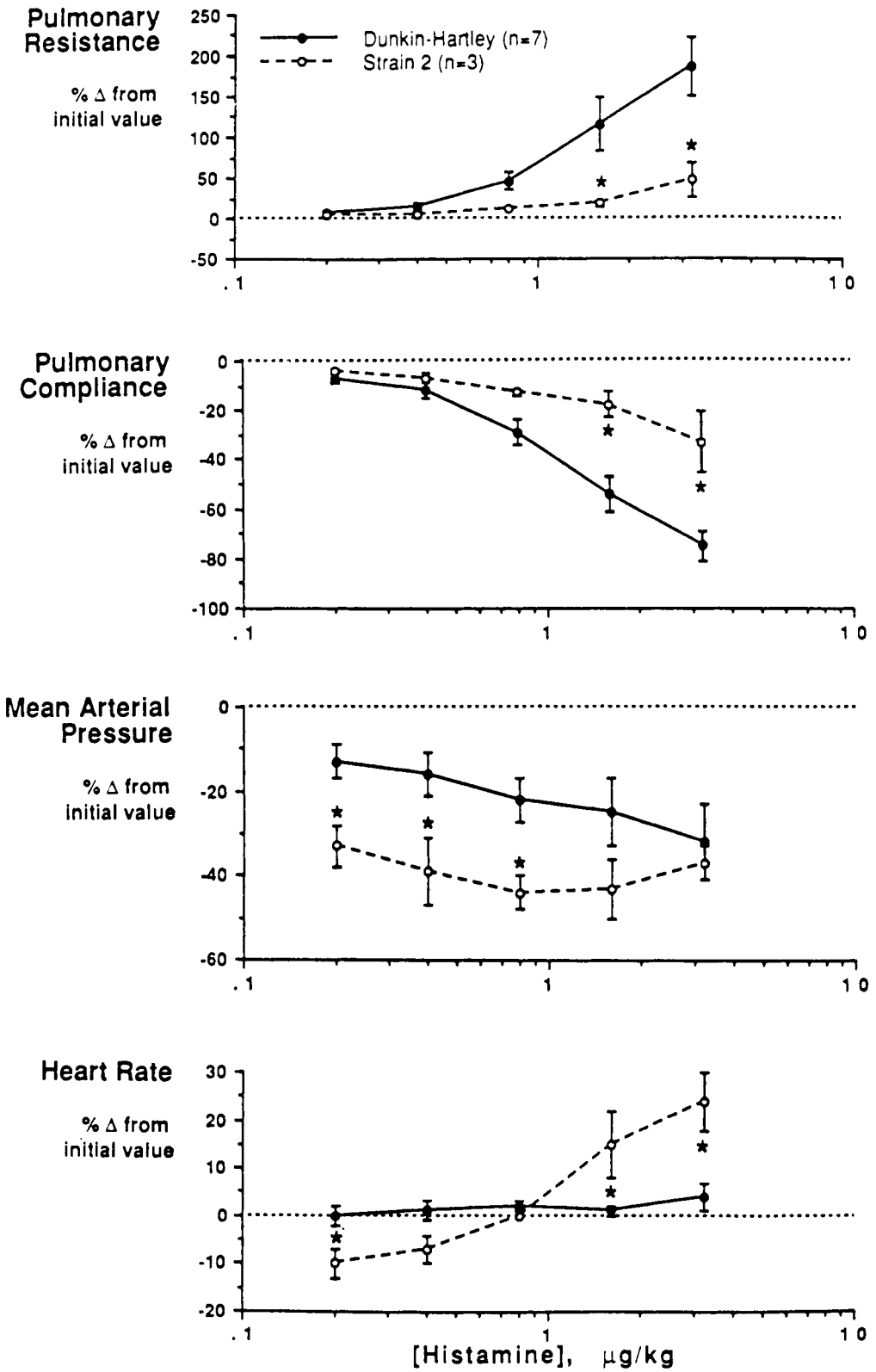

Figure 3. Peak systemic responses of anesthetized guinea pigs to intravenous bolus injections of exogenous histamine as percentage of change from initial values. Mean ± SE. *$P < 0.05$, as compared with value obtained in the Dunkin-Hartley guinea pigs in response to the same histamine concentration.

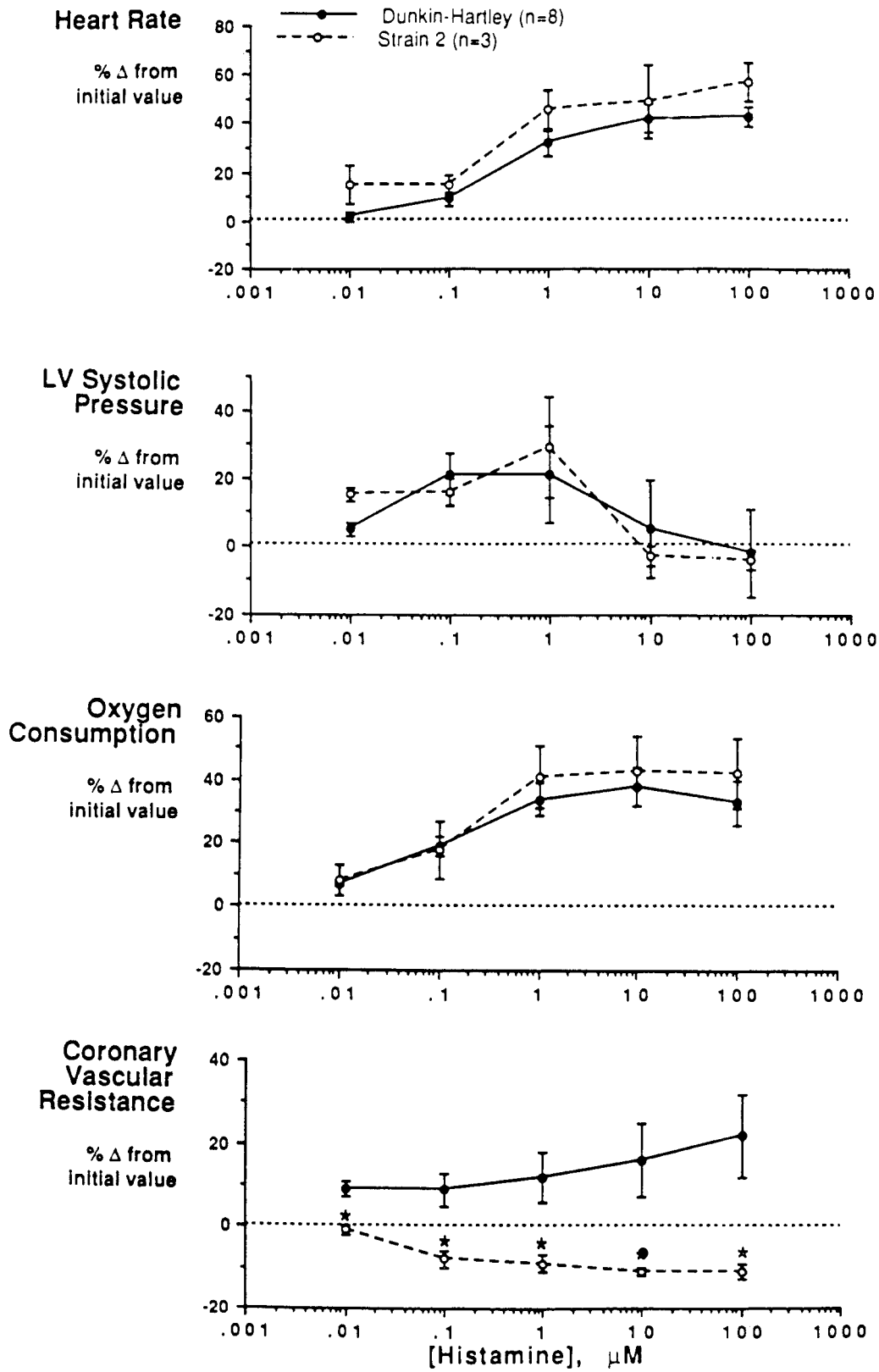

Figure 4. Cardiac responses of isolated perfused guinea pig hearts after 5-min infusions of increasing concentrations of exogenous histamine as percentages of change from initial values. Mean ± SE. *$P < 0.05$, as compared with value obtained in the Dunkin-Hartley guinea pigs in response to the same histamine concentration.

In contrast, antigen challenge evoked significant histamine release from the Dunkin-Hartley strain hearts, but no detectable histamine release from the Strain 2 hearts. The releasability of histamine from pulmonary tissues was not assessed in these studies.

Responses to Exogenous Histamine. The systemic responses of the two strains of anesthetized guinea pigs to bolus intravenous injections of increasing doses of histamine are indicated in Figure 3. Initial values for the Dunkin-Hartley ($n = 7$) and Strain 2 ($n = 3$) groups, respectively, are as follows: pulmonary airway resistance in cm H_2O/ml/sec, 0.26 ± 0.01 and 0.31 ± 0.0.02 ($P = 0.01$); dynamic airway compliance in ml/cm H_2O, 0.47 ± 0.06 and 0.48 ± 0.07 ($P = 0.88$); mean arterial pressure in mm Hg, 46 ± 4 and 39 ± 4 ($P = 0.34$); and heart rate in beats/min, 218 ± 12 and 209 ± 10 ($P = 0.63$). The data in Figure 3 indicate that the histamine-induced increases in pulmonary resistance and the decreases in dynamic compliance are significantly attenuated in the Strain 2 guinea pigs as compared with those in the Dunkin-Hartley strain, whereas the histamine-induced decreases in mean arterial pressure and the changes in heart rate are more pronounced in the Strain 2 guinea pigs than in the Dunkin-Hartley strain. Since the changes in mean arterial pressure and heart rate may be complicated by reflex influences, any interpretation of these interesting but unexplained observations will not be included in this report.

The responses of the isolated perfused hearts from the two strains to infusions of increasing concentrations of exogenous histamine are indicated in Figure 4. Initial values for the Dunkin-Hartley ($n = 8$) and Strain 2 ($n = 3$) groups, respectively, are as follows: heart rate in beats/min, 185 ± 6 and 158 ± 7 ($P = 0.03$); left ventricular systolic pressure in mm Hg, 94 ± 8 and 116 ± 8 ($P = 0.14$); myocardial oxygen consumption in μl O_2/min/g, 101 ± 4 and 80 ± 9 ($P = 0.05$); and coronary vascular resistance in mm Hg/ml/min/g, 5.70 ± 0.40 and 8.17 ± 0.44 ($P = 0.01$). As shown in Figure 4, except for the changes in coronary vascular resistance, there were no significant differences between the two strains in the cardiac responses to histamine infusion. Because of the differences in initial vascular resistance and the fact that histamine tended to lower the vascular resistance in the Strain 2 preparations and to raise it in the Dunkin-Hartley preparations, the final coronary vascular resistance was similar in the two groups after histamine infusion. The significance of this finding is not clear.

Discussion

The results of this study clearly show that, in the presence of adequate levels of antibody to achieve passive sensitization, the Strain 2 guinea pig is still highly resistant to anaphylactic reactions when compared with the random-bred Dunkin-Hartley descendants from Bio-lab. This is demonstrated by the near absence of systemic and cardiac anaphylactic responses (Figs. 1 and 2) and the significant attenuation of the cutaneous anaphylactic responses under conditions that evoke vigorous reactions in the outbred Dunkin-Hartley strain. Since passive sensitization techniques were used in these studies, no conclusions about antibody production by the Strain 2 guinea pigs can be made. However, it can be concluded that the lack of responsiveness in *these* experiments must be a result of a failure of the antibody to fix to tissue sites, a failure of the antigen-antibody interaction to trigger mediator release, and/or a failure of the mediator to cause the anaphylactic response.

The results of this study provide support for the second of these possibilities, i.e., that the severely diminished anaphylactic responsiveness of the Strain 2 guinea pigs may result from a failure of the antigen to trigger mediator (e.g., histamine) release from the sensitized tissues. This conclusion is based upon the observations that in the Strain 2 guinea pigs: (i) there are significant amounts of histamine in the lungs and heart tissue; (ii) histamine can be released from cardiac tissue by compound 48/80; and (iii) exogenous histamine evokes significant systemic and cardiac responses. It must be pointed out, however, that the severe attenuation of the pulmonary components of the anaphylactic reaction in the Strain 2 guinea pigs might be partially accounted for by the significantly lower histamine content of the lungs. These data are consistent with the unpublished findings referred to by Clausen *et al.* (22) of an abnormally low pulmonary histamine content in a different anaphylaxis-resistant guinea pig strain. In addition, the reduced sensitivity of the airways of the Strain 2 guinea pigs to histamine (Fig. 3) might also contribute to the diminished respiratory anaphylactic responses in this strain. On the other hand, since there were no discernible differences in the histamine content of the hearts or in the sensitivity of the hearts to histamine between the two strains tested, it is likely that the differences in cardiac anaphylactic responsiveness are primarily a result of the failure of antigen to release histamine.

The reasons for the failure of the antigen to cause histamine release in Strain 2 guinea pigs are not clear. Several possible explanations might include: (i) a reduced binding of the IgG antibody to the mast cells; (ii) a diminished interaction of the antigen with mast cell-bound IgG; and (iii) alterations in the signal transduction events following antigen-antibody interaction resulting in reduced histamine releasability. Subsequent studies addressing these issues using this strain of guinea pigs may provide important insight about the causes of varying susceptibility to immediate hypersensitivity reactions within the human population.

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