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Failure of enhancement by labetalol of bronchopulmonary effects of histamine in guinea-pigs: independence of α-adrenoceptor antagonism

Christine Clerici, [1]Alain Harf & *Isabelle Macquin-Mavier

Inserm U 296, and *Service de Pharmacologie Clinique, Faculté de Médecine de Créteil, 94010 Créteil, France

1 The effect of labetalol on histamine-induced bronchoconstriction was studied in anaesthetized guinea-pigs. Unlike propranolol (1 mg kg^{-1}), the same dose of labetalol did not enhance histamine-induced bronchoconstriction.

2 To determine whether the absence of enhancement of the respiratory effects of histamine by labetalol was due to its α_1-blocking properties or to its partial agonist activity at β_2-adrenoceptors, the effects of propranolol plus prazosin and of propranolol plus labetalol on histamine-induced bronchoconstriction were examined. In both cases, the bronchoconstrictor effects of histamine were enhanced to the same extent as with propranolol alone.

3 These data support the hypothesis that the non impairment of respiratory mechanics by labetalol is not due to antagonism at α-adrenoceptors and may be mediated by its partial agonist activity at β_2-adrenoceptors.

Introduction

Many antagonists at β-adrenoceptors, both β_1-selective and non selective, have been reported to worsen ventilatory function in patients with asthma or obstructive airway diseases (Shand, 1983). In recent years, several clinical studies have shown that labetalol, a non selective antagonist at β-adrenoceptors that is also an antagonist at α-adrenoceptors, is safer for these patients (Skinner *et al.*, 1975; Larsson, 1982; George *et al.*, 1985). Since α-adrenoceptor blockade has been reported to reduce or abolish the bronchospasm in asthmatics (Gaddie *et al.*, 1972; Patel & Kerr, 1975), it was postulated that the α-blocking properties of labetalol were responsible for this absence of ventilatory impairment, although this assumption has never been demonstrated. As recent pharmacological studies have shown that labetalol is a partial agonist at β_2-adrenoceptors (Carey & Whalley, 1979; Carpenter, 1981), the absence of deterioration in respiratory function seen with labetalol might be ascribable either to the α-blocking properties of labetalol or to its partial agonist activity.

The aim of our study was to investigate the effects of labetalol on histamine-induced bronchoconstriction in guinea-pigs, and to determine whether the lack of deterioration in respiratory function was due to its α_1-blocking properties or to its intrinsic sympathomimetic activity.

[1] Author for correspondence

Methods

Protocol

Male Hartley guinea-pigs weighing 300 ± 20 g (Charles River, France) were anaesthetized with pentobarbitone sodium (30 mg kg^{-1}, i.p.). The dose was selected because it allowed all surgical procedures to be performed without pain for the animal, since it remained insensitive throughout the experiment. A jugular vein was cannulated for drug administration. A tracheal cannula was inserted just below the larynx. The animals were paralyzed with pancuronium bromide (4 mg kg^{-1}, i.p.) and mechanially ventilated with a tidal volume of 8 ml kg^{-1} at a frequency of 60 breaths min^{-1} in order to obtain normocapnia (Clerici *et al.*, 1985). The heart rate was monitored by continuous electrocardiographic recording (ECG Biotach, Gould). Body temperature was measured with a rectal probe and maintained constant at 38°C by means of a thermostatically controlled heating blanket (Harvard, les Ulis, France).

The animals were placed in a whole body-plethys-

mograph; box pressure was measured with a Schlumberger CH5112 ± 2 cmH_2O transducer and was considered to be proportional to the change in thoracic volume. Flow was obtained by electrical differentiation of the volume signal. Tracheal pressure was measured with a Schlumberger CH5022 ± 50 cmH_2O transducer. The volume, flow and pressure signals were used to compute the conductance and dynamic compliance of the respiratory system using the methods of Mead & Whittenberger (1953) as previously described (Clerici *et al.*, 1985).

Basal measurements of conductance (G) and dynamic compliance (C_{dyn}) were made 20 min after guinea-pigs had been placed in the body plethysmograph. The drugs (see below) were infused intravenously over 10 min and control measurements repeated after 15 and 30 min.

A dose-response curve was then drawn. For this purpose histamine was infused i.v. for 3 min until respiratory parameters reached a steady state. G and C_{dyn} were then calculated. The histamine infusion was stopped and 15 to 30 min allowed for the parameters to return to basal values. The same procedure was repeated three times with larger doses of histamine until G and C_{dyn} fell by about half. Thus, four doses of histamine were used to construct the dose-response curves. (The concentration in the infused fluid was adjusted so that the infusion rate was about 0.2 ml min^{-1}).

The dose of histamine required to reduce C_{dyn} to 50% of the baseline level (termed the D_{50} C_{dyn}) was obtained by linear interpolation of the dose-response curve, as was the dose required to reduce G to half its baseline value (termed the D_{50} G).

Six groups of six guinea-pigs each were studied. They were treated i.v. with saline, labetalol at two doses (1 and 4 mg kg^{-1}), propranolol (1 mg kg^{-1}), propranolol (1 mg kg^{-1}) plus prazosin (0.3 mg kg^{-1}), and propranolol (1 mg kg^{-1}) plus labetalol (1 mg kg^{-1}) respectively.

In order to determine equiactive doses of labetalol and propranolol, two groups of four guinea-pigs each were given bolus injections of two different doses of isoprenaline (0.1 and 0.3 μg kg^{-1}) before and after three cumulative doses of propranolol or labetalol and the increases in heart rate were recorded. For both drugs, the doses tested were 0.3, 1 and 3 mg kg^{-1}. The results are shown in Figure 1. Labetalol (1 mg kg^{-1}) was as effective as propranolol (1 mg kg^{-1}) in inhibiting the tachycardia produced by isoprenaline. However, as in previous studies, labetalol was four times less potent than propranolol at β_1-adrenoceptors (Brittain & Levy, 1976; Baum & Sybertz, 1983). We

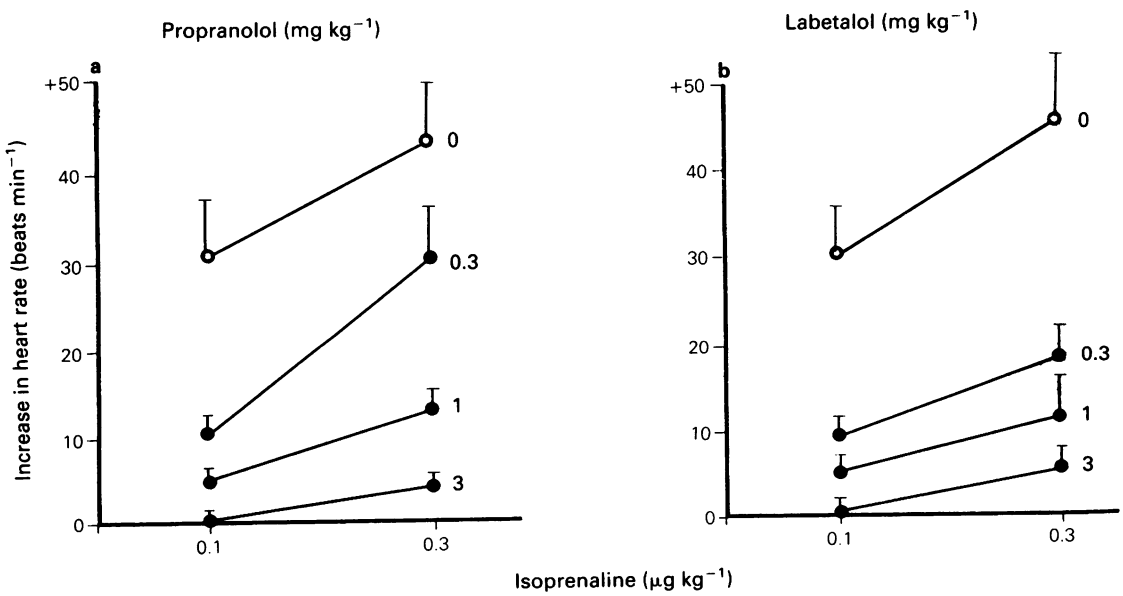

Figure 1 Increase in heart rate in response to i.v. injection of 0.1 and 0.3 μg kg^{-1} isoprenaline before (○) and after (●) three cumulative i.v. doses (0.3, 1 and 3 mg kg^{-1}) of propranolol (a) or labetalol (b). Analysis of variance revealed no significant differences between the effects on the heart rate of identical doses of labetalol and propranolol.

also assessed the effects of labetalol (4 mg kg^{-1}) on histamine-induced bronchoconstriction.

Drugs

The following drugs were used: histamine dihydrochloride (Sigma), isoprenaline hydrochloride (Sigma), propranolol hydrochloride (ICI), labetalol hydrochloride (Glaxo), prazosin (Pfizer), pentobarbitone sodium (Clin Midy), and pancuronium bromide (Organon).

Solutions of histamine dihydrochloride, isoprenaline hydrochloride and propranolol hydrochloride were freshly made up in 0.9% saline. Labetalol and prazosin were dissolved in 0.9% saline in the presence of 0.5% ethanol. All doses are expressed as the base.

Statistics

Results are expressed as mean ± s.d. A Kolmogorof-Smirnov test was used to demonstrate that D_{50} values were log-normally distributed (Siegel, 1956). Statistical analysis were performed using log (D_{50}) values (Fleming *et al.*, 1972). One way analysis of variance was used to compare the D_{50} for C_{dyn} and G.

Results

Mean control values for C_{dyn} and G before drug administration were respectively 0.42 ± 0.07 ml cmH_2O^{-1} and 4.88 ± 0.69 ml s^{-1} cmH_2O^{-1}.

Propranolol, labetalol, propranolol plus prazosin, or propranolol plus labetalol did not significantly alter C_{dyn} or G, which were measured 15 and 30 min after drug administration.

Effects of labetalol and propranolol on histamine-induced bronchoconstriction

As regards the effects of propranolol (1 mg kg^{-1}) and labetalol (1 or 4 mg kg^{-1}) on histamine-induced bronchoconstriction, labetalol had no significant effect on D_{50} C_{dyn} or D_{50} G compared to the control group. In contrast, propranolol markedly potentiated histamine effects, since the D_{50} for C_{dyn} and G dropped to about half compared to the control group (Figure 2).

Effects of propranolol plus prazosin and propranolol plus labetalol on histamine-induced bronchoconstriction

To determine whether the α_1-blocking properties of labetalol were responsible for the absence of enhancement by labetalol of histamine-induced bronchoconstriction, prazosin (0.3 mg kg^{-1}) was given together with propranolol (1 mg kg^{-1}) before histamine administration. Results are shown in Figure 3. This dose of prazosin failed to prevent the propranolol-induced potentiation of histamine. There was no significant difference between the effects of propranolol alone and of propranolol plus prazosin.

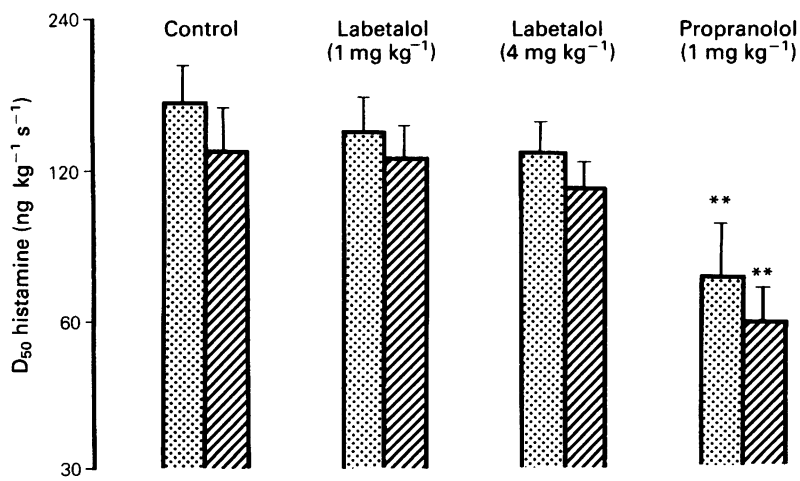

Figure 2 Interpolated doses of histamine required to induce a 50% decrease in C_{dyn} (stippled columns) and a 50% decrease in G (hatched columns) in the control group, and the three groups respectively pretreated with labetalol (1 mg kg^{-1}), labetalol (4 mg kg^{-1}) or propranolol (1 mg kg^{-1}). Significant differences versus the control group are denoted by $^{**}P < 0.001$. Values are means; vertical lines represent s.d. Each group included 6 animals.

Similarly, labetalol (1 mg kg^{-1}) failed to overcome the propranolol-induced potentiation of histamine (Figure 3).

Discussion

Labetalol is a compound which combines α- and β-adrenoceptor blocking effects and also possesses partial agonist activity at β_2-adrenoceptors. Although it produces a non selective β-adrenoceptor blockade, ventilatory function impairment, when reported, has been small or insignificant (Skinner *et al.*, 1975; Maconochie *et al.*, 1977; Larsson, 1982; George *et al.*, 1985).

Under the conditions of our experiments, labetalol did not potentiate histamine as a bronchoconstrictor, whereas the same dose of propranolol clearly did on both small airways (dynamic compliance) and large airways (conductance). Similar results have been reported in asthmatic patients by Skinner *et al.* (1975) who showed that 5 mg i.v. propranolol induced bronchoconstriction whereas 20 mg i.v. labetalol did not. Larsson (1982) found some decrease in FEV_1 after 20 mg labetalol i.v. although it was much smaller than after 5 mg propranolol. In our study we found 1 mg kg^{-1} labetalol to be as potent as 1 mg kg^{-1} propranolol on the heart. In order to compare our results with those of previous studies, we also tested 4 mg kg^{-1} labetalol, but did not find that it enhanced histamine-induced bronchoconstriction. This absence of respiratory effects caused by labetalol might be due to its possession of pharmacological properties that propranolol does not have, i.e. α_1-selective antagonism and/or partial agonist activity at β_2-adrenoceptors.

Labetalol has selective α_1-antagonist properties without inhibitory activity at α_2-adrenoceptors and is similar in this regard to prazosin, one of the most potent and selective antagonists at α_1-adrenoceptors (Graham & Pettinger, 1979). Labetalol has also been reported to be four to eight times less potent at α than β-adrenoceptors in isolated tissues, and some studies have indicated an intravenous β to α potency ratio of 16:1 (Brittain & Levy, 1976).

To assess the role of the α_1-adrenoceptor antagonist properties of labetalol, we tried to mimic its properties by giving propranolol plus prazosin. Although 0.3 mg kg^{-1} prazosin is likely to be more potent than 1 mg kg^{-1} labetalol at α-adrenoceptors (Tung *et al.*, 1982), the results of this treatment were not significantly different from those of propranolol alone in potentiating histamine. These results show that α_1-adrenoceptor blocking properties are not involved in preventing the enhancement of histamine-induced bronchoconstriction by non selective β-blockers. Our results are not consistent with most of the previous findings for the effects of α-blockade in asthmatic patients. In these patients, it has been reported that antagonists at α-adrenoceptors might prevent a bronchospasm induced by histamine, (Gaddie *et al.*, 1972; Prime *et al.*, 1972) exercise (Bianco *et al.*, 1974; Beil & de Kock, 1978), or allergic challenge (Patel & Kerr,

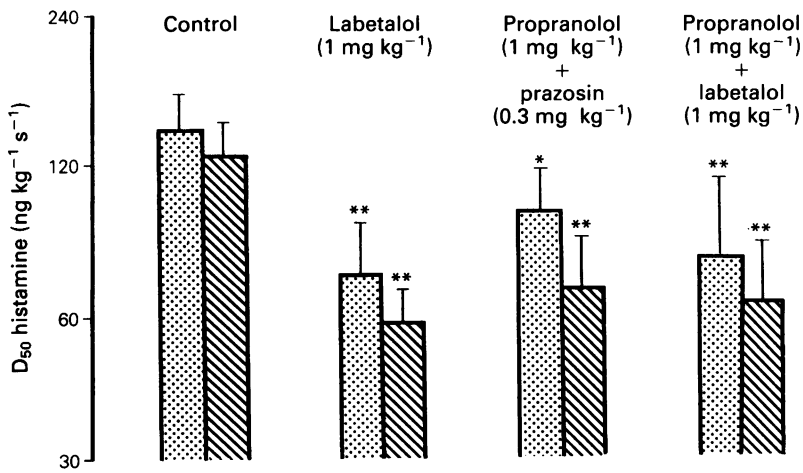

Figure 3 Interpolated doses of histamine required to induce a 50% decrease in C_{dyn} (stippled columns) and a 50% decrease in G (hatched columns) in 4 groups of guinea-pigs respectively pretreated with labetalol (1 mg kg^{-1}), propranolol (1 mg kg^{-1}), propranolol (1 mg kg^{-1}) plus prazosin (0.3 mg kg^{-1}), propranolol (1 mg kg^{-1}) plus labetalol (1 mg kg^{-1}). Significant differences versus the labetalol group are denoted $*P < 0.01$ and $**P < 0.001$. D_{50} histamine for G and C_{dyn} were not significantly different among the groups pretreated with propranolol, propranolol + prazosin or propranolol + labetalol. Values are means; vertical lines represent s.d. Each group included 6 animals.

1975; Walden *et al.*, 1984). However, the antagonists that were used in the above studies (such as phentolamine and thymoxamine) also display anti-histamine activity, influence catecholamine release and uptake (Hoffman & Lefkowitz, 1980) and induce relaxation of smooth muscle (Paterson *et al.*, 1979).

Our results are in keeping with those of certain other authors. Barnes *et al.* (1981) demonstrated that inhalation by asthmatic patients of 0.5 mg prazosin did not change the airway response to histamine compared to placebo. In cats, Blaber & Fryer (1985) showed that i.v. administration of 1 mg kg^{-1} prazosin did not affect this response, either in untreated animals or those pretreated with propranolol. In the guinea-pig, Advenier *et al.* (1984) found that 0.03 mg kg^{-1} prazosin had no significant effect, either on basal airway resistance or acetylcholine-induced bronchoconstriction.

Labetalol was originally believed to be devoid of intrinsic sympathetic activity (Brittain & Levy, 1976) but recent studies have clearly demonstrated that it does possess this property. Labetalol has intrinsic activity at β_2-adrenoceptors that relax guinea-pig tracheal smooth muscle (Carpenter, 1981; Brion *et al.*, 1985) and rat uterine preparations (Carey & Whalley, 1979). No intrinsic activity at β_1-adrenoceptors has been reported (Farmer *et al.*, 1972; Levy & Richards, 1980).

Various clinical benefits have been claimed for antagonists at β-adrenoceptors with intrinsic sympathomimetic activity, as several studies have shown that drugs like pindolol, oxprenolol, alprenolol and practolol are less likely to induce bronchospasm. Oh *et al.* (1978) demonstrated that during exercise-challenge, patients pretreated with propranolol displayed greater airway obstruction than patients pretreated with pindolol or oxprenolol. In comparative clinical trials, Morgan *et al.* (1974) reported a higher incidence of bronchospasm during treatment with propranolol and timolol, two drugs without intrinsic activity, than during treatment with pindolol and alprenolol. In other studies (Frishman *et al.*, 1979), patients whose lung function deteriorated with propranolol exhibited no deterioration with pindolol during either acute or chronic treatment. It is possible that the intrinsic activity of labetalol at β_2-adrenoceptors may be responsible for the absence of respiratory effects. Such a hypothesis is strongly supported by our present observations that administration of labetalol after propranolol enhances histamine-induced bronchoconstriction to the same extent as propranolol alone. This result further suggests that the effects of labetalol were not mediated through its being an antagonist at α-adrenoceptors.

This study gives further support to the view that the lack of bronchopulmonary effects of labetalol may be due largely to its intrinsic activity at β_2-adrenoceptors. By contrast its α_1-adrenoceptor antagonist properties do not appear to play a significant role.

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