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INCREASED AIRWAY REACTIVITY IN THE GUINEA-PIG FOLLOWS EXPOSURE TO INTRAVENOUS ISOPRENALINE

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SUMMARY

1. Intravenous infusion of (±)isoprenaline (1–100 $\mu g\ kg^{-1}\ h^{-1}$) enhanced airway responses (resistance, R_L; and compliance, C_{dyn}) to histamine (1·0–1·8 $\mu g\ kg^{-1}$) and bombesin (100–240 $ng\ kg^{-1}$), whereas airway responses to vagal stimulation remained unchanged.

2. Bilateral vagotomy before intravenous infusion of (±)isoprenaline (100 $\mu g\ kg^{-1}\ h^{-1}$) prevented development of airway hyperreactivity to histamine or bombesin, yet vagotomy after infusion of isoprenaline was without effect.

3. Prior treatment with atropine (1 $mg\ kg^{-1}$) did not influence the capacity of (±)isoprenaline (100 $\mu g\ kg^{-1}\ h^{-1}$) to increase airway reactivity to bombesin.

4. Despite a 500-fold difference in spasmolytic potency *in vivo*, infusion of (+)isoprenaline (100 $\mu g\ kg^{-1}\ h^{-1}$) or (−)isoprenaline (100 $\mu g\ kg^{-1}\ h^{-1}$) increased reactivity of the airways to histamine or bombesin to a comparable extent.

5. Neither adrenaline (100 $\mu g\ kg^{-1}\ h^{-1}$) nor forskolin (600 $\mu g\ kg^{-1}\ h^{-1}$) increased reactivity of the airways to histamine or bombesin.

6. Intravenous infusion of dopamine (100 $\mu g\ kg^{-1}\ h^{-1}$) or noradrenaline (100 $\mu g\ kg^{-1}\ h^{-1}$) increased reactivity of the airways to histamine or bombesin.

7. Intravenous infusion of (±)propranolol (100 $\mu g\ kg^{-1}\ h^{-1}$) increased reactivity of the airways to histamine or bombesin which was partially inhibited by bilateral vagal section.

8. Depletion of circulating platelets by lytic anti-platelet serum or concomitant infusion of an antagonist of platelet-activating factor (PAF), ginkgolide B (1 $mg\ kg^{-1}\ h^{-1}$), did not diminish the capacity of (±)isoprenaline (100 $\mu g\ kg^{-1}\ h^{-1}$) to induce hyperreactivity of the airways to histamine or bombesin.

9. These observations indicate that (±)isoprenaline can induce airway hyperreactivity by a mechanism unrelated to β-adrenoceptor activation, but which is dependent upon intact vagus nerves.

INTRODUCTION

The potency of (±)isoprenaline as a relaxant of airway smooth muscle is the basis for use of this drug in asthma therapy. Concentrations greater than 10^{-8} M relax

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isolated human bronchus or lung parenchymal strips (Goldie, Paterson & Wale, 1982; Goldie, Spina, Henry, Lulich & Peterson, 1986). Whether administered by intravenous infusion or as an inhalation, (±)isoprenaline effects a reduction of airway obstruction in almost all asthma patients. In some patients, however, increased airway obstruction has been observed and referred to as paradoxical bronchospasm (Trautlein, Allegra, Field & Gillin, 1976) or rebound bronchospasm (Paterson, Courtenay Evans & Prime, 1971). Furthermore, clinical studies indicate that the use of this class of compound may lead to increased reactivity of airways to spasmogens such as histamine or methacholine (Kraan, Koeter, Mark, Sluiter & De Vries, 1985; Kerrebijn, van Essen-Zandvliet & Neijens, 1987). The basis for the anomalous action of such drugs remains uncertain.

In the anaesthetized guinea-pig, intravenous infusion of platelet-activating factor (PAF) causes a platelet-dependent increased responsivity of the airways to spasmogens such as histamine or bombesin (Mazzoni, Morley, Page & Sanjar, 1985*a*; Sanjar, Smith, Schaeublin, Kristersson, Chapman, Mazzoni & Morley, 1989). Several anti-asthma drugs can diminish this effect of PAF, yet co-infusion of (±)isoprenaline consistently increased the intensity of airway hyperreactivity (Mazzoni, Morley, Page & Sanjar, 1985*b*). This unexpected finding led to a study of the effect of (±)isoprenaline upon airway reactivity, which revealed the capacity of this material to increase the sensitivity of guinea-pig airways to spasmogens. Preliminary findings have been presented to the Physiological Society (Morley & Sanjar, 1987).

METHODS

Animals

Dunkin–Hartley guinea-pigs (male, 450–600 g) were used for lung function experiments and New Zealand White rabbits (Male, 3·5–5·0 kg) were used for raising anti-sera.

Preparation of antibodies to guinea-pig platelets

Blood was removed from ether-anaesthetized guinea-pigs by cardiac puncture. Coagulation was prevented by addition of a citrate buffer (Mollison, 1967) to blood (1:10 v/v). Normally two guinea-pigs provided 50 ml of blood and platelets from this quantity of blood were used to sensitize one rabbit. Blood was centrifuged at 200 *g* for 10 min. Platelet-rich plasma (PRP) was removed and centrifuged (1000 *g*, 10 min). Supernatant was discarded and the platelet pellet was resuspended in 1 ml of saline (0·9% NaCl). This suspension was frozen and thawed (three cycles) to fragment the platelets. The brei was mixed with 3 ml of Freund's complete adjuvant (FCA) and homogenized to produce a uniform emulsion, which was injected subcutaneously at three separate sites on the neck and flank of a rabbit. Four weeks later, the above procedure was repeated, and 2 weeks later rabbits were anaesthetized with sodium pentothal (30–50 mg kg^{-1} I.V.) and exsanguinated by cardiac puncture. Clotted blood was stored for 24 h, after which serum containing antibodies to guinea-pig platelets (APS) was separated and heated at 56 °C for 1 h to deactivate complement proteins. Antibodies to erythrocytes were removed by addition of washed guinea-pig erythrocytes to the serum; erythrocytes were sedimented by low-speed centrifugation (200 *g* for 10 min) and serum was aliquoted and stored at −20 °C.

Guinea-pigs were rendered thrombocytopenic by intravenous injection (1 ml kg^{-1}) of APS. A selective reduction (*ca.* 95%) of circulating platelets was observed 24 h after treatment. Untreated guinea-pigs had $451 \pm 64 \times 10^3$ platelets μl^{-1} ($n = 5$) and APS-treated animals had $22 \pm 2 \times 10^3$ platelets μl^{-1} ($n = 5$). Neutrophil numbers were not affected by treatment with APS (control, 5100 ± 543 μl^{-1}, $n = 5$; APS, 4970 ± 610 μl^{-1}, $n = 5$).

Measurement of pulmonary function

Guinea-pigs were anaesthetized with a combination of sodium phenobarbitone (100 mg kg^{-1}, I.P.) and sodium pentobarbitone (30 mg kg^{-1}, I.P.) and paralysed with gallamine (10 mg kg^{-1}, I.M.). Animals were ventilated (8 ml kg^{-1}, 1 Hz) via a tracheal cannula with a mixture of air and oxygen (1:1 v/v) at an end-inspiratory pressure of 60–80 mmH_2O. Respiratory air flow was measured with

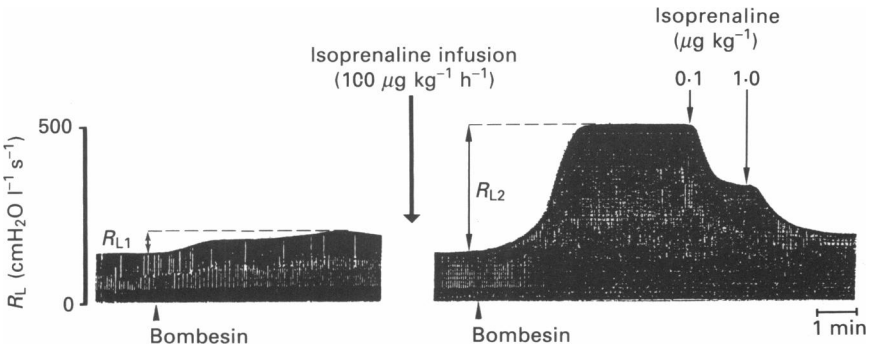

Fig. 1. Increased airway resistance results from an intravenous injection of bombesin (240 ng kg^{-1}) in the anaesthetized ventilated guinea-pig. The left-hand panel shows the response in an animal prior to an intravenous infusion of (±)isoprenaline (100 μg kg^{-1} h^{-1}) and the right-hand panel shows the response to bombesin 30 min after (±)isoprenaline. Increased airway reactivity is expressed as incremental increase in R_L (ΔR_L) which is estimated as the difference between R_{L1} and R_{L2}. In such animals the increased airway resistance can be reversed by intravenous injection of (±)isoprenaline (0·1–1·0 μg kg^{-1}).

a pneumotachograph (type 0000, Fleisch, Switzerland), connected to a differential pressure transducer (type PM45, Validyne, USA), in line with the respiratory pump. Changes of intrapleural pressure with respect to the trachea were measured by an intrathoracic cannula connected to the negative port of a differential pressure transducer (type PM45-24, Validyne, USA) whose positive port was connected, via a T-piece, to the tracheal cannula. Airway resistance (R_L, cmH_2O l^{-1} s^{-1}) and compliance (C_{dyn}, ml cmH_2O^{-1}) were calculated according to the principles of Amdur & Mead (1958), for each breath, with a respiratory analyser (Model 6, Buxco, USA) and displayed on a chart recorder (type R411, Beckman, USA). Airway resistance was measured at an isovolumetric point at 70% of inspiration. Both jugular veins were cannulated for infusion of drugs; a carotid artery was cannulated so that blood pressure and pulse rate could be monitored by a pressure transducer (type PD23Gb, Gould, USA).

Bronchodilator responses

Intravenous injection of bombesin induced a protracted increase in R_L which lasted for 10–15 min, both in untreated and (±)isoprenaline-treated animals. In guinea-pigs which had not received an infusion of (±)isoprenaline, higher doses of bombesin (up to 1·2 μg kg^{-1}) were required to produce an increase in R_L (300–500 cmH_2O l^{-1} s^{-1}) comparable to (±)isoprenaline-treated animals. Bronchodilator efficacy was evaluated during the plateau phase of bronchospasm by bolus intravenous injections, at 1 min intervals, of isoprenaline (Fig. 1) or other β-adrenoceptor agonists at increasing concentrations. The bronchodilator efficacy of each substance was calculated from cumulative dose–effect curves constructed from groups of five animals and a concentration (ng kg^{-1}) which inhibited the increased R_L by 50% (ED_{50}) was estimated.

Vagus nerve stimulation

Vagal nerve stimulation was used as a spasmogenic stimulus before and after infusion of (±)isoprenaline (100 μg kg^{-1} h^{-1}). Guinea-pigs were anaesthetized with urethane (1·6 g kg^{-1} I.P.) and paralysed with suxamethonium (60 μg kg^{-1} I.V. bolus followed by an infusion of

30 μg kg^{-1} min^{-1} throughout the experiment). The vagus nerve on the right side was isolated, tied cranially and placed on platinum electrodes connected to an electrical stimulator (type S48, Grass, USA). Bronchoconstriction was elicited with stimuli of 5 V of 2·5 ms duration at 5 Hz for between 5 and 15 s depending on the responsiveness of individual animals.

Measurement of airway hyperreactivity

After preparation, a period of 10–15 min was allowed to elapse prior to recording pulmonary function. Lungs were hyperinflated by increasing the volume of each inspiration to 15 ml kg^{-1} for three to four breaths, which ensured that airway resistance (basal R_L) was at a low, yet stable, value prior to injection of spasmogens. Basal R_L values normally ranged between 90 and 140 cmH_2O l^{-1} s^{-1}; animals with higher basal R_L values were excluded. Bolus injections of histamine (1–1·8 μg kg^{-1}) and bombesin (100–240 ng kg^{-1}) at 10 min intervals were used to define doses which caused a small increase of R_L. (±)Isoprenaline (1, 10 or 100 μg kg^{-1}), (+)isoprenaline (100 μg kg^{-1}), (−)isoprenaline (100 μg kg^{-1}), adrenaline (100 μg kg^{-1}), noradrenaline (100 μg kg^{-1}), dopamine (100 μg kg^{-1}), forskolin (600 μg kg^{-1}) or (±)propranolol (10 or 100 μg kg^{-1}) were infused for 1 h (2 ml h^{-1}) into a cannulated jugular vein by use of an infusion pump (type 5003, Precidor, Switzerland). Guinea-pigs infused with saline (0·9% NaCl, w/v) served as controls. Thirty minutes after cessation of the infusion, test doses of histamine or bombesin, which gave a marginal response prior to infusion, were injected at 10 min intervals in order to detect changed airway reactivity. A convenient index of airway hyperreactivity was provided by the difference between the maximal R_L or C_{dyn} to spasmogens before, and after, the 1 h infusion of test substance. Hence, airway hyperreactivity has been expressed as incremental increase of airway resistance (ΔR_L) (Fig. 1) or as incremental decrease in compliance (ΔC_{dyn}). Although two spasmogens have been used throughout this study, results recorded with bombesin are presented most frequently, since no cardiovascular effects resulted from intravenous injection of bombesin and since increased R_L was sustained, thereby permitting estimation of bronchodilator activity of β-adrenoceptor agonists and forskolin.

To ascertain the role of the vagus nerve in changed airway responses, both vagus nerves were isolated and transected without severing the sympathetic ganglia. Vagotomy was performed prior to lung function measurements and (±)isoprenaline infusion in a group of ten animals, and after (±)isoprenaline infusion in a group of five animals.

RESULTS

Bronchodilator efficacy

Bronchodilator efficacy of (±)isoprenaline has been attributed to occupancy and activation of β-adrenoceptors, with the (−)isomer being largely responsible for this effect. Bronchodilator efficacy of (±)isoprenaline, (+)isoprenaline, (−)isoprenaline, (±)adrenaline and forskolin was determined in groups of guinea-pigs ($n = 5$) whose airways had been constricted by intravenous injection of bombesin (0·5–1·2 μg kg^{-1}). The observed potency ranking was: (−)isoprenaline (ED_{50} = 1·5 ng kg^{-1}) > (±)isoprenaline (ED_{50} = 10·5 ng kg^{-1}) > adrenaline (ED_{50} = 22·8 ng kg^{-1}) > (+)isoprenaline (ED_{50} = 816·8 ng kg^{-1}) > forskolin (ED_{50} = 2350·4 ng kg^{-1}) (Fig. 2*A*). Bronchodilator efficacy of (±)isoprenaline was also determined in guinea-pigs after infusion of (±)isoprenaline (100 μg kg^{-1} h^{-1}), and was slightly less (ED_{50} = 49·5 ng kg^{-1}) than that observed in untreated animals, with a difference being most prominent at the lowest concentration (Fig. 2*B*). Infusion of propranolol (100 μg kg^{-1} h^{-1}) reduced substantially the bronchodilator efficacy of (±)isoprenaline (ED_{50} = 326·7 ng kg^{-1}), whereas a lower dose of propranolol (10 μg kg^{-1} h^{-1}) had a marginal effect upon bronchodilator responses to (±)isoprenaline (ED_{50} = 22·9 ng kg^{-1}).

Infusion of racemic isoprenaline

Airway resistance in response to intravenous injections of histamine (1–1·8 μg kg^{-1}) or bombesin (240 ng kg^{-1}) had an increased amplitude after animals had been infused with (±)isoprenaline (100 μg kg^{-1} h^{-1}) (Fig. 1). This effect could be detected within

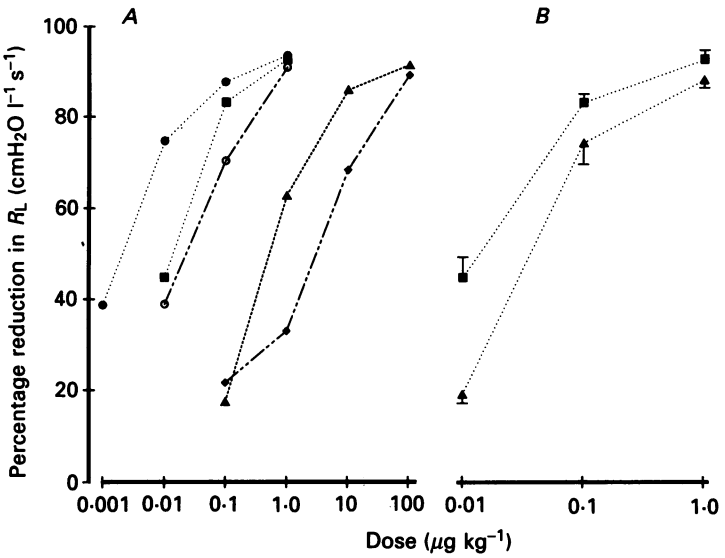

Fig. 2. Inhibition of increased airway resistance (R_L, cmH$_2$O l^{-1} s^{-1}) due to intravenous injection of bombesin (0·5–1·2 μg kg^{-1}) in anaesthetized ventilated guinea-pigs after intravenous injection of (−)isoprenaline (●), (+)isoprenaline (▲), (±)isoprenaline (■), adrenaline (○) or forskolin (◆) (*A*). Comparable inhibition was observed in animals which were untreated (■) or exposed to (±)isoprenaline (▲) (*B*).

30 min of cessation of (±)isoprenaline infusion, and persisted for several hours (> 4 h); the effect was dose-related (1–100 μg kg^{-1}) (Table 1). Airway reactivity to vagal nerve stimulation did not change (ΔR_L, 29±13; ΔC_{dyn} −0·16±0·1, $n = 6$) after infusion of (±)isoprenaline (100 μg kg^{-1} h^{-1}), despite increased reactivity to histamine (ΔR_L, 238±25; ΔC_{dyn}, −0·47±0·1, $n = 8$) or acetylcholine (ΔR_L, 168±36; ΔC_{dyn}, −0·42±0·2, $n = 5$) being evident in these animals (Fig. 3).

Vagal section and atropine

Bilateral vagal section ($n = 10$) prior to infusion of (±)isoprenaline (100 μg kg^{-1} h^{-1}) prevented development of airway hyperreactivity, whereas no inhibition was observed when vagus nerves were sectioned after infusion (Fig. 4). On the other hand, prior treatment with atropine (1 mg kg^{-1}) did not impair the development of airway hyperreactivity that resulted from infusion of (±)isoprenaline (100 μg kg^{-1} h^{-1}) (Fig. 4).

Infusion of (−)- or (+)isoprenaline and related compounds

To ascertain whether induction of airway hyperreactivity by (±)isoprenaline was related to β-adrenoceptor occupancy, infusions of (+)isoprenaline or (−)iso-

TABLE 1. Increases in airway reactivity of anaesthetized ventilated guinea-pigs to bombesin after intravenous infusion of increasing doses of (±)isoprenaline and other substances

Treatment	Dose (μg kg^{-1})	n	Increase in R_L in response to bombesin			Decrease in C_{dyn} in response to bombesin		
			Before	After	ΔR_L	Before	After	ΔC_{dyn}
Saline	—	15	79±20	110±23	32±21	−0·50±0·13	−0·53±0·09	−0·03±0·13
Isoprenaline	1	10	41±9	113±24	72±21	−0·27±0·04	−0·46±0·11	−0·19±0·10
	10	10	53±13	168±37	115±27*	−0·30±0·08	−0·47±0·09	−0·16±0·10
	100	38	74±8	333±19	259±17*	−0·37±0·07	−0·89±0·09	−0·52±0·10*
(+)Isoprenaline	100	10	77±19	288±72	212±65*	−0·45±0·09	−0·86±0·09	−0·42±0·16
(−)Isoprenaline	100	10	137±28	455±95	318±76*	−0·05±0·12	−1·00±0·14	−0·50±0·12*
Adrenaline	100	10	125±33	116±28	−9±22	−0·55±0·17	−0·65±0·16	−0·10±0·11
Forskolin	600	10	81±24	145±26	65±28	−0·30±0·11	−0·45±0·13	−0·14±0·12
Noradrenaline	100	5	83±22	233±44	150±31*	−0·31±0·14	−0·69±0·16	−0·38±0·12
Dopamine	100	5	42±11	208±42	166±37*	−0·16±0·11	−0·64±0·19	−0·47±0·10
Propranolol	10	10	54±14	221±52	166±41*	−0·33±0·09	−0·65±0·12	−0·32±0·07
	100	10	94±21	404±49	310±52*	−0·58±0·14	−0·92±0·17	−0·34±0·18

Racemic mixtures of each compound have been used for infusion, unless otherwise indicated. Dose indicates the amount of substance (μg kg^{-1}) that was infused intravenously over a 1 h period. R_L values are in cmH_2O l^{-1} s^{-1} and C_{dyn} in ml cmH_2O^{-1}. Student's t test for groups of unequal size was used to assess statistical significance of differences between treatment and saline; * indicates at least $P < 0{\cdot}025$.

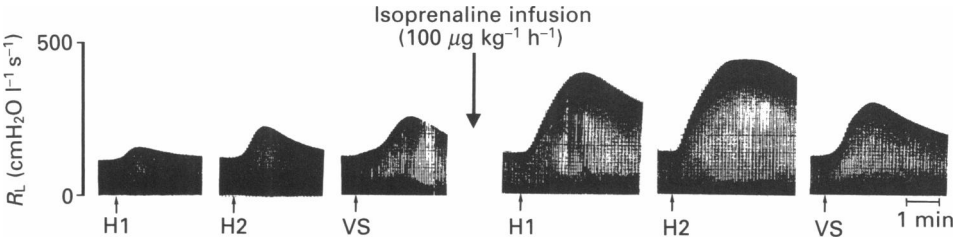

Fig. 3. Increased airway resistance in response to intravenous injection of histamine (H1, 1·0 μg kg^{-1}; H2, 1·8 μg kg^{-1}) and vagal nerve stimulation (VS; 5 V, 5 Hz, 2·5 ms for 10 s) before (left-hand panel) and after (right-hand panel) an intravenous injection of (±)isoprenaline (100 μg kg^{-1} h^{-1}). Whereas histamine responses are substantially increased, bronchoconstriction due to vagal nerve stimulation is only slightly affected.

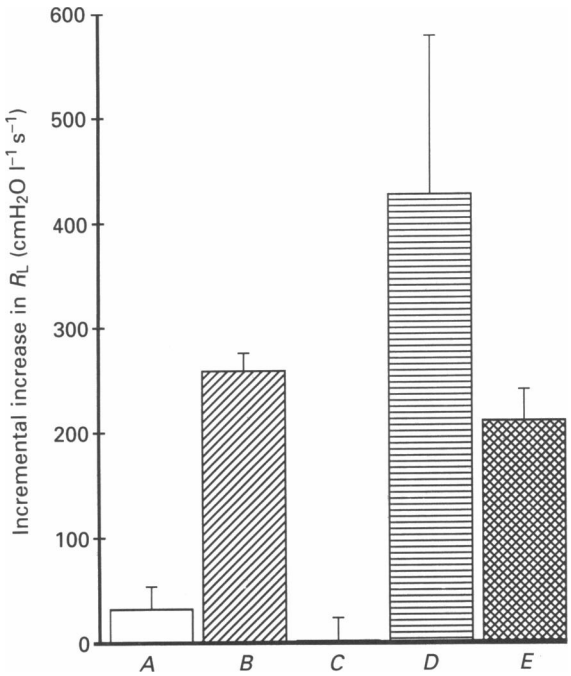

Fig. 4. Incremental increase of airway resistance (ΔR_L) in response to intravenous bombesin (240 ng kg^{-1}) in guinea-pigs exposed to an infusion of saline (*A*), (±)isoprenaline (100 μg kg^{-1} h^{-1}) alone (*B*), bilateral vagal section prior to (±)isoprenaline (*C*), bilateral vagal section after (±)isoprenaline (*D*) and pre-treatment with atropine (1 mg kg^{-1}) prior to (±)isoprenaline (*E*).

prenaline, adrenaline, noradrenaline, dopamine or forskolin were employed. Despite the superior potency of (−)isoprenaline as a relaxant of airway smooth muscle, infusions of (+)isoprenaline or (−)isoprenaline were similarly effective in causing airway hyperreactivity (Table 1). These observations are not consistent with a thesis that development of airway hyperreactivity is due to changed occupancy of

β-adrenoceptors. This conclusion is strengthened by the observation that adrenaline, a potent stimulant of β-adrenoceptors, and forskolin (600 μg kg^{-1}), a stimulant of adenylate cyclase, do not induce airway hyperreactivity (Table 1). Yet noradrenaline and dopamine, which are not categorized as β-adrenoceptor stimulants, none the less induce airway hyperreactivity (Table 1).

Infusion of racemic propranolol

Inhibition of the spasmolytic action of (±)isoprenaline upon airway smooth muscle can be achieved by (±)propranolol, a β-adrenoceptor antagonist. However, co-infusion of (±)propranolol with (±)isoprenaline led to an enhancement of airway hyperreactivity (Mazzoni, Morley, Sanjar & Schaeublin, 1987). This anamolous interaction is due to the capacity of propranolol (10 or 100 μg kg^{-1} h^{-1}) to induce increased airway reactivity (Table 1), even at a dose (10 μg kg^{-1} h^{-1}) which lacked antagonistic effects for bronchodilator actions of (±)isoprenaline. Development of airway hyperreactivity following infusion of (±)propranolol was only partially inhibited by bilateral vagal section (ΔR_L 163±23; ΔC_{dyn}, −0·57±0·1; $n = 5$).

Platelet depletion and selective antagonism of platelet-activating factor

Development of airway hyperreactivity following an infusion of PAF can be prevented by depletion of platelets or concomitant infusion of PAF receptor antagonists (Mazzoni *et al.* 1985*a*; Sanjar *et al.* 1989). To ascertain whether airway hyperreactivity caused by intravenous infusion of (±)isoprenaline might be dependent upon generation of PAF, animals were depleted of circulating platelets by injection of anti-platelet serum, or given an infusion of a selective PAF antagonist, ginkgolide B (1 mg kg^{-1} h^{-1}), during infusion of (±)isoprenaline. The incremental increases of airway resistance produced by intravenous injection of bombesin (240 ng kg^{-1}) in animals treated with (±)isoprenaline (100 μg kg^{-1} h^{-1}) after platelet depletion (ΔR_L, 213±55; ΔC_{dyn}, −0·45±0·14; $n = 10$) or infusion of ginkgolide B (ΔR_L, 210±44; ΔC_{dyn}, −0·75±0·37; $n = 5$) were comparable with effects in control animals (ΔR_L, 259±17; ΔC_{dyn}, 0·52±0·1; $n = 38$).

DISCUSSION

Paradoxical effects of β-adrenoceptor agonists, which can result in airway obstruction or increased airway reactivity in asthma, have long been recognized by clinicians (Keighly, 1966; Paterson *et al.* 1971; Trautlein *et al.* 1976; Kraan *et al.* 1985; Kerrebijn *et al.* 1987). Hitherto, mechanisms related to β-receptor occupancy by metabolic products of isoprenaline (Paterson, Conolly, Davies & Dollery, 1968) or β-adrenoceptor desensitization (Szentivanyl, 1968) have been proposed to account for such phenomena. These proposals were supported by observations that propranolol could induce acute bronchospasm in asthma patients (McNeill & Ingram, 1966) and lead to increased airway reactivity in normal subjects or asthma patients (Orehek, Gayrard, Grimaud & Charpin, 1975; Maconochie, Woodings & Richards, 1977; Carpentiere, Castello & Marino, 1988). The present study suggests that the comparable effects of β-adrenoceptor agonists and antagonists may be subordinate to a common mechanism which is unrelated to β-adrenoceptor occupancy and which lacks stereo-specificity.

The phenomenon of (±)isoprenaline-induced airway hyperreactivity in experimental animals has not been reported previously, although increased sensitivity to allergen (Izard, Henson, Collins & Brunson, 1971) or histamine (Conolly, Davies, Dollery & George, 1971; Bouhuys, Douglas & Lewis, 1972) in guinea-pigs that have been exposed to isoprenaline or other β-adrenoceptor agonists has been previously reported. In acute experiments the phenomenon of increased airway reactivity may have been overlooked, since bronchoconstriction is not demonstrable during the period of bronchodilatation that results from exposure to (±)isoprenaline.

The present experiments show that intravenous infusion of (±)isoprenaline (100 $\mu g\ kg^{-1}$) over 1 h into anaesthetized ventilated guinea-pigs can produce a substantially increased responsivity of airways to intravenous injections of spasmogens such as histamine, bombesin or acetylcholine. Surprisingly, bronchoconstriction due to vagal stimulation was only slightly enhanced after (±)isoprenaline infusion. This observation may be explained by inhibition of ganglionic transmission by (±)isoprenaline, as has been reported for the ferret (Skoogh, 1986).

Tissue responses to (±)isoprenaline or other β-adrenoceptor agonists can become diminished in amplitude and duration following repeated application of (±)isoprenaline (Benoy, El-Fellah, Schneider & Wade, 1975; Holgate, Stubbs, Wood, McCaughey, Alberti & Tattersfield, 1980) or after administration of a large dose (Avner & Noland, 1978). Such tachyphylaxis has been attributed to a loss of β-adrenoceptor sensitivity, in consequence of diminished numbers of β-adrenoceptors (Mukherjee, Caron & Lefkowitz, 1975). A loss of β-adrenoceptor binding sites is readily demonstrated in peripheral blood leukocytes (Galant, Duriseta, Underwood & Insel, 1978; Tashkin, Conolly, Deutsch, Hui, Littner, Scarpace & Abrass, 1982), but airway smooth muscle is relatively resistant to tachyphylaxis (Harvey & Tattersfield, 1982; Tashkin *et al.* 1982) which accords with the evidence that maximal relaxation of airway smooth muscle can be achieved without detectable receptor occupancy (Kaumann & Lemoine, 1984). β-Adrenoceptor desensitization cannot account for increased responsivity to airway spasmogens due to infusion of (±)isoprenaline, since only a modest reduction in sensitivity to bronchodilator action of (±)isoprenaline was detected with the high infusion dose (100 $\mu g\ kg^{-1}\ h^{-1}$). Furthermore, development of airway hyperreactivity also followed lower doses of (±)isoprenaline (10 $\mu g\ kg^{-1}\ h^{-1}$), when no desensitization was detectable. The effect of intravenous infusions of (−)- and (+)isomers of isoprenaline reinforces this conclusion. (+)Isoprenaline, which is a weak stimulant of β-adrenoceptors, induced airway hyperreactivity that was comparable with that produced by infusion of (−)isoprenaline. These results imply that (+)- and (−)isomers of isoprenaline cause airway hyperreactivity by acting at a site other than the β-adrenoceptor, which is unrelated to adenylate cyclase activation, since forskolin did not induce airway hyperreactivity. Moreover, (±)adrenaline, which is a potent β-adrenoceptor agonist, failed to effect such changed behaviour of the airways, whereas both noradrenaline and dopamine, which lack β-adrenoceptor agonist activity, did induce airway hyperreactivity. These observations are incompatible with β-adrenoceptor activation accounting for the induction of airway hyperreactivity.

(±)Propranolol, a β-adrenoceptor antagonist, cannot be used to determine the mechanism of (±)isoprenaline-induced airway hyperreactivity, since it induces airway hyperreactivity *per se* (Maclagan & Ney, 1979). Interestingly, both (−)- and

(+)isomers of propranolol cause airway hyperreactivity in the guinea-pig (Ney, 1983), which indicates an effect unrelated to adrenoceptor occupancy. This conclusion is reinforced by the present study which reveals that (±)propranolol can cause hyperreactivity when infused at doses that are insufficient to effect detectable antagonism of the spasmolytic effects of (±)isoprenaline. It has previously been reported that infusion of PAF in the guinea-pig can induce airway hyperreactivity of comparable amplitude to that induced by (±)isoprenaline (Mazzoni *et al.* 1985*a*). The induction of airway hyperreactivity by an infusion of PAF can be prevented by prior infusion of PAF-receptor antagonists (Sanjar *et al.* 1989) or by depletion of platelets (Mazzoni *et al.* 1985*a*). Neither platelet depletion nor co-administration of ginkgolide B, a selective antagonist of PAF, diminished the hyperreactivity that resulted from infusion of (±)isoprenaline. These characteristics distinguish hyperreactivity due to (±)isoprenaline from that produced by PAF, a conclusion that can be extended to (±)propranolol (Dixon, Wilsoncroft, Robertson & Page, 1989). Such distinction has been reinforced by the effect of vagal section. Thus, hyperreactivity due to PAF infusion is not diminished by vagal section (Mazzoni *et al.* 1985*a*), whereas the hyperreactivity due to (±)isoprenaline is abolished when vagal section precedes exposure to (±)isoprenaline. This consequence of vagal section cannot be attributed to a loss of cholinergic vagal reflexes, since atropine was without influence on development of airway hyperreactivity following exposure to (±)isoprenaline. Moreover, vagal section after infusion of (±)isoprenaline failed to inhibit development of airway hyperreactivity. Although activation of β-adrenoceptors can enhance ganglionic transmission (Langer, 1981), this phenomenon is stereo-specific and cannot explain the current findings since both (+)- and (−)isoprenaline induce airway hyperreactivity.

It is known that the pulmonary vasculature extracts amines and their derivatives with considerable efficacy, as exemplified by isoprenaline (Herting, 1964; Foster, 1969; Bryan, Cole & O'Donnell, 1980), noradrenaline (Hughes, Gillis & Bloom, 1969), propranolol (Hayes & Cooper, 1971), 5-hydroxytryptamine (Junod, 1972*a*; Anderson, Orton, Pickett & Eling, 1974) or imipramine (Junod, 1972*b*). This process has been attributed to an amine uptake mechanism in vascular endothelium that may lack stereo-specificity. The possibility that an amine uptake mechanism or the products of amine metabolism may influence airway reactivity has yet to be excluded.

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