β -adrenoceptor subtypes mediating the airways response to BRL 35135 in man

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- 1 The purpose of the study was to assess the bronchorelaxant effects of the β_3 -adrenoceptor agonist BRL 35135 in normal human airways.
- 2 Eight healthy male subjects were studied, having previously demonstrated airways responsiveness to inhaled salbutamol 200 μ g, with a group mean ($\pm$ s.e. mean) fall in airways resistance (R_{aw}), from baseline, of $37 \pm 5\%$.
- 3 Subjects attended the laboratory on 3 separate days, having fasted and taken placebo (PL) or nadolol 20 mg (N20), 2 h previously.
- 4 After 30 min rest, baseline measurements of R_{aw} , serum potassium, glucose and free fatty acid were performed before subjects were given single oral doses of BRL 35135 8 mg (BRL) or placebo BRL. Measurements were repeated 60 min after the BRL or placebo BRL were given.
- 5 There was a significant ($P < 0.05$) fall in R_{aw} (% change from baseline, as means and 95% CI) with PL/BRL: $-32(-18, -46)$, compared with either PL/PL: $-8(5, -21)$, or N20/BRL: $-11(2, -24)$. There was no significant difference between PL/PL and N20/BRL.
- 6 A similar pattern to R_{aw} was observed for both of the β_2 -mediated metabolic responses which were also antagonised by nadolol. For the potassium response (mmol l^{-1}), there was a significant ($P < 0.05$) difference between PL/BRL: $-0.50(-0.31, -0.69)$, in comparison with either PL/PL: $0.08(-0.11, 0.27)$ or N20/BRL: $0.09(-0.10, 0.28)$, but values for PL/PL and N20/BRL were not significantly different. In contrast, with the free fatty acid response (nmol l^{-1}), the increase seen with N20/BRL: $85(1.0, 171.0)$ was significantly ($P < 0.05$) different from PL/PL: $3.7(-82.3, 89.8)$, but was not different from PL/BRL: $132.5(46.5, 218.5)$.
- 7 In conclusion, BRL 35135 produced airways, potassium and glucose responses which were antagonised by nadolol, whereas the lipolysis response was not. This suggests that there are not functional β_3 -adrenoceptors in human airways.

Keywords β_3 -adrenoceptor airways response BRL 35135

Introduction

Pharmacological effects of β -adrenoceptor stimulation have been observed recently, which could not be adequately explained by β_1 - or β_2 -adrenoceptor mediation, leading to the concept of a third (β_3) atypical sub-population of β -adrenoceptors [1–3]. Furthermore, the gene encoding for the human β_3 -adrenoceptor has since been identified and sequenced, allowing characterisation of the pharmacological activity of this receptor subtype in transfected cloned cell lines [4].

β_3 -adrenoceptors have been identified in a variety of tissue types in experimental animals, including rat adipocytes [5] and skeletal muscle [6], as well as in gut [7, 8] and guinea pig heart and trachea [9]. However, there is little known regarding the distribution and function of the β_3 -adrenoceptor in man. *In vitro* studies have demonstrated their presence in human adipocytes [10], and neuroblastoma cell lines [11]. Recent studies in man with isoprenaline have suggested that β_3 -adreno-

ceptors play a role in mediating thermogenic responses [12], and may also be present in the heart [13]. Previous experiments with rat adipocytes and human β_3 -adrenoceptor cloned cell lines have demonstrated that novel non-catecholamine type β -adrenoceptor agonists such as BRL 35135 are potent stimulants of β_3 -adrenoceptors [4, 5].

The aim of the present study was to evaluate the airways response to the β_3 -adrenoceptor agonist BRL 35135 (SmithKline Beecham Pharmaceuticals, Great Burgh, UK) in normal subjects using nadolol 20 mg to antagonise β_2 -but not β_3 -adrenoceptors [4], in an attempt to dissect out any β_3 -adrenoceptor mediation of the airways response.

Methods

Subjects

Eight healthy male subjects, mean $\pm$ s.e. mean age, 21 $\pm$ 1 years with normal spirometry, mean FEV₁ as % predicted (and in litres), 90 $\pm$ 4%, range 83–115% (4.22 $\pm$ 0.24, range 3.31–5.65 l), were recruited to the study. All subjects were required to have a normal physical examination, full blood count, biochemical profile, urinalysis and 12-lead electrocardiogram prior to entry into the study. Those recruited did not have asthma or atopic conditions, were non-smokers and were not taking any concurrent medication. At an initial screening visit, all subjects demonstrated airways responsiveness to a dose of 200 μ g inhaled salbutamol, with a group mean fall in airways resistance (Raw) of 37 $\pm$ 5% from baseline, measured by constant volume body plethysmography. The study was approved by the Tayside Medical Ethics Committee, and written informed consent was provided by all subjects.

Protocol

Subjects attended the laboratory on 3 separate days, 1 week apart, having fasted for at least 6 h, and taken pre-treatment with either placebo (PL) or nadolol 20 mg (N20) 2 h previously. Strenuous physical exertion and caffeine consumption were avoided for 24 h before each study day. A cannula was inserted and kept patent with bolus injections of heparinised saline. Following a 30 min supine rest period, baseline measurements of airway resistance (Raw), serum potassium, glucose and free fatty acids were performed with 2 ml of cannula dead space being removed before blood samples were taken. Subjects then received single oral doses of either BRL 35135 8 mg (BRL) or placebo BRL, with all treatments being given in a randomised, double-blind crossover fashion. Measurements were repeated 60 min after the BRL was given, with subjects remaining seated throughout.

Measurements

Raw was measured by constant volume body plethysmography using the method described by Du Bois *et al.* [14, 15]. A Bodystar FG90 plethysmograph (Fenyes

and Gut, Basle, Switzerland) was used with on-line computer assisted determination of Raw. The mean of 5 consistent measurements was used for the purpose of analysis.

All biochemical variables were assayed in batches after completion of the study, and all samples were analysed in duplicate. Serum potassium was measured by flame photometry (IL943 analyser, Instrumentation Laboratory Ltd, Warrington, UK). The coefficients of variability for analytical imprecision within and between assays were 0.38% and 0.44% respectively. The normal reference range for our laboratory is 3.5–5.0 mmol l⁻¹. Serum glucose was measured using a GM7 analyser (Analox Instruments Ltd, London, UK). The inter- and intra-assay coefficients of variability for analytical imprecision were 7.2% and 2.7% respectively. Serum free fatty acid concentration was determined using an enzymatic colorimetric assay ('Half-micro test', Boehringer Mannheim, East Sussex, UK). The inter- and intra-assay coefficients of variability were 8.1% and 8.3%.

Statistical analysis

All data were calculated as change from baseline with Raw being expressed as a percentage change, and was analysed using a 'Stratgraphics' software package (STSC Software Publishing Group, Bethesda, USA). Multi-factorial analysis of variance (ANOVA) was used to compare treatments, with subjects, treatment and periods as within factors for the analysis. Duncan's multiple range testing, with confidence limits set at 95% was used to identify where differences between treatments were significant [16]. A probability value of $P < 0.05$ (two-tailed) was considered as being of significance. Values are expressed in the text as means and 95% confidence intervals, and in the figures as means and pooled standard errors generated from the ANOVA.

Results

Baseline values for Raw, serum potassium and free fatty acid were not significantly different between the 3 study days (Table 1). For serum glucose, there was a significant ($P < 0.05$) difference between baseline values for PL/PL and N20/BRL.

Airways and metabolic responses (Figure 1)

There was a small fall in Raw after placebo (PL/PL) which was not significant. At 60 min after BRL 35135 was given, there was a significant ($P < 0.05$) fall in Raw (% change) with PL/BRL: -32(-18, -46), compared with either PL/PL: -8(5, -21), or N20/BRL: -11(2, -24). The small fall in Raw with N20/BRL was not of significance, and there was no significant difference in Raw response between PL/PL and N20/BRL.

A similar pattern to Raw occurred with both potassium and glucose responses. For potassium (mmol l⁻¹), there was a significant ($P < 0.05$) difference after PL/BRL: -0.50(-0.31, -0.69), compared with PL/PL: 0.08(-0.11, 0.27), or N20/BRL: 0.09(-0.10, 0.28). No

Table 1 Baseline values for airway and metabolic parameters

	PL/PL	N20/BRL	PL/BRL
Raw (kPa l ⁻¹ s ⁻¹)	0.37 (0.33, 0.41)	0.37 (0.33, 0.41)	0.34 (0.30, 0.38)
K (mmol l ⁻¹)	4.25 (4.07, 4.43)	4.24 (4.05, 4.42)	4.13 (3.95, 4.31)
Gluc (mmol l ⁻¹)	4.83 (4.63, 5.03)	4.45 (4.24, 4.66)*	4.61 (4.41, 4.81)
FFA (nmol l ⁻¹)	146.2 (80.0, 212.4)	113.7 (47.5, 179.9)	181.2 (115.0, 247.4)

Raw = airways resistance, K = serum potassium, Gluc = serum glucose, FFA = serum free fatty acid. PL/PL = placebo + placebo BRL, N20/BRL = nadolol 20 mg + BRL 35135 8 mg, PL/BRL = placebo + BRL 35135 8 mg. Values shown are means and 95% confidence intervals.

*Denotes a significant ($P < 0.05$) difference from PL/PL.

Figure 1 Responses as change from baseline ($\Delta = 'd'$), for (a) airways resistance (Raw), (b) free fatty acid (FFA), (c) serum potassium (K) and (d) serum glucose (Gluc) measured 60 min after treatment with placebo + placebo BRL (PL/PL, $\square$), nadolol 20 mg + BRL 35135 8 mg (N20/BRL, $\square$), or placebo + BRL 35135 8 mg (PL/BRL, $\blacksquare$). Asterisks denote a significant ($P < 0.05$) difference from PL/PL. Values shown are means and s.e. means.

significant difference in the potassium response was found between PL/PL and N20/BRL. For glucose (mmol l⁻¹), there was a significant ($P < 0.05$) difference after PL/BRL: 0.75(0.46, 1.04), compared with PL/PL: -0.03(-0.32, 0.26), or N20/BRL: -0.01(-0.30, 0.28). As with the potassium response, there was no significant difference in the glucose response between PL/PL and N20/BRL.

In contrast, the free fatty acid (nmol l⁻¹) response to N20/BRL: 85.0(1.0, 171.0) was significantly ($P < 0.05$) different from PL/PL: 3.7(-82.3, 89.8). There was no significant difference between the fatty acid responses to PL/BRL: 132.5(46.5, 218.5) and N20/BRL.

Discussion

The results from the current study showed that the airways response to BRL 35135, like the potassium and glucose responses, were antagonised by nadolol 20 mg, when compared with placebo. In contrast, the lipolysis response to BRL 35135 was different in that it was not antagonised by nadolol in comparison with placebo. Taken together, these findings would suggest that there are not functional airway β_3 -adrenoceptors in humans.

In order to evaluate the β -adrenoceptor subtypes responsible for producing the airways effects of BRL 35135, it is important to consider the respective *in*

vitro β -adrenoceptor selectivity of BRL 35135 and nadolol. Firstly, in terms of intrinsic activity and potency, it has been shown in cloned cell lines expressing human β_3 -adrenoceptors, that BRL 37344 (the free acid metabolite of BRL 35135 produced *in vivo*) produces cyclic AMP accumulation which is 87% of the maximal response to (–)-noradrenaline, with activation constants (K_{act}) for BRL 37344 and (–)-noradrenaline of 5.9 nM and 6.3 nM respectively [4]. Salbutamol, whilst also a partial agonist producing 77% of the noradrenaline response, was considerably less potent with a K_{act} value of 266 nM. Secondly, in terms of relative β -adrenoceptor subtype potency (i.e. selectivity), it has been demonstrated that the selectivity ratio of BRL 37344 for lipolysis (in rat) over atrial chronotropy (in rat) was 400, with a selectivity ratio of 20.5 over tracheal relaxation (guinea pig) [3]. In contrast, selectivity ratios for isoprenaline induced lipolysis were 0.14 and 0.23 *vs* atria and trachea, and for salbutamol were 0.35 and 0.01 respectively. In terms of relative β_2 -adrenoceptor potency in guinea-pig trachea, BRL 37344 was 6.3-fold less potent than isoprenaline, and 1.35-fold less potent than salbutamol. The potency of BRL 37344 for adipocyte lipolysis was 5.5-fold greater than isoprenaline and 739-fold greater than salbutamol. Thus, in terms of relative potency, BRL 37344 (and hence BRL 35135) is selective for β_3 -compared with β_2 -adrenoceptor mediated effects *in vitro*, although it is a potent stimulant of β_2 -adrenoceptors in its own right.

Blockade of β_2 - but not β_3 -adrenoceptors with nadolol was essential, in order to dissect out any possible β_3 -adrenoceptor mediated effects of BRL 35135 in the airway. *In vitro* data from Emorine *et al.* [4] using cloned cell lines expressing human β_1 -, β_2 - and β_3 -adrenoceptors, showed that nadolol in a concentration of 10^{-4} M completely blocked isoprenaline-induced cyclic

AMP accumulation at the β_1 - and β_2 -adrenoceptors, but not at the β_3 -adrenoceptor subtype. Furthermore, dose-ranging studies in man [12, 13] have shown that nadolol 20 mg completely blunts known β_1 - and β_2 -mediated effects of isoprenaline, but only partially attenuates the metabolic rate response, which is thought to be partially β_3 -mediated. Hence low-dose nadolol was considered to be a suitable drug to antagonise β_2 - but not the β_3 -responses to BRL 35135.

Our results showed that the potassium and glucose responses to BRL 35135 were antagonised by nadolol, which is consistent with previous data showing that these effects are mediated by β_2 -adrenoceptors [17, 18]. The lack of effect of nadolol on the free fatty acid response to BRL 35135 would be in keeping with stimulation of β_3 -adrenoceptors [3, 5]. In another separate study evaluating the thermogenic effects of BRL 35135 8 mg and salbutamol 8 mg, it was found that there was a significant increase in basal metabolic rate with both drugs [19]. In the case of salbutamol, the basal metabolic rate response was completely blunted by nadolol, whereas with BRL 35135, the response was only partially attenuated inferring that BRL 35135 stimulates β_3 -adrenoceptors in man. Thus, using a dose of BRL 35135 which is known to stimulate β_3 -adrenoceptors, the airways response to BRL 35135 was antagonised by nadolol when compared with placebo, a finding which was mirrored by β_2 -mediated metabolic responses. This suggests that the airway response, like the glucose and potassium responses, was caused by stimulation of β_2 -adrenoceptors alone.

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