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INVESTIGATION OF THE ROLE OF HISTAMINE IN ANTIGEN-INDUCED BRONCHOCONSTRICTION IN THE ASCARIS-HYPERSENSITIVE DOG

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1 Aerosol administration of ascaris antigen to the airways of ascaris-hypersensitive dogs provoked increases in pulmonary resistance (Rp) and decreases in dynamic lung compliance (Cdyn). These changes in pulmonary mechanics were not inhibited by the histamine H_1-receptor antagonists, diphenhydramine or mepyramine.

2 Increases in Rp and decreases in Cdyn induced by a histamine aerosol were markedly or totally inhibited by comparable doses of these H_1-antihistamines.

3 Doses of antigen which produced pathophysiological pulmonary responses failed to produce a detectable histamine release from the cardiopulmonary system *in vivo*. Aerosol antigen provocation, equivalent to 5 to 9 times greater than that which produced substantial pathophysiological pulmonary responses, did cause histamine release *in vivo*.

4 The canine cardiopulmonary system showed only a modest ability to remove and/or degrade circulating histamine.

5 It is concluded that histamine may not play a major role in mediating the acute antigen-induced bronchoconstriction in the ascaris-hypersensitive dog.

Introduction

Aerosol administration of a partially purified extract of *Ascaris suum* round-worms to the airways of dogs bronchially hypersensitive to this antigen provokes pathophysiological pulmonary responses (Booth, Patterson & Talbot, 1970; Gold, Kessler, Yu & Frick, 1972; Patterson, Mellies, Kelly & Harris, 1974; Krell, Chakrin & Wardell, 1975). These changes in pulmonary mechanics resemble, in many respects, those documented clinically in allergic asthmatic patients undergoing an acute episode (Yu, Gallant & Gold, 1972; Rosenthal, Norman & Summer, 1975). Furthermore, this response to ascaris antigen appears to be mediated by a reaginic antibody (Kessler, Frick & Gold, 1974) which possesses physicochemical and biological characteristics similar to human reaginic antibody of the immunoglobulin E class (Patterson & Sparks, 1962; Patterson, Pruzansky & Chang, 1963; Schwartzman, Rockey & Halliwell, 1971; Kessler *et al.*, 1974).

More recently attempts have been made to determine whether there are biochemical and pharmacological similarities between canine and human models of this disease. Studies with an *in vitro* fragmented canine lung system have established that the nature of the mediators released, as well as the ability of a variety of pharmacological agents to influence this release, compared favourably to those previously documented for human isolated lung (Krell & Chakrin, 1976b; Krell & Chakrin, 1978). Hence, the provisional addition of biochemical and pharmacological similarities to the established immunological and physiological similarities suggested that the *in vivo* canine model of allergic asthma might represent a useful experiment tool.

As a further characterization of the *in vivo* canine model, the role of histamine in mediating the acute bronchoconstriction induced by administration of aerosol ascaris antigen has been investigated and forms the basis of this paper.

Parts of this work were presented at the American Congress of Allergy and Immunology (Krell, 1977).

Methods

Adult male mongrel dogs weighing 10 to 20 kg were used. Standard ascaris antigen (SAA) was prepared from *Ascaris suum* worms as described previously (Krell & Chakrin, 1976b). Dogs were screened for cutaneous sensitivity and bronchial reactivity to SAA by techniques previously described (Krell, Chakrin & Wardell, 1975; 1976). Aerosols of SAA or histamine were delivered to the airway with a Bird positive pressure respirator, fitted with an inline aerosolizer. Respirator parameters were as follows: inspiratory pressure limit, 20; flow rate, 20; inspiratory sensitivity, 20. Particle size generated by the respirator ranged from 0.01 to 4.0 μm. Student's *t* test was used to evaluate differences between means.

Measurement of pulmonary mechanics, ascaris antigen challenge and analysis of drug effects

These techniques have been described in detail previously (Krell *et al.*, 1975; 1976). Briefly, dogs were anaesthetized with pentobarbitone (40 mg/kg, i.p., or 35 mg/kg, i.v.) and the trachea intubated. Airflow was measured with a heated Fleisch pneumotachograph (Instrumentation Associates, Inc., New York) connected to a Statham PM5E differential pressure transducer (Statham Instruments, Inc., Calif.). Transpulmonary pressure was determined with a Statham PM5E differential pressure transducer which monitored the pressure difference between a side-arm adapter and an intrapleural cannula placed in the fifth or sixth intercostal space. Pulmonary resistance (Rp), dynamic lung compliance (Cdyn) and tidal volume (TV) were calculated on a breath-by-breath basis by an on-line analog computer (Buxco Electronics, Inc., Sharon, Conn.) and recorded on either a Beckman Type RM 8-channel dynograph (Beckman Instruments, Inc., Schiller Park, Ill.) or a Gould 260 6-channel recorder (Gould Inc., Cleveland, Ohio).

Dogs cutaneously sensitive to ascaris antigen were challenged at two week (biweekly) intervals with appropriate dilutions of aerosol SAA. SAA was diluted in phosphate-buffered saline (PBS, 10 mM phosphate buffer, pH 7.4 in 0.9% w/v NaCl solution). Animals demonstrating reproducible pulmonary responses to aerosol antigen on three successive biweekly challenges were considered suitable for drug evaluation. Considerable variability between animals in responsiveness to antigen required that biweekly antigen challenges in the presence of the drug be alternated with vehicle controls in order that each animal could serve as its own control.

The time interval between antigen provocation and peak increase in Rp varies considerably from challenge to challenge within the same animal (Krell *et al.*, 1975). Further, Rp and Cdyn do not consistently reach maximum responses at the same time. To overcome this source of variability responses were analyzed as a function of peak Rp increase without regard to time of antigen administration or peak decrease in Cdyn. Peak Rp increases occurred 5.5 (2 to 9) min (mean and range of 100 responses in 10 dogs) after antigen administration.

Aerosol histamine dose-response curves

Dose-response curves to aerosol histamine were obtained in control dogs by sequentially increasing the number of inhalations of a 5 mg/ml solution of the agonist in a step-wise manner. Each dose of histamine was administered after the effect of the previous dose had waned. In a separate series of experiments, dose-response curves to aerosol histamine were obtained after the intravenous administration of diphenhydramine 2 mg/kg or mepyramine 3.5 mg/kg.

Determination of systemic arterial-venous plasma histamine differences across the cardiopulmonary system during anaphylaxis

Dogs were prepared for measurement of pulmonary mechanics as described above. Bilateral 4 cm craniocaudal incisions were made 1 cm lateral to the ventral cervical midline. The right jugular vein and left carotid artery were dissected free of connective tissue and cannulated with, respectively, a 4 cm or 6 cm 20-gauge and a 2 cm 18-gauge, teflon-coated nonobstructive cannula for sampling systemic venous and arterial blood. At various time intervals before, during and after antigen provocation, 1 ml arterial and venous blood samples were simultaneously withdrawn for extraction and assay of histamine.

Determination of the removal of histamine from the circulation by the cardiopulmonary system

Dogs were prepared for measurement of pulmonary mechanics as described above. After measurement of baseline pulmonary function, the animals were artificially ventilated with a Harvard Respiration Pump (Harvard Apparatus Co., Dover, Mass.) at the original baseline respiratory frequency but with twice the measured tidal volume. A midline thoracotomy was then performed and nonobstructive cannulae placed in the right atrium to sample venous blood and in the right common carotid artery to sample systemic arterial blood. Histamine, at an infusion rate of 100 ng kg^{-1} min^{-1}, was infused into the femoral vein with a Harvard Infusion Pump (Harvard Apparatus Co., Dover, Mass.). Blood samples (1 ml) were simultaneously withdrawn from the venous and arterial cannulae immediately before the start of the infusion and at 30 s intervals during the 2 min infusion.

The process was repeated at 30 min intervals for histamine infusions of both 500 and 1000 ng kg^{-1} min^{-1}. Histamine was extracted from the blood and assayed as described below.

Histamine extraction and assay

Histamine was extracted from the blood by a modification of the technique of Snyder, Axelrod & Bauer, 1964. All procedures were carried out in the cold (0 to 4°C). Blood samples of approximately 1.0 to 1.3 ml were placed in 1.5 ml plastic centrifuge tubes containing 20 µl of heparin and kept on ice until all samples were collected. Samples were centrifuged (500 *g* for 20 min) after which 500 µl of the plasma supernatant was removed and placed in a tube containing 500 µl 0.8 N $HClO_4$. After mixing and standing for 30 min the samples were centrifuged (2000 *g* for 30 min) and the resulting supernatant removed and made alkaline by the addition of 500 µl of 1.0 N NaOH. After salt saturation, 5 ml of butanol:$CHCl_3$ (3:2, freshly prepared) was added, mixed vigorously for 20 s and centrifuged for 3 min at 500 *g*. The organic layer was removed, placed in a fresh tube to which 1 ml of 0.1 N HCl was added and mixed vigorously for 20 seconds. The mixture was again centrifuged (500 *g* for 3 min), the aqueous layer removed, placed in a 1.5 ml plastic centrifuge tube and evaporated to dryness in a microrotary evaporator (Omicron Industries, Troy, Ill.). The dried powder was washed with 100 µl of absolute ethanol to remove traces of butanol and again dried. Samples were stored at −20°C until assayed.

Samples were reconstituted on the day of assay by the addition of 100 µl 0.05 M sodium phosphate buffer (pH 7.9). Histamine was assayed, in triplicate, by the microradio-enzymatic double label technique of Taylor & Snyder (1972). All values have been corrected for a histamine recovery of 44% from plasma. A three-point histamine standard curve, in triplicate, was included in each assay. Samples were counted in a Mark III Liquid Scintillation Counter, Searle-Analytical, Chicago, Ill.

Chemicals and solutions

S-adenosyl-L methionine [methyl-^{14}C] sp. act 57.8–59.7 mCi/mmol and histamine-[^{3}H (G)], sp. act. 10.5–12.7 Ci/mmol were purchased from New England Nuclear, Boston, Massachusetts. Histamine dihydrochloride was purchased from Calbiochem, San Diego, California.

Histamine dihydrochloride was dissolved in PBS for aerosol administration. Diphenhydramine and mepyramine were dissolved in an appropriate volume of sterile 0.9% w/v NaCl solution (saline) for intravenous administration. Doses refer to the base form.

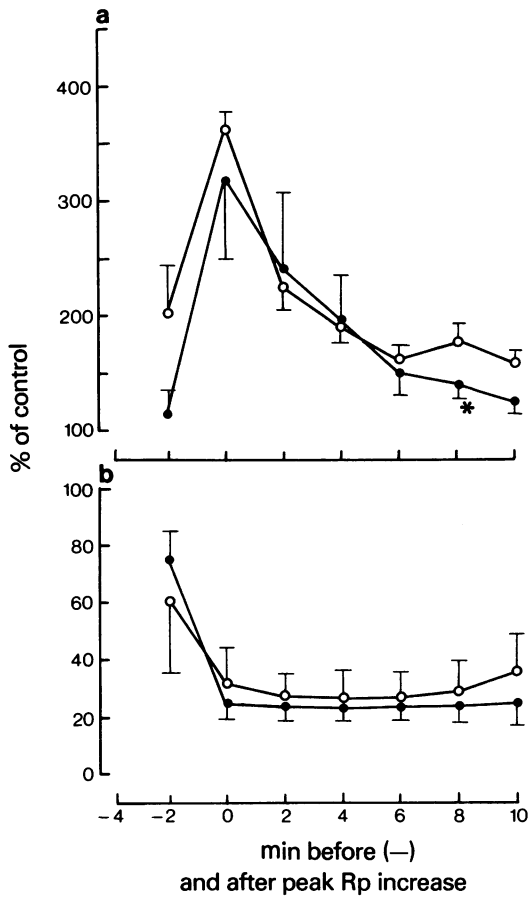

Figure 1 Effect of diphenhydramine on antigen-induced changes in (a) pulmonary resistance (Rp) and (b) dynamic lung compliance (Cdyn) in dog 1. Diphenhydramine (2 mg/kg i.v.) was administered 15 min before 7 inhalations of a 1:10 dilution of SAA. Mean ± s.e. mean baseline values for Rp (cmH_2O/LPS) and Cdyn (ml/cmH_2O), determined immediately before antigen administration, were, respectively: 1.93 ± 0.14 and 68.1 ± 2.30 for control (●; n = 3); 1.78 ± 0.19 and 61.8 ± 11.2 for diphenhydramine (○; n = 3). Symbols with vertical lines represent the mean ± s.e. mean. * $P < 0.05$.

Results

Effect of histamine H_1-receptor antagonists on ascaris antigen-induced pulmonary anaphylaxis

Administration of the H_1-antihistamine diphenhydramine (2 mg/kg, i.v.) prior to an aerosol of ascaris antigen provocation did not attenuate antigen-induced increases in Rp or decreases in Cdyn (Figure 1). Similarly, in a second series of experiments,

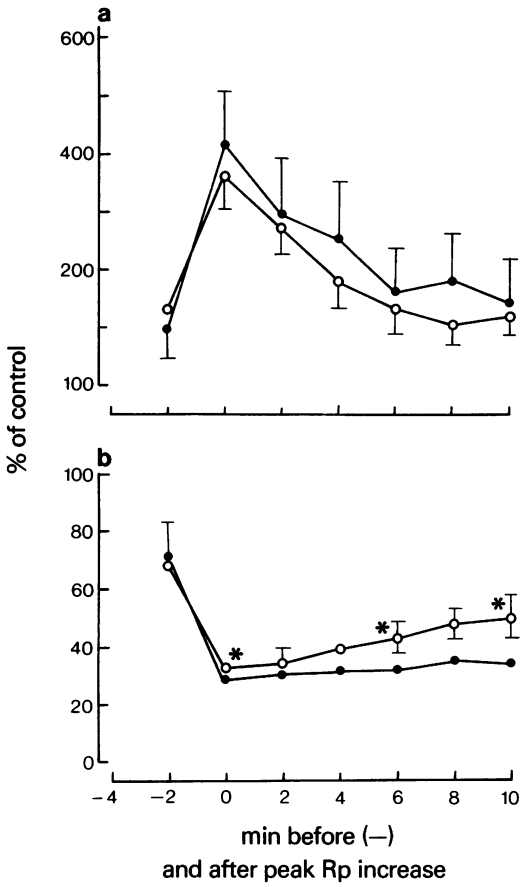

Figure 2 Effect of diphenhydramine on antigen-induced changes in (a) pulmonary resistance (Rp) and (b) dynamic lung compliance (Cdyn) in dog 2. Diphenhydramine (2 mg/kg i.v.) was administered 15 min before 10 inhalations of a 1:10 dilution of ascaris antigen. Mean ± s.e. mean baseline values for Rp (cmH_2O/LPS) and Cdyn (ml/cmH_2O), determined immediately before antigen administration were, respectively: 1.64 ± 0.20 and 60.5 ± 10.0 for control (●; $n = 3$); 1.63 ± 0.08 and 68.3 ± 4.04 for diphenhydramine (○; $n = 3$). Symbols with vertical lines represent the mean ± s.e. mean.*$P < 0.05$.

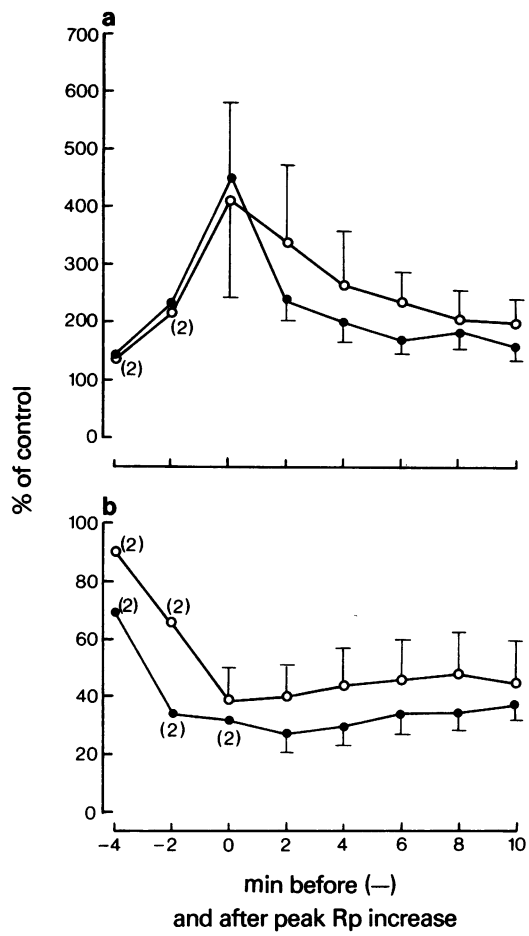

Figure 3 Effect of mepyramine on antigen-induced changes in (a) pulmonary resistance (Rp) and (b) dynamic lung compliance (Cdyn) in dog 3. Mepyramine (3.5 mg/kg i.v.) was administered 15 min before 7 inhalations of a 1:10 dilution of ascaris antigen. Mean ± s.e. mean baseline values for Rp (cmH_2O/LPS) and Cdyn (ml/cmH_2O), determined immediately before antigen administration, were, respectively; 1.22 ± 0.10 and 136 ± 9.4 for control (●; $n = 3$–4); 1.06 ± 0.18 and 115 ± 13.6 for mepyramine (○; $n = 3$). Symbols with vertical lines represent the mean ± s.e. mean. Numbers in parentheses associated with data points refer to the number of observations for that point only.

diphenhydramine did not influence antigen-induced increases in Rp (Figure 2), but did provide a small, significant ($P < 0.05$), inhibition of Cdyn decreases at 3 of 7 time periods. The modest effect of diphenhydramine on Cdyn decreases may suggest more of a statistical than physiological significance.

A second H_1-antihistamine, mepyramine (3.5 mg/kg, i.v.) administered before the antigen, similarly failed to inhibit ascaris-induced increases in Rp or decreases in Cdyn (Figure 3). In a second animal, the effect of mepyramine appeared to be one of enhancement ($P < 0.05$) of antigen-induced changes in Rp and Cdyn at all time periods (Figure 4). However, in this animal, mepyramine administration resulted in a significant ($P < 0.05$) decrease in baseline Rp values. Since such baseline alterations can markedly influence data calculated as percentage of control, an

attempt was made to 'correct' for this effect by using the Rp baseline values recorded immediately before mepyramine administration to calculate Rp changes. This baseline, which was 1.80 ± 0.10 cmH_2O/LPS (mean ± s.e. mean) did not differ significantly ($P > 0.05$) from the value of 1.87 ± 0.08 cmH_2O/LPS (mean ± s.e. mean) obtained with the drug vehicle. However, when the data were 'corrected' mepyramine still enhanced ($P < 0.05$) Rp changes at most time periods although the extent was diminished. In addition, Cdyn baseline values obtained after mepyramine differed significantly ($P < 0.05$) from the vehicle controls. However, in this instance, the difference appeared to be unrelated to mepyramine administration as baseline values determined immediately before mepyramine were 100 ± 4.87 ml/cmH_2O (mean ± s.e. mean) and were not significantly ($P > 0.05$) different from vehicle control or post-mepyramine values (Figure 4). The significant difference between baselines appears to be the result of the abnormally small variability of the control values; therefore, the data were not corrected. Thus, mepyramine appeared to enhance significantly ($P < 0.05$) antigen-induced decreases in Cdyn in this dog.

Effects of diphenhydramine and mepyramine on histamine-induced changes in Rp and Cdyn

Aerosol administration of a variable number of inhalations of a 5 mg/ml solution of histamine to the airways caused marked increases in Rp and decreases in Cdyn (Figure 5). Rp increases produced by 5 and 8 inhalations of histamine were approximately 2 to 4 fold greater than Rp increases produced by administration of ascaris antigen aerosol (cf. Figures 1 to 4).

Diphenhydramine at a dose equivalent to that which failed to inhibit antigen-induced changes in pulmonary mechanics, produced a marked attenuation of histamine-induced increases in Rp and decreases in Cdyn (Figure 5). Similarly, mepyramine, also at the dose lacking efficacy in the antigen provocation studies, abolished the effect of aerosol histamine on both Rp and Cdyn (Figure 5). The increase in Cdyn which accompanied aerosol administration of histamine after treatment with mepyramine is related to lung hyper-inflation by the respirator.

Correlation of histamine release in vivo *with pulmonary anaphylaxis*

Provocation of bronchially responsive dogs with ascaris antigen aerosol produced marked changes in Rp, Cdyn and TV (Figure 6). During anaphylaxis both systemic venous and arterial plasma histamine appeared to demonstrate a modest, but insignificant ($P > 0.05$) rise above prechallenge baselines. There was no significant difference ($P > 0.05$) between arterial and venous plasma levels at any time during anaphylaxis.

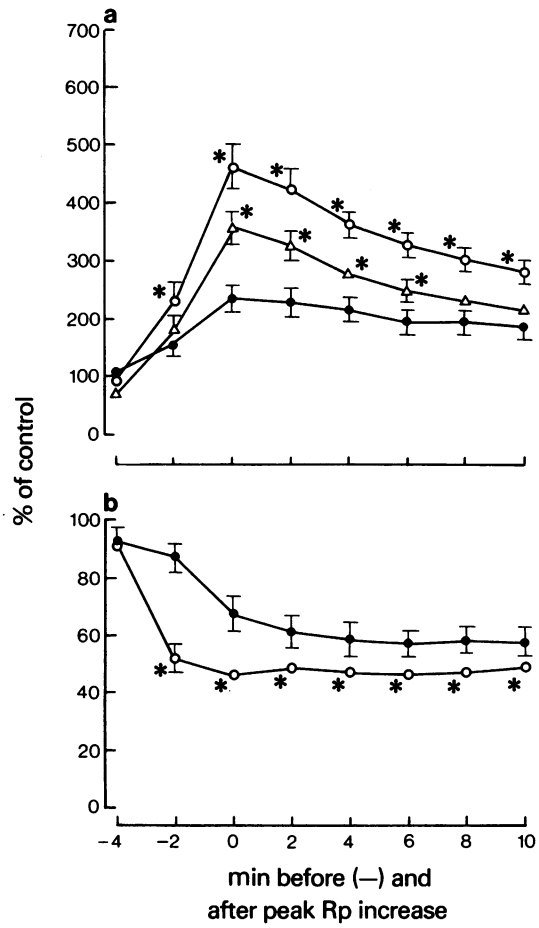

Figure 4 Effect of mepyramine on antigen-induced changes in (a) pulmonary resistance (Rp) and (b) dynamic lung compliance (Cdyn) in dog 4. Mepyramine (3.5 mg/kg i.v.) was administered 15 min before 2 inhalations of a 1:10 dilution of ascaris antigen. Mean ± s.e. mean baseline values for Rp (cmH_2O/LPS) and Cdyn (ml/cmH_2O), determined immediately before antigen administration, were, respectively: 1.87 ± 0.08 and 91 ± 1.2 for control (●; $n = 3$); 1.39 ± 0.09 and 108 ± 5.5 for mepyramine (○; $n = 3$). In this study mepyramine altered baseline Rp, consequently, Rp changes in the presence of mepyramine have been 'corrected' (△) by using the Rp baseline value obtained immediately before drug administration which was 1.80 ± 0.10 cmH_2O/LPS. Symbols with vertical lines represent the mean ± s.e. mean. * $P < 0.05$.

When a second antigen provocation was administered 30 min after the first, using twice the original

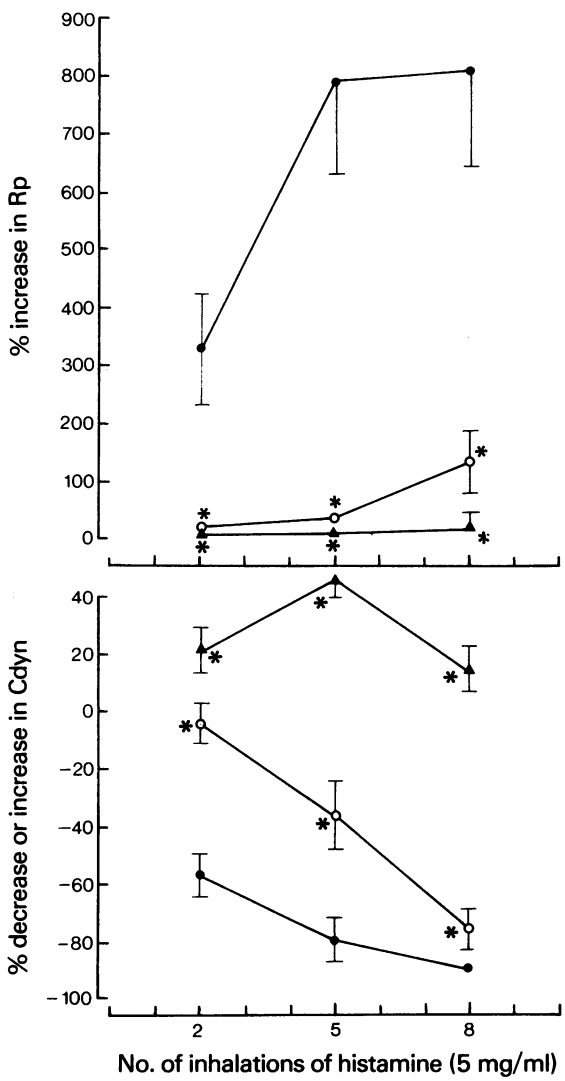

Figure 5 Effect of diphenhydramine and mepyramine on histamine-induced changes in Rp (pulmonary resistance) and Cdyn (dynamic lung compliance). Sequential aerosol histamine dose-response curves were obtained in control animals (●; n = 9–11) and after intravenous administration of diphenhydramine 2 mg/kg (○; n = 6) or mepyramine 3.5 mg/kg (▲; n = 6). *,$P < 0.05$.

number of antigen inhalations, comparatively modest increases in Rp resulted with virtually no further change in Cdyn or TV. The absence of change in Cdyn or TV in response to the second provocation may be related to the incomplete return to baseline which Cdyn and TV achieved during the interval between challenges. In this experiment, arterial histamine appeared to be elevated somewhat, but not significantly ($P > 0.05$), above venous levels. The arterial histamine profile observed with the second provocation suggested that, under the appropriate experimental conditions, ascaris antigen could release histamine *in vivo*.

In an effort to establish whether the antigen could cause histamine release *in vivo*, another series of experiments were undertaken. Antigen was administered for 5 min (52 to 92 inhalations of a 1:10 dilution of SAA) which was equivalent to about 5 to 9 times the challenge dose, as established in this laboratory, for provoking pathophysiological pulmonary responses. Pulmonary mechanics were not determined in these experiments as the severity of the antigen challenge resulted in respiratory arrest requiring artificial ventilation to prevent death. Figure 7 illustrates that, under these conditions, systemic arterial histamine increased substantially above systemic venous histamine during and immediately following challenge. However, significant differences ($P < 0.05$) were obtained at only one time period (5 minutes).

Removal of histamine from the circulation by the cardiopulmonary system

To determine if the canine cardiopulmonary system was capable of removing and/or metabolizing circulating histamine, the amine was infused into the femoral vein and systemic venous-arterial plasma differences across the heart and lung were determined. At the lowest infusion rate, 100 ng kg^{-1} min^{-1} (Figure 8), a significant ($P < 0.05$) difference between arterial and venous levels was noted only at the earliest sampling period. When the infusion rate was increased to 500 ng kg^{-1} min^{-1}, arterial plasma histamine levels were significantly ($P < 0.05$) lower than venous levels at three of the four time periods (Figure 8). Increasing the infusion rate to 1000 ng kg^{-1} min^{-1} abolished the significant differences between arterial and venous plasma levels seen at the lower rate although arterial histamine was consistently below venous histamine at all sampling points (Figure 8).

Discussion

Ascaris antigen challenge of canine lung fragments prepared from dogs cutaneously hypersensitive to this antigen caused a concentration-related histamine release *in vitro* (Krell & Chakrin, 1976b). This release appeared to be immunologically mediated as it could be enhanced by passive sensitization of the fragments with a heat-labile (56°C for 4 h) antibody obtained from dogs with a high serum reaginic titre directed against ascaris antigen (Krell & Chakrin, 1976b). The

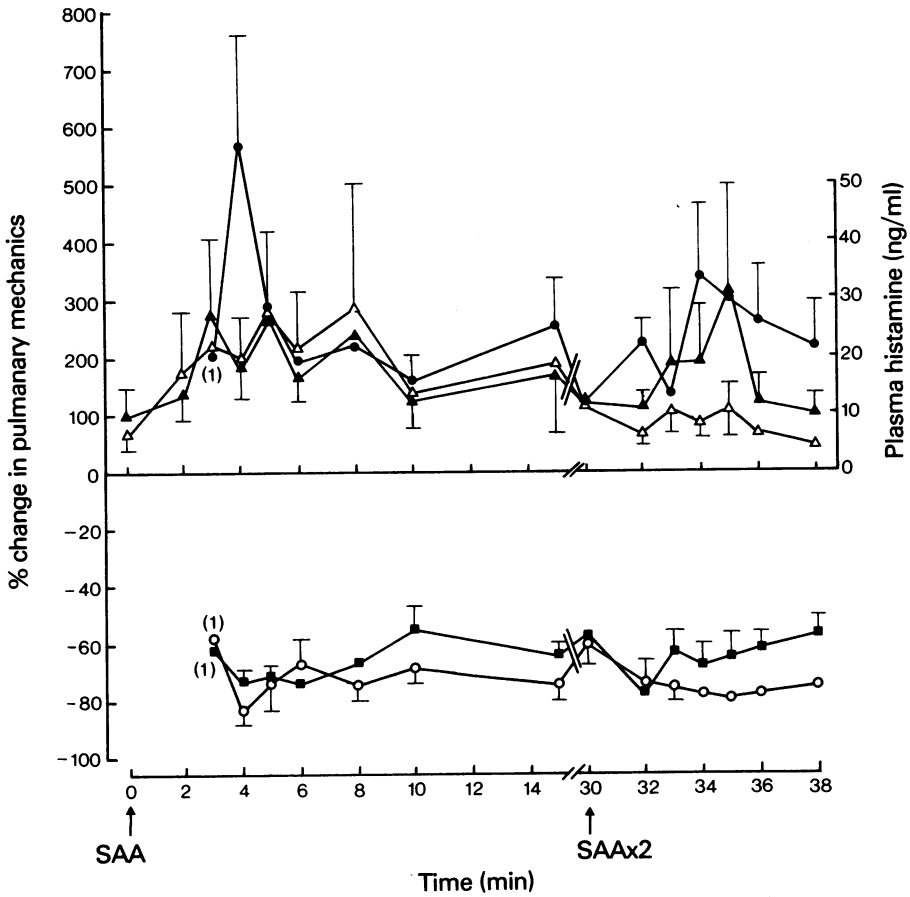

Figure 6 Correlation of plasma histamine levels with antigen-induced alterations in pulmonary mechanics in bronchially responsive dogs. Arterial and venous blood was sampled across the cardiopulmonary system following ascaris antigen-induced pulmonary anaphylaxis as described in Methods. The number of inhalations of antigen each animal received was determined from previously established pulmonary responses and varied from 5 inhalations of a 1:20 dilution of standard ascaris antigen to 10 inhalations of a 1:10. Thirty minutes after the initial challenge, each animal received a second aerosol administration of ascaris antigen at double the original number of inhalations. (●) Pulmonary resistance (n = 3–6); (○) dynamic lung compliance (n = 3–6); (■) tidal volume (n = 3–6); (▲) arterial histamine (n = 4–6); (△) venous histamine (n = 4–6). Symbols with vertical lines represent the mean ± s.e. mean. Numbers in parentheses associated with data points refer to the number of observations for that point only.

ability of ascaris antigen to release histamine from canine lung *in vitro* coupled with the well-known bronchoconstrictive action of this amine in the dog (Krell *et al.*, 1976; Krell & Chakrin, 1976a) provided circumstantial support for the hypothesis that histamine may be involved in ascaris antigen-induced bronchoconstriction in the hypersensitive dog.

The administration of two potent histamine H_1-receptor antagonists, diphenhydramine and mepyramine, before aerosol ascaris challenge failed to inhibit antigen-induced constriction of large (Rp) or small (Cdyn) airways. Indeed, in one animal mepyramine caused a significant enhancement of the pulmonary pathophysiology. Since neither diphenhydramine or mepyramine influenced antigen-induced release of histamine or slow reacting substance of anaphylaxis (SRS-A) from canine lung *in vitro* (Krell & Chakrin, unpublished) it is, at present, difficult to account for this observation. Nevertheless, the inability of the H_1-antihistamines to inhibit antigen effects on the airways cannot be attributed to ineffective doses as both agents markedly attenuated aerosol his-

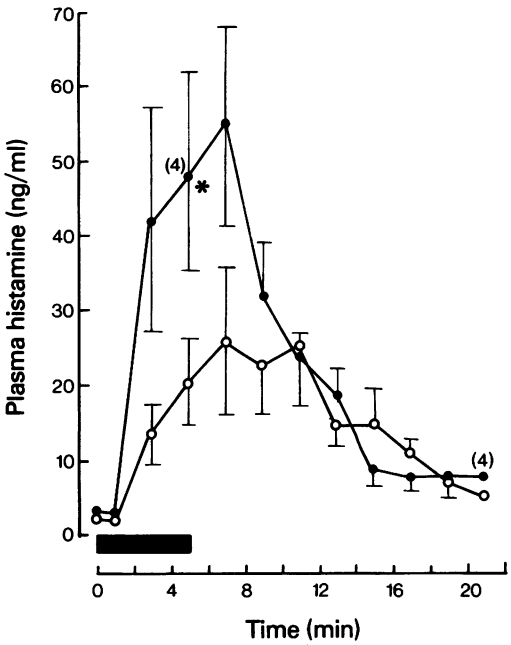

Figure 7 Histamine release from lung by aerosol ascaris antigen challenge of artificially ventilated dogs. Ascaris antigen was aerosolized into the airways for 5 min (solid bar). Systemic arterial (●) and venous (○) blood across the cardiopulmonary system was sampled as described in Methods. Symbols with vertical lines represent the mean ± s.e. mean of 5–6 observations. Numbers in parentheses associated with data points refer to the number of observations for that point only. * $P < 0.05$.

tamine-induced changes in pulmonary mechanics that were 2 to 4 times greater than those obtained with antigen. These observations are in agreement with similar studies conducted in isolated tissues (Hawkins & Rosa, 1956). The lack of effect of these H_1-antihistamines suggests that histamine, at least through interaction with H_1-receptors may not mediate acute antigen-induced bronchoconstriction in the dog.

Previous observations (Krell & Chakrin, 1977) have established that the histamine H_2-receptor antagonist, metiamide (Black, Duncan, Emmett, Ganellin, Hesselbo, Parsons & Wyllie, 1973), administered before aerosol antigen challenge at doses ranging from 3 to 16 times the ED_{50} for inhibition of histamine-induced gastric acid secretion, was likewise without effect on the antigen-induced pulmonary pathophysiology. Hence, using pharmacological techniques, a role for histamine in the acute antigen-induced bronchospasm could not be demonstrated.

In an effort to establish a biochemical correlate to these pharmacological observations an attempt was

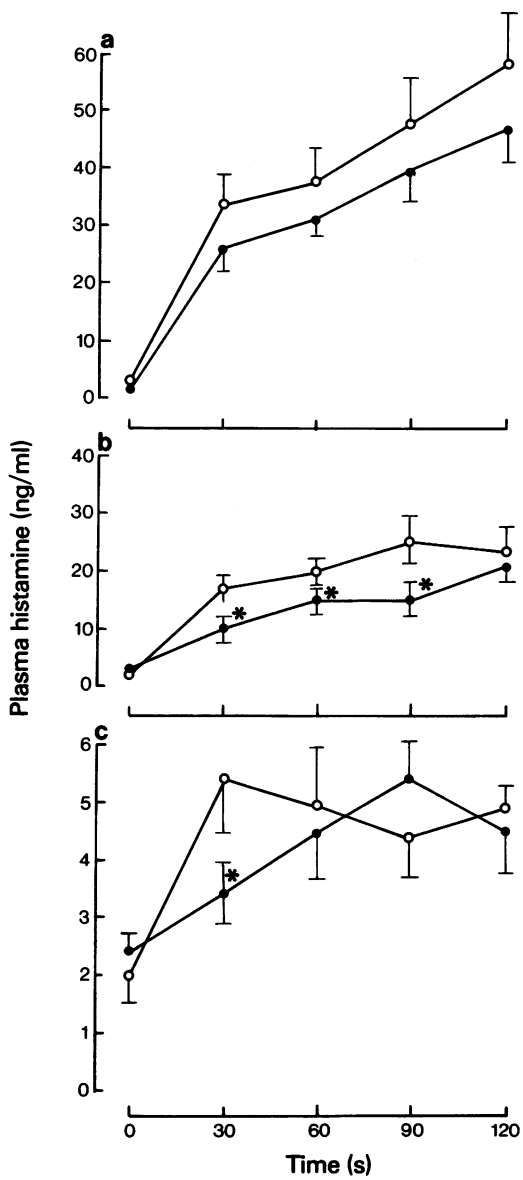

Figure 8 Removal of histamine from the circulation by the cardiopulmonary system. Histamine was infused into the femoral vein during which time arterial (●) and venous (⊙) blood was sampled across the cardiopulmonary system as described in Methods. Each animal received 3 infusions: (a) 1,000 ng kg^{-1} min^{-1} (n = 5–6); (b) 500 ng kg^{-1} min^{-1} (n = 5–7) and (c) 100 ng kg^{-1} min^{-1} (n = 6–7), beginning with the lowest and proceeding to the highest, 30 min apart. Symbols with vertical lines represent the mean ± s.e. mean. * $P < 0.05$.

made to determine whether ascaris antigen could evoke histamine release *in vivo*. Antigen doses which provided substantial pathophysiological pulmonary responses, and which are routinely used as standard provocation doses in this laboratory, did not result in the detectable release of histamine from the cardiopulmonary system. While these results were consistent with the lack of effect of H_1- and H_2-antihistamines, they were clearly incongruous with the demonstrated *in vitro* ability of ascaris antigen to release histamine from fragmented canine lung. In an attempt to clarify this apparent contradiction additional experiments were undertaken in which larger doses of the antigen were used. At these elevated doses, which ranged from 5 to 9 times standard provocation regimens, histamine release from the cardiopulmonary system was evident.

The absence of significant histamine release *in vivo* by standard aerosol doses of antigen could be accounted for by efficient processes of removal and/or metabolism by the cardiopulmonary system subsequent to release. However, infusion of histamine and measurement of the disappearance of the amine across the cardiopulmonary system failed to provide evidence consistent with this concept. At best, the cardiopulmonary system of the dog displays a modest ability to remove and/or degrade circulating histamine and would therefore be unlikely to account for the apparent absence of the amine in arterial plasma during anaphylaxis. The modest ability of the cardiopulmonary system to effect the removal of circulating histamine is consistent with previous observations (Ferreira, Ng & Vane, 1973; Krell & Chakrin, 1978). It would appear from these observations that *in vitro* conditions established to provoke histamine release may not be qualitatively appropriate to the *in vivo* model.

One criticism of the present experimental design is that the failure to detect histamine release *in vivo*, with standard doses of antigen, may relate to the route of antigen administration. For example, aerosol administration of the antigen may be presumed to cause histamine release on or near the mucosal surface of the airways. Amine released in this proximity may be degraded and/or removed by lumenal or submucosal processes which prevent the appearance of the unchanged amine in the circulation. Such processes may not have been adequately assessed by the histamine infusion experiments. However, if such were the case, studies with H_1-receptor antagonists would be expected to have provided positive results since H_1-antihistamines clearly antagonized the effect of aerosol histamine on the airways. As this was not the case, it must be concluded that ascaris antigen is capable of producing substantial pathophysiological pulmonary responses at doses considerably below those necessary to provoke histamine release. Consequently, it would appear that under these conditions histamine may not be involved in the pulmonary pathophysiological responses produced by ascaris antigen aerosol.

The results obtained on the ability of ascaris antigen to provoke histamine release *in vivo* are virtually identical to those obtained by Chiesa, Dain, Myers, Kessler & Gold (1975) although the conclusions drawn are opposite. The difference in interpretation relates principally to experimental design. These investigators routinely employ a prolonged antigen exposure, 5 to 10 min, to induce the pulmonary anaphylaxis, conditions under which histamine release appeared to correlate with the extent of the airways response. However, they also noted that less severe antigen provocation resulted in significant changes in pulmonary mechanics that were not accompanied by detectable histamine release *in vivo*. Although these authors did not evaluate the effect of H_1-antihistamines, they concluded that histamine, presumably released in undetectable amounts, may have accounted for the pulmonary responses to low dose antigen. While this concept bears relationship to Dale's (1948) hypothesis that 'intrinsic' histamine released on anaphylaxis may be more effective in stimulating tissues than exogenous, 'extrinsic', histamine, an alternative conclusion may be that other putative mediators such as SRS-A, prostaglandins etc. should be evaluated for a possible role in this response.

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