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DUAL EFFECTS OF ASPIRIN IN GUINEA-PIG LUNGS

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1 Dual effects of aspirin were demonstrated in guinea-pig lungs: (a) aspirin (3.3 mg/kg i.v.) antagonized bronchoconstriction induced by slow reacting substance of anaphylaxis (SRS-A); (b) aspirin produced bronchoconstriction when injected in the presence of propranolol into guinea-pigs *in vivo* at 330 mg/kg, or into guinea-pig isolated lungs *in vitro* as a 4% solution (40 mg/ml).

2 The severity of bronchoconstriction following administration of aspirin was directly related to the degree of β-adrenoceptor blockade and to the age of the guinea-pigs. Aspirin-induced bronchoconstriction was prevented *in vivo* and *in vitro* by atropine and it could be reversed *in vivo* by atropine. Aspirin-induced bronchoconstriction was not inhibited by vagotomy or phenoxybenzamine.

3 These data suggest that the mechanism involved in aspirin-induced bronchoconstriction may be local cholinergic stimulation and that reduced β-adrenergic drive may be a predisposing factor.

Introduction

Hypersensitivity to aspirin in man has been described as a triad of symptoms: skin reactions, nasal polyps, and the most serious symptom, asthma. The estimated incidence of hypersensitivity to aspirin ranges from 0.2% in the total population (Giraldo, Blumenthal & Spink, 1969), to 10–20% in the asthmatic population (Von Maur, Adkinson, Van Metre, Marsh & Norman, 1974).

Other analgesic and/or anti-inflammatory agents, such as mefenamic acid, indomethacin, paracetamol and phenylbutazone may also produce asthmatic episodes in man (Samter & Beers, 1968; Smith, 1971; Szczeklik, Gryglewski & Czerniawska-Mysik, 1975; Delaney, 1975). Paradoxically, most of these agents counteract bronchoconstriction induced in experimental animals by slow reacting substance of anaphylaxis (SRS-A) or bradykinin (Berry & Collier, 1964). The numerous attempts to elucidate the mechanism of aspirin-induced asthma have not revealed specific immunological reactions due to aspirin itself (Pearson, 1963; Giraldo *et al.*, 1969; Yurchak, Wicher & Arbesman, 1970; Vatanasuk, Hornbrook & Kohler, 1971; Schlumberger, Lobbecke & Kallos, 1974). However, de Weck (1971) has suggested that the anhydride of aspirin could act as an immunogen. Samter & Beers (1968) have postulated that altered responses of peripheral chemoreceptors initiate a series of reflexes in which mild analgesics act as agonists rather than antagonists. Lately, inhibition of biosynthesis of prostaglandins by aspirin and other mild analgesics has been associated with their ability to induce asthmatic episodes (Szczeklik *et al.*, 1975). However, there is little clinical or experimental evidence suggesting that prostaglandins are directly involved in aspirin-induced bronchoconstriction (Von Maur *et al.*, 1974; Delaney, Smith & Silver, 1975). Fisherman & Cohen (1974), measuring mainly bleeding time, found that patients intolerant to aspirin were over-responsive to α-adrenergic stimulation.

There is general agreement that intolerance to aspirin is more frequent in middle-aged than in juvenile asthmatics, and that the mechanism of intolerance to aspirin is not immunological.

In order to study the different aspects of aspirin-induced asthma, a model of aspirin-induced bronchoconstriction was developed in guinea-pigs. This paper describes a possible mechanism of aspirin-induced bronchoconstriction in guinea-pigs, and compares the bronchoconstrictory effects of aspirin with its ability to inhibit SRS-A-induced bronchoconstriction in guinea-pigs.

Methods

Hartley strain guinea-pigs of either sex weighing 250 to 800 g were used. The weight range was kept within 150 g in any given experiment.

Measurement of bronchoconstriction

Bronchoconstriction induced by SRS-A. SRS-A was harvested as described by Orange & Austen (1968).

Briefly, rats were passively sensitized by intraperitoneal injections of rabbit anti-bovine serum albumen (BSA) antibody. Two hours later, 10 mg of BSA in 5 ml of Tyrode solution was injected intraperitoneally and the peritoneal fluid harvested 5 min later. This fluid constituted the crude SRS-A preparation; it contained an insignificant amount of or no histamine.

Guinea-pigs were anaesthetized with urethane (1.5 g/kg i.p.) and the trachea and jugular vein were cannulated. Respiration was maintained with a rodent pump at 40 strokes/min; the stroke volume was adjusted to guinea-pig weight, and the resistance to pulmonary inflation monitored continuously (Konzett & Roessler, 1940). Immediately after the start of the artificial respiration many of the guinea-pigs were fairly refractory to bronchoconstrictory stimuli. The reactivity of the respiratory tract was therefore repeatedly checked by intravenous injections of histamine 5 μg/kg. When this increased the resistance to inflation by more than 15 mmHg the contractile reactivity of the respiratory tract was considered satisfactory.

In order to enhance the bronchoconstrictor response to SRS-A and to antagonize possible effects of histamine, each guinea-pig received 1.0 mg/kg of propranolol hydrochloride and 0.2 mg/kg of thenyldiamine hydrochloride intravenously 3 min before the injection of SRS-A. The dose (volume) of crude SRS-A was chosen so as to produce an optimal range of bronchoconstriction, viz. 15 to 35 mmHg increases in resistance to pulmonary inflation. Ten parts of aspirin were mixed with three parts of Na_2CO_3 in warm water. The soluble sodium salt was kept on ice and used within 4 hours. It was injected slowly intravenously 3 min before propranolol. Rapid injections occasionally produced immediate bronchospastic reactions. Because SRS-A is labile, the effects of aspirin upon SRS-A-induced bronchoconstriction were studied simultaneously in one guinea-pig medicated with aspirin and in one control guinea-pig. Percentage inhibition of SRS-A-induced bronchoconstriction was calculated from: 1 − (ratio in mmHg of increase in resistance to inflation between medicated and control guinea-pigs) × 100.

Bronchoconstriction induced by analgesics in vivo. Guinea-pigs prepared according to Konzett & Roessler (1940) were pretreated with 1.0 mg/kg of propranolol intravenously unless indicated otherwise. Analgesics were injected intravenously 3 min later. Slow intravenous injections of aspirin or meclofenamate were immediately followed by bronchoconstriction; the peak increase in resistance to pulmonary inflation was recorded as the severity of bronchoconstriction.

Bronchoconstriction in vitro. The thoracic organs of exsanguinated guinea-pigs were removed *in toto*, and the pulmonary artery and trachea were cannulated. Oxygenated Krebs-Henseleit bicarbonate buffer solution (pH 7.0, 40°C) entered the lung at 7.5 ml/min through the trachea (Luduena, Miller & Wilt, 1955) and left it through scarifications on the lung surface. The perfusion pressure in cmH_2O was monitored continuously. Sodium aspirin was injected via the pulmonary artery in Krebs-Henseleit buffer adjusted to pH 7.0, in a volume sufficient to fill the arteriovenous system of each lung-heart preparation. The peak increase in perfusion pressure after the injection of aspirin was recorded as aspirin-induced bronchoconstriction.

Since perfusion pressure in perfused guinea-pig lungs can increase spontaneously, the average rate of spontaneous increases for each minute of perfusion was obtained from ten non-treated lungs. The adjusted increase in perfusion pressure due to aspirin was calculated as A − B, where A = peak increase in perfusion pressure caused by aspirin, and B = average spontaneous increase in perfusion pressure at the time at which A occurred (up to 10 min after aspirin injection).

Significances of differences between the means of medicated and control groups were calculated using the *t* test for independent samples.

Drugs

The following drugs were used in this study: aspirin (Glenbrook Laboratories, 'Bayer needles'), histamine phosphate (Lilly), propranolol hydrochloride (Ayerst Laboratories), thenyldiamine hydrochloride (Sterling-Winthrop Research Institute), sodium meclofenamate (Parke-Davis & Co.), phenoxybenzamine hydrochloride (Smith, Kline & French), and atropine sulphate (Merck & Co., Inc.). Krebs Henseleit bicarbonate buffer solution (pH 7.0) contained the following chemicals (g/l): NaCl 6.19, KCl 0.353, $CaCl_2$ 0.282, KH_2PO_4 0.162, $MgSO_4$ 0.294, $NaHCO_3$ 2.10, and dextrose 1.0.

Results

SRS-A-induced bronchoconstriction

Intravenous administration of aspirin at doses ranging from 3.3 to 330 mg/kg to 300–370 g guinea-pigs resulted in 70 to 90% inhibition of SRS-A-induced bronchoconstriction (Figure 1). The ED_{50} was 1.5 mg/kg intravenously. The same doses of aspirin administered to 400 to 550 g guinea-pigs were, in general, less effective and a dose of 330 mg/kg had no significant inhibitory effect in 400 to 550 g guinea-pigs (Figure 1).

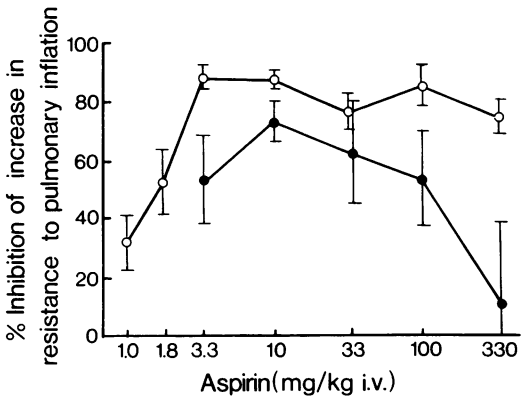

Figure 1 Inhibition of SRS-A-induced bronchoconstriction by aspirin in anaesthetized guinea-pigs: (○) 300–370 g guinea-pigs; (●) 400–550 g guinea-pigs. Mean of 5–10 guinea-pigs/group are shown. Vertical lines show s.e. mean. Inhibition was significant ($P<0.01$) at all doses, except for the 330 mg/kg dose in 400–550 g guinea-pigs ($P>0.1$).

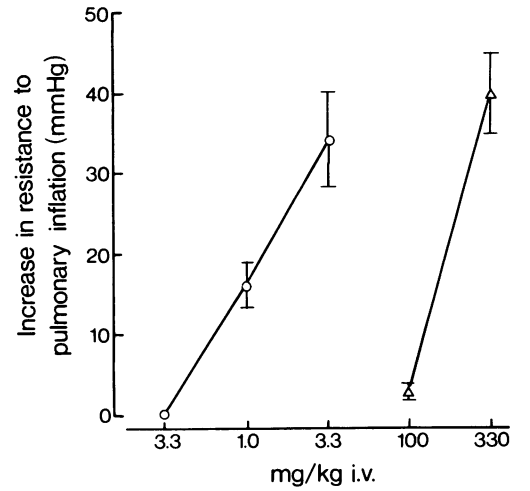

Figure 2 Bronchoconstriction in guinea-pigs following intravenous administration of aspirin (△) or meclofenamate (○). 3 min after propranolol (1 mg/kg i.v.). Means of 5–6 guinea-pigs (500–600 g each)/group are shown. Vertical lines show s.e. mean.

Drug-induced bronchoconstriction in vivo

When relatively high doses of aspirin or meclofenamate were administered to 500 to 600 g guinea-pigs after injections of propranolol (1 mg/kg i.v.), consistent and significant bronchoconstriction developed (Figure 2) which attained peak values in most cases within 1 to 2 minutes. The resistance to inflation increased for short periods (1–5 min) if the reaction was mild (5–10 mmHg), but greater increases

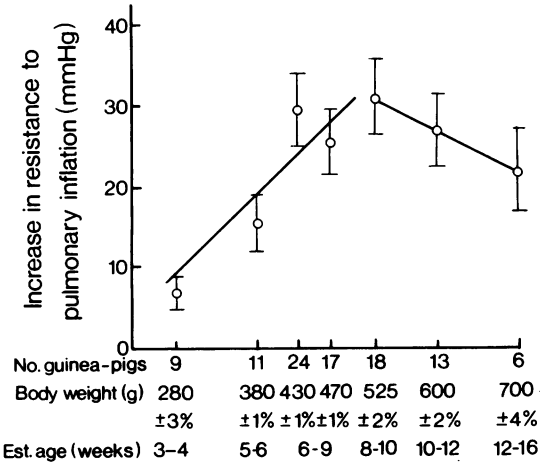

Figure 3 Effect of age upon aspirin-induced bronchoconstriction in guinea-pigs. Propranolol (1 mg/kg i.v.) was given 3 min before aspirin (330 mg/kg i.v.). All increases in resistance to inflation were significant at $P>0.01$.

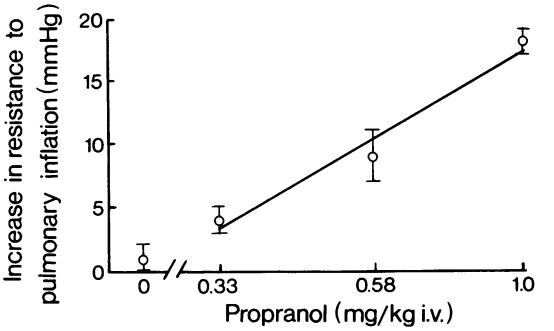

Figure 4 Relationship of β-adrenoceptor blockade to bronchoconstriction following aspirin (330 mg/kg i.v.). Means of 6 guinea-pigs (400–450 g each)/group are shown. Vertical lines show s.e. mean.

in resistance to inflation lasted in most cases proportionately longer. Severe and prolonged bronchoconstriction led to death in most cases. Neither meclofenamate 33 mg/kg nor aspirin 330 mg/kg was lethal in the absence of propranolol.

The age of the guinea-pigs influenced significantly aspirin-induced bronchoconstriction which was related to age up to 8–10 weeks (expressed as body weight; Figure 3).

The role of the β-adrenergic system in aspirin-induced bronchoconstriction was studied further by administering graded intravenous doses of propranolol before the injection of aspirin. Increases in resistance to pulmonary inflation due to aspirin were related to the dose of propranolol and the resulting degree of β-adrenoceptor blockade (Figure 4).

The effect of the α-adrenoceptor system was studied by administering phenoxybenzamine at its maximal tolerated dose (50 mg/kg i.p.) 30 min before the intravenous injections of propranolol and aspirin. This dose of phenoxybenzamine blocked bronchoconstriction induced by a low dose of the α-adrenoceptor stimulant metaraminol (3.3 mg/kg i.v.) but not by a high dose (10 mg/kg). The peak increase

Table 1 Effects of atropine, phenoxybenzamine, or vagotomy upon aspirin-induced bronchoconstriction in guinea-pigs *in vivo*

Treatment before propranolol	*Body wt. (g) (mean ± s.e.)*	*Peak increase in resistance to inflation after aspirin (mmHg) (mean ± s.e.)*
Atropine (1.0 mg/kg i.v.)	485 ± 26	4 ± 2*
Controls	480 ± 20	29 ± 6
Phenoxybenzamine (50 mg/kg i.p.)	428 ± 12	12 ± 6
Controls	411 ± 8	18 ± 4
Vagotomy	496 ± 26	19 ± 4
Controls	490 ± 22	19 ± 5

Propranolol (1.0 mg/kg i.v.) was given 3 min before sodium aspirin (400 mg/kg i.v.) 10–13 guinea-pigs/group.
* $P < 0.01$.

Table 2 Reversal of aspirin-induced bronchoconstriction in guinea-pigs by atropine

Body wt. (g) (mean ± s.e.)	*Peak increase in resistance to iflation after aspirin (mmHg†) (mean ± s.e.)*	*Injection at peak of bronchoconstriction*	*Resistance to inflation after atropine or saline (mmHg†) (mean ± s.e.)*	
			3 min	*7 min*
740 ± 78	36 ± 4	Saline (i.v.)	33 ± 3	32 ± 4
723 ± 40	26 ± 3	Atropine (1 mg/kg i.v.)	1 ± 1*	0 ± 1*

There were five guinea-pigs/group. Propranolol 1.0 mg/kg i.v. 3 min before sodium aspirin 400 mg/kg i.v.
† Above basal value.
* P value of the effect of atropine vs. control < 0.01.

Table 3 Effects of propranolol and/or atropine on bronchoconstriction induced in guinea-pig isolated lungs by 4% sodium aspirin intra-arterially

Group	*Drug in perfusate*	*Body wt. (g) (mean ± s.e.)*	*Adjusted peak increase in perfusion pressure after Na aspirin (cmH_2O) (mean ± s.e.)*	P *values*	
A	—	323 ± 11	6 ± 4		—
B	Propranolol (4 μM)	306 ± 18	>21 ± 4*	*vs* A	<0.05
C	Propranolol (4 μM) and atropine (2 μM)	309 ± 7	8 ± 5	*vs* A	>0.1
D	—	506 ± 15	>30 ± 6*	*vs* A	<0.01
E	Atropine (2 μM)	492 ± 22	10 ± 4	*vs* D	<0.05

There were five or six guinea-pig lungs/group.
* One increase in perfusion pressure exceeded the measuring capacity of the apparatus.

in resistance to inflation in guinea-pigs pre-treated with phenoxybenzamine was slightly lower than in control guinea-pigs but the difference was not significant (*t* test; Table 1).

The participation of the parasympathetic nervous system in aspirin-induced bronchoconstriction was studied by severing both vagus nerves 15 min before each experiment and by administering atropine (1.0 mg/kg i.v.) either 3 min before the injection of propranolol, or at the peak of aspirin-induced bronchoconstriction. Aspirin-induced bronchoconstriction was largely prevented or completely reversed by atropine, but not affected by vagotomy (Tables 1 and 2). The small difference between the average peak increases in resistance to inflation in the guinea-pigs to be injected with atropine and in the control guinea-pigs (Table 2) was not statistically significant.

Drug-induced bronchoconstriction in vitro

Intra-arterial injections of 4% sodium aspirin solutions increased perfusion pressure in lungs dissected from guinea-pigs of 475 to 550 g, but not in lungs dissected from younger guinea-pigs (300–350 g) (Table 3). The increases in the lungs from older guinea-pigs were inhibited by the presence of 2 μM atropine in the perfusion fluid. If 4 μM propranolol was added to the fluid perfusing of lungs from younger guinea-pigs, intra-arterial injections of aspirin produced increases in perfusion pressure, which could be abolished by the addition of 2 μM atropine to the perfusion fluid.

Discussion

The results show the contrast between the effectiveness of aspirin against SRS-A-induced bronchoconstriction and the bronchoconstriction induced by aspirin itself. It is apparent that the declining effectiveness of high doses of aspirin against SRS-A-induced bronchoconstriction in older guinea-pigs could be attributed to its own bronchoconstrictor action. A similar reversal of the antibronchoconstrictor effect of the analgesic fenamate when administered at higher doses, has been described by Collier, James & Piper (1968).

The present test system was not suitable for studying bronchoconstriction following oral administration of aspirin or meclofenamate. However, in previous studies (unpublished data), we found that aspirin given orally counteracted SRS-A-induced bronchoconstriction in guinea-pigs with an approximate ED_{50} of 50 mg/kg (given orally). It is not known whether the described action of aspirin against SRS-A in guinea-pigs is related to the occasionally observed anti-asthmatic activity in man (Pearsons, 1963; Stresemann, 1963; Laborie, 1964; Clarke, 1969).

In vivo and *in vitro* studies suggest that aspirin-induced bronchoconstriction may be attributable to local stimulation of cholinoceptors in the presence of a reduced β-adrenergic drive in the lungs of older animals. Thus the sensitivity of β-receptors to isoproterenol is reduced in older rats (Ericsson, 1974). The incidence of aspirin intolerance is higher in middle-aged than in young asthmatics (Pearsons, 1963; Samter & Beers, 1968; Farr, 1970; Falliers, 1973; Von Maur *et al.*, 1974). The slightly decreased intensity of reactions to aspirin of older guinea-pigs (12–16 weeks) as compared to guinea-pigs of 8–10 weeks, may correspond to the decline in aspirin intolerance in patients past middle age (Pearsons, 1963).

Our finding that atropine could block and reverse aspirin-induced bronchoconstriction in guinea-pigs is in agreement with the observations of Fisherman & Cohen (1974) that atropine could block aspirin-induced increases in bleeding time and respiratory symptoms in patients sensitive to aspirin. However, our results differ from Fisherman & Cohen's (1974) in that we could not inhibit aspirin-induced bronchoconstriction by phenoxybenzamine. This failure could be due to a relatively weak blocking effect by phenoxybenzamine, as shown by its lack of activity against a higher dose of metaraminol. Hence, we cannot rule out the possibility that aspirin in a high dose has α-adrenoceptor effects, in addition to its cholinoceptor stimulation.

The present data obtained in guinea-pigs, and Fisherman & Cohen's (1974) findings in patients intolerant to aspirin suggest that the autonomic nervous system is involved in aspirin-induced bronchoconstriction, and that cholinoceptor blockade might be an effective means of preventing and/or reversing aspirin-induced asthmatic attacks.

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