INVOLVEMENT OF HISTAMINE H1- AND H2-RECEPTORS IN INDUCED ASTHMAS IN DOGS

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Abstract—Participation of histamine H1- and H2-receptors in both asthmas, i.e. experimentally induced bronchoconstriction and bronchosecretion, with ascaris suum and histamine in anesthetized dogs was investigated. Dogs given 0.2% histamine solution or ascaris antigen (3 mg protein) by inhalation showed increases in respiratory resistance (Rrs) and respiratory rate to 2.5–5.0 fold. Airway secretion volume was also significantly increased 3–4 fold. The increase in Rrs by histamine inhalation was effectively inhibited or abolished by a histamine H1-receptor antagonist, chlorpheniramine (0.3–1 mg/kg i.v.), but not by a H2-receptor antagonist, cimetidine (1–3 mg/kg i.v.). The increase in Rrs by antigen inhalation was reduced by relatively high doses of chlorpheniramine (1–3 mg/kg i.v.), and not by cimetidine. In contrast, hypersecretion of tracheobronchial fluid in both asthmatics was significantly prevented by either chlorpheniramine or cimetidine. Combinations of both antagonists abolished the hypersecretion. Atropine (2 mg/kg i.v.) significantly inhibited the occurrence of responses in both asthmatics. The results suggest that histamine is involved in the allergic asthma produced by ascaris suum and that histamine directly evokes airway constriction through H1-receptors and hypersecretion of tracheobronchial fluid through H1- and H2-receptors, and, in a part, indirectly activates the cholinergic pathway.

There has been much evidence that histamine is the chief chemical mediator released from mast cells during anaphylaxis (1–9). Experimental bronchial asthma in guinea pigs is prominently inhibited by classical antihistamines (10–12). Schild et al. (3) showed unequivocally that histamine release occurs in bronchial tissue of an asthmatic patient.

Ash and Schild (13) demonstrated two types of histamine receptors and Black et al. (14) introduced specific antagonists of these receptors which made it possible to characterize and locate these receptors in the body. Data of the presence of H1- and H2-receptors in airway smooth muscle in different species have been documented, and are cited in the review of Chand and Eyre (15). There are, however, specifically no reports on the distribution of these receptors in the dog airway smooth muscle or on the role of these receptors in bronchosecretion. We attempted to characterize histamine receptors responsible for airway constriction and hypersecretion which occurs in ascaris- and histamine-induced asthmas, using histamine H1- and H2-receptor antagonists.

MATERIALS AND METHODS

Male mongrel dogs weighing between 6 and 17 kg were anesthetized with sodium...
pentobarbital (30 mg/kg i.v.). Ascaris suum antigen (Asc-Ag) was prepared with veronal buffered saline (VBS) as described in a previous paper (16). Allergically asthmatic responses were produced with Asc-Ag inhalation in dogs naturally sensitive to Asc-Ag. Dogs that showed positive skin reaction to Asc-Ag solution at a low concentration (below 10^{-5} g protein/ml) were used as naturally sensitized ones; the incidence ratio being 88% of all dogs used herein.

To produce allergic asthma, Asc-Ag, 3 mg protein, was given by inhalation for 10 min, and to produce histamine asthma, 0.2% histamine was for 15 min (total histamine dose inhaled, 3 mg) using an ultrasonic nebulizer (TUR-3000, Nihon Kohden, Co., Ltd.) via a branch of inserted tracheal cannula. The resistance of the total respiratory system (Rrs) was measured by a forced oscillation method using a respiratory resistance meter (MRP-6, Nihon Kohden, Co., Ltd.; oscillation frequency, 4 Hz) connected with an endotracheal tube, every 5-10 min during 70 min after the antigen or histamine inhalation. Respiratory movement was recorded as impedance change using a respiratory pick-up (MCR-2TA, Nihon Kohden, Co., Ltd.) applied around the thorax of animals. Femoral arterial pressure and heart rate were also recorded using a pressure transducer and a tachometer. Sixty min after termination of antigen or histamine inhalation, the volume of airway secretion accumulating in the trachea (about 20 cm between the incised area for insertion of the endotracheal cannulae and the bifurcatio trachea) was collected with a spatula after making a midline incision in the trachea, and was then sucked up and measured with a syringe of 0.25 or 0.5 cc. Pretreatment drugs such as chlorpheniramine, cimetidine and atropine were injected i.v. into the cephalic vein 10 min before start of the antigen or histamine inhalation. None of the three drugs produced any change in Rrs.

Results are given as the mean with S.E.. Student's t-test was used for statistical analysis, and differences were considered significant if P < 0.05.

The drugs uses were: histamine dihydrochloride (Wako Pure Chemicals); chlorpheniramine maleate (Sankyo); cimetidine (Smith, Kline & French); atropine sulfate (Tokyo Kasei). Solutions of cimetidine were prepared by dissolving the base in a small volume of 0.1 N HCl, neutralizing the solution with 0.1 N NaOH and making up to required volume with 0.9% NaCl solution immediately before use. All doses were expressed as salts except for cimetidine.

RESULTS

Asthmatic bronchoconstrictions induced by histamine and Asc-Ag

Typical recordings of the effects of inhaled Asc-Ag, 3 mg protein for 10 min, and 0.2% histamine for 15 min are shown in Fig. 1. An Asc-Ag aerosol caused significant increases in Rrs and respiratory rate, accompanied by an increase in pulse pressure, bradycardia and sometimes arrhythmias. Similar responses were observed with histamine aerosol. Fig. 2 shows the time course of changes in Rrs during both asthmas in five to eight dogs. The increase in Rrs in either asthma reached a peak immediately after the end of inhalation, the change being from 5.0 to 14.7 in allergic asthma and from 3.7 to 14.5 cm H2O/L/sec in histo-
Effects of inhalation administrations of ascaris antigen (Asc-Ag) and histamine in anesthetized dogs. B.P., femoral arterial blood pressure; H.R., heart rate; Rrs, respiratory resistance; Resp., respiratory movement. Asc-Ag, 3 mg protein for 10 min, and 0.2% histamine for 15 min were inhaled in two different animals.

FIG. 2. Effects of ascaris antigen (Ag) and histamine (Hist) inhalations on respiratory resistance (Rrs) in dogs. Each point represents the mean of eight dogs for antigen group, and of five dogs for either veronal buffered saline (VBS) control or histamine group.

Effects of antihistamines on asthmatic bronchoconstrictions

Effects of pretreatment drugs were estimated with the total effect on respiratory resistance (JRrs) evoked by Asc-Ag or histamine aerosol expressed as the area delineated by the graph and over the pre-inhalation control level for 70 min after inhalation.

In histamine induced asthma: Chlorpheniramine (CP), a histamine H1-receptor antagonist, in a dose of 0.3 mg/kg significantly inhibited, and in a dose of 1 mg/kg abolished the increase in JRrs (22± 4) by histamine. On the other hand, cimetidine (CM), a histamine H2-receptor antagonist, in doses of 1–3 mg/kg rather tended to potentiate the increase in JRrs induced by histamine. Atropine (2 mg/kg) inhibited the airway constriction by histamine by 50% (Fig. 3).

In Asc-Ag induced asthma: CP in doses of 1–3 mg/kg which were about 10 times as
high doses as being used in histamine asthma, inhibited the increase in JRrs (30 ± 5) by Asc-Ag dose-dependently, but did not abolish the response as was seen in histamine asthma. CM in doses of 1–3 mg/kg had almost no effect on Asc-Ag induced bronchoconstriction. Combinations of both antagonists (CP 1 mg/kg plus CM 3 mg/kg, CP 3 mg/kg plus CM 3 mg/kg) had similar inhibitory effects as were seen in administration of CP alone. Atropine (2 mg/kg) inhibited the airway constriction by antigen by 73%; (Fig. 4).

Hypersecretion of tracheobronchial fluid in asthma

In histamine induced asthma: In the control group given VBS aerosol instead of histamine or Asc-Ag, the volume of tracheobronchial fluid secreted for 60 min was 0.07 ± 0.03 ml. Histamine inhalation increased airway secretion to 0.33 ± 0.07 ml. Pretreatment with either CP or CM significantly inhibited the hypersecretion by histamine. Atropine also showed a significant inhibition against histamine (Fig. 5).
FIG. 5. Effects of antihistamines and atropine on the increase in airway secretion produced by histamine (Hist) inhalation in dogs. Ordinate: airway secretion volume for 60 min after the end of 15 min inhalation of 0.2% histamine solution. VBS, veronal buffered saline; CP, chlorpheniramine; CM, cimetidine; and Atro, atropine. Figures in parenthesis express the number of experiments. *Significant at P<0.05, and **at P<0.01.

FIG. 6. Effects of antihistamines and atropine on the increase in airway secretion produced by ascaris antigen (Ag, 3 mg protein) inhalation in dogs. Explanations as in Fig. 5.

In Asc-Ag induced asthma: Asc-Ag inhalation increased airway secretion to 0.27±0.03 ml. Pretreatment with either CP or CM markedly inhibited the hypersecretion by Asc-Ag. CM appeared to have a stronger effect than CP. A combination of CP and CM almost abolished the increase in secretory activity evoked by antigen inhalation. Atropine also showed a significant inhibition against the antigen (Fig. 6).

DISCUSSION

Experimental bronchial asthma produced by ascaris suum was found to result from reaginic antibodies in dogs (17) and in monkeys (9, 18). Inhalation of cromolyn prominently inhibits the increase in respiratory resistance produced by exposure of Asc-Ag aerosol (16). Thus it is suggested that histamine released from mast cells plays an important role in the asthmatic reactions by Asc-Ag. The significance of histamine in ascaris-induced asthma was confirmed from the present study in which antihistamines were used. Respiratory anaphylactic responses caused by antigen inhalation such as increases in airway resistance,
respiratory rate and secretory activity resembled the responses caused by histamine inhalation. The sustained maintenance of antigen-induced responses may be ascribed to continuous release of chemical mediators including histamine from mast cells during antigen-antibody reactions. The role of SRS-A which has a delayed action cannot of course be ruled out.

A part of airway reactions evoked by histamine are known to occur as the result of the vagal reflex in humans (19-21), in rabbits (22) and in dogs (23, 24) as well as by direct musculotropic action. The present result that histamine-induced asthma was significantly inhibited by pretreatment of atropine parallels the above findings. Asthmatic responses evoked by ascaris antigen in dogs (16, 25) and by grass pollen or house dust allergen in humans (26) were found to be also, at least in a part, mediated via the vagal reflex. The effects of histamine and ascaris antigen challenge which could not be blocked by an adequate dose (2 mg/kg) of atropine may be the direct action of histamine on the airway smooth muscle and secretory gland.

There are reports concerning characterization of histamine receptors on the airway tissues. Eyre (27) found that the relaxation responses of the isolated sheep bronchus and cat trachea to histamine were antagonized by burimamide and not by mepyramine, suggesting the presence of H₂-receptors on those tissues. An increase in bronchial resistance by histamine in guinea pigs is inhibited by promethazine, and potentiated by burimamide (28). Thus, in some species, histamine constricts the airway smooth muscle through histamine H₁-receptors and dilates it through H₂-receptors. In our results, the increase in respiratory resistance produced by histamine inhalation was effectively inhibited or abolished by chlorpheniramine, and tended to be potentiated by cimetidine. The increase in respiratory resistance produced by ascaris antigen was also reduced by chlorpheniramine, but not significantly influenced by cimetidine. The findings suggest that bronchoconstriction evoked by antigen-antibody reactions is, at least in a part, mediated through histamine H₁-receptors, and there may be H₂-receptors which mediate bronchodilation in dogs.

As to tracheobronchial secretions, the secretory activity was enhanced by ascaris antigen challenge (16) and histamine which was effectively antagonized by either chlorpheniramine or cimetidine, suggesting that both H₁- and H₂-receptors are responsible for the hypersecretion. The action of histamine on the glands may, however, be mediated via a cholinergic pathway, since atropine significantly inhibited the hypersecretion by histamine.

Itkin and Anand reported that inhalation of diphenhydramine could block the airway obstruction by allergen inhalation challenge in most cases of bronchial asthmatic patients (29). From the present findings, it is considered that administration of antihistamines to asthmatics may well be reinvestigated; H₁-receptor antagonist for attenuation of bronchoconstriction and H₂- and H₁-receptor antagonists for that of hypersecretion in asthma.

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