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C5a INDUCED TRACHEAL CONTRACTION: A HISTAMINE INDEPENDENT MECHANISM[1]

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C5a, a peptide derived from the fifth component of complement, caused significant prolonged smooth muscle contraction in isolated guinea pig trachea. Diphenhydramine, a histamine receptor antagonist of the H_1 type, had no effect on either the rate, amplitude or duration of C5a induced tracheal contraction, whereas it significantly inhibited the tracheal response to exogenous histamine. Diphenhydramine also caused a significant delay in the response to compound 48/80 in normal guinea pig trachea and to antigen in actively sensitized trachea, indicating that the antihistamine was capable of inhibiting tracheal contractions to endogenously released histamine. C5a induced tracheal smooth muscle contraction was also unaffected by antagonists of H_2, muscarinic cholinergic and *alpha* adrenergic receptors. These results indicate that C5a induced tracheal contraction is independent of histamine and is potentially a mediator of allergic bronchospasm.

Cleavage of the complement (C) proteins C3 and C5 by activation of the C system yields two polypeptide fragments, C3a and C5a (anaphylatoxins), which when purified from human serum have m.w. of 9000 and 11,000, respectively (1). Biologic activities of C3a and C5a include histamine release from mast cells, an increase in vascular permeability, as well as contraction of smooth muscle from various organs including respiratory smooth muscle. The ability of crude guinea pig anaphylatoxin to cause respiratory obstruction and death on i.v. injection in guinea pigs was documented in 1922 (2). More recently, Bodammer and Vogt (3, 4) quantitated the bronchospastic effect of i.v. injected hog anaphylatoxin in spontaneously breathing, urethane-anesthetized guinea pigs. They found that the anaphylatoxin-induced bronchospasm was reduced by tripelennamine,, a histamine antagonist of the H_1 type. In addition, anaphylatoxins purified from human serum are reported to contract the isolated guinea pig trachea (1). Although anaphylatoxin has been shown to release histamine as well as a substance with prostaglandin-like activity from perfused guinea pig lung (5–7), the involvement of such released substances in the anaphylatoxin-induced bronchospasm is unclear. In the present studies we have quantitated the contraction of isolated guinea pig tracheal muscle by C5a and demonstrated that neither the rate, amplitude, nor duration of the C5a-induced tracheal contraction is affected by a histamine antagonist of the H_1 type.

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MATERIALS AND METHODS

Purification of C5a. Guinea pig anaphylatoxin was partially purified from yeast-activated guinea pig serum in the presence of 1 M ϵ-aminocaproic acid by the method of Fernandez and Hugli for human C5a (8). The guinea pig anaphylatoxin isolated was chemotactic for mouse peritoneal macrophages with a modified Boyden method (9, 10). Disc electrophoresis of partially purified anaphylatoxin in 6% polyacrylamide gels, pH 4.3 (11) revealed five major bands. Ileum-contracting activity was eluted from only one of these bands. Anaphylatoxin was identified as a C5 derivative (C5a) by using rabbit anti-guinea pig C5 and rabbit anti-guinea pig C3 as described by Snyderman *et al.* (12). The ileum-contracting activity of anaphylatoxin was inactivated by rabbit anti-guinea pig C5 but not by rabbit anti-bovine serum albumin or rabbit anti-guinea pig C3. This partially purified guinea pig C5a was utilized in the following studies with isolated guinea pig trachea.

Preparation of Tissue. Male Albany strain guinea pigs (a closed colony maintained by the New York State Department of Health, Division of Laboratories and Research) of 300 to 500 g were anesthetized by pentobarbital injection (90 mg/kg i.p.). Tracheas were removed and cut into rings approximately 2-mm wide. The ventral cartilage of the rings was cut and strip-like preparations of tracheal smooth muscle were mounted in a 37°C tissue bath containing 5 ml Krebs-Henseleit solution vigorously bubbled with 95% O_2, 5% CO_2. The composition of the Krebs-Henseleit solution was (in mM): NaCl, 122.4; KCl, 4.56; KH_2PO_4, 1.15; $MgSO_4 \cdot 7H_2O$, 1.17; $CaCl_2 \cdot 2H_2O$, 2.53; $NaHCO_3$, 24.7; glucose, 5.5. An initial resting tension of 1.5 to 2.5 g was applied and the tracheal strips were equilibrated for 90 min.

RESULTS

Except in tachyphylaxis experiments, each tracheal strip was exposed to C5a only once. Isometric contractions were measured and results were expressed as the maximum change in tension in grams after C5a or agonist addition (mean ± S.E. of three to eight experiments in different tracheal strips). The maximum response to acetylcholine (1.1×10^{-5} M), histamine (1.7×10^{-5} M), and serotonin (6.4×10^{-6} M) in the tracheal strips was 1.60 ± 0.16 g, 1.49 ± 0.12 g, and 0.68 ± 0.06 g, respectively.

As little as 0.18 μg/ml C5a resulted in a detectable contraction of the tracheal strips (0.25 ± 0.11 g). The maximum response that occurred within 6 min after addition of C5a (1.08 ± 0.25 g) was attained by using 1.8 μg/ml C5a and represents 57.5 ± 10.7% of a maximum response to acetylcholine. The smooth muscle tone spontaneously returned to pre-C5a values in 40 ± 3.2 min, and washing 10 min after exposure to C5a did not significantly affect the duration of the tracheal contraction. A second exposure of the same tracheal strip to C5a (1.8 μg/ml) caused a contraction that was 43.2 ± 10.4% less than the initial response.

The effect of an antihistamine on the contraction of the isolated trachea by C5a was examined (Fig. 1) in order to determine if released histamine was responsible for the contraction. Although diphenhydramine (10^{-5} M), an H_1 receptor antagonist, significantly reduced the response of the trachea to a supramaximal dose of histamine (4.3×10^{-5} M), it failed to affect the amplitude or rate of the tracheal response to C5a (Figs. 1 and 2). This is in contrast to our own and results of others (13), which indicates that C5a-induced contraction of guinea pig ileum is prevented by H_1 receptor antagonists.

To ensure that the antihistamine employed was capable of inhibiting smooth muscle contraction due to locally released histamine, the effect of diphenhydramine (10^{-5} M) on the contraction of trachea by antigen and by compound 48/80, a condensation product of *N*-methyl *p*-methoxyphenethylamine with formaldehyde, was investigated (Fig. 2). Histamine release by antigen from sensitized guinea pig lung and by compound 48/80 from normal guinea pig lung has been demonstrated (14–17). In addition, compound 48/80 has been shown to release a lipid soluble acid, i.e., a "slow reacting substance" from cat paw (18), which is similar in its properties to slow reacting substance of anaphylaxis produced by antigen challenge of sensitized tissues. The concentrations of C5a, compound 48/80, and antigen that were employed produced contractions that were near the maximum attainable for each agent. The rate and amplitude of the tracheal contraction to C5a was unaffected by diphenhydramine (Fig. 2). However, the antihistamine did cause a significant delay in the onset of the contraction induced by compound 48/80 in normal guinea pig trachea and by ovalbumin in actively sensitized guinea pig trachea. A similar effect of diphenhydramine on the initial phase of antigen-induced bronchoconstriction in passively sensitized guinea pig tracheal rings has been reported (19, 20). At higher concentrations, diphenhydramine was no longer a specific antagonist of the histamine response. The H_2 receptor antagonist metiamide (10^{-4} M) also failed to affect significantly the response of the trachea to C5a.

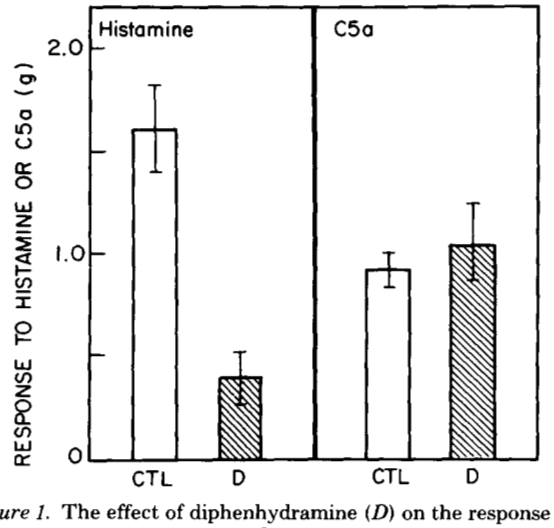

Figure 1. The effect of diphenhydramine (*D*) on the response of the trachea to histamine (4.3×10^{-5} M) or C5a (1.8 μg/ml). Each bar represents the mean ± S.E. of three to eight experiments in different tracheal strips. CTL, no antagonist; D, diphenhydramine (10^{-5} M). D was added to the tissue bath 10 min before C5a or histamine, and the maximum contractile response in grams (*g*) determined in the presence of the antagonist. D itself did not significantly alter the smooth muscle tone.

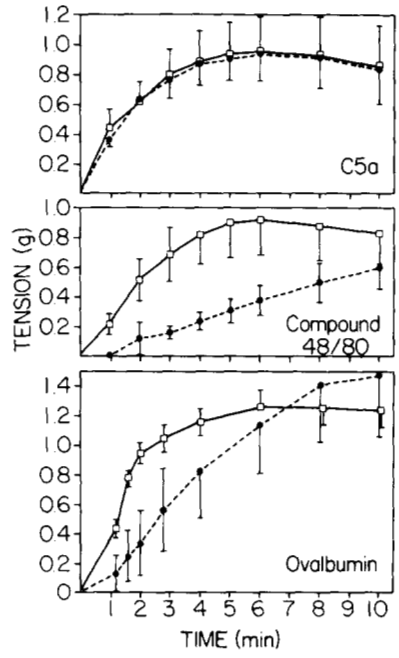

Figure 2. The effect of diphenhydramine (10^{-5} M) on the response of the guinea pig trachea to C5a, compound 48/80, and antigen. Each bar represents the mean ± S.E. of three to eight experiments in different tracheal strips. Tissues were incubated with (●-----●) or without (□——□) diphenhydramine for 10 min and the response to C5a (1.8 μg/ml), compound 48/80 (20 μg/ml), or ovalbumin (50 ng/ml) was determined. Ovalbumin-induced tracheal contraction was determined on day 26 in animals that had received i.p. injections of ovalbumin (1 mg/kg) on days 1, 3, and 5.

The muscarinic cholinergic receptor antagonist atropine and the α adrenergic antagonist phentolamine were ineffective in preventing the contraction of the trachea induced by C5a. These results indicate that the C5a-induced contraction is not due to the action of released acetylcholine or catecholamines or to interaction of C5a with the muscarinic cholinergic or α adrenergic receptors.

DISCUSSION

Diphenhydramine was capable of inhibiting tracheal contractions to exogenous histamine, as well as locally released histamine, but was ineffective in reducing C5a-induced tracheal contraction. C5a has been demonstrated, under optimal conditions, to release up to 50% of total histamine from human basophils, whereas antigen-induced release can reach 80% (21). Also, C5a has been reported to release up to 88% of the total histamine content of guinea pig mast cells (22). Thus, one would predict that the amount of histamine released by C5a in the trachea would be comparable to that released by antigen, and that the concentration of diphenhydramine employed would be adequate to antagonize the C5a-induced contraction if it were due to histamine. Thus, although histamine may be released during tracheal exposure to C5a, the failure of diphenhydramine

to block the resultant smooth muscle contraction indicates that histamine is not the sole mediator.

The lowest concentration of partially purified guinea pig C5a capable of producing tracheal contraction is 0.18 μg C5a/ml. Since the estimated maximum attainable C5a concentration in guinea pig serum is approximately 5 μg/ml (23), local production of C5a in the lung could be sufficient to cause significant prolonged smooth muscle contraction. Although the IgE-mast cell system is currently viewed as the major mechanism mediating allergic reactions, there are some reports suggesting that C activation occurs after antigen challenge in some asthmatics (24–27). The fact that this apparent C activation occurs concurrently with the decreased forced expiratory volume, combined with the observation that anaphylatoxin induces bronchospasm in experimental animals, implicates C5a and/or C3a as possible additional mediators of bronchospasm in asthma.

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