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Pharmacological study of chicken airway smooth muscle

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Isolated chicken bronchus contracts to carbachol, $PGF_{2\alpha}$, histamine, 5-HT, bradykinin and phenylephrine. The bronchial strips from the horse plasma sensitized domestic fowl contracted to specific sensitizing antigen *in vitro* (Schultz-Dale anaphylactic reaction) and were 5 to 10 fold more reactive to $PGF_{2\alpha}$, histamine and 5-HT compared with the bronchi from normal chickens. Second antigen challenge was inactive or produced a weak response (desensitization). Allowing the bronchi to rest for 1 h resulted in partial recovery of anaphylactic response. Bronchi which had partially contracted submaximally to carbachol, antigen or $PGF_{2\alpha}$, relaxed to isoprenaline, adrenaline and PGE_1 and E_2.

The antigen-induced contraction of isolated smooth muscle has become a standard technique for demonstrating anaphylactic hypersensitivity and is commonly known as the 'Schultz-Dale phenomenon.' Anaphylactic contractions have been demonstrated in a wide variety of actively and passively sensitized smooth muscle organs from numerous mammalian species (reviewed by Chand & Eyre, 1978). In contrast, a unique relaxant response to antigen has been reported in sheep pulmonary vein (Eyre, 1975).

Chickens are highly sensitive to acute systematic anaphylactic reactions (Makinodan, Wolfe & others, 1952) which are always characterized by severe respiratory distress (Lecomte & Beaumariage, 1958; Pedersoli, 1973; Chand & Eyre, unpublished). The Schultz-Dale reaction has been demonstrated in avian pulmonary blood vessels.

Chickens also suffer from an acute cardiorespiratory disease usually referred to as the 'Sudden Death Syndrome' (Howell, 1972). This condition is strikingly similar to acute systemic anaphylaxis (Carlson & Riddell, personal communication). This study was undertaken as part of an extensive investigation of chicken anaphylaxis. We report the reactivity of isolated chicken bronchus to chemical mediators of anaphylaxis and to specific sensitizing antigens *in vitro*.

MATERIALS AND METHODS

Adult domestic fowl were sensitized to horse plasma (1 ml kg^{-1}, i.v.) (Chand & Eyre, 1976). Seven days later the chickens were killed with pentobarbitone (30 mg kg^{-1}, i.v.). Both bronchi were dissected out immediately and cut spirally. Two such bronchial strips from each chicken were suspended in 30 or 50 ml isolated organ baths, containing Krebs solution maintained at 37°, and gassed with 5% CO_2 in oxygen. The bronchial strips were allowed to equilibrate for 1 h under a resting tension of 1 g. Isotonic contractions were recorded with linear motion transducers and an E & M Physiograph (Desk Model: Type DMP-4A).

The following drugs were used: isoprenaline hydrochloride (Wintrobe, New York), histamine diphosphate, 5-hydroxytryptamine creatinine sulphate (5-HT), phenylephrine HCl, adrenaline HCl, bradykinin triacetate (Sigma Chemical Co., St. Louis, Mo.); carbamylcholine chloride (carbachol) (Nutritional Biochemical Corp., Cleveland, Ohio); PGE_1, E_2 and $F_{2\alpha}$ (Upjohn Co., Kalamazoo, Mich.).

RESULTS

Threshold dose (M) ranges of bronchoactive agents are presented in Table 1. Carbachol (10^{-7} to 10^{-5} M),

Table 1. *Threshold dose ranges of some bronchoactive agents on isolated bronchial strips of the adult domestic fowl.*

Bronchoactive agent		Theshold dose ranges (M)	
Carbachol	(C)	10^{-7} to 10^{-6}	(24)
Histamine	(C)	10^{-4} to 10^{-3}	(24)
5-HT	(C)	10^{-4} to 10^{-3}	(5)
$PGF_{2\alpha}$	(C)	10^{-6} to 5×10^{-6}	(18)
PGE_1	(R)	10^{-6} to 10^{-5}	(9)
PGE_2	(R)	10^{-6} to 10^{-5}	(9)
Isoprenaline	(R)	10^{-7} to 5×10^{-6}	(17)
Noradrenaline	(R)	10^{-5} to 5×10^{-5}	(7)
Phenylephrine	(C)	10^{-5} to 10^{-4}	(10)
Bradykinin	(C)	10^{-5} to 10^{-4}	(5)

C = Contraction taken from rest.
R = Relaxation taken from partially (carbachol or $PGF_{2\alpha}$) contracted strips.
Values in parentheses indicate number of observations.

* Correspondence.

histamine and 5-HT (10^{-4} to 5×10^{-3} M) and bradykinin (10^{-5}–10^{-4} M) invariably contracted the chicken isolated bronchial strips in a dose-dependent manner. Thus histamine and 5-HT were 100 to 1000 fold less active than carbachol and $PGF_{2\alpha}$ about 2 to 20 fold less potent. PGE_1 and E_2 (10^{-6} to 5×10^{-5} M) produced weak relaxations of bronchi submaximally contracted to $PGF_{2\alpha}$. PGE_1 and E_2 either produced a weak (n = 7) or no (n = 5) relaxation of carbachol-contracted strips. Strips which were partially contracted to carbachol relaxed to adrenaline but contracted to phenylephrine. Isoprenaline was 10 to 100 times more potent as a bronchodilator than adrenaline. Typical responses of some of the bronchoactive agents on chicken isolated bronchus are depicted in Fig. 1.

The responses of bronchi from sensitized and non-sensitized chickens to the above drugs were in general similar, but 'sensitized' bronchi were found to be approximately 5 to 10 fold more reactive to $PGF_{2\alpha}$, histamine and 5-HT compared with bronchi of the non-sensitized birds.

Schultz-Dale reaction

Twelve of 18 isolated bronchi from actively-sensitized chickens produced strong contractions (20–80% of the histamine maximum) to specific antigen (horse plasma: 0·3 to 0·5 ml in 30 ml bath) with a delay of 1 to 5 min. Three other bronchi produced a threshold contraction only, while the remaining three did not respond at all to antigen challenge. A second antigen challenge of the bronchi with the same or a higher dose of the antigen produced little or no response (desensitization). Washing the tissues frequently and allowing them to rest for 1 to 2 h resulted in a partial (approximately 50%) recovery of the original antigen response (Fig. 1) with no change in the responsiveness of the tissues to spasmogenic drugs. Nine bronchi from non-sensitized birds all failed to respond to horse plasma (0·3 to 0·5 ml in 30 ml bath). Five bronchial strips from the horse plasma sensitized chickens did not contract to bovine plasma (0·3 to 0·5 ml in 30 ml bath).

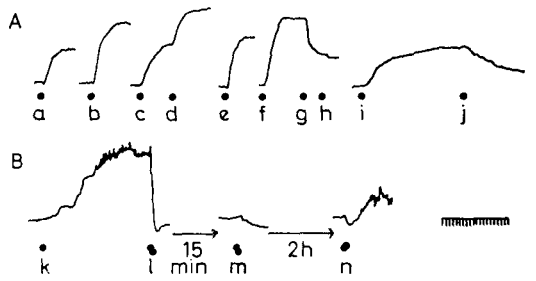

FIG. 1. (A) Isolated chicken bronchial strips at resting tension of 1 g, contracting to histamine (H), 5-HT, phenylephrine (PE), carbachol (carb) and $PGF_{2\alpha}$. PGE_1, adrenaline (adren) and isoprenaline (isop) relaxes the contracted strip. (B) Schultz-Dale anaphylactic reaction of isolated bronchus obtained from horse plasma (1 ml kg^{-1}, i.v.) sensitized chicken. Second antigenic challenge produced weak relaxation (desensitization). Washing the strip frequently with Krebs solution during a 2 h period resulted in partial recovery of antigen response. Doses of agonists are in molar (M) concentration; horse plasma (HP) in ml. Horizontal scale: min. Concn (M) except where shown otherwise. a, H 5×10^{-4}; b, H 10^{-3}; c, 5-HT 10^{-4}; d, PE 10^{-4}, e, carb 5×10^{-6}; f, carb 10^{-5}; g, adren 5×10^{-5}; h, adren 10^{-4}; i, $PGF_{2\alpha}$ 10^{-5}; j, PGE_1 10^{-5}; k, HP 0·3ml; l, isop 5×10^{-7}; m, HP 0·3ml; n, HP 0·3ml.

DISCUSSION

In the present investigation, chicken bronchus contracted strongly to specific antigen, carbachol, $PGF_{2\alpha}$, histamine and 5-HT but weakly to bradykinin and phenylephrine. Strong relaxation to isoprenaline and adrenaline and weak relaxation to PGE_1 and PGE_2 were also recorded. The observed increased reactivity of the sensitized bronchial smooth muscles to $PGF_{2\alpha}$, 5-HT and histamine compared with controls is in accord with similar hyperreactivity of 'asthmatic' airways to these drugs (Itkin, 1967). It has been proposed that β-adrenoceptor (Szentivanyi, 1968) or H_2-histamine receptor (Busse & Sosman, 1977) deficiency might exist in hypersensitivity (allergic) states.

When the effects of histamine are compared with mammalian airway smooth muscles, chicken bronchus appears to occupy a 'middle' position between tissues which have extremely low sensitivity to histamine, e.g. tracheal muscle of cat (Lulich, Mitchell & Sparrow, 1976; Chand & Eyre, 1977b, c), pig (Main, 1964), bronchus of sheep (Eyre, 1969; 1973) and those which are highly sensitive to its spasmogenic action e.g. trachea and bronchus of guinea-pig, dog, horse and man (Persson & Ekman, 1976; Chand & Eyre, 1977c).

Also, compared with the typical strong constrictor action of 5-HT on the airway (tracheal and bronchial) smooth muscles of the cat, sheep, horse and dog (Eyre, 1969; Chand & Eyre, 1977c), chicken bronchus is poorly responsive to this biogenic amine. The bronchoconstrictor action of 5-HT in chicken appears to be similar to its action on trachea of rabbit and rat and bronchus of man and rabbit (Mathé, Astrom & Persson, 1971; Fleisch & Calkins, 1976; Chand & Eyre, 1977c).

Bradykinin is a weak bronchoconstrictor in the chicken. This observation is in sharp contrast to the

potent constriction by kinins of trachea and bronchus of guinea-pig, horse and ferret and relaxation in the trachea of cat, guinea-pig, rabbit, dog and bronchi of man, rabbit, cat and dog (Mathé & others, 1971; Chand & Eyre, 1977c, d).

$PGF_{2\alpha}$ was 10 to 100 times more potent in causing bronchoconstriction in chicken than histamine, 5-HT and bradykinin. $PGF_{2\alpha}$ has been reported earlier to contract airway smooth muscles of man and animals (Main, 1964; Lulich & others, 1976; Chand & Eyre, 1977b,c,d).

PGE_1 and E_2 have been extensively reported to produce relaxation in a number of respiratory smooth muscle preparations. In the present study, PGE_2 produced either weak or no relaxation of carbachol-contracted chicken bronchus but effectively relaxed $PGF_{2\alpha}$-contracted bronchus. PGE_1 however relaxed both carbachol and $PGF_{2\alpha}$-contracted bronchus.

Despite the relatively weak inhibitory activity of PGE_1 and E_2 on chicken bronchus, the generally accepted view of opposite actions of prostaglandins of the E and F series on airway smooth muscles appears to be supported by this study.

The chicken bronchus may now be added to the long list of tissues of several species which give the Schultz-Dale reaction.

The demonstration of Schultz-Dale reactions in the intestine (Chand & Eyre, 1976), pulmonary blood vessels (Chand & Eyre, 1977a) and bronchi (this study) of actively sensitized chickens suggest the presence of tissue fixed antibodies, capable of interacting with specific sensitizing antigens on challenge, and resulting in the release of pharmacologically-active substances (most probably from mast cells) and which contract smooth muscle.

In this study the following criteria, characteristic of Schultz-Dale phenomenon were satisfied: (i) tissues from *non-sensitized* chickens did not react to specific sensitizing antigen even at high bath concentrations; (ii) tissues from sensitized birds did not react to heterologous *non-specific* antigen; (iii) specific antigen-induced contractions were invariably preceded by a latent period of at least 30 s: probably indicative of antigen-antibody reaction and formation and release of active substances from the mast cells; (iv) tachyphylaxis or desensitization was observed on second antigen challenge; and (v) the antigen response partially recovered after 1 to 3 h of resensitization 'rest' interval.

It would not be unreasonable to suggest that the Scultz-Dale anaphylaxis may contribute to bronchospasm (Aronson, Bilstad & Wolfe, 1961) producing dyspnoea and apnoea, the characteristic feature of systemic anaphylaxis in chickens (Lecomte & Beaumariage, 1958; Pedersoli, 1973; Chand & Eyre, unpublished observations). It is a matter of speculation but certainly a possibility, that local bronchial anaphylaxis may contribute to the etiology of important diseases of the airways of the domestic fowl. There is considerable similarity in the symptoms of anaphylaxis and a disease causing sudden death in growing chickens, commonly known as 'flips', 'flip-over', 'lung oedema' or 'heart attack' (Howell, 1972). Acute pulmonary venoconstriction (Chand & Eyre, 1977a) and bronchospasm (Aronson & others, 1961; this study) resulting from immediate hypersensitivity reactions (Makinodan & others, 1952; Lecomte & Beaumariage, 1958; Aronson & others, 1961; Pedersoli, 1973) may be considered as one of several possible causes of this avian disease of unknown etiology.

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