Effects of cardioselective beta blockade on the peripheral lung in guinea pigs

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**ABSTRACT:** Impairment of lung function with selective beta-1 blocking drugs has been repeatedly demonstrated in guinea pigs, normal subjects and asthmatic patients. The effects of several beta blockers, propranolol (non-selective), atenolol (beta-1 selective), IPS 339 (beta-2 selective) on histamine-induced bronchoconstriction have been investigated in 30 anaesthetized and mechanically ventilated guinea pigs, measuring changes in conductance and dynamic compliance. Their effects on peripheral lung, where only beta-2 adrenoceptors are present, were more specifically assessed using changes in lung distensibility by means of static pressure-volume curves. Atenolol (1 mg·kg⁻¹), IPS 339 (2 mg·kg⁻¹) and propranolol (2 mg·kg⁻¹) enhanced histamine-induced decrease in lung distensibility, conductance and dynamic compliance. The decrease was of the same order of magnitude for all three parameters. Atenolol (1 mg·kg⁻¹) and propranolol (2 mg·kg⁻¹) decreased lung distensibility to the same extent. By contrast low dose atenolol (0.1 mg·kg⁻¹) did not potentiate histamine-induced bronchoconstriction although this dose did produce a significant cardiac beta blockade. These results demonstrate that 1) beta blockers have a clear effect on the peripheral lung, 2) beta-1 adrenoceptors are not involved in pulmonary effects of cardioselective drugs. They suggest that dose dependent loss of selectivity is the major mechanism behind impairment of lung function following such drugs.

It is well known that beta adrenoceptor blocking drugs can cause bronchoconstriction. Although a non specific property of the drugs has been invoked [10], this effect is generally ascribed to removal of the beta-2 mediated sympathetic bronchodilator drive of the airways. The use of cardioselective beta blockers is therefore recommended in asthmatic patients. However impairment of ventilatory function has also been reported with these drugs and has been tentatively ascribed to the presence of beta-1 adrenoceptors in the airways [16].

Recently a high density of adrenoceptors in the peripheral lung (alveolar septa and terminal bronchi- oles) has been demonstrated by means of autoradiographic studies in guinea pigs [7]. These receptors were exclusively of the beta-2 subtype in contrast to those of the central airways where some beta-1 receptors have been found [19]. We postulated that if cardio­

At usual doses of cardioselective beta blockers, we found a potentiation of the decrease in lung distensibility during the histamine challenge indicating a clear effect on the peripheral lung. Since this effect was equivalent to that obtained after non-selective or beta-2 selective beta blockers, it suggests that beta-1 adrenoceptors are not involved. In contrast, when a low dose of cardioselective beta blocker was used there was no such potentiation. These results suggest that a dose dependent loss of selectivity is a major mechanism behind impairment of lung function with these drugs.

**Methods**

Experiments were performed on a total of 36 Hartley strain guinea pigs (body weight 300 ± 20 g) (Charles River, France). The guinea pigs were anaesthetized with pentobarbital sodium (30 mg·kg⁻¹ i.p.). 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 mechanically ventilated. The tidal volume was maintained at 8 mg·kg⁻¹ at a rate of 60 breaths min⁻¹ in order to obtain normocapnia [4]. The heart rate was monitored by continuous electrocardiographic recordings (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 Ealing, Les Ulis, France).

The animals were placed in a pressure bodyplethysmograph. Box pressure was measured with a Schumonber CH5112 ±0.2 kPa transducer, and flow was obtained by electrical differentiation of the volume signal. Tracheal pressure was measured with a Schumonber CH5022 ±5 kPa transducer. The volume, flow and pressure signals were used to compute the conductance and the dynamic compliance of the respiratory system using the method of MEAD and WHITTENBERG (12) as previously described [4].

Quasi static pressure-volume curves were obtained by inflating and then deflating the lungs with a constant flow pump (Watson Marlow, Falmouth, England) connected to the tracheal cannula and delivering a constant flow of 18 ml·min⁻¹. The inflation was performed from the volume of relaxation up to a tracheal pressure of 3 kPa. Deflation was then immediately performed by using a three way valve which switches the tracheal tube from the outlet to the inlet of the pump. The duration of the manoeuvre was standardized to 45 s. Since a constant flow was used, the volume increased during inflation and decreased during deflation proportionally to the duration of the cycle. Volume and tracheal pressure were recorded on an XY recorder (Ifelec 3802, Paris, France). Distensibility was assessed by analyzing the deflation limb of the curve and static compliance was computed as the slope of linear part above the volume of relaxation [8].

Protocol

After control determination of conductance (G), dynamic compliance (Cdyn) and quasi-static compliance (Cst), histamine was infused until a steady state of respiratory parameters was reached (3–5 min). G and Cdyn were computed at the end of this steady state. The histamine infusion was discontinued and sufficient time was allowed for the parameters to return to basal values (15–30 min). A step-wise increase in histamine mass flow was performed until a fall of 50% in the parameters was achieved. The concentration in the infused fluid was adjusted so that at an infusion rate of 0.1–0.3 ml·min⁻¹ the required mass flow of histamine was obtained. Pressure-volume curves were obtained for the two last doses of histamine.

The beta blocker was then administered intravenously over 10 min and values of G, Cdyn and Cst were determined 30 min after. A second histamine dose-response curve using different histamine infusion rates as shown in figure 1 was then performed using the same procedure. From the dose-response curve, the interpolated doses that would have been required to cause a decrease of 50% in Cdyn (D₅₀ Cdyn) and in G (D₅₀ G) and of 60% in Cst compared to control values were calculated. The ratio of these doses, after and before beta blockade, was computed and defined as the dose ratio (DR).

Five groups of six guinea pigs were studied, pretreated with either saline, a non-selective beta blocker propranolol 2 mg·kg⁻¹ (7.7 × 10⁻⁶ mol·kg⁻¹), a beta-2 selective beta blocker IPS 339 2 mg·kg⁻¹ (5.5 × 10⁻⁶ mol·kg⁻¹) or a beta-1 selective beta blocker atenolol at two doses 1 and 0.1 mg·kg⁻¹ (3.8 × 10⁻⁶ and 3.8 × 10⁻⁷ mol·kg⁻¹).

Drugs

The following drugs were used: histamine dihydrochloride (Sigma), isoproterenol oproterenol hydrochloride (Sigma), atenolol and propranolol hydrochloride (ICI), IPS 339 (hydrochloride of (t-buty1-amino-3-ol-2-propyl) oximino-9 fluorene) kindly supplied by Pr G. Leclerc, Strasbourg.

Drug solutions were freshly prepared using 0.9% w/v NaCl solution (saline). All doses are expressed as the base.

Statistics

Results are expressed as mean ± standard deviation (σ0). One-way and two-way analysis of variance were used to compare respectively the dose ratio and the dose-response curves for the different parameters. The level of significance was p < 0.05.

Results

Mean control values for Cdyn, Cst and G were respectively: 3.6 ± 0.4 ml·kPa⁻¹, 5.7 ± 0.9 ml·kPa⁻¹ and 42 ± 9.7 ml·s⁻¹·kPa⁻¹.

Administration of propranolol and IPS 339 was followed by a decrease in G, Cdyn and Cst. This effect was maximal between 15 and 20 min after the infusion. With propranolol the parameters returned within 10% of the control values after 30 min while with IPS 339 a 25% decrease persisted. No effect was observed with atenolol at 0.1 mg·kg⁻¹ nor 1 mg·kg⁻¹.
During histamine infusion (without pretreatment) there was a dose related fall in Cdyn and G and Cst (figs 1 and 2). The comparison of the dose-response curve for Cdyn and G (two way analysis of variance) showed a larger decrease in conductance than in Cdyn (p < 0.001). The comparison between Cdyn and Cst showed that when Cdyn decreased to 50% there was a 30% fall in Cst.

There was a clear potentiation in histamine-induced bronchoconstriction with propranolol, IPS 339 and atenolol 1 mg·kg⁻¹ as shown in figure 1. The histamine dose ratios for the different experimental groups and for the three parameters studied (G, Cdyn and Cst) are presented in figure 3. It can be seen that: 1) there was a major effect of propranolol, IPS 339, and atenolol at the dose of 1 mg·kg⁻¹, IPS 339 being responsible for a slightly but significantly more pronounced effect; 2) the dose ratios for Cst were similar to those for Cdyn and G, for all drugs, including atenolol at the higher dose. To determine a low dose of atenolol that still had a significant cardiac effect we studied, in a group of six animals, the increase in heart rate induced by a bolus infusion of two doses of isoprenaline (0.1 and 0.3 µg·kg⁻¹) before and after cumulative doses of atenolol. Three doses of atenolol (0.1 mg·kg⁻¹, 0.3 mg·kg⁻¹ and 1 mg·kg⁻¹) were tested. The results are shown in figure 4. A significant inhibition (p < 0.01) of the effect of isoprenaline on heart rate was seen after the dose of 0.1 mg·kg⁻¹ indicating that this dose produces a significant beta blockade. At this dose atenolol did not potentiate histamine-induced bronchoconstriction as compared to the control group (fig. 2).

**Figure 2.** Histamine dose-response curves for dynamic compliance (Cdyn) plotted as percentage of control value, before (open circles) and after other saline or beta blockade by atenolol 0.1 mg·kg⁻¹ (solid circles). Values are mean, vertical lines represent SD. Each group contained six animals. The effects of atenolol 0.1 mg·kg⁻¹ on histamine-induced bronchoconstriction were not significantly different from control group (saline).

**Figure 3.** Dose ratios for effects of histamine on Cdyn, G and Cst without antagonist (C). and with atenolol 0.1 mg·kg⁻¹ (A 0.1), atenolol 0.01 mg·kg⁻¹ (A 0.01), propranolol 2 mg·kg⁻¹ (P), IPS 339 2 mg·kg⁻¹ (I). Propranolol, IPS 339 and atenolol 1 mg·kg⁻¹ enhanced histamine-induced bronchoconstriction. The decrease was of the same order of magnitude for the three parameters (Cdyn, G, Cst). By contrast atenolol 0.1 mg·kg⁻¹ had no significant effect as compared to the control group.

**Figure 4.** Increase in heart responses to i.v. isoprenaline (0.1 and 0.3 µg·kg⁻¹) before (open columns) and after cumulative doses of atenolol i.v. 0.1 mg·kg⁻¹ (hatched columns), 0.3 mg·kg⁻¹ (stippled columns) and 1 mg·kg⁻¹ (cross-hatched columns). *p < 0.05, **p < 0.01. Analysis of variance revealed a significant difference between atenolol 0.1 mg·kg⁻¹ and the control group for the two doses of isoprenaline.

**Discussion**

Impairment of lung function with beta-1 selective beta blockers has been repeatedly demonstrated in guinea pigs, normal humans and asthmatic patients [16]. To explain these observations three hypotheses have been put forward: 1) involvement of airway beta-1 adrenoceptors; 2) dose dependent loss of selectivity; 3) non-specific effect of the drugs as suggested by MACLAQAN and NEY [10]. In this study we have investigated the first two hypotheses by studying the effects of different doses of a cardioselective beta blocker on the peripheral lung where beta-2 adrenoceptors are exclusively present.

The distensibility of the respiratory system has been chosen as a functional index of the peripheral lung. Since lung distensibility is assessed in static (or quasi static) conditions any influence of large or small airways can be ruled out and it explores only the peripheral lung. Although this index is usually taken as representative of the connective tissue of the lung [10], it has been clearly demonstrated that acute and reversible changes of distensibility can be observed when histamine is administered [4, 5]. Anatomic studies using tantalum bronchography [3] and rapid
Investigation of the mechanism of distribution and quantitative developmental changes in physical performance can be explained on the basis of a dose dependent loss of selectivity. Such an hypothesis is consistent with the data reported by Wale and Fitzgerald [18]. Comparing the effects of low doses of propranolol and atenolol they have shown that while propranolol 0.2 mg·kg⁻¹ shifted the isoprenaline increase in heart rate and airway pressure dose response curves to the right, atenolol 0.1 mg·kg⁻¹ modified only cardiovascular effect. A similar conclusion was drawn by Smith et al. [17] who analysed the cardiovascular (heart rate vs vasomotion) and metabolic (free fatty acids vs glucose, insulin and lactate) responses of 0.3 and 1 mg·kg⁻¹ of atenolol. The particularity of our findings is the loss of specificity that occurs for a dose which is in the range required for efficient cardiac beta blockade; cardioselectivity is only observed for lower doses.

In conclusion we have shown that: 1) the peripheral lung is involved to a large extent in the potentiation of histamine-induced bronchoconstriction by beta blocking agents and such an observation is against the responsibility of beta-1 adrenoceptors; 2) dose dependent loss of selectivity is the major mechanism behind impairment of lung function following cardioselective beta blocking drugs.

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References
RESUME : La survenue d'une bronchoconstriction après l'administration de beta bloquants cardioselectifs a été rapportée à de nombreuses reprises chez le cobaye, les sujets sains et les patients asthmatiques. Nous avons étudié les effets bronchopulmonaires de différents beta bloquants: propranolol (non sélectif), aténolol (beta-1 sélectif) et IPS 339 (beta-2 sélectif) lors d'une bronchoconstriction induite par l'histamine i.v. chez trente cobayes anesthésiés et ventilés artificiellement. Leurs effets sur le système respiratoire ont été évalués par les mesures de conductance (G) et de compliance dynamique (Cdyn). Le poumon périphérique, où ne sont présents que des beta-2 récepteurs, a été plus particulièrement étudié par la mesure de la distensibilité pulmonaire au moyen de courbe pression-volume obtenues en conditions semi statiques (Cat). L'aténolol (1 mg·kg⁻¹), le propranolol (2 mg·kg⁻¹) et l'IPS 339 (2 mg·kg⁻¹) potentiaient les effets de l'histamine, la baisse de G, Cdyn et Cat étant de même importance. L'aténolol (0.1 mg·kg⁻¹) ne potentiait pas les effets de l'histamine bien que cette dose entraîne un blocage significatif des beta-1 récepteurs cardiaques. Ces résultats montrent que 1) les beta bloquants ont un effet majeur sur le poumon périphérique 2) le blocage des beta-1 récepteurs n'est pas responsable de l'effet bronchopulmonaire des beta bloquants cardioselectifs. Ils suggèrent que ces effets sont essentiellement le fait d'une perte de cardioselectivité en fonction de la dose.