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THE EFFECT OF CATECHOLAMINES ON THE *in vivo* AND *in vitro* RESPONSES OF THE CAT LUNG DURING ANAPHYLAXIS

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1 Anaphylaxis in the lung of cats actively sensitized to *Ascaris* antigen has been investigated *in vivo* and *in vitro*.

2 *In vivo* there was a 100% increase in airways resistance and a 50% decrease in dynamic lung compliance following intravenous challenge with *Ascaris* antigen. Prostaglandin $F_{2\alpha}$ induced similar changes but with histamine only dynamic lung compliance was affected. (–)-Isoprenaline prevented these prostaglandin $F_{2\alpha}$- and histamine-induced changes and caused a delay of about 2 min in the onset of the mechanical changes following anaphylactic challenge.

3 *In vitro* the isolated lung strip contracted within seconds of challenge whereas there was a delay of 2 to 3 min in the onset of the tracheal anaphylactic response. (–)-Isoprenaline, (–)-adrenaline and (±)-noradrenaline reduced the magnitude of anaphylactic contractions of the isolated trachea but did not significantly affect those of the isolated lung strip. This indicated lack of inhibition of mediator release from the lung parenchyma.

4 Histamine was released from sensitized lung fragments following challenge with the *Ascaris* extract. This release constituted 6.3% of the total tissue histamine and was not inhibited by (–)-isoprenaline (1 μM).

5 (–)-Isoprenaline abolished 5-hydroxytryptamine (5-HT)-induced contractions of the isolated trachea but not those elicited in response to acetylcholine. The isolated lung strip responses to histamine, prostaglandin $F_{2\alpha}$ and 5-HT were highly resistant to inhibition by (–)-isoprenaline.

Introduction

The effects of anaphylaxis on the lungs have been described in most laboratory species. Inhaled antigen causes an increase in airways resistance and a reduction in dynamic lung compliance in the conscious sensitized guinea-pig (Popa, Douglas & Bouhuys, 1973) and increases overflow when given intravenously in the rat Konzett-Rossler preparation (Farmer, Richards, Sheard & Woods, 1975). However, there have been few studies describing anaphylaxis in cats. In spite of earlier reports to the contrary (Akcasu, 1963), cats can be actively sensitized to albumin (Barch & Talbott, 1976) and to an *Ascaris* antigen (Lulich, Mitchell & Sparrow, 1976). Barch & Talbott (1976) found that, on challenge, there was a 50% increase in airways resistance and *in vitro* Lulich *et al.* (1976) reported that both central and peripheral airways contract.

Drugs which stimulate β-adrenoceptors are widely used in the treatment of bronchial asthma in man and are able to prevent anaphylactically induced bronchospasm in animals. For example, in the conscious dog isoprenaline aerosol reverses anaphylactic bronchoconstriction (Dain & Gold, 1975) and *in vitro* terbutaline reduces anaphylactic contractions in the guinea-pig trachea (Sorenby, 1975). Not only do these agents relax tracheal and bronchial smooth muscle (McDougal & West, 1953) but they also partially inhibit anaphylactic histamine release from guinea-pig lung (Schild, 1936; Assem & Schild, 1971a). Similarly, inhibition of mediator release from the lungs of man (Assem & Schild, 1969) and monkey (Ishizaka, Ishizaka, Orange & Austen, 1970) has been reported.

The isolated lung strip of the cat (Mitchell & Sparrow, 1975; Lulich *et al.*, 1976) is an *in vitro* preparation of peripheral airways which allows the mechanical responses to drugs and anaphylactic challenge to be measured. We describe here the effects of β-adrenoceptor agonists on the mechanical responses of the cat isolated lung strip and trachea and on histamine release from parenchymal lung fragments. *In vivo* changes in airways resistance and dynamic lung compliance during anaphylaxis have also been followed.

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Methods

Isolated lung strips and trachea were prepared from cats sensitized to an *Ascaris* extract essentially as described by Lulich *et al.* (1976). The procedure is briefly described here.

Preparation of isolated lung strips and trachea of cat

Adult cats of either sex were anaesthetized with intraperitoneal sodium pentobarbitone and exanguinated. The chest was opened and the lungs flushed free of blood with 30 ml warmed Krebs solution injected into the right ventricle (both superior and inferior venae cavae being clamped). The trachea and lungs were then removed. A pair of isolated tissues comprising a lung strip and tracheal segment were suspended in a 6 ml tissue bath containing gassed Krebs solution at 37°C.

Three tissue baths were run concurrently. Following equilibration the tissues were challenged with *Ascaris* extract 1 mg/ml which was washed out when the peak response had been obtained. The peak contraction to a maximal concentration of acetylcholine (10 mM) was then elicited, and the anaphylactic contraction expressed as a percentage of this response. In experiments with the 3 catecholamines, tissues from 4 sensitized cats were used. Each drug was added to the bath 5 min before challenge; after the ensuing response the bath was washed out. The reference acetylcholine contractions were repeated at 10 min intervals until the responses were consistent. The 3 catecholamines were tested on tissues from all cats. Each pair of tissues was used once because of the rapid desensitization of the anaphylactic response. Two pairs of tissues from each cat were used to measure control anaphylactic contractions, i.e. in the absence of catecholamines, and control responses were compared with the contractions in the presence of the drugs. Except where indicated otherwise, statistical analysis was carried out with the paired *t*-test, $P < 0.05$ being regarded as significant.

Active sensitization of cats to Ascaris *antigen*

Cats were sensitized to an aqueous extract of *Ascaris* worms that were obtained from pigs' intestines. Initially each cat was injected subcutaneously with 10 mg extract made up in 1 ml distilled water and 10^{10} organisms of *B. pertussis* as an adjuvant. Subsequently the cats received 5 mg extract and adjuvant at weekly intervals for 4 to 6 weeks.

Measurement of histamine release from cat lung

The lungs from sensitized cats were removed as described above, then placed in Krebs solution at 4°C. That the lungs were sensitized was first established by eliciting Schultz-Dale contractions in a pair of lung strips. The lung was then coarsely chopped with scissors and the parenchyma finely minced into fragments of about 1 mm^3. The lung tissue was placed in 150 ml Krebs solution at 37°C and gassed with 95% O_2 and 5% CO_2 mixture. The Krebs solution was drawn off at 15 min intervals and replaced with fresh solution. After 1 h approximately 300 mg aliquots of lung were distributed to test tubes containing between 1.6 and 2.0 ml Krebs solution and mounted in a water bath at 37°C with a mechanical agitator. After 4 min appropriate tubes were challenged with 0.2 ml *Ascaris* solution to give a final concentration of 1 mg/ml.

In each experiment the minced lung tissue was divided in 3 groups to measure histamine release (a) spontaneous (b) in the presence of *Ascaris* antigen and (c) in the presence of antigen and (−)-isoprenaline. Duplicate or quadruplicate samples were prepared in each group. The (−)-isoprenaline was added 3 min before the *Ascaris* extract (1 mg/ml) and the reaction was allowed to proceed for a further 10 min before stopping the reaction by placing the tubes in ice. The incubation solution was then drawn off and frozen at −20°C until assayed. The residual tissue histamine was extracted by boiling the lung fragments in 5 ml Krebs solution for 10 minutes. Preliminary experiments had shown that there was no advantage in either acidifying the solution or in homogenizing the lung before boiling it. The boiled lung fragments were then blotted dry on filter paper and weighed. The boiled tissue was weighed rather than the freshly incubated fragments in order to avoid the loss of tissue histamine from the mechanical trauma of blotting tissues dry. Boiled lung was found to weigh 16% less than the freshly incubated minced lung.

Assay of histamine

Histamine was assayed biologically on the superfused guinea-pig ileum in the presence of atropine (0.3 μM) and propranolol (3 μM). Responses were first elicited to injections of 10, 30 and 100 ng of histamine standard until consistent contractions were obtained. In this range the contractile response was linear with respect to the log-dose. The volume of histamine standards and unknowns ranged from 0.1 to 0.5 ml. The unknown samples were administered in random order, a standard dose of histamine being injected at intervals to ascertain that the sensitivity of the ileum had not altered. To determine the total lung histamine content the released histamine was added to the residual tissue histamine. Mepyramine (1 μM) abolished the contractions of the ileum in response to the unknown samples indicating that histamine was the spasmogen.

Measurement of airways resistance (R_A) and dynamic lung compliance (C_D)

Cats were anaesthetized with chloralose (80 mg/kg) and sodium pentobarbitone (10 mg/kg, i.p.). A

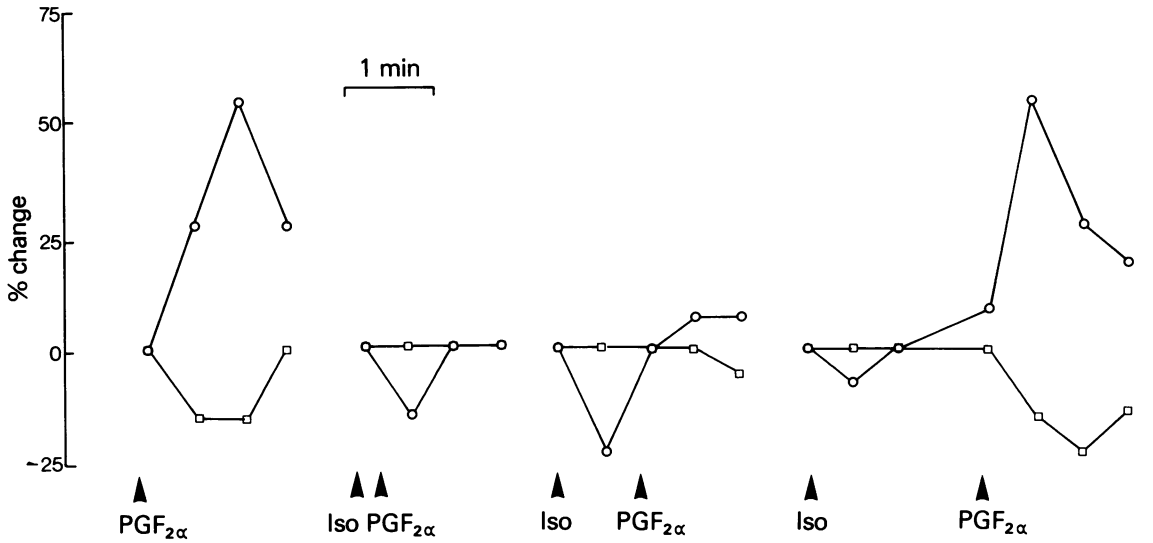

Figure 1 An example of the protection by (–)-isoprenaline (Iso, 0.1 μg/kg) on the airways response to prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$, 0.3 μg/kg) in an anaesthetized cat: airways resistance (○); dynamic lung compliance (□). From left to right: control response to prostaglandin $F_{2\alpha}$, repeated 15 s, 1 min and 2 min after injection of (–)-isoprenaline which itself produced a small decrease in airways resistance.

femoral artery was cannulated for measurement of blood pressure. Drugs were administered via a cannula inserted in the right jugular vein and advanced to the right auricle. Air flow was measured with a Fleisch pneumotachograph head (type 00) connected to a tracheal cannula and a Statham PM5 differential gas pressure transducer. Intrapleural pressure was obtained using a Malecot catheter (size 12) placed in the right thorax via the 5th interspace and was measured with a Grass volumetric pressure transducer (type PT5A). The air flow signal was integrated to give tidal volume. Airways resistance (R_A) and dynamic lung compliance (C_D) were obtained by the method of electrical subtraction (Mead & Whittenberger, 1953) using 2 oscilloscopes with calibrated axes. R_A and C_D were read directly from the closed hysteresis loops in terms of cmH_2O ml^{-1} s^{-1} and ml/cmH_2O respectively. A period of 30 min was allowed after completion of surgery before starting drug administration. Drugs were tested at a minimum interval of 10 min with readings of R_A and C_D made before and after their administration. The sensitivity of the animals to histamine and prostaglandin $F_{2\alpha}$ was established before challenging animals with the *Ascaris* extract (50 mg in 2 ml 0.9% w/v NaCl saline solution).

Drugs

The following drugs were used: prostaglandin $F_{2\alpha}$ (Upjohn Co.), histamine acid phosphate (Koch-Light), acetylcholine chloride (Roche), propranolol hydrochloride (ICI), atropine sulphate (DHA), (–)-isoprenaline hydrochloride (Sigma), (–)-adrenaline hydrochloride (Sigma), (±)-noradrenaline bitartrate (Sigma), chloralose (Koch-Light), sodium pentabarbitone (Nembutal, Abbot Labs.) and 5-hydroxytryptamine creatinine sulphate (5-HT, Sigma). Stock solutions of the catecholamines contained 1% ascorbic acid to minimize oxidation.

Results

Anaphylaxis in vivo

A mean airways resistance (R_A) of 0.17 ± 0.03 cmH_2O ml^{-1} s^{-1} and dynamic lung compliance (C_D) of 2.28 ± 0.35 ml/cmH_2O was obtained in a series of experiments on 13 anaesthetized cats. Changes in these parameters were readily elicited by drugs and autacoids. Prostaglandin $F_{2\alpha}$ (0.3 to 5 μg/kg) increased R_A by $23.7 \pm 3.0\%$ and reduced C_D by $22.6 \pm 2.2\%$ (6 tests). Histamine (1 to 10 μg/kg) had no significant effect on R_A ($-1.5 \pm 4.8\%$) but decreased C_D by $26.4 \pm 7.2\%$ (7 tests). These drug-induced responses were completely prevented by prior intravenous injection of (–)-isoprenaline (0.1 μg/kg) but the protection lasted not more than 2 min after its administration (Figure 1).

In 4 sensitized cats 50 mg *Ascaris* (i.v.) caused a rapid and prolonged increase in R_A and decrease in C_D. The average maximal changes during anaphylaxis were $+108.4 \pm 35.6\%$ in R_A and $-55.3 \pm 8.1\%$ in C_D

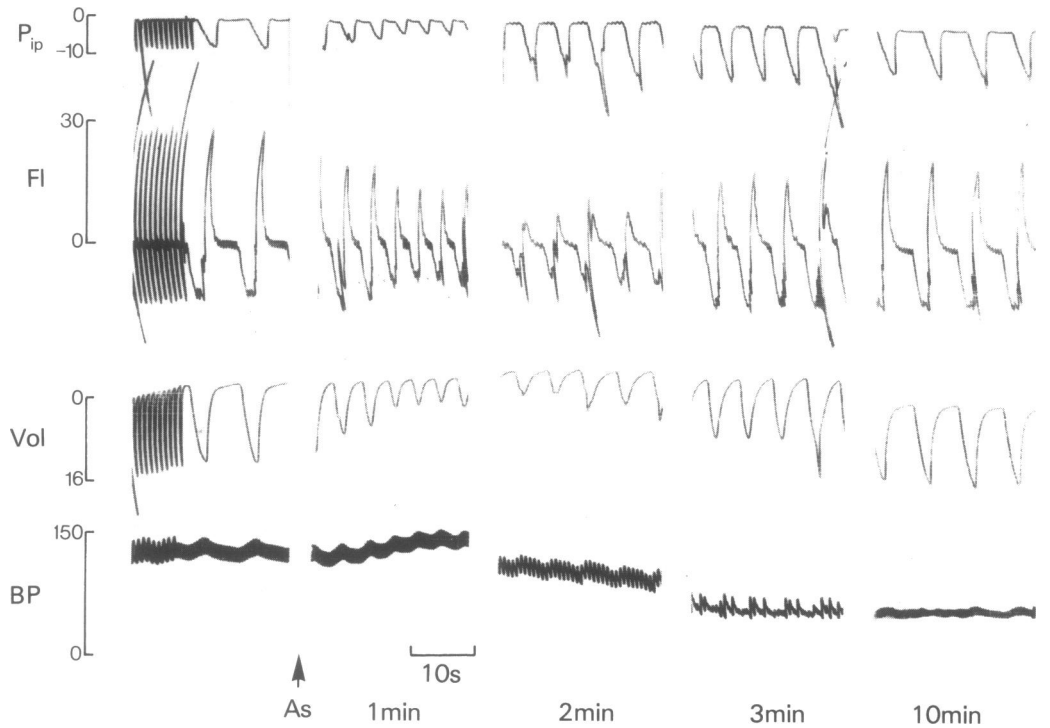

Figure 2 A chart recording the dynamic responses to anaphylactic challenge in a sensitized, anaesthetized cat. P_{ip}, intrapleural pressure (cmH_2O); Fl, air flow (ml/s); Vol, tidal volume (ml); BP, blood pressure (mmHg). Traces shown are during control respiration then 1, 2, 3 and 10 min after injection of 50 mg *Ascaris* (As).

and occurred about 5 min after challenge. The respiratory rate increased in some cats and decreased in others, but not by more than a factor of 2 and blood pressure was consistently reduced by 50 to 80 mmHg. The onset of the anaphylactic responses occurred within 1 min (Figure 2). In a further 4 cats pretreated with 0.1 μg/kg (–)-isoprenaline 15 s before being challenged, there was a consistent delay of up to 2 min before the onset of the anaphylactic changes. In these cats the maximal changes in R_A and C_D were $+99.6 \pm 37.9\%$ and $-57.0 \pm 8.1\%$ respectively and occurred at about 3 min after injection of the extract. Two cats (one from each group) stopped breathing and died after approximately 20 minutes. Death did not appear to be associated with an acute increase in R_A. The remainder of the cats showed partial recovery of the respiratory parameters over the ensuing hour.

The effect of β-adrenoceptor agonists on anaphylactic contractions of the isolated lung strip and trachea of the cat

Isolated lung strips and trachea from sensitized cats contracted on administration of the *Ascaris* extract (1 mg/ml). The lung strips responded within seconds of challenge whereas there was a consistent delay of 2.5 ± 0.5 min ($n=9$) in the onset of the tracheal contraction (Figure 3a, b).

The mean control anaphylactic contractions of lung strips and trachea from 4 cats were $76.4 \pm 2.4\%$ and $53.8 \pm 5.7\%$ respectively of the maximal acetylcholine response. Lung strips were relaxed by the catecholamines, the effect being maximal by 5 minutes. (–)-Isoprenaline, (–)-adrenaline and (±)-noradrenaline reduced anaphylactic contractions of the trachea (Figures 3a and 4a) but had no significant effect on the lung strip with incubation times of either 15 s or 15 min (Figures 3b and 4b). In the trachea the inhibition ranged from 54.2% with (–)-adrenaline (10 μM) to 90.5% with (–)-isoprenaline (1 μM), but there was no statistically significant difference between the maximum inhibitions attainable with the three catecholamines (Student's t test). The rank order of potency with the 3 catecholamines on the trachea was (–)-isoprenaline > (–)-adrenaline $\simeq$ (±)-noradrenaline. (–)-Isoprenaline was approximately 13 times more potent on a molar basis than the other 2 stimulants.

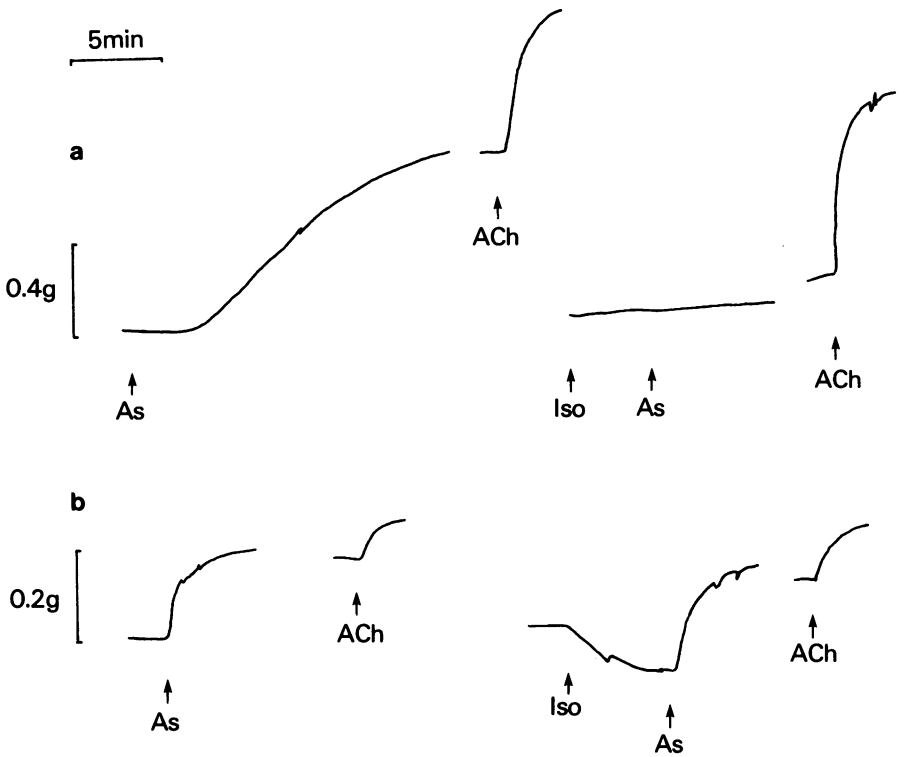

Figure 3 Traces showing the anaphylactic contractions of isolated (a) trachea and (b) lung strips of the cat following challenge with *Ascaris* extract (As, 1 mg/ml). The left hand traces show control anaphylactic responses and those on the right when (–)-isoprenaline (Iso, 1 μM) was included in the tissue bath. In this example the tracheal response is completely abolished whereas there is no change in the lung strip contraction. In each case the magnitude of the contractile responses is compared with the tension developed to a maximal concentration of acetylcholine (ACh, 10 mM) injected after washout.

No change was seen after exposure to catecholamines in the latent period between antigenic challenge and onset of the contraction in the trachea (control 2.5 ± 0.5 min, $n=9$; treated 2.4 ± 0.4 min, $n=10$).

The inability of the β-adrenoceptor agonists to inhibit completely contractile responses due to antigenic challenge suggested that responses to other stimuli might also be refractory. Experiments were therefore undertaken to investigate the effect of (–)-isoprenaline 1 μM on the responses of lung strips and trachea to some contractile drugs. Concentrations of the contracting drugs that elicited 50% to 70% of their maximum effect were chosen. A 5 min exposure to (–)-isoprenaline completely abolished 5-HT-induced contractions on the trachea but only inhibited acetylcholine responses by $44.3 \pm 10.4\%$ ($n=5$). Increasing the concentration of (–)-isoprenaline to 10 μM caused no further diminution in the acetylcholine contractions. On the lung strip preparation pretreatment with (–)-isoprenaline (up to 10 μM) did not prevent contractions elicited by submaximal concentrations of histamine, prostaglandin $F_{2\alpha}$ or 5-HT. Examples of the effect of (–)-isoprenaline on some contractile responses of lung strips and trachea are shown in Figure 5.

Effect of (–)-isoprenaline on anaphylactic mediator release from cat minced lung

The effect of (–)-isoprenaline (1 μM) on anaphylactic histamine release was determined in lung from 5 sensitized cats. Sensitivity was confirmed by first challenging isolated lung strips from each cat with 1 mg/ml *Ascaris* extract. Tissues from all cats contracted by 50 to 100% of the maximal acetylcholine response.

There was a significant ($P<0.05$) increase in histamine release in the presence of antigen compared with spontaneous release and this was not significantly reduced by (–)-isoprenaline. The spontaneous histamine release was 67.7 ± 7.8 ng/g ($n=4$). In the presence of *Ascaris* extract this increased to

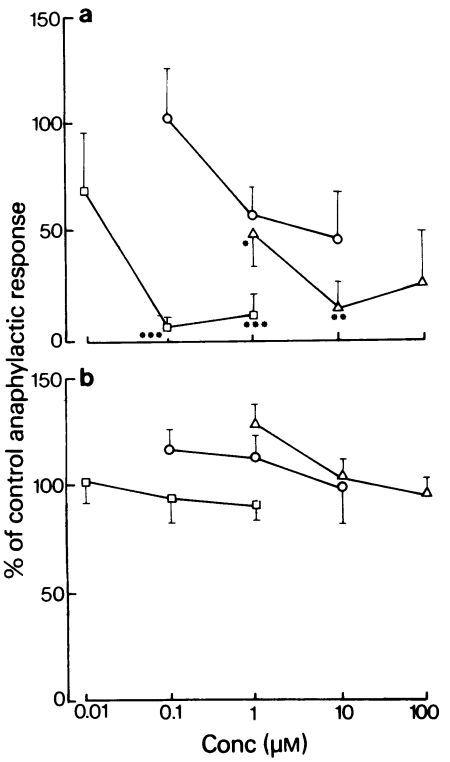

Figure 4 Concentration-response curves for (–)-isoprenaline (□), (–)-adrenaline (○) and (±)-noradrenaline (△) on anaphylactic contractions of the isolated (a) trachea and (b) lung strip of the cat. The tissues were pre-treated with the catecholamines 5 min before antigen challenge. In each tissue the magnitude of the anaphylactic contraction was expressed as the ratio of the tension developed to a maximal concentration of acetylcholine (10 mM). In tissues exposed to catecholamine this ratio was compared with the ratio of the control responses and expressed as a percentage, e.g. in control experiments the anaphylactic contraction of the trachea was 0.75 that of its acetylcholine response and in (–)-isoprenaline-treated tissues was 0.5, thus % of control response = 0.5/0.75 of 100 = 66%. Each point is the mean of 4 tissues; vertical lines show s.e. means. Asterisks indicate a significant difference between anaphylactic contractions in the presence of catecholamines and the control responses: $*P<0.05$; $**P<0.02$; $***P<0.001$.

283.6 ± 108.9 ng/g ($n=5$) and in controls when (–)-isoprenaline had been added to 219.0 ± 57.3 ng/g ($n=5$). In terms of percentage tissue histamine the spontaneous release was $2.2 \pm 1.3\%$ ($n=3$), with *Ascaris* $6.3 \pm 1.6\%$ ($n=4$) and with *Ascaris* plus (–)-isoprenaline $5.3 \pm 1.6\%$ ($n=4$).

Discussion

In vivo anaphylactic challenge caused an increase in airways resistance (R_A) and a decrease in dynamic compliance (C_D). The anaphylactic responses, typically an increase of 100% in R_A and a decrease of 50% in C_D are comparable with those reported for the guinea-pig (Popa *et al.*, 1973). The reduction in C_D by histamine and prostaglandin $F_{2\alpha}$ may reflect constriction of the peripheral airway smooth muscle; these substances contract the isolated lung strip preparation but not the trachea (Lulich *et al.*, 1976). *In vitro* isolated lung strips contract within seconds of challenge whereas there is a delay of some 2 to 3 min before the onset of anaphylactic contraction in the trachea. A comparable delay was not observed in the anaesthetized cats, suggesting differences in response or access rates. (–)-Isoprenaline prevented histamine and prostaglandin $F_{2\alpha}$-elicited changes in R_A and C_D but the effect was short lived, paralleling its action on cardiovascular parameters. Similarly (–)-isoprenaline consistently delayed the onset of the anaphylactic responses by up to 2 min *in vivo*.

In vitro the catecholamines reduced the magnitude of anaphylactic contractions in the trachea. The rank order of potency of the 3 catecholamines in the trachea is similar to that reported for relaxation of the tone of tracheal smooth muscle by McDougal & West (1953) and Lulich *et al.* (1976). The latter authors also demonstrated that adrenoceptors in the cat trachea were predominantly of the β_1-subtype. The inhibitory effect of the catecholamines on the anaphylactic responses of the trachea may have been due, at least in part, to inhibition of mediator release. However, in the lung strip preparation anaphylactic responses were not significantly reduced by the catecholamines suggesting that mediator release was not being affected. This was supported by the observation that (–)-isoprenaline had no effect on histamine release from the minced lung from the sensitized cats. In guinea-pig and human lung, anaphylactic histamine release has been shown to be inhibited by drugs that stimulate β-adrenoceptors (Assem & Schild, 1969; 1971a, b). Holyrode & Eyre (1976) however reported that histamine release from bovine granulocytes was inhibited by α-adrenoceptor agonists. These authors found that at 100 μM (–)-isoprenaline did not affect histamine release from bovine granulocytes and proposed that this was due to equal and opposite effect of isoprenaline on β- and α-adrenoceptors at this particular concentration. This is unlikely to be the explanation for our results in the cat lung strip preparation because there was no qualitiative difference between the 3 catecholamines. The lack of a demonstrable effect of (–)-isoprenaline may be related to the small amount of histamine released from cat lung, which was 6.3% of the total histamine content. In the guinea-pig lung about 20% of total tissue histamine is released during anaphylaxis

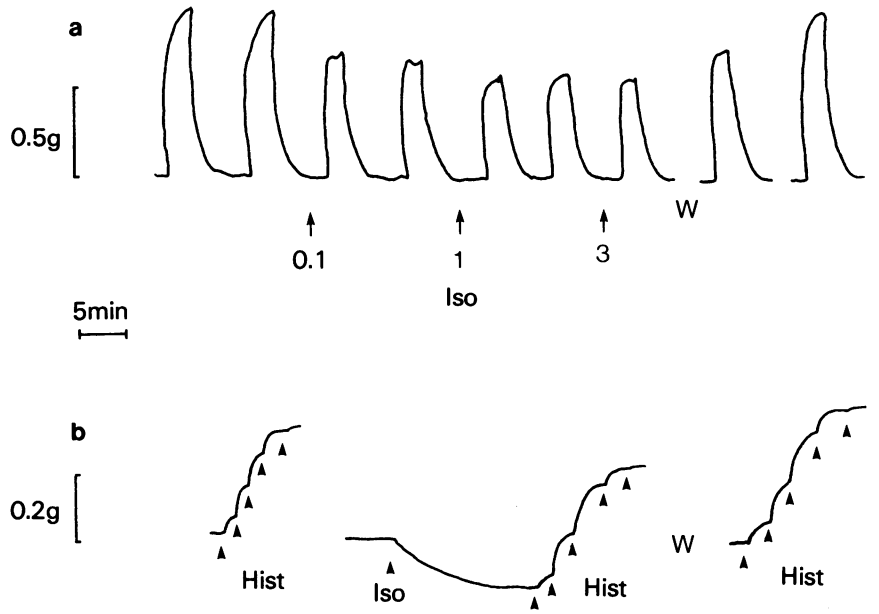

Figure 5 The effect of (–)-isoprenaline on the contractile responses of the isolated trachea and lung strip of the cat. (a) Trachea: contractions to acetylcholine 1.0 μM were elicited repeatedly using a 7-min time cycle. (–)-Isoprenaline (Iso) was then included in the Krebs solution in increasing concentrations (μM). At W it was washed from the tissue bath. (b) Lung strip: a cumulative concentration-response curve was first obtained to histamine (Hist), which was injected at arrows, and then repeated when the tissue was maximally relaxed with (–)-isoprenaline (Iso, 1 μM). The concentration of histamine ranged, in ascending order, from 0.3 to 600 μM. Control contractions were then repeated after washout (W) of the (–)-isoprenaline.

(Sorenby, 1974a, b) and in humans it may be as high as 50% (Assem & Schild, 1971a, b). We also observed that the histamine content of the cat lung was 6 μg/g which is about half that reported for guinea-pigs. Interpretation of the histamine release values obtained in different species is difficult when the proportion of tissue histamine contained in their mast cells (presumed to be the target cells) is not established.

Other chemical mediators may be important in anaphylaxis of cat lung. The isolated trachea of the cat is not contracted by histamine or by the prostaglandins $F_{2\alpha}$, E_1 and E_2 (Horton & Main, 1965; Lulich *et al.*, 1976) but does contract following antigen challenge, a response that is partially inhibited by isoprenaline. β-Adrenoceptor agonists have been shown to inhibit the non-histamine component of anaphylaxis in man and guinea-pig. Kaliner, Orange & Austen (1972) found that the release of slow reacting substance of anaphylaxis (SRS-A) from human lung fragments was inhibited by β-adrenoceptor stimulants and similarly, Liebig, Bernauer & Peskar (1974) and Mathé & Levine (1973) reported a concomitant inhibition of histamine and prostaglandin $F_{2\alpha}$ release from guinea-pig lung. The nature of the non-histamine component of anaphylaxis in the cat trachea would need to be determined before modulation of its release by catecholamines could be investigated.

Relatively high concentrations of β-adrenoceptor agonists are needed to antagonize anaphylactic and drug-induced contractions of the isolated trachea of the cat. The lung strip responses to antigen, histamine, prostaglandin $F_{2\alpha}$ and 5-HT were yet more highly resistant to inhibition by (–)-isoprenaline. We have also found that contractions of the circular smooth muscle from the cat duodenum can be only partially inhibited by (–)-isoprenaline in response to acetylcholine or alternating current field stimulation (unpublished observation). Nevertheless, the intrinsic tone of these tissues is completely abolished by low concentrations of catecholamines (Lulich *et al.*, 1976). Inhibition of spontaneous tone (Bowman & Hall, 1970) and relaxation of near maximal drug-induced contractions by catecholamines (Edman & Schild, 1963) appear to involve different mechanisms. Hence caution should be exercised, in drawing comparisons between the effect of (–)-isoprenaline on small changes in tone of the airways smooth muscle *in vivo* and on the near maximal contraction of the isolated lung strip elicited by *Ascaris* antigen.

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