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THE EFFECTS OF HAEMOPHILUS INFLUENZAE VACCINATION ON ANAPHYLACTIC MEDIATOR RELEASE AND ISOPRENALINE-INDUCED INHIBITION OF MEDIATOR RELEASE

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The influence of Haemophilus influenzae on anaphylactic mediator release from ovalbumin-sensitized isolated guinea pig lungs was investigated. Lungs from H. influenzae-vaccinated animals released prostaglandins and thromboxanes following a smaller dose of ovalbumin than was effective in non-vaccinated animals. Histamine release was significantly increased in 4 day-vaccinated animals but not 1 or 10 days after vaccination, while bronchoconstriction was potentiated in 1 and in 4 day-vaccinated animals. This increased histamine release was achieved following 2 μg ovalbumin. In contrast, doses of 10 μg and 1 mg ovalbumin respectively did not affect and decreased histamine release in the vaccinated group. The inhibition of anaphylactic mediator release by an infusion of 6×10^{-9} M isoprenaline was significantly attenuated by H. influenzae vaccination. These results indicate an increased sensitivity to antigenic challenge and suggest that the functioning of β-adrenoceptors was decreased as a result of H. influenzae vaccination.

Haemophilus influenzae Anaphylactic shock Prostaglandins Thromboxane A_2 Histamine

1. Introduction

Some of the clinical manifestations of asthma may be ascribed to the anaphylactic release of endogenous chemical mediators. In the guinea pig, mediators of anaphylaxis are histamine, slow reacting substance of anaphylaxis (SRS-A), prostaglandins (PGs), prostaglandin endoperoxides and thromboxane A_2 (Brocklehurst, 1960; Dawson et al., 1976; Piper and Vane, 1969; Svensson et al., 1975).

Besides immunological hypersensitivity, another fundamental characteristic of asthma is the bronchial hyperreactivity. Szentivanyi (1968) postulated, in this respect, that the pathogenesis of atopy was due to a malfunctioning of β-adrenergic receptors resulting in an imbalance of α- and β-adrenoceptors. Furthermore the atopic abnormality in bronchial asthma was found to be closely associated with respiratory infection. Several other investigators also consider infection as an important aetiological factor in the pathogenesis of asthma (Aas, 1969; Williams et al., 1958). Experimental evidence supporting this hypothesis is partly based on the fact that vaccination with Bordetella pertussis shows symptoms generally observed in asthmatic patients, like eosinophilia (Morse, 1974; Szentivanyi, 1968), hypersensitivity to a number of pharmacological mediators, and a decreased hyperglycemic response to epinephrine (Fishel and Szentivanyi, 1963). However, the limitation on the use of B. per-

tussis is that a causative relation with human bronchial asthma is lacking. On the other hand Haemophilus influenzae can be isolated from the upper airways of normal individuals and also from the deeper airways of asthmatic patients (see Hirshmann and Everett, 1979; Van der Zwan, 1976).

We studied the influence of H. influenzae on the anaphylactic release of prostaglandins, thromboxanes and histamine, and also on the effect of isoprenaline in isolated guinea pig lungs.

2. Materials and methods

2.1. Lung perfusion and bioassay

Male guinea pigs weighing between 300 and 350 g were sensitized with ovalbumin, 100 mg given subcutaneously (s.c.) and 100 mg intraperitoneally (i.p.). After three weeks the animals were anesthetized with pentobarbital and the lungs were removed, suspended in a chamber and perfused through the pulmonary artery with Krebs bicarbonate at 10 ml/min as previously described (Piper and Vane, 1969). For the bioassay of prostaglandins, prostaglandin endoperoxides and thromboxanes, the effluent from the lung was used to superfuse a strip of rabbit aorta, rat stomach and rat colon (Piper and Vane, 1969). The assay tissues were treated with a combination of antagonists (Gilmore et al., 1968) which prevented the actions of histamine, acetylcholine, catecholamines and 5-hydroxytryptamine. Indomethacin (1 μg/ml) was also added to the lung effluent to prevent endogenous prostaglandin generation. Contraction of the tissues was detected with a Harvard smooth muscle transducer and displayed on a multichannel Watanabe pen recorder.

2.2. Vaccination with H. influenzae

The stock suspensions of H. influenzae contained 5×10^9 killed cells per ml in sterile, non-pyrogenic saline (0.9% NaCl solution). The guinea pigs were injected with the suspension, 0.1 mg/100 g body weight (i.p.) given 1, 4 and 10 days prior to the experiments.

2.3. Measurement of anaphylactic release of prostaglandins, thromboxanes and histamine

Arachidonic acid injected in the pulmonary artery leads to the formation of prostaglandins, prostaglandin endoperoxides and thromboxanes (Hamberg and Samuelsson, 1974; Piper and Vane, 1969; Vargaftig and Dao, 1971). We constructed a dose-response curve for arachidonic acid injected through the pulmonary artery and the subsequent release of prostaglandins and thromboxanes. In addition a dose-response curve was made for the direct effect of PGE_2, on the rat stomach.

Starting with an injection of 50 ng ovalbumin, the dose of ovalbumin through the sensitized lungs was gradually increased until the anaphylactic release of prostaglandins and thromboxanes was detectable and could be compared to the release induced by the dose of arachidonic acid causing the same degree of contraction of the aorta or the amount of PGE_2 causing the same contraction of the stomach. In order to exclude variations in individual lungs and tissues, results are also presented as the ratios of the dose of ovalbumin and the dose of arachidonic acid or PGE_2, that have comparable effects on the assay tissues, to correct for differences in sensitivity in lungs and tissues.

The threshold dose in H. influenzae-vaccinated and control animals was determined and related to the standards.

For measuring histamine release, lungs were superfused for 20 min and shocked with 2 μg ovalbumin. Ten or five 1 min superfusate fractions were collected and histamine measured in the perfusate by means of a enzymatic double isotopic assay (Beaven et al., 1972).

Pressure in the column connected to the pulmonary artery was determined with a

Statham transducer (model P23Ac) and displayed on a Grass polygraph. The increase in pressure after ovalbumin reflects bronchoconstriction.

2.4. *Inhibition by isoprenaline*

The threshold dose for ovalbumin was detected as described in *2.3.* Accordingly an infusion of isoprenaline (0.1 ml/min) in the pulmonary artery was started and after 15 min the threshold dose was again measured. The ratio 'threshold dose after isoprenaline and threshold dose before isoprenaline' is called the factor of inhibition.

2.5. *Analysis of data*

Unless stated otherwise, levels of significance were calculated using Student's *t*-test. Means ± S.E.M. are presented. Outliers were ruled out by using the Dixon test (De Jonge, 1963).

2.6. *Drugs*

The drugs used were: isoprenaline hydrochloride (OPG); histamine hydrochloride (BDH chemicals); mepyramine hydrochloride (OPG); atropine sulfate (OPG); propranolol hydrochloride (ICI); cyproheptadine (Merck, Sharp and Dohme); phenoxybenzamine hydrochloride (Smith, Kline and French); ovalbumin (Merck); arachidonic acid (Sigma); PGE_2 (gift of Dr. J. Pike, Upjohn, Michigan); indomethacin (Merck, Sharp and Dohme) and pentobarbital sodium (Abbott). Drug solutions were made up freshly and kept on ice during experiments. The mixture of antagonists was made in saline; indomethacin was dissolved in 50 mM Tris-HCl buffer pH 8.0. Arachidonic acid was stored as a stock solution of 10 mg/ml in n-hexane at −20° C. Just before starting the experiment, the solvent was evaporated rapidly under nitrogen and the acid was redissolved in 50 mM Tris-HCl buffer (pH 7.5) to give the sodium salt. PGE_2 was stored as 10 mg/ml stock solution in ethanol at −20° C, was evaporated under nitrogen before the experiment then redissolved in Krebs buffer. All other drug solutions were made in Krebs medium.

3. Results

3.1. *The effect of H. influenzae on the threshold dose for mediator release*

Fig. 1 shows the threshold dose for ovalbumin-induced mediator release in H. influenzae-vaccinated and control animals. Lungs from vaccinated animals released thromboxanes and prostaglandins of the E_2 and $F_{2\alpha}$ type simultaneously following a significantly lower dose than did lungs from control animals ($P < 0.01$). The threshold doses were 0.4 ± 0.1 μg ovalbumin for the vaccinated animals and 1.9 ± 0.5 μg for the controls ($n = 8\text{-}11$, $P < 0.01$). In a second series of experiments there was again a significant difference in threshold dose. Means for H. influenzae-vaccinated and control animals were 0.4 ± 0.1 and 1.2 ± 0.4 μg ovalbumin, respectively ($n = 7$, $P < 0.05$). Ratios of the dose of ovalbumin and the dose of arachidonic acid with the same contraction of the aorta in H. influenzae-vaccinated and

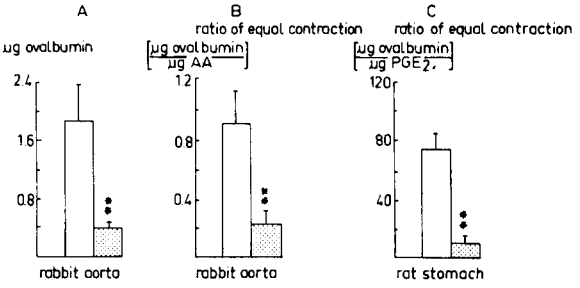

Fig. 1. The threshold dose for ovalbumin-induced thromboxane-A_2 and prostaglandin release in control (open columns) and H. influenzae-vaccinated (shaded columns) lungs, isolated from sensitized guinea pigs (A). The ratio of the dose of ovalbumin and the dose of arachidonic acid (B) inducing the same contraction of the aorta and the ratio of ovalbumin and PGE_2 (C) with the same contraction of the stomach respectively, are also shown to correct for differences in sensitivity of lungs and tissues ($n = 8$, $** P < 0.01$).

TABLE 1

Release of thromboxane A_2 and prostaglandins induced by histamine and bradykinin in control and 4 days H. influenzae-vaccinated, isolated, sensitized guinea pig lungs. Ratios of the doses of histamine and bradykinin and the doses of arachidonic acid and PGE_2 giving the same contraction of the assay tissues are presented to correct for differences in sensitivity of lungs and tissues (n = 8-11; * $0.05 < P < 0.01$).

	Histamine		Bradykinin	
	Control	H. infl.	Control	H. infl.
Arachidonic acid	2.88 ± 1.40	1.71 ± 0.23	0.224 ± 0.095	0.096 * ± 0.021
PGE_2	1.03 ± 0.43	1.69 ± 1.06	18.95 ± 5.07	9.66 * ± 2.69

control animals were: 0.23 ± 0.09 and 0.94 ± 0.22 ($P < 0.01$), respectively.

Ratios of the dose of ovalbumin and the dose of PGE_2, with the same contraction of the stomach were: 10.4 ± 4.2 and 76.1 ± 11.2 ($P < 0.01$), respectively. The correction for differences in sensitivity of lungs and tissues did not alter the significance levels.

The release of prostaglandins and thromboxanes by histamine and bradykinin (table 1) did not differ significantly between vaccinated and control animals. However, with bradykinin the release was somewhat increased in H. influenzae-vaccinated animals ($0.05 < P < 0.10$). The doses of arachidonic acid administered through the lung, which caused a contraction of the rat stomach similar to that with 50 ng PGE_2 were, in vaccinated and non-vaccinated animals: 7.4 ± 2.5 and 6.0 ± 2.2 μg (n = 10, difference not significant) respectively. Doses of arachidonic acid causing a 20 mm contraction of the rabbit aorta were 6.7 ± 2.1 and 7.1 ± 1.9 μg (n = 10, difference not significant) respectively. From these data it can be concluded that the conversion of arachidonic acid to prostaglandins and thromboxanes did not differ between vaccinated and control groups.

3.2. Effect of H. influenzae on histamine release and bronchoconstriction

Table 2 shows the effect of 4 days' vaccination with H. influenzae on histamine release following different doses of ovalbumin. The χ^2 test of significance was used as not every lung responded with histamine release and bronchoconstriction after antigenic challenge. Vaccination with H. influenzae resulted in a significant increase of histamine release following 2 μg ovalbumin ($P < 0.025$). After 10 μg the responses of the two groups did not differ greatly but after 1 mg ovalbumin, histamine release was reduced in the vaccinated group ($P < 0.05$).

The time course of histamine release of the control and the 4 day-vaccinated group, following challenge with 2 μg ovalbumin, is presented in fig. 2. As shown in this figure maximal histamine release was measured 2 min after the antigen challenge. The difference in histamine release between vaccinated and control animals was significant in this superfusion fraction only ($P < 0.05$).

The effect of vaccination with H. influenzae on different days prior to the experiments on antigen-induced histamine release is shown in fig. 3. The histamine release from animals vaccinated four days earlier was increased as shown before, but was not significantly

TABLE 2

Total amount of histamine (ng) released in five minutes following various doses of ovalbumin (* $P < 0.05$, ** $P < 0.025$).

	2 μg	10 μg	1000 μg
Controls	96.3	364.6	4327.6
H.infl.-vaccinated (4 days)	159.5 **	428.2	1629.0 *
n	15—8	5	6

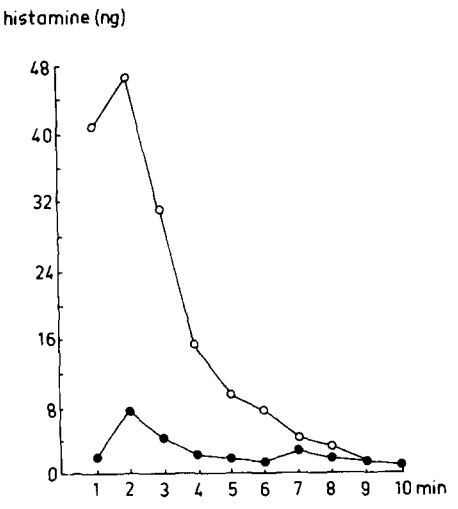

Fig. 2. Time course of the histamine release following 2 μg ovalbumin, in isolated lungs from sensitized guinea pigs. Release was measured in ten 1-min superfusion fractions from 4 days H. influenzae-vaccinated (○———○) and control (●———●) animals (n = 8).

altered in animals vaccinated 1 or 10 days before the experiment.

Bronchoconstriction as a result of a 2 μg ovalbumin injection in 1 day-vaccinated lungs was 2.9 ± 0.7 mm Hg, while there was no detectable bronchoconstriction in control lungs (n = 5, $P < 0.01$). Bronchoconstriction was also potentiated in the 4 day-vaccinated group as compared to the control group (2.0 ± 0.7 vs. 0.1 ± 0.1 mm Hg, n = 10, $P < 0.02$).

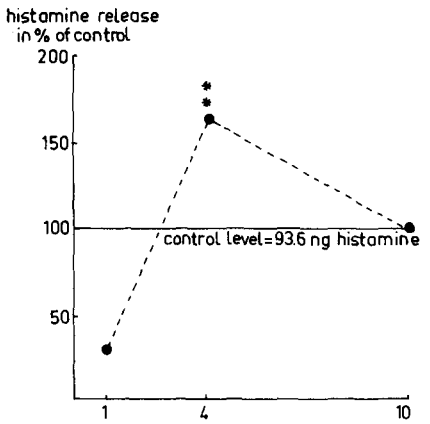

Fig. 3. The total amount of histamine (ng) released in the first five 1-min superfusion fractions following an injection of 2 μg ovalbumin in the pulmonary artery of sensitized lungs. Control lungs (n = 15) were compared to lungs of animals vaccinated with H. influenzae 1, 4 and 10 days prior to the experiments (n = 5, 8 and 5, respectively, ** $P < 0.025$). *Abscissa:* days after vaccination.

3.3. The effect of H. influenzae on the inhibition of mediator release by isoprenaline

Prostaglandin and thromboxane release in H. influenzae-vaccinated as well as in control animals was completely inhibited by a concentration of 2×10^{-8} M isoprenaline (not shown). The inhibition by doses of 6×10^{-9} and 2×10^{-9} M isoprenaline is presented in fig. 4.

In the H. influenzae-vaccinated groups there was no significant inhibition with 2×10^{-9} and 6×10^{-9} M isoprenaline (not signifi-

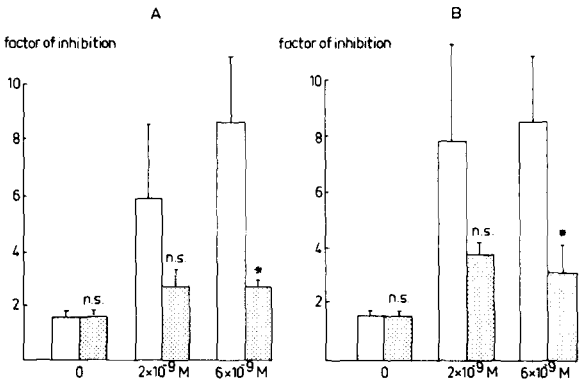

Fig. 4. Inhibition of prostaglandin and thromboxane release by doses of 2×10^{-9} and 6×10^{-9} M of isoprenaline infused into the pulmonary artery. Control (open columns) and H. influenzae-vaccinated lungs (shaded columns). The factor of inhibition is the ratio of the dose of ovalbumin needed to give an anaphylactic contraction after an infusion of isoprenaline compared to the dose of ovalbumin before the isoprenaline infusion. The dose of ovalbumin is compared to the dose of arachidonic acid inducing the same contraction of the rabbit aorta (A) and to the dose of PGE_2 with the same contraction of the rat stomach (B) (for details see *2.3.*). Significance was determined for the difference between control and vaccinated groups (n = 5-8, * $P < 0.05$).

cantly different from an infusion of physiological saline).

In contrast, in the control lungs, 6×10^{-9} M isoprenaline caused a significant inhibition of prostaglandin and thromboxane release ($P < 0.01$). Differences between control and vaccinated group were significant ($P < 0.05$, $n = 8$). The factor for inhibition related to the effect of arachidonic acid and PGE_2 was 8.7 ± 2.8 and 8.6 ± 2.4 respectively. With 2×10^{-9} M isoprenaline there was also inhibition in the control group, but this inhibition was not significantly different from that observed in the H. influenzae-vaccinated group. The inhibition factors were 5.8 ± 2.7 and 7.9 ± 3.4 ($n = 5$) respectively.

4. Discussion

Several animal models have been used to investigate asthmatic processes, e.g. 'atopic dogs' or the anaphylactic state (Middleton, 1972; Patterson, 1974), hypothalamic lesions (Macris et al., 1970) and impairment of β-adrenoceptor function (Townley et al., 1967) whereas vaccination with B. pertussis has also been used as a model of atopy (Szentivanyi, 1968). However, Terpstra et al. (1979) described vaccination with H. influenzae as a better model for human atopy because H. influenzae is one of the most common bacteria found in the lower respiratory tract of patients with chronic asthmatic bronchitis (see Hirschmann and Everett, 1979; Van der Zwan, 1976).

Because some of the clinical manifestations of asthma are due to the release of endogenous chemical mediators, we first investigated the influence of H. influenzae on the antigen-induced release of a number of these mediators. The threshold dose of ovalbumin for mediator release was lowered in isolated H. influenzae-vaccinated guinea pig lungs. In other words, in sensitized lungs there was a release of thromboxanes and prostaglandins after a smaller antigenic challenge than was needed for normal lungs. This effect was reproducible. The prostaglandins of the E-series which are bronchodilators, as well as the prostaglandins of the F-series which are bronchoconstrictors, were released and their effects counteract each other. However, thromboxanes (Svensson et al., 1977) and prostaglandin endoperoxides (Hamberg et al., 1975) are potent stimulators of respiratory smooth muscle and they are also released after a still smaller dose of antigen. In analogy, patients with chronic asthmatic bronchitis are also more sensitive to environmental stimuli than are normal individuals (Jenne et al., 1977; Townley et al., 1975).

After 2 μg ovalbumin, both histamine release and bronchoconstriction were potentiated in H. influenzae-vaccinated animals. Terpstra et al. (1979) showed that in rats 4 days after vaccination, both lung and plasma histamine levels were increased and mast cell histamine content was elevated so it seems logical that more histamine would also be released after ovalbumin shock.

With the higher dose i.e., 10 μg ovalbumin, the effect of H. influenzae on histamine release and bronchoconstriction was no longer significant. After a challenge with 1 mg ovalbumin the effect was reversed: the vaccinated group released less histamine.

In vaccinated animals there was an increased release of prostaglandins and thromboxanes. Tauber et al. (1973) showed that exogenous prostaglandins depressed the release of histamine and SRS-A during antigen challenge of human lungs and this negative feedback mechanism may explain the fact that in an anaphylactic shock with maximal mediator release less histamine is liberated from vaccinated lungs.

Szentivanyi (1968) described a decreased β-adrenoceptor function as a possible cause underlying atopic abnormalities and respiratory infection as an entity that makes the latent abnormality clinically manifest. In our experiments the inhibition of mediator release with isoprenaline was more effective in control lungs than in H. influenzae-vaccinated lungs. These results therefore suggest decreased

functioning of the β-adrenoceptors or altered functioning of mechanisms mediating the biological activity.

Nijkamp et al. (1976) and Blackwell et al. (1978) suggested that several agents such as antigen, histamine and RCS-RF that release prostaglandin endoperoxides and thromboxane A_2 from guinea pig perfused lungs probably do so by liberating the substrate from intracellular lipid stores through activation of phospholipase A_2. H. influenzae-vaccinated lungs released more prostaglandins and thromboxanes following antigen challenge and stimulation of phospholipase A_2 is one of the possible explanations. Beside antigen and histamine, bradykinin is known to release prostaglandins and thromboxanes.

There is a tendency for bradykinin to release more prostaglandins and thromboxanes in vaccinated animals. For histamine, however, no significant difference between the groups was observed. These results are therefore difficult to interpret with respect to phospholipase A_2 stimulation.

The present results emphasize the importance of further elucidation of the role of H. influenzae in asthmatic processes.

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