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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 maguitude 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.

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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 bronchioles) 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 cardioselective beta blockers indeed act only on beta-1 adrenoceptors they would have a minimal effect on the peripheral lung. We therefore studied several beta antagonists (non-selective, beta-1 and beta-2 selective) during histamine challenge, assessing their effects on the respiratory system (using changes in conductance and dynamic compliance) and more specifically on the peripheral lung (using changes in lung distensibility measured by static pressure-voume curve).

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 body-plethysmograph. Box pressure was measured with a Schlumberger CH5112 ±0.2 kPa transducer, and flow was obtained by electrical differentiation of the volume signal. Tracheal pressure was measured with a Schlumberger 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 WHITTENBERGER [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 $ml \cdot min^{-1}$. 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 $ml \cdot min^{-1}$ 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 (D_{50}Cdyn) and in G (D_{50}G) and of 60% in Cst compared to control

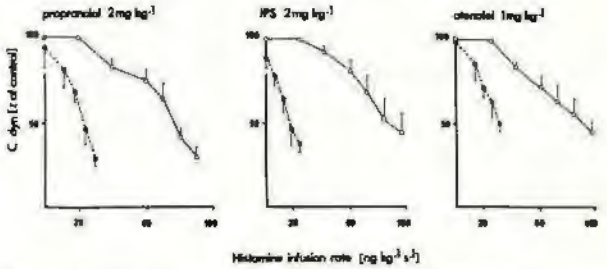

Fig. 1. Histamine dose-response curves for dynamic compliance (Cdyn) plotted as percentage of control value, before (open circles) and after beta blockade (solid circles), by propranolol (2 $mg \cdot kg^{-1}$), IPS 339 (2 $mg \cdot kg^{-1}$), and atenolol (1 $mg \cdot kg^{-1}$). Each group contained six animals. Values are mean, vertical lines represent SD. A clear potentiation of histamine-induced bronchoconstriction was observed after selective and non-selective beta blockers.

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 $mg \cdot kg^{-1}$ ($7.7\ 10^{-6}$ $mol \cdot kg^{-1}$), a beta-2 selective beta blocker IPS 339 2 $mg \cdot kg^{-1}$ ($5.5\ 10^{-6}$ $mol \cdot kg^{-1}$) or a beta-1 selective beta blocker atenolol at two doses 1 and 0.1 $mg \cdot kg^{-1}$ ($3.8\ 10^{-6}$ and $3.8\ 10^{-7}$ $mol \cdot kg^{-1}$).

Drugs

The following drugs were used: histamine dihydrochloride (Sigma), is isoproterenol oproterenol hydrochloride (Sigma), atenolol and propranolol hydrochloride (ICI), IPS 339 (hydrochloride of (t-butyl-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 (SD). 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 $ml \cdot kPa^{-1}$, 5.7±0.9 $ml \cdot kPa^{-1}$ and 42±9.7 $ml \cdot s^{-1} \cdot kPa^{-1}$.

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 $mg \cdot kg^{-1}$ nor 1 $mg \cdot kg^{-1}$.

During histamine infusion (without pretreatment) there was a dose related fall in Cdyn, 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 $mg \cdot kg^{-1}$ 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 $mg \cdot kg^{-1}$, IPS 339 being responsible for a slightly but significantly ($p<0.01$) 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 $\mu g \cdot kg^{-1}$) before and after cumulative doses of atenolol. Three doses of atenolol (0.1 $mg \cdot kg^{-1}$, 0.3 $mg \cdot kg^{-1}$ and 1 $mg \cdot kg^{-1}$) 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 $mg \cdot kg^{-1}$ 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).

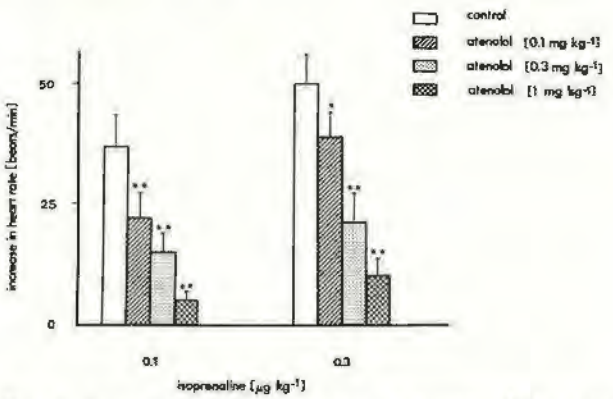

Fig. 4. Increase in heart responses to i.v. isoprenaline (0.1 and 0.3 $\mu g \cdot kg^{-1}$) before (open columns) and after cumulative doses of atenolol i.v.: 0.1 $mg \cdot kg^{-1}$ (hatched columns), 0.3 $mg \cdot kg^{-1}$ (stippled columns) and 1 $mg \cdot kg^{-1}$ (cross-hatched columns). * $p<0.05$, ** $p<0.01$. Analysis of variance revealed a significant difference between atenolol 0.1 $mg \cdot kg^{-1}$ and the control group for the two doses of isoprenaline.

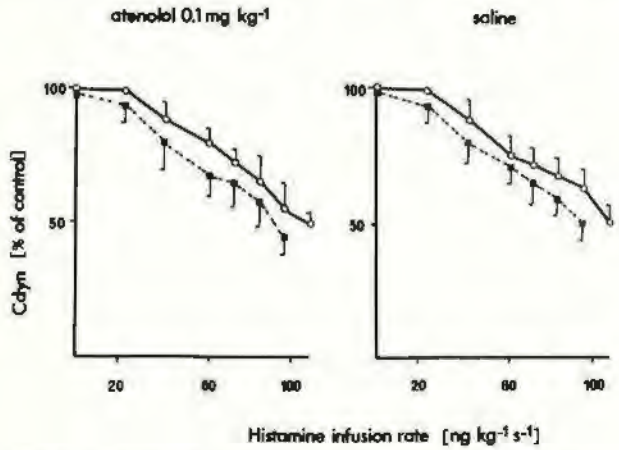

Fig. 2. Histamine dose-response curves for dynamic compliance (Cdyn) plotted as percentage of control value, before (open circles) and after either saline or beta blockade by atenolol 0.1 $mg \cdot kg^{-1}$ (solid circles). Values are mean, vertical lines represent SD. Each group contained six animals.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

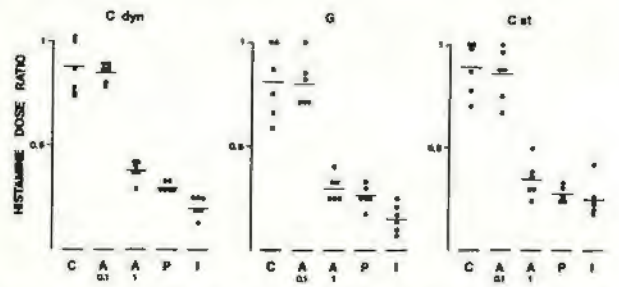

Fig. 3. Dose ratios for effects of histamine on Cdyn, G and Cst without antagonist (C), and with atenolol 0.1 $mg \cdot kg^{-1}$ (A 0.1), atenolol 1 $mg \cdot kg^{-1}$ (A 1), propranolol 2 $mg \cdot kg^{-1}$ (P), IPS 339 2 $mg \cdot kg^{-1}$ (I). Propranolol, IPS 339 and atenolol 1 $mg \cdot kg^{-1}$ 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 $mg \cdot kg^{-1}$ had no significant effect as compared to the control group.

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 MacLagan 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

freezing techniques [5] have shown that the decreased lung distensibility was due to constriction of smooth muscle in alveolar ducts and terminal bronchioles.

Pharmacological and radioligand binding studies have supported the general idea that both subtypes of beta adrenoceptors are present in the same organ, although generally one type predominates [16]. In the lung a large number of beta adrenoceptors are present. Autoradiographic methods, in the guinea pig, have demonstrated a beta adrenoceptor density gradient, with a high density in the peripheral lung (alveoli and bronchioles) *vs* a lower density in the central lung (bronchi, pulmonary vessels and connective tissue) [7]. In the peripheral lung these beta adrenoceptors have been shown to be exclusively of beta-2 subtype in contrast to the airways where both beta-1 and beta-2 subtypes have been found [19]. It can be assumed that after beta blockade changes in lung distensibility (representative of the peripheral lung) indicate a beta-2 adrenoceptor involvement.

We have found a clear effect of propranolol in enhancing histamine-induced bronchoconstriction, confirming previous reports [10, 13]. In addition to the fall in Cdyn and G our results show a large decrease in Cst. The effect of propranolol was approximately of the same order of magnitude for Cst as for Cdyn and G, indicating that the peripheral lung is largely involved in the response to beta blockers.

In order to assess a potential role of beta-1 adrenoceptors we first investigated the effect of a beta-2 blocker. We did not find fewer respiratory effects for IPS 339 than for propranolol, suggesting that the beta-1 blocking component of propranolol enhancement of bronchoconstriction was of minor importance. However, since the equivalence of the beta-2 effects for the two drugs was difficult to assess, we looked for more evidence on the role of beta-1 adrenoceptors.

Atenolol is known to be a highly cardioselective beta blocking agent [1, 2]. Several clinical studies have demonstrated that bronchospasm was of lesser importance with cardioselective beta blockers [6,11], while in other studies, during histamine or cholinergic bronchial challenge, equivalent potentiation was seen after cardioselective and non-selective beta blockers [14,15]. In our study atenolol appeared to be as potent as propranolol in enhancing the histamine-induced bronchoconstriction. Since lung distensibility decreased as much as did the other parameters, it can be postulated, as discussed above, that beta-2 adrenoceptors were involved in these effects of atenolol. A dose dependent loss of selectivity therefore could be responsible for this respiratory effect.

In order to evaluate this hypothesis we studied the respiratory effect of a lower dose of atenolol. A dose of 0.1 $mg \cdot kg^{-1}$ was chosen since it exerts a significant beta blockade as shown by the reduction in the isoprenaline-induced tachycardia. In contrast to the dose of 1 $mg \cdot kg^{-1}$, atenolol at a dose of 0.1 $mg \cdot kg^{-1}$ had no effect on the respiratory system. This result 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 FITZERALD [18]. Comparing the effects of low doses of propranolol and atenolol they have shown that while propranolol 0.2 $mg \cdot kg^{-1}$ shifted the isoprenaline increase in heart rate and airway pressure dose response curves to the right, atenolol 0.1 $mg \cdot kg^{-1}$ 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 $mg \cdot kg^{-1}$ 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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RÉSUMÉ: La survenue d'une bronchoconstriction après l'administration de beta bloquants cardiosélectifs 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 recepteurs, a été plus particulièrement étudié par la mesure de la distensibilité pulmonaire au moyen de courbe pression-volume obtenues en conditions semi statiques (Cst). L'aténolol (1 $mg \cdot kg^{-1}$), le propranolol (2 $mg \cdot kg^{-1}$) et l'IPS 339 (2 $mg \cdot kg^{-1}$) potentialisent les effets de l'histamine, la baisse de G, Cdyn et Cst étant de même importance. L'aténolol (0.1 $mg \cdot kg^{-1}$) ne potentialise 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 cardiosélectifs. Ils suggèrent que ces effets sont essentiellement le fait d'une perte de cardiosélectivité en fonction de la dose.