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PHARMACOLOGY OF SCHULTZ-DALE REACTION IN CANINE LUNG STRIP *in vitro*: POSSIBLE MODEL FOR ALLERGIC ASTHMA

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1 Isolated lung parenchymal strips of the dog contracted in response to histamine > carbachol > prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) > bradykinin (Bk) > 5-hydroxytryptamine (5-HT). The order of the relative activity of these agents on the tracheobronchial smooth muscles (TBSM) was carbachol > 5-HT > histamine; $PGF_{2\alpha}$ and Bk were inactive. Thus there are marked differences in the responsiveness of the smooth muscle of central (trachea and bronchus) and peripheral (lung strip) airways to autonomic and autacoid agents.

2 Lung strips and TBSM partially contracted by carbachol, histamine or horse plasma, were relaxed by isoprenaline, PGE_1 and PGE_2.

3 Lung strips from dogs sensitized to horse-plasma contracted in response to antigen (Schultz-Dale anaphylactic reaction). Tachyphylaxis or desensitization to subsequent antigen challenge was invariably observed; it was followed after 1 to 2 h of rest by partial recovery of the anaphylactic response.

4 Mepyramine selectively antagonized responses to histamine without altering responses to carbachol and antigen.

5 Metiamide, an H_2-receptor antagonist, did not influence responses to histamine, carbachol or horse plasma.

6 Indomethacin was found to be ineffective as an inhibitor of the Schultz-Dale anaphylactic reaction.

7 The results showed the presence of H_1-histamine receptors mediating constriction in the peripheral airways of the dog. Histamine and $PGF_{2\alpha}$ appear to have no important role in the anaphylactic reaction in this tissue. The involvement of slow reacting substance of anaphylaxis (SRS-A) and endoperoxides (thromboxanes) in allergic reactions of canine lung is strongly suggested.

Introduction

The Schultz-Dale phenomenon i.e. *in vitro* contraction of sensitized smooth muscles to antigens (Schultz, 1910; Dale, 1913) has frequently been used to demonstrate immediate hypersensitivity in a number of tissues in man and animals (Chand & Eyre, 1978a). Anaphylactic contractions to specific antigens have been demonstrated in the bronchus of guinea-pig (Grover, 1932), man (Schild, Hawkins, Mongar & Herxheimer, 1951; Brocklehurst, 1960; Dunlop & Smith, 1975; 1977), cat and chicken (Chand & Eyre, 1977a; 1978b); in the trachea of guinea-pig (Joiner, Wall, Davis & Hahn, 1974), cat (Lulich, Mitchell & Sparrow, 1976; Chand & Eyre, 1977a), monkey (Patterson, Miyamoto, Reynolds & Pruzansky, 1967) and dog (Antanissen, Mitchell, Kroeger & Stephens, 1978); and in the cat lung parenchymal strip (Lulich *et al.*, 1976).

Dogs have frequently been used as a model for allergic asthma in man (Booth, Patterson & Talbot, 1970; Gold, Kessler, Yu & Frick, 1972; Meyers, Dain, Gold, Miller & Bourne, 1977; Krell & Chakrin, 1976; 1977; 1978; Krell, 1978). In the present investigation we evaluated the lung strip as an *in vitro* model for canine asthma and studied its reactivity to antigen and some autonomic and autacoid agents.

Methods

Seventeen adult dogs of mixed breed and either sex were used. Eight dogs were used as controls and nine dogs were sensitized to horse plasma. On day 1, an emulsion of 1 ml of horse plasma and 1 ml of Freund's incomplete adjuvant was injected subcutaneously (Dhaliwal, Arkins & Berger, 1971); subsequently alternative intravenous and subcutaneous injections of 1 ml of horse plasma were given daily for 5 days (Thomas & Essex, 1949). On day 21, dogs

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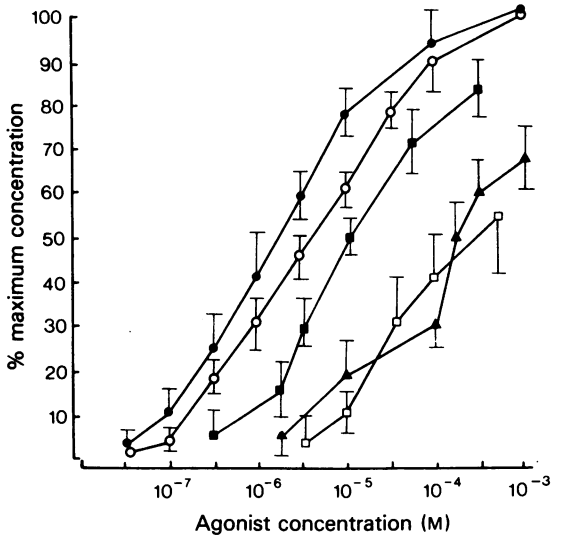

Figure 1 Dose–response curves showing the relative spasmogenic activity of histamine (●), carbachol (○), prostaglandin $F_{2\alpha}$ (■), 5-hydroxytryptamine (▲) and bradykinin (□) on isolated lung strips of the dog in Krebs solution mixed with 5% CO_2 in O_2, at 37°C. Mean results are shown; vertical lines indicate s.d. The number of observations with each dose of each agonist was 7 to 12.

were skin tested with intradermal injections of serial dilutions of horse plasma and histamine in 0.9% w/v NaCl solution (saline). On days 28, 29 or 30, dogs were given pentobarbitone sodium (50 mg/kg, i.v.), exsanguinated and their lungs removed into ice-cold oxygenated Krebs solution.

Isolated lung strips of dogs were prepared as described for cat strips (Lulich *et al.*, 1976). The strips (25 × 3 × 2 mm) were dissected from the apical and cardiac lobes. Isotonic responses to drugs and antigen on paired lung strips were recorded as described by Eyre (1971) and Chand & Eyre (1977a; 1978b).

Results

The relative spasmogenic activity of histamine, carbachol, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), bradykinin (Bk) and 5-hydroxytryptamine (5-HT) on isolated lung strips of the dog is presented in Figure 1. Typical responses of a dog lung strip to some spasmogens are shown in Figure 2a.

Lung strips that were partially contracted (50 ± 10% of maximum) by histamine, carbachol or horse plasma, were relaxed by isoprenaline, PGE_1 and PGE_2 (Figure 2b). The EC_{50} concentration ranges (μmol/l) of these relaxants on histamine-contracted lung strips were as follows: isoprenaline 1 to 5 (n = 17); PGE_1 4 to 10 (n = 14) and PGE_2 5 to 10 (n = 15). In general, isoprenaline was 4 to 10 times more potent as a relaxant than PGE_1 or PGE_2.

Strips stored at 4°C, in oxygenated Krebs solution for 6 to 14 h showed no change in the responsiveness to drugs and antigen. The lung strips from both sensitized and non-sensitized dogs exhibited similar responses to the agonists used.

Schultz-Dale reaction

None of the lung strips obtained from control (unsensitized) dogs (n = 8) showed any reaction to horse plasma (1:100 to 1:50). Two out of nine sensitized dogs exhibited poor reactivity to intradermal injections of the antigen. The lung strips obtained from these two dogs showed only threshold contractions to antigen. However, the lung strips obtained from other sensitized dogs (n = 7) exhibited strong anaphylactic contractions (63 ± 27%, mean ± s.d., of histamine maximum) to horse plasma (1:100) (Figure 2c) with a delay of 30 to 120 s, reaching a maximum in 3 to 10 min. The subsequent antigen challenge, within 15 min, invariably produced tachyphylaxis or desensitization of the anaphylactic response (Figure 2c). After 1 to 2 h of rest with frequent washings the antigen response often recovered partially (25 to 50%). Anaphylactic responses were not affected by indomethacin (5 μM).

Effects of blocking agents on Schultz-Dale reaction

Mepyramine (0.2 μM) significantly ($P < 0.05$) inhibited histamine-induced contractions (dose-ratio = 15 ± 6, mean ± s.d., n = 11) without altering responses to carbachol (dose-ratio = 1, n = 11) and antigen (n = 5).

Metiamide (5 to 10 μM) did not influence responses to histamine, to carbachol (dose-ratio = 1; n = 7 with each dose of metiamide) or to antigen (n = 5).

Indomethacin (5 μM) slightly (15 ± 7%) enhanced contractile responses to histamine, but did not alter responses to carbachol and antigen.

Discussion

Dog lung strips were contracted by histamine > carbachol > $PGF_{2\alpha}$ > Bk > 5-HT and relaxed by isoprenaline > PGE_1 > PGE_2. Cat, dog and guinea-pig lung strips exhibit similar responses to some of these agonists (Lulich *et al.*, 1976; Kleinstiver & Eyre, 1978; Schneider, Drazen, Snapper, Loring & Ingram, 1978; Drazen & Schneider, 1978; Chand & DeRoth, 1978; Yen, 1978). In a similar way, canine bronchus and trachea are contracted by carbachol > 5-HT > histamine and relaxed by isoprenaline > PGE_1 > PGE_2

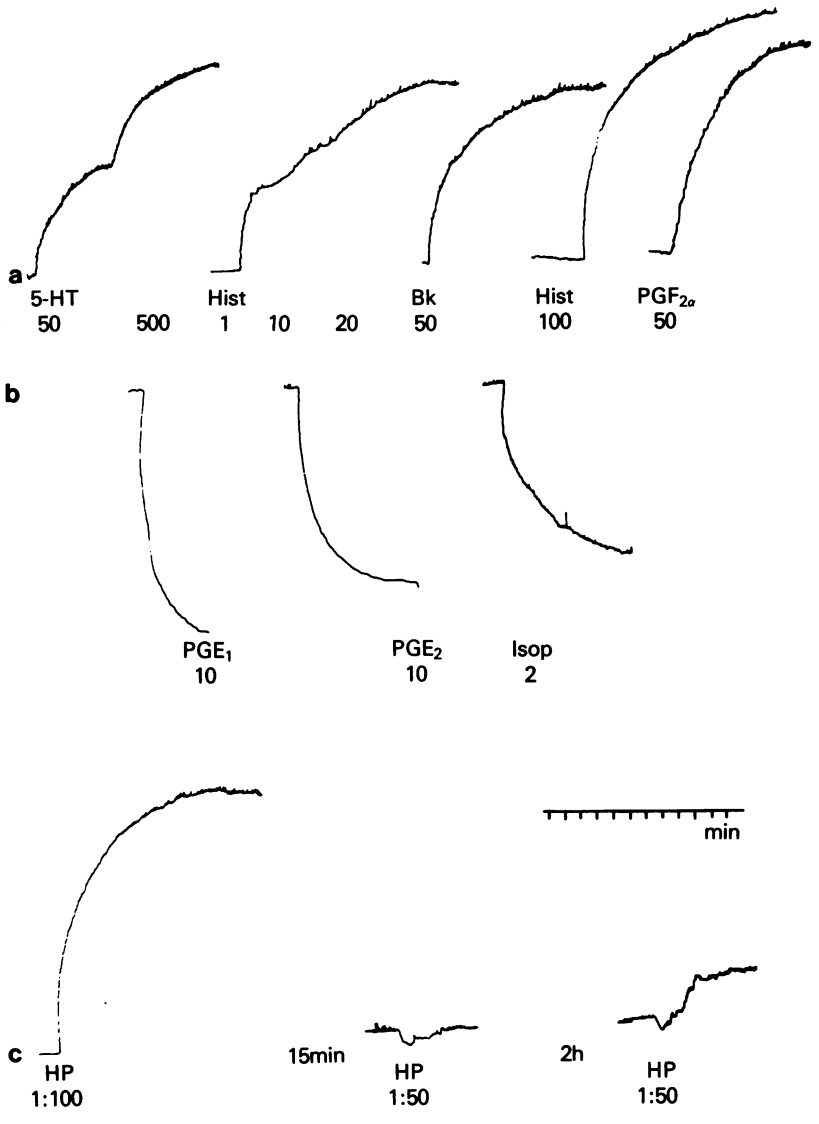

Figure 2 Isolated lung strip of dog (25 × 3 × 2 mm) in Krebs solution mixed with 5% CO_2 in O_2, maintained at 37°C. (Resting tension = 3 g). (a) Contractile responses to 5-hydroxytryptamine (5-HT), histamine (Hist), bradykinin (Bk) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) are recorded from rest. (b) Lung strip contracted (50 ± 10% of maximum) by histamine, relaxed by PGE_1, PGE_2 and isoprenaline (Isop). (c) Schultz-Dale anaphylactic contraction induced by horse plasma (HP 1:100 bath concentration). After 15 min of rest, the response to antigen was abolished due to tachyphylaxis. After a further 2 h of rest with frequent washings, the response to antigen had partially (25%) recovered. The responses in presence of 5 μM indomethacin (shown in figure) did not differ from those in absence of the drug. The doses of agonists are expressed as final bath concentration (μM). Time marker indicates minutes.

(Joiner, Minor, Davis, Kadowitz & Hyman, 1975; Chand & Eyre, 1977b). In contrast to the lung strip, the dog bronchus is either unresponsive or weakly contracts or relaxes in response to $PGF_{2\alpha}$ and Bk (Joiner *et al.*, 1975; Chand & Eyre, 1977c). Thus there are significant qualitative and quantitative differences in the reactivity of the central (trachea and bronchus) and peripheral airways (lung strip: bronchioles and bronchio-alveolar ducts) to autonomic and autacoid agents in dogs (Joiner *et al.*, 1975; Chand & Eyre,

1977b, c; Schneider *et al.*, 1978; this study). The lung strip is not only useful for the study of effects of chemical mediators on the peripheral airways (Lulich *et al.*, 1976) but also for screening anti-asthmatic (anti-allergic) agents and possibly for measuring the cyclic nucleotide changes associated with contraction and relaxation induced by drugs and antigens in the peripheral airways of man and animals.

The canine lung strip may now be added to the growing list of tissues which produce a Schultz-Dale reaction (Chand & Eyre, 1978a). The exact mechanisms of initiation, tachyphylaxis and recovery of the Schultz-Dale reaction are still largely unknown (Chand & Eyre, 1978a). However, the release of potent chemical mediators like slow reacting substance of anaphylaxis (SRS-A, Brocklehurst, 1960; Krell & Chakrin, 1977; 1978), $PGF_{2\alpha}$ (Vane, 1971), endoperoxides or thromboxanes (Chijimatsu, Nguyên & Said, 1977; Piper, 1977) and bradykinin from the sensitized mast cells which are found in abundance in canine peripheral airways (Meyer *et al.*, 1973), may account for the anaphylactic reaction in the lung strip.

Metiamide, an H_2-receptor antagonist (Black, Duncan, Emmett, Ganellin, Hesselbo, Parsons & Wyllie, 1973), has been shown to inhibit histamine-induced relaxations or to enhance histamine-induced airway constrictions in the bronchus of sheep, man, guinea-pig, horse and chicken; cat trachea; trachea and lung strip of guinea-pig (Eyre, 1973; Chand & Eyre, 1977b, d; 1978c, d; Dunlop & Smith, 1977; Okpako, Chand & Eyre, 1978; Chand & DeRoth, 1978; Yen, 1978). H_2-inhibitory histamine receptors have been demonstrated in the tracheobronchial smooth muscles of these species. However, dog trachea (Antonissen *et al.*, 1978) and lung strip (this study) appear to possess only H_1-histamine receptors.

Mepyramine, an H_1-receptor antagonist (Ash & Schild, 1966) exhibits variable effects on anaphylactic contractions of airways *in vitro* (Chand & Eyre, 1978a). The drug only attenuates anaphylactic contractions in guinea-pig trachea (Joiner *et al.*, 1974); cat lung strip (Lulich *et al.*, 1976) and human bronchus (Dunlop & Smith, 1977), but completely inhibits them in dog trachea (Antonissen *et al.*, 1978). In contrast to its actions in the trachea, mepyramine was ineffective in anaphylactic reactions of dog lung strip. This discrepancy may be due to differences in the doses of mepyramine, or it may show that in the lung strip other chemical mediators (eg. SRS-A or endoperoxides) play a more important role than histamine.

Metiamide enhances immunological release of histamine without affecting the release of SRS-A from the canine lung (Krell & Chakrin, 1977). It also potentiates *in vitro* anaphylactic constriction of the human bronchus (Dunlop & Smith, 1977), systemic anaphylaxis in the calf and horse (Eyre & Wells, 1973; Eyre, 1976) and domestic fowl (Chand & Eyre, 1978e) and pulmonary anaphylactic response in the guinea-pig (Drazen, Venugopalan & Soter, 1978). However, metiamide is ineffective in the *in vivo* immediate hypersensitivity reactions of the dog (Krell & Chakrin, 1977) as well as *in vitro* models of canine asthma, i.e. Schultz-Dale reaction in trachea (Antonissen *et al.*, 1978) and lung strip (this study).

Indomethacin, a potent inhibitor of prostaglandin synthesis (Vane, 1971), attenuates *in vitro* anaphylactic reactions of the human bronchus implicating the role of $PGF_{2\alpha}$ in allergic bronchoconstriction (Dunlop & Smith, 1975). Indomethacin enhances the immunological release of SRS-A from dog lung (Krell & Chakrin, 1978). However, it did not enhance the contractile responses to the antigen of dog lung strip (this study) and of human bronchus (Dunlop & Smith, 1975). As indomethacin did not inhibit the anaphylactic reaction in canine lung strips, it seems unlikely that $PGF_{2\alpha}$ is an important mediator of the anaphylactic response in this tissue. Therefore other chemical mediators (eg. SRS-A) may be playing a more important role than biogenic amines and prostaglandins in the mediation of the anaphylactic response in the peripheral airways of dogs.

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