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β_2-Adrenoceptor blockade is the basis of guinea-pig bronchial hyper-responsiveness to leukotriene C_4 and other agonists

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1 Four β-adrenoceptor antagonists, namely (−)-propranolol, (+)-propranolol, ICI-118551 and (±)-practolol, were investigated for their effects on leukotriene C_4 (LTC_4)-induced bronchoconstriction in the anaesthetized guinea-pig. (−)-Propranolol was also investigated for its effects on acetylcholine and histamine bronchospasm in the anaesthetized guinea-pig, and on LTC_4-induced contractions of guinea-pig isolated trachea and lung parenchyma.

2 The various β-adrenoceptor antagonists potentiated, dose-dependently, the bronchoconstriction induced by threshold doses of LTC_4 and the intensity of the potentiation correlated with the β_2-blocking capacity possessed by the drugs.

3 (−)-Propranolol potentiated the bronchospasm induced by threshold doses of acetylcholine and histamine but to a lesser degree than the LTC_4-induced bronchospasm.

4 The airway hyper-responsiveness induced by (−)-propranolol was unaffected by pretreatment with mepyramine, cyproheptadine, phenoxybenzamine, atropine or indomethacin.

5 The airway hyper-responsiveness induced by (−)-propranolol persisted even in adrenalectomized or reserpine-treated guinea-pigs, although adrenalectomy induced some increase in airway responsiveness.

6 (−)-Propranolol had no effect on LTC_4, histamine and acetylcholine-induced contractions of isolated trachea and lung parenchyma.

7 The results show that the airway hyper-responsiveness induced by β-adrenoceptor antagonists generally correlates with their β_2-blocking activity. The possibility remains that some other unknown mechanism(s) may also be implicated.

Introduction

The ability of β-adrenoceptor blocking drugs to cause bronchospasm has been reported in asthmatic (McNeill, 1964; Von Meier, Lydtin & Zöllner, 1966) and in normal subjects (McNeill & Ingram, 1966; MacDonald, Ingram & McNeill, 1967).

In experimental animals also, these drugs cause a hyper-reactive bronchomotor state accompanied by a potentiation of the bronchoconstriction induced by several agents: a dose-dependent increase in airway resistance by various β-adrenoceptor antagonists was observed in the guinea-pig by Advenier, Boissier & Giudicelli (1972) together with a significant correlation between this increase and the degree of bradycardia, while Diamond (1972) demonstrated that propranolol, sotalol and oxprenolol were equiactive in enhancing the bronchomotor response to histamine in the guinea-pig at dose levels producing equivalent degrees of negative chronotropism. Other investigators found that β-adrenoceptor antagonists potentiate the bronchoconstriction caused by other agonists such as acetylcholine (McCulloch, Proctor & Rand, 1967), bradykinin (Collier, James & Piper, 1965), 5-hydroxytryptamine (MacLagan & Ney, 1979) and leukotriene E_4 (Welton, Crowley, Miller & Yaremko, 1981). However, there is disagreement as to whether blockade of the β-receptor is the mechanism responsible for this effect: Diamond (1972) suggested that the potentiation by β-blockers might be 'related to intervention with compensatory adrenergic mechanisms or abatement of tonic sympathetic activity'; MacLagan & Ney (1979), on the other hand, denied that β-blockade is the mechanism

responsible for the airway hyper-reactivity, suggesting that endogenous bronchoactive substances may be involved.

The present study is an attempt to determine whether bronchial hyper-responsiveness is β-dependent, and to verify if the release of some endogenous bronchoactive substance is implicated. We investigated in the guinea-pig, both *in vitro* and *in vivo*, the effects of β-blockade on bronchoconstriction induced by leukotriene C_4(LTC_4), a component of the 'slow reacting substance of anaphylaxis', which is probably a significant primary mediator of bronchospasm in allergic asthma, whose bronchoconstrictor action in guinea-pigs seems to depend mainly on the formation of thromboxane A_2 (Engineer, Morris, Piper & Sirois, 1978; Schiantarelli, Bongrani & Folco, 1981; Omini, Folco, Viganò, Rossoni, Brunelli & Berti, 1981).

Methods

Konzett & Rössler preparation in the guinea-pig

Male Dunkin-Hartley guinea-pigs, weighing 350–440 g and fasted for 15 h, were anaesthetized with urethane (1.5 g kg^{-1} i.p.), tracheotomized and subjected to forced ventilation by means of a Starling pump (Miniature Ideal Pump, Bioscience) calibrated at 4–5 ml and 54 rev min^{-1}. Gallamine triethiodide (5 mg kg^{-1}) was given intravenously in order to abolish spontaneous respiration; body temperature was maintained at 37°C by Homoeothermic Blanket Control, Bioscience. Bronchoconstriction was evaluated according to the Konzett & Rössler (1940) method which, according to Diamond (1967), mainly indicates 'bronchiolar resistance': ventilation was regulated so as to counterbalance a pressure of 10 cmH_2O: the overflow air was diverted to a 4 ml Bioscience piston recorder and registered on a smoked drum. Bronchoconstriction was evaluated as a percentage of the maximum overflow obtained by total occlusion of the tracheal cannula. In some experiments, together with bronchiolar resistance, heart rate and systemic blood pressure were also evaluated. The overflow air was diverted to a Hewlett-Packard (HP) 21069/B pneumotachograph connected to an HP 47304/A flow transducer; the overflow volume was obtained by integration (HP 8815/A respiratory integrator) of the instantaneous flow. The mean systemic blood pressure was measured through a carotid catheter connected to an HP 1290/A pressure transducer. The heart rate was detected by an HP 8811/D cardiotachograph by processing of the ECG signal. All the parameters were monitored and registered on an HP 7758/B multichannel polygraph. All the drugs were given by bolus injection (via the previously cannulated jugular vein), dissolved in saline (1 ml kg^{-1} for β-blockers and antagonist drugs; for the agonists the constant volume of 0.1 ml was used). Bronchoconstrictor agonists (LTC_4, histamine, acetylcholine) were administered at intervals of not less than 20 min. Beta-adrenoceptor antagonists were given 10 min before the bronchoconstrictors. The remaining drugs (mepyramine, cyproheptadine, phenoxybenzamine, atropine and indomethacin) were administered 10 min before the β-blockers. The bronchoconstrictor agonists were administered several times to each animal, and β-blockers and other drugs once only.

The bronchoconstrictor effect of LTC_4 and its interaction with (−)-propranolol was also assessed in animals previously adrenalectomized or reserpine-treated. Bilateral adrenalectomy was performed 15 min before the experiment; reserpine 5 mg kg^{-1} was given intraperitoneally 24 h before the experiment. The catecholamine content of the adrenal glands was assayed by high pressure liquid chromatography (h.p.l.c.), according to Felice, Felice & Kissinger (1978).

Guinea-pig uterus in situ

Female guinea-pigs weighing 350–400 g and fasted for 15 h were anaesthetized, ventilated and thermoregulated as previously described. A uterine horn was prepared for recording of its isotonic shortening. Briefly, the horn was cut at the tubal end, connected to a 7006 Basile isotonic transducer with a resting load of 0.5 g and kept moist with paraffin oil. After an equilibration period of 30 min, the basal tone was increased by an intravenous infusion of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) 50 μg kg^{-1} min^{-1}. The selective β_2-adrenoceptor agonist, fenoterol, given intravenously at 10 nmol kg^{-1} caused in all animals tested an almost complete suppression of the $PGF_{2\alpha}$-induced contraction. (−)-Propranolol, (+)-propranolol and ICI-118551 were given intravenously 10 min before fenoterol, and their capacity to antagonize its relaxant effect was investigated.

In vitro *experiment*

Zigzag tracheal strips prepared according to Emmerson & MacKay (1979) and lung parenchymal strips prepared according to Lulich, Mitchell & Sparrow (1976) from male guinea-pigs, were suspended in 20 ml organ baths containing Krebs-Henseleit solution (mM: NaCl 118, KCl 4.75, $CaCl_2$ 2.50, $MgSO_4$ 1.19, KH_2PO_4 1.19, $NaHCO_3$ 25.0, glucose 11.5, ascorbic acid 1.1) kept at 37°C and aerated with 95% O_2 plus 5% CO_2. The contractions of tracheal strips were measured isometrically by a DYO Basile

isometric transducer; the contractions of lung parenchymal strips were measured isotonically with a 7006 Basile isotonic transducer. The effects of different drugs on both preparations were assessed after an equilibration period of 60 min; for both preparations the resting tension was 1 g.

Drugs

The drugs used and their sources were as follows: synthetic leukotriene C_4(LTC_4) (Merck-Frosst); acetylcholine chloride (Merck); histamine dihydrochloride (C.Erba); $PGF_{2\alpha}$ (Prostin $F_{2\alpha}$, Upjohn); (−)-propranolol hydrochloride, (+)-propranolol hydrochloride, erythro-DL-1(7-methylindan-4-yloxy)-3-isopropylaminobutan-2-ol (ICI-118551) hydrochloride and (±)-practolol hydrochloride (I.C.I. Ltd.); FPL-55712 (Fisons Ltd.); fenoterol hydrobromide (Boehringer); indomethacin meglumine salt (Chiesi Farmaceutici S.p.A.); atropine sulphate (Merck); mepyramine maleate (Sigma); cyproheptadine hydrochloride (Sigma); phenoxybenzamine hydrochloride (S.K. & F. Ltd.); reserpine (B.D.H.); tyramine monohydrochloride (Nutrit.Biochem.Corp.); gallamine triethiodide (Sigma); urethane (Fluka).

Results

Under our experimental conditions intravenous administration of (−)-propranolol (up to the dose of 3 μmol kg^{-1}) to anaesthetized guinea-pigs induced no large variations of basal bronchiolar resistance, there being a small increase in only 30% of the animals. In contrast, (−)-propranolol consistently enhanced the response to bronchoconstrictor agents.

The bronchospasm induced by a threshold spasmogenic dose of LTC_4 (0.16 nmol kg^{-1}, i.v.) was potentiated dose-dependently by intravenous pretreatment with the non-specific β-adrenoceptor blocking agent, (−)-propranolol. The potentiation was already evident at doses of the β-blocker as low as 30 nmol kg^{-1} becoming maximal at 3 μmol kg^{-1}, and remaining unaltered at least up to 1 h (Figure 1). A dose-dependent enhancement of the threshold effects of LTC_4 was also observed following pretreatment with the β_2-specific (O'Donnel & Wanstall, 1980) antagonist ICI-118551 and, even though to a much smaller extent, with the selective β_1-blocker (±)-practolol and the isomer (+)-propranolol, which possesses weak β-adrenoceptor blocking activity (Figure 2). (−)-Propranolol also potentiated the bronchoconstriction caused by threshold doses of histamine (3 nmol kg^{-1}) and acetylcholine (30 nmol kg^{-1}); however, acetylcholine- and histamine-induced bronchoconstriction was potentiated significantly less than LTC_4 bronchoconstriction (Figure 3).

In order to verify that the doses of β-adrenoceptor antagonists used were sufficient to produce β_2-blockade, in additional experiments, performed in the guinea-pig *in vivo*, their capacity to antagonize a specific β_2-challenge was investigated. These trials

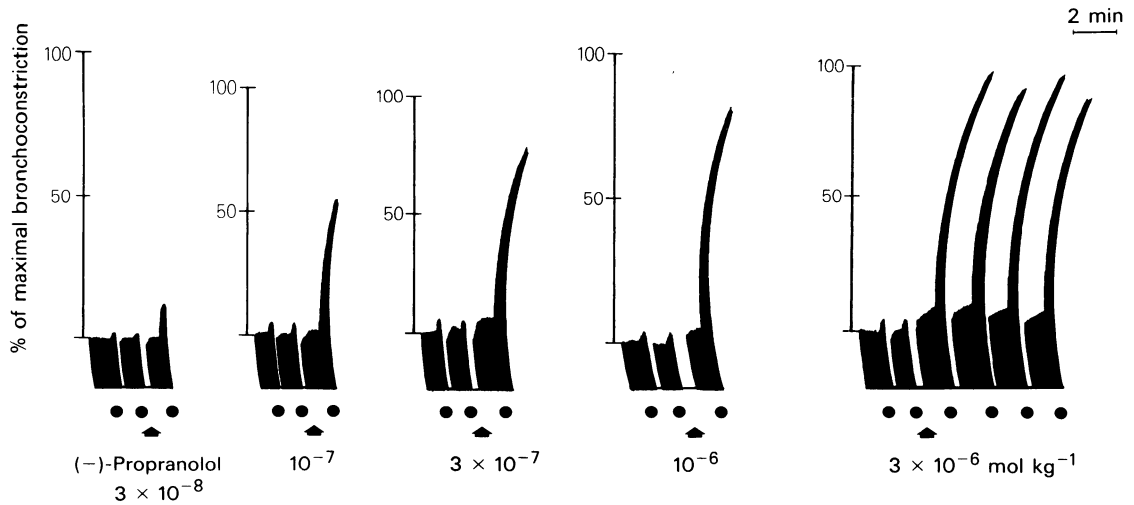

Figure 1 Potentiation by intravenous (−)-propranolol at the indicated doses (arrows) of bronchoconstriction induced in anaesthetized guinea-pigs by leukotriene C_4, 0.16 nmol kg^{-1} i.v. (black dots). The response illustrated for each dose of (−)-propranolol is typical of 3 to 8 separate experiments.

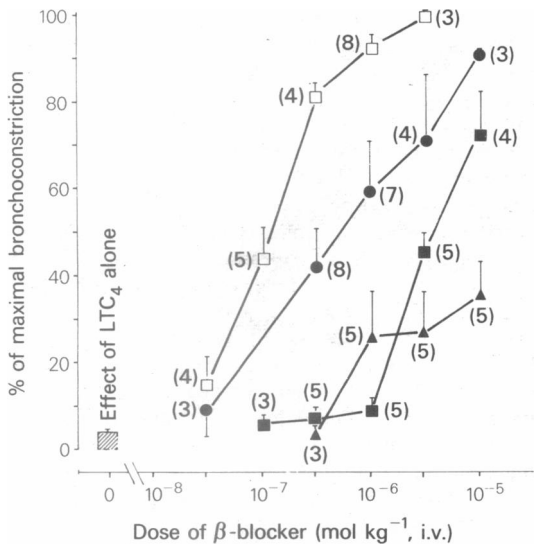

Figure 2 β-Adrenoceptor antagonists potentiate dose-dependently the bronchoconstriction caused by a threshold dose of leukotriene C_4 (LTC_4, 0.16 nmol kg^{-1}) in anaesthetized guinea-pigs. (−)-Propranolol (□); (+)-propranolol (■); ICI-118551 (●); (±)-practolol (▲). Numbers in parentheses indicate number of separate experiments at each dose. The vertical bars indicate s.e.mean. The column represents the effect of LTC_4 (0.16 nmol kg^{-1}) alone ($n = 89$).

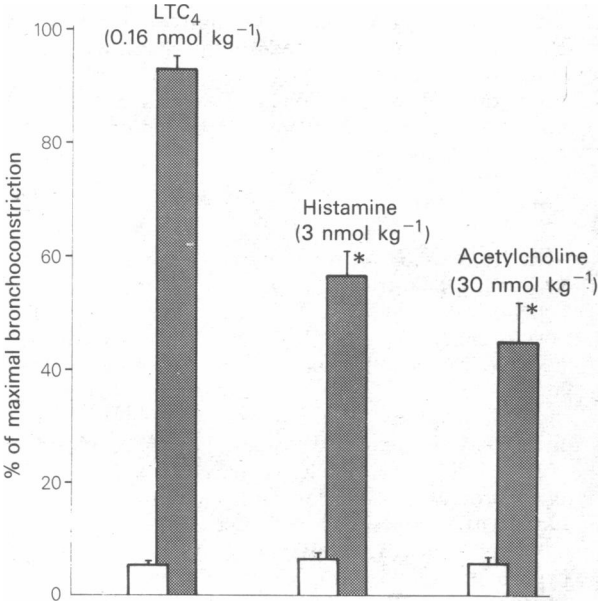

Figure 3 The brochoconstriction induced in anaesthetized guinea-pigs by threshold i.v. doses of leukotriene C_4 (LTC_4), histamine and acetylcholine before (open columns) or after (shaded columns) i.v. (−)-propranolol 1 μmol kg^{-1}. Values represent the mean of 8 experiments; vertical bars indicate s.e.mean. Statistical difference versus the LTC_4 value (Student's t test): $^*P < 0.001$.

were carried out on systems other than the respiratory, since, as reported by James (1969), the Konzett & Rössler preparation does not respond directly to the effects of bronchodilator drugs, such as fenoterol. Furthermore, even if the β_2-agonist is used to prevent the effect of e.g. acetylcholine, the supervening potentiation by β-blockers masks any inhibition exerted by these compounds on the protective effects of the β_2-agonist. Experiments on the vascular system of our preparation turned out to be hardly practicable, because of the weak and erratic hypotension obtained with β_2-agonists. On the other hand, significant evidence has been obtained in experiments performed on the guinea-pig uterus *in situ*, an organ rich in β_2-adrenoceptors (O'Donnell, Persson & Wanstall, 1978). In fact, doses of β-blockers able to trigger bronchial hyper-responsiveness were also able to antagonize the utero-relaxant effects of fenoterol (10 nmol kg^{-1}, i.v.) which suppressed almost completely the increased basal tone caused by the i.v. infusion of $PGF_{2\alpha}$. Such an effect of fenoterol was abolished by intravenous pretreatment with (−)-propranolol 3×10^{-7} mol kg^{-1}, and partially but appreciably inhibited by a lower dose (10^{-7} mol kg^{-1}) (Figure 4). The compound ICI-118551 behaved similarly, whereas (+)-propranolol antagonized the utero-relaxant effect of fenoterol at much higher doses (2×10^{-5} mol kg^{-1}).

In a series of separate experiments arterial blood pressure and heart rate were also monitored in order to ascertain if any change of cardiovascular parameters took place during bronchial hyper-responsiveness in the guinea-pig. Doses of LTC_4, histamine and acetylcholine, which promoted a threshold bronchoconstriction, were also able to cause appreciable changes of arterial blood pressure and heart rate. The results shown in Table 1 express the effects of the agonists, rated at the peak of their activity. Pretreatment with (−)-propranolol, at the dose-range producing hyper-responsiveness, did not affect such cardiovascular modifications (Table 1). Nevertheless, (−)-propranolol *per se*, caused an appreciable reduction of the basal values of both arterial blood pressure and heart rate, even at the lowest dose employed of 3×10^{-8} mol kg^{-1} (Table 1).

Indomethacin 1 mg kg^{-1} (as acid) intravenously abolished the bronchospasm induced by the threshold dose of LTC_4 even after pretreatment with (−)-propranolol (Figure 5); on the other hand, the same dose of indomethacin failed to modify his-

tamine and acetylcholine-induced bronchoconstriction and its potentiation by (−)-propranolol (Figure 5).

In a limited number of experiments, the capacity of indomethacin (5 mg kg^{-1}) to prevent the bronchoconstrictor effects of a maximally effective dose of LTC_4 (3.2 nmol kg^{-1}) has been explored both in the absence and in the presence of (−)-propranolol (1 μmol kg^{-1}). Indomethacin abolished the bronchoconstriction caused by LTC_4 in the absence of (−)-propranolol, whereas a maximal bronchoconstriction following LTC_4 administration was evident in animals pretreated with (−)-propranolol. These results suggest that (−)-propranolol is able to evoke, and potentiate, the bronchoconstrictor component of LTC_4, since the thromboxane A_2-dependent component is likely to be abolished by such a dose of indomethacin.

The chromone compound FPL-55712, described as a selective SRS-A antagonist *in vitro* (Augstein, Farmer, Lee, Sheard & Tattersall, 1973), at a dose of 1 mg kg^{-1} intravenously, abolished the bronchoconstriction induced by the threshold dose of LTC_4, but inhibited only partially (58% ± 17% s.e.mean, $n = 4$) its potentiation by (−)-propranolol 1 μmol kg^{-1}. Such partial protection, however, was evident provided that FPL-55712 was given shortly before (1 min) challenge with LTC_4; this is probably due to the well-known short half-life of FPL-55712. (−)-Propranolol-potentiated LTC_4 bronchoconstriction was in no way affected by previous intravenous administration (1 mg kg^{-1} as bases) of mepyramine, cyproheptadine, phenoxybenzamine or atropine ($n = 3$–5).

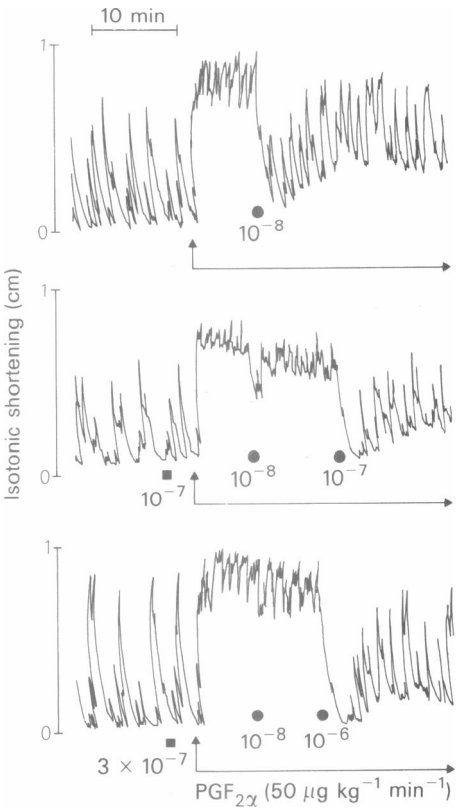

Figure 4 Guinea-pig uterus *in situ*: antagonism by (−)-propranolol of fenoterol relaxation of uterine tone enhanced by intravenous infusion of prostaglandin $F_{2\alpha}$: (●) = fenoterol (mol kg^{-1} i.v.); (■) = (−)-propranolol (mol kg^{-1} i.v.). The responses illustrated are typical of 5 to 7 experiments.

Neither the contractile response nor the β-blocker potentiation was observed when acetylcholine or histamine were given after their specific antagonist, i.e. atropine or mepyramine (1 mg kg^{-1}) ($n = 4$).

In order to understand the mechanism by which β-blockers cause airway hyper-responsiveness, some experiments were performed in adrenalectomized or reserpine-treated guinea-pigs; animals subjected to both reserpinization and adrenalectomy could not be used because of an exceedingly high lethality.

Bilateral adrenalectomy *per se* enhanced the bronchoconstriction induced by LTC_4 0.16 nmol kg^{-1}: the bronchoconstriction (% of maximal) observed before adrenalectomy was 3.7 ± 1.6 (mean ± s.e.mean), while after adrenalectomy the bronchoconstriction in the same animals ($n = 11$) became 48.7 ± 6.5 (mean ± s.e.mean) (Figure 6a). In subsequent experiments the effect of (−)-propranolol on LTC_4 bronchoconstriction was tested in adrenalectomized guinea-pigs: in these experimental conditions the dose of LTC_4 was reduced to 0.08 nmol kg^{-1} in order to obtain threshold bronchoconstrictor effects as well. Also in adrenalectomized animals, (−)-propranolol markedly potentiated the bronchoconstrictor effect of LTC_4: in fact the response was 3.6 ± 0.7 (mean ± s.e.mean) before and 68.7 ± 6.4 (mean ± s.e.mean) ($n = 5$) after (−)-propranolol 1 μmol kg^{-1}. An example of this experiment is depicted in Figure 6b. Pretreatment with reserpine (5 mg kg^{-1}, i.p. 24 h before the experiment) did not induce appreciable changes in the response to a threshold dose of LTC_4 (0.16 nmol kg^{-1}) as compared with untreated animals. In these experimental conditions, (−)-propranolol 1 μmol kg^{-1} greatly enhanced the bronchoconstriction induced by LTC_4 0.16 nmol kg^{-1}: the % of maximal bronchoconstriction was in fact 5.5 ± 1.9 (mean ± s.e.mean; $n = 4$) before, and 67.0 ± 9.5 (mean ± s.e.mean) after (−)-propranolol. An example of this experiment is represented in Figure 6c.

The effectiveness of reserpine pretreatment was checked in separate experiments: atria isolated from

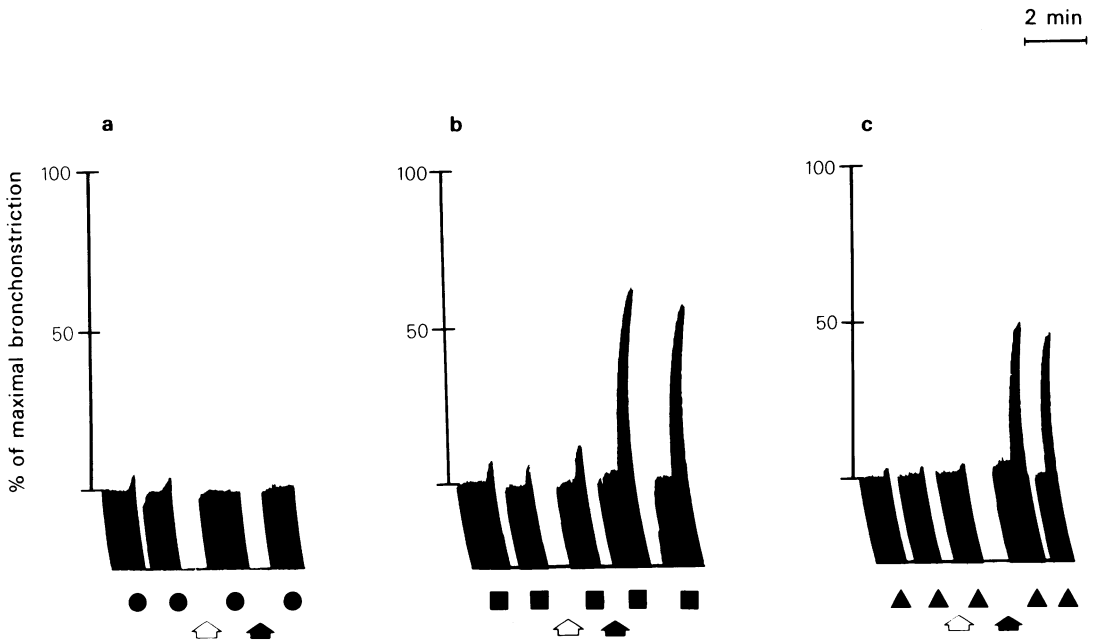

Figure 5 Effects of intravenous indomethacin 1 mg kg^{-1} (open arrows) on bronchoconstriction induced in anaesthetized guinea-pigs by threshold intravenous doses of (a) leukotriene C_4 (LTC_4, 0.16 nmol kg^{-1}, ●), (b) histamine (3 nmol kg^{-1}, ■), and (c) acetylcholine (30 nmol kg^{-1}, ▲) before and after intravenous (−)-propranolol 1 μmol kg^{-1} (solid arrows). The responses illustrated are typical of 5 separate experiments for each bronchospasm-inducing agent.

Table 1 Effects on bronchiolar resistance, arterial blood pressure and heart rate induced in anaesthetized guinea-pigs by intravenous leukotriene C_4 (LTC_4, 0.16 nmol kg^{-1}), histamine (Hist, 3 nmol kg^{-1}) and acetylcholine (ACh, 30 nmol kg^{-1}) in the absence or in the presence of different doses of (−)-propranolol (Prop)

Treatment	*No. of experiments*	*Bronchoconstriction* (% of maximal overflow)	*Arterial blood pressure* (Δ% from basal value)	*Heart rate* (Δ% from basal value)
Leukotriene C_4	7	2.8 ± 1.1	16.7 ± 7.3	−2.7 ± 1.6
			(−12.0 ± 2.8)	
Histamine	9	3.2 ± 1.4	−17.1 ± 1.4	5.6 ± 1.0
Acetylcholine	9	2.9 ± 0.9	−33.4 ± 1.5	−11.4 ± 1.2
Propranolol (0.03 μmol kg^{-1})	9	2.8 ± 0.6	−11.4 ± 2.3	−19.3 ± 1.6
LTC_4 + Prop (0.03 μmol kg^{-1})	4	16.0 ± 3.2**	12.3 ± 3.4	−3.3 ± 1.5
			(−9.8 ± 2.7)	
Hist + Prop (0.03 μmol kg^{-1})	3	12.7 ± 3.7*	−10.8 ± 2.2	6.7 ± 0.7
ACh + Prop (0.03 μmol kg^{-1})	3	5.9 ± 2.1	−29.0 ± 0.4	−10.0 ± 2.6
Propranolol (1 μmol kg^{-1})	11	7.4 ± 0.8	−12.0 ± 1.6	−19.2 ± 1.4
LTC_4 + Prop (1 μmol kg^{-1})	3	82.0 ± 11.3**	9.0 ± 4.6	−5.0 ± 1.2
			(−10.1 ± 3.3)	
Hist + Prop (1 μmol kg^{-1})	6	49.3 ± 8.8**	−15.2 ± 2.0	10.8 ± 5.3
ACh + Prop (1 μmol kg^{-1})	6	36.5 ± 7.5**	−28.3 ± 2.1	−7.5 ± 2.1

(−)-Propranolol was given i.v. 10 min before the agonists; also shown are the changes induced by (−)-propranolol alone. Since LTC_4 caused a biphasic pressor effect (transient hypertension followed by a prolonged hypotension), both changes are given. The basal values of arterial blood pressure and heart rate were 45.2 ± 1.6 mmHg and 272 ± 8 beats min^{-1}, respectively (n = 18). The results are expressed as mean ± s.e.mean. Statistical difference versus the agonist without (−)-propranolol (Student's t test): *$P < 0.05$; **$P < 0.001$

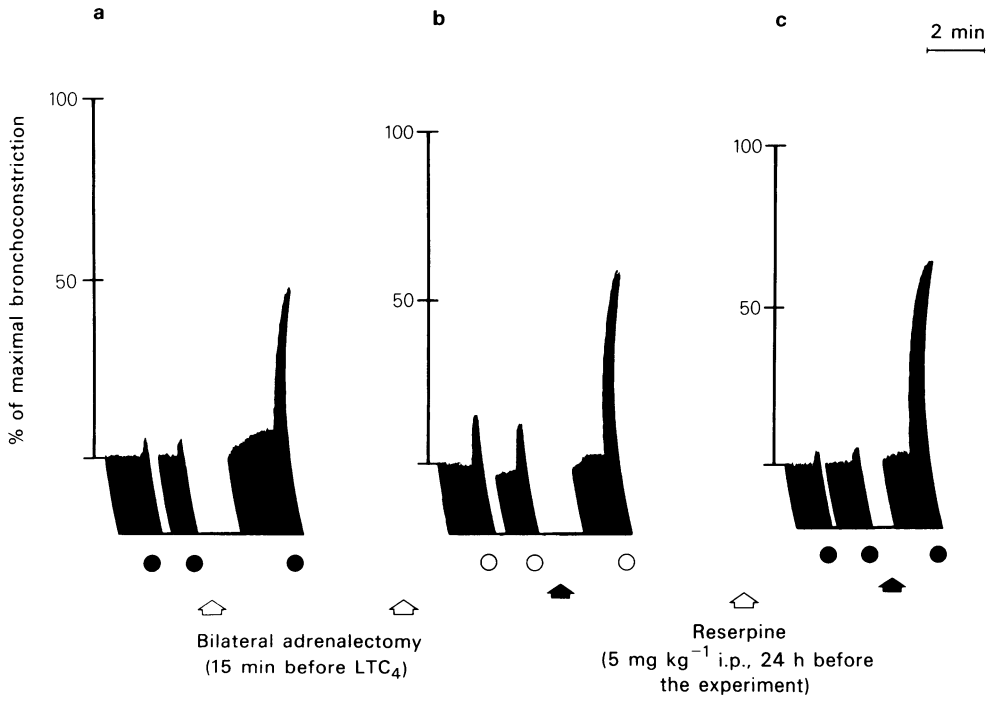

Figure 6 Effects of bilateral adrenalectomy (a and b) or reserpine-treatment (c) on bronchoconstriction induced in anaesthetized guinea-pigs by threshold doses of leukotrience C_4 (LTC_4) in the absence or in the presence of (−)-propranolol 1 μmol kg^{-1} (solid arrows). The responses illustrated are typical of 4 to 11 separate experiments. (a) Leukotriene C_4, 0.16 nmol kg^{-1} (●); (b) LTC_4, 0.08 nmol kg^{-1} (○); (c) LTC_4, 0.16 nmol kg^{-1} (●).

reserpine-treated (24 h before) guinea-pigs and prepared for recording of inotropic and chronotropic responses were unresponsive to tyramine (10^{-5} to 10^{-3} M), whereas those isolated from untreated guinea-pigs did respond with a dose-dependent increase in force of contraction and heart rate. In parallel experiments the contents of catecholamines was determined in adrenal glands of control and reserpine-treated guinea-pigs: the content of adrenaline (μg g^{-1} of tissue; mean ± s.e.mean, $n = 6$) dropped from 234.0 ± 10.5 to 23.4 ± 3.6 and that of noradrenaline from 14.2 ± 1.0 to 3.39 ± 0.01.

Finally, experiments carried out *in vitro* on guinea-pig isolated tracheal strips or lung parenchymal strips showed that (−)-propranolol, in the concentration range 3×10^{-9} M to 3×10^{-6} M, did not induce appreciable changes of basal tone, and in no way affected the contractions induced by LTC_4 (Figure 7). The existence of β_2-blockade was verified because (−)-propranolol 10^{-7} M was able to prevent the relaxation induced by the β_2-selective agonist fenoterol, 10^{-8} M, in guinea-pig isolated tracheae precontracted with carbachol 3×10^{-7} M. Similarly, no potentiation by the same concentrations of (−)-propranolol was observed on the contractions induced by histamine and acetylcholine given at the concentration corresponding approximately to their EC_{30} on the two preparations (data not shown).

Discussion

The results of this study show that β-adrenoceptor blocking agents dramatically potentiate the bronchomotor response of the guinea-pig to LTC_4 as well as to other agonists such as histamine and acetylcholine.

The potentiation by β-blocker of LTC_4 bronchoconstriction, seems to occur both on the thromboxane-dependent component, and on the component due to the direct action of LTC_4, since a maximal bronchospasm was evoked by (−)-propranolol even in the presence of indomethacin (cyclo-oxygenase block) when a sufficient dose of LTC_4 was given.

In our experimental conditions, the potentiation of the bronchoconstriction induced by threshold doses of LTC_4 appeared significantly higher than that obtained with threshold doses of histamine and acetylcholine. This could be due to the different site of

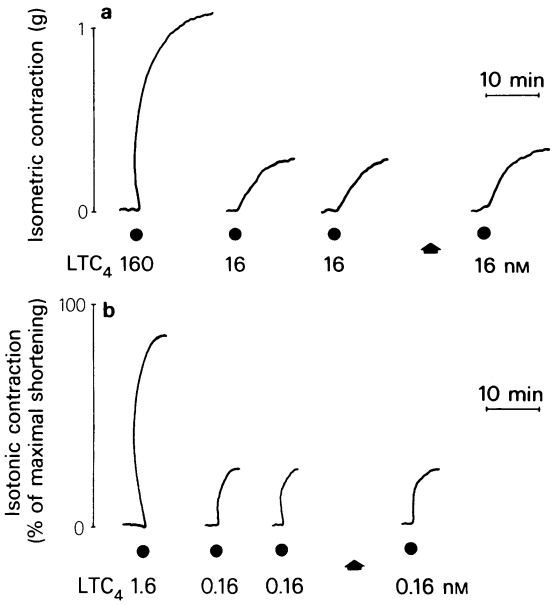

Figure 7 Inactivity of (−)-propranolol 10^{-6}M (solid arrows) on the contraction induced by leukotriene C_4 (LTC_4, ●) on guinea-pig isolated tracheal strip (a) and lung parenchymal strip(b). (−)-Propranolol (solid arrows) was added 10 min before LTC_4. On both preparations in 5 separate experiments (−)-propranolol, in concentrations ranging from 3×10^{-9}M to 3×10^{-6}M, did not modify the contractions induced by LTC_4.

action, within the respiratory tree, of the agonists considered, since LTC_4 acts chiefly on the pulmonary compliance, i.e. on peripheral airways (Drazen, Lewis, Austen, Toda, Brion, Marfat & Corey, 1981), while the other agonists affect preferentially the larger airways or act on both levels (Drazen & Austen, 1974; Hensley, O'Cain, McFadden & Ingram, 1978). In fact, the Konzett & Rössler preparation is more sensitive to changes of the tone of smaller than larger airways (Diamond, 1967). It is important to emphasize that bronchial hyper-responsiveness is not accompanied by significant changes of arterial blood pressure and heart rate, indicating that such features of the β-blockers concern selectively bronchial smooth muscle.

A potentation by β-blockers of histamine and 5-hydroxytryptamine-induced bronchoconstriction has already been reported by MacLagan & Ney (1979) who concluded that the potentiation was unrelated to blockade of β-adrenoceptors.

Our results, by contrast, suggest that the observed hyper-reactivity is somehow related to β_2-adrenoceptor blockade. In fact, both (±)-practolol and (+)-propranolol cause less hyper-reactivity and at higher doses, than do the β_1-β_2-blocker, (−)-propranolol, and the specific β_2-blocker ICI-118551. As shown in Figure 2, the dose-hyper-reactive response curve of (+)-propranolol is shifted to the right by about 1.5 and 1 logarithmic units compared with the (−)-propranolol and ICI-118551 curves. In addition, the maximal potentiating effect of the selective β_1-blocker, (±)-practolol, is appreciably weaker than that of the remaining compounds. It also seems likely that the potentiation induced by (+)-propranolol and by (±)-practolol is due to a residual β_2-blocking activity of these drugs. In fact, the β-blocking activity of (+)-propranolol is reportedly 10 to 100 times less than that of (±)-propranolol (Howe & Shanks, 1966; Kaumann & Blinks, 1967; Levy, 1968).

A potency ratio of the same order between the two optical isomers is evident from the shift between the respective log dose-hyper-reactive response curves (Figure 2). Some degree of β_2-blocking activity of the selective β_1-blocker, practolol, is also reported by Miesch, Bieth, Leclerc & Schwartz (1978), who found pA_2 values in guinea-pig isolated trachea of 5.13 and 8.47 for practolol and propranolol respectively. The potency ratio of approximately 2200, calculated from these values is consistent with that roughly deducible from our results (Figure 2), although the lack of parallelism and the difference in maximal response between the curves for (±)-practolol and (−)-propranolol do not allow quantitative comparison.

A further, although indirect, proof that bronchial hyper-responsiveness is related to the β_2-blocking component of β-blockers, is provided by their capacity to antagonize the utero-relaxant properties of fenoterol. Such antagonism occurs at doses of the same order as that causing bronchial hyper-reactivity, and versus doses of fenoterol large enough to protect the guinea-pig completely against acetylcholine bronchospasm (Schiantarelli, Bongrani, Papotti & Cadel, 1982).

In the *in vitro* experiment the increased tone induced by LTC_4 and by the other agonists, histamine and acetylcholine, was not modified by β-blockade. This *in vivo-in vitro* discrepancy suggests the presence in the *in vivo* situation of a bronchodilator sympathetic tone that a β-blocker can inhibit. Such a hypothetical tone must be humoral in nature since an adrenergic innervation in guinea-pig lung can be excluded (O'Donnel, Saar & Wood, 1978).

The experiments using adrenalectomized animals support the hypothesis of a humoral sympathetic tone because removal of the adrenal glands caused *per se* a certain potentiation of the LTC_4 bronchoconstriction; on the other hand, the (−)-propranolol potentiation observed both in adrenalectomized and in reserpine-treated guinea-pigs requires more thorough comment. As far as reserpine-treated ani-

mals are concerned, it is noteworthy that bronchial hyper-activity is present in spite of a substantial (−90%) depletion of adrenal catecholamines; it is difficult to appreciate the relative importance of the remaining 10% which might be responsible for a residual adrenergic humoral tone whose abatement causes airway hyper-responsiveness. Moreover, the fact that potentiation persists in adrenalectomized animals as well, could be ascribed to the existence of a bronchodilator tone derived from neuronally-released noradrenaline within the lung which probably overflows from well-innervated vasculature adjacent to sparsely innervated airways, as suggested by Ainsworth, Garland & Payne (1982). Anyway, the involvement of mechanism(s) unrelated to β-blockade cannot be ruled out.

A possible participation of different bronchoactive substances, which might be released by β-blockers was suggested by MacLagan & Ney (1979). Moreover, Terpstra, Raaijmakers & Wassink (1981) have pointed out that rat peritoneal mast-cells incubated with (+)-propranolol, as well as with the racemic form, release histamine. Our results rule out any dependence of β-blocker potentiation upon a release of histamine because mepyramine did not affect (−)-propranolol potentiation of either LTC_4 or acetylcholine bronchospasm, while, as expected, it abolished the response to histamine. Similarly, the involvement of cyclo-oxygenase products such as thromboxane A_2 or $PGF_{2\alpha}$ can be ruled out, because pretreatment with the cyclo-oxygenase inhibitor, indomethacin, did not affect the potentiation induced by (−)-propranolol of histamine or acetylcholine bronchospasms; on the other hand the abolition by indomethacin of the response to a threshold dose of LTC_4 and of its potentiation by (−)-propranolol accords with the hypothesis that LTC_4 triggers the release of thromboxane A_2 (Engineer *et al.*, 1978; Schiantarelli *et al.*, 1981; Omini *et al.*, 1981). Likewise a participation of 5-hydroxytryptaminergic, cholinergic or α-adrenergic pathways is unlikely because cyproheptadine, atropine and phenoxybenzamine failed to modify (−)-propranolol potentiation when a non-specific agonist was used. Finally, the finding that (−)-propranolol potentiation was fully prevented when administration of histamine or acetylcholine was preceded by that of their specific antagonist (mepyramine or atropine) rules out the possibility that this potentiation is in some way sustained by the release of other bronchial contractants, such as PAF.

In conclusion, airway hyper-reactivity by β-blockers seems to be linked to their β_2-blocking activity, although there is, as yet, no fully satisfactory evidence that this is the only mechanism involved.

As an explanation of the bronchial hyper-responsiveness, it could be argued that the inhibition of β_2- tone by the β-blocker may contribute functionally, and hence synergistically, to the bronchoconstrictor effect of the agonist. Indeed, according to Ariens, Simonis & van Rossum (1964), a functional interaction between two drugs, i.e. a combination of two drugs which produce their effect by means of a common effector system but interacting with different independent receptor systems, gives rise to an over-additive effect.

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