Evaluation of the β2 adrenoceptor agonist/antagonist activity of formoterol and salmeterol

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Abstract

Background – Salmeterol and formoterol have a lower intrinsic activity at β2 receptors than isoprenaline in human bronchus in vitro. The aim of the present study was to evaluate in vivo the β2 agonist/antagonist activity of salmeterol and formoterol at rest with low endogenous adrenergic tone, on exercise with raised endogenous adrenergic tone, and in the presence of fenoterol, an exogenous full β2 receptor agonist.

Methods – Eight normal subjects were randomised to receive single doses of placebo, salmeterol 300 µg, formoterol 72 µg, or propranolol 80 mg at weekly intervals. β2 adrenoceptor responses were evaluated at rest, at peak exercise, and after treatment with fenoterol 2·4 mg.

Results – At rest salmeterol and formoterol exhibited equivalent β2 agonist activity with regard to decrease in serum potassium levels and increase in finger tremor, with propranolol having no effect. Salmeterol and formoterol, like propranolol, potentiated the hyperkalaemic delta response to exercise compared with placebo, consistent with β2 antagonism: (mean difference and 95% confidence interval (CI) compared with placebo) salmeterol 0·20 (0·02 to 0·38) mmol/l, formoterol 0·17 (0·00 to 0·34) mmol/l, propranolol 0·45 (0·08 to 0·82) mmol/l. Propranolol blunted the heart rate delta response to exercise, consistent with β1 blockade, whilst salmeterol and formoterol had no effect. Salmeterol and formoterol, like propranolol, attenuated the hypokalaemic, tremor, and heart rate delta responses to fenoterol compared with placebo, in keeping with β2 blockade: potassium, salmeterol 0·18 (0·0 to 0·36) mmol/l, formoterol 0·17 (−0·03 to 0·37) mmol/l, propranolol 0·80 (0·54 to 1·06) mmol/l; tremor, salmeterol −0·69 (−1·26 to −0·12) log units, formoterol −0·71 (−1·53 to 0·11) log units, propranolol −0·85 (−1·66 to −0·04) log units; heart rate, salmeterol −6 (−13 to 1) beats/min, formoterol −10 (−19 to −1) beats/min, propranolol −18 (−29 to −7) beats/min.

Conclusions – At rest with low endogenous adrenergic tone salmeterol and formoterol showed equivalent β2 mediated agonist activity in terms of serum potassium and finger tremor responses. In the presence of raised endogenous adrenergic tone at peak exercise and in the presence of fenoterol (an exogenous full β2 receptor agon-

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ist), salmeterol and formoterol, like propranolol, exhibited β2 receptor antagonism as evidenced by their attenuation of β2 receptor mediated responses. The degree of β2 blockade with formoterol and salmeterol was comparable but less than with propranolol. The relevance of these findings at extrapulmonary β2 receptors with regard to airway β2 responses remains unclear and warrants further investigation.

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Salmeterol and formoterol are potent long acting β2 receptor agonists. The potency of these drugs will determine the dose required to produce a given effect, but it is important to appreciate that factors other than potency may influence the expression of their β2 agonist activity. Both salmeterol and formoterol have a lower intrinsic activity than isoprenaline in human bronchus in vitro, and therefore at saturating concentrations will produce a lower maximal response, with salmeterol having a lower efficacy than formoterol.[1] In other words, they are less efficient than a full agonist at activating the receptor transduction mechanism required to produce a maximal cellular response. Thus, as partial agonists the expression of their β2 agonist or antagonist activity may be modulated by the presence of a full β2 agonist such as isoprenaline or adrenaline. It could be predicted from first principles that, in the presence of a low concentration of a full agonist, salmeterol and formoterol would be expected to behave as β2 agonists, occupying additional receptors and therefore augmenting the overall response. However, in the presence of high concentrations of a full agonist they may behave as β2 antagonists because, by occupying receptors that would otherwise have been occupied by the full agonist, they will reduce the overall response.[2]

By evaluating the β2 receptor mediated modulation of exercise-induced hyperkalaemia due to raised endogenous levels of adrenaline, we have previously shown that oral salbutamol exhibits β2 antagonist effects at extrapulmonary β2 receptors in vivo.[3] We were therefore interested to investigate whether similar effects occurred with inhaled long acting β2 agonists given that they, like salbutamol, are not full β2 receptor agonists.

The aim of the present study was to evaluate in vivo β2 agonist/antagonist activity of sal-

meterol and formoterol at extrapulmonary β_2 receptors at rest when there is low endogenous adrenergic tone, and during exercise when endogenous adrenergic tone is high.

Furthermore, recent in vitro data have shown that salmeterol inhibits fenoterol and salbutamol induced relaxation in precontracted guinea pig airways.[4] Thus, β_2 receptor mediated responses were also evaluated in the presence of fenoterol, an exogenous full β_2 receptor agonist.[5-7] Propranolol was included as a positive control for the effects of β_1 and β_2 receptor antagonism.

Methods

SUBJECTS

Eight healthy volunteers (two women) with a mean (SE) age of 27 (1·9) years completed the study. Each volunteer had a normal physical examination including 12-lead ECG, haematological and biochemical screen prior to inclusion in the study. None of the subjects was taking regular medication. The study was approved by the local ethics committee, and subjects gave informed written consent.

PROTOCOL

A single blind randomised (Latin square) crossover, placebo controlled design was used. Subjects attended the laboratory on four occasions at least one week apart.

An intravenous cannula was inserted into a forearm vein to allow blood sampling. After 30 minutes of supine rest baseline measurements (t_0) of heart rate, postural finger tremor, and serum potassium were made. Subjects then received inhaled salmeterol 300 µg (Serevent metered dose inhaler, 25 µg per actuation, Allen and Hanburys, Uxbridge, Middlesex, UK), or inhaled formoterol 72 µg (Foradil metered dose inhaler, 12 µg per actuation, Ciba-Geigy, Basel, Switzerland), or inhaled placebo or oral propranolol, 80 mg, with double dummies as appropriate. One hour after administration of the drugs measurements of potassium, heart rate, and tremor were repeated (t_1) and subjects then underwent a standardised three minute exercise step test in order to produce a maximal heart rate response.[8] Peak exercise heart rate was recorded, and a blood sample was taken immediately on completion of the exercise for estimation of serum potassium (t_2) (tremor was not recorded on completion of the exercise). Subjects then rested supine for 30 minutes. Further measurements of potassium, heart rate and tremor were made (t_3) before subjects received inhaled fenoterol 2·4 mg (Berotec 200 metered dose inhaler, 200 µg per actuation, Boehringer Ingelheim, Bracknell, UK). This was administered as 12 sequential puffs over a period of four minutes. Final measurements (t_4) were made 30 minutes after the administration of the fenoterol. At the end of each of the four study days subjects received 32 mmol effervescent potassium (Sando K, Sandoz Pharmaceuticals, Camberley, UK).

MEASUREMENTS

Heart rate was monitored and recorded using a 12-lead electrocardiograph with stress test adaptor (HP4700A, Hewlett Packard, Palo Alto, California, USA). Heart rate was calculated from the mean of five consecutive R–R intervals. Postural finger tremor was measured by a previously validated method[9] with an accelerometer transducer (Entran Ltd, Ealing, UK) attached to the distal phalanx of the middle finger. Each measurement consisted of four recordings, the results of which were stored on computer disc for subsequent spectral analysis of total tremor power >2 Hz (units mg²/s) using computer assisted autocovariance. The mean of three consistent recordings was used in subsequent analysis. Serum potassium was analysed by flame photometry (IL943 analyser, Instrumentation Lab Ltd, Warrington, UK), samples being analysed in batches at the end of the study with each sample analysed in duplicate. The normal reference range for our laboratory is 3·5–5·5 mmol/l, and the coefficients of variability for analytical imprecision within and between assays are 0·41% and 1·04%, respectively.

STATISTICAL ANALYSIS

Finger tremor data were transformed using logarithm to base 10 to achieve conformation to a normal distribution prior to analysis. Data were then analysed using the Statgraphics software package (STSC Software, Rockville, USA). Effects at rest and in response to exercise and fenoterol were all analysed as delta responses – that is, the difference between the response before and after drug administration (t_1-t_0), before and after exercise (t_2-t_1), and before and after fenoterol (t_4-t_3). Comparisons were made by multifactorial analysis of variance (MANOVA) using subjects, treatments, and time as within factors for the analysis. Where the overall MANOVA was significant, Duncan's multiple range testing was used to establish where differences between treatments were significant. Differences from placebo, where significant, were calculated as means and 95% confidence intervals. A probability value of $p < 0.05$ (two tailed) was considered significant for all tests.

Results

SUPINE REST

There were no significant differences in baseline (t_0) measurements between each of the four study days for any of the measured parameters.

One hour after dosing (t_1) salmeterol and formoterol significantly ($p < 0.05$) lowered serum potassium levels (as delta response, t_1-t_0) compared with placebo, an effect consistent with β_2 agonism: (mean differences and 95% CI versus placebo) salmeterol −0·38 (−0·65 to −0·11) mmol/l, formoterol −0·36 (−0·63 to −0·09) mmol/l. Likewise, the finger tremor delta response was significantly ($p < 0.05$) greater with salmeterol and formoterol than with placebo: salmeterol 0·56 (−0·16 to 1·28) log units, formoterol 0·61 (−0·19 to 1·41) log

Mean (SE) absolute values

	Treatment	Pre-drug (t_0)	Post-drug/pre-exercise (t_1)	Post-exercise (t_2)	Pre-fenoterol (t_3)	Post-fenoterol (t_4)
Potassium (mmol/l)	Placebo	3·74 (0·08)	3·84 (0·07)	4·07 (0·11)	3·86 (0·07)	2·93 (0·07)
	Salmeterol	3·74 (0·08)	3·48 (0·07)*	3·91 (0·11)	3·50 (0·07)*	2·75 (0·07)
	Formoterol	3·81 (0·08)	3·56 (0·07)*	3·91 (0·11)	3·58 (0·07)*	2·80 (0·07)
	Propranolol	3·81 (0·08)	3·95 (0·07)	4·60 (0·10)*	4·08 (0·07)	3·95 (0·07)*
Heart rate (beats/min)	Placebo	72 (3)	73 (5)	162 (4)	84 (2)	99 (3)
	Salmeterol	70 (3)	81 (5)	162 (4)	95 (2)*	105 (3)
	Formoterol	73 (3)	74 (5)	165 (4)	90 (2)	97 (3)
	Propranolol	73 (3)	71 (5)	129 (4)*	73 (2)*	71 (3)*
Finger tremor (log units)	Placebo	1·73 (0·12)	1·58 (0·27)		1·70 (0·18)	2·68 (0·11)
	Salmeterol	1·88 (0·11)	2·20 (0·18)		2·27 (0·12)*	2·60 (0·11)
	Formoterol	1·93 (0·10)	2·28 (0·35)		2·28 (0·18)*	2·38 (0·16)
	Propranolol	1·78 (0·10)	1·58 (0·24)		1·67 (0·18)	1·69 (0·20)*

t_0 = before treatment; t_1 = one hour after treatment; t_2 = immediately after exercise; t_3 = before treatment with 2·4 mg fenoterol; t_4 = 30 minutes after treatment with fenoterol.
* Significant difference from placebo at a given time point.

units. Absolute values for potassium levels but not finger tremor or heart rate were significantly different with salmeterol and formoterol compared with placebo (table). Propranolol had no significant effect on resting serum potassium level, finger tremor, or heart rate compared with placebo.

EXERCISE RESPONSES

Propranolol significantly (p<0·05) attenuated the β_1 receptor mediated heart rate response to exercise (t_2–t_1) compared with placebo, consistent with β_1 antagonism: (mean difference and 95% CI compared with placebo) −31 (−54 to −8) beats/min (fig 1). Salmeterol

and formoterol had no effect on this response. Propranolol significantly (p<0·05) potentiated the rise in potassium on exercise (t_2–t_1) consistent with β_2 antagonism. Salmeterol and formoterol, like propranolol, also potentiated the potassium response to exercise compared with placebo: salmeterol 0·2 (0·02 to 0·38) mmol/l, formoterol 0·17 (0·0 to 0·34) mmol/l, propranolol 0·45 (0·08 to 0·82) mmol/l. Propranolol produced significantly greater potentiation of the delta potassium response than either salmeterol or formoterol. The absolute post exercise potassium level (t_2) was significantly higher (p<0·05) with propranolol than with placebo, but salmeterol and formoterol were not significantly different from placebo (table).

RESPONSES TO FENOTEROL

Propranolol significantly (p<0·05) attenuated the potassium, heart rate, and tremor response to fenoterol (t_4–t_3) compared with placebo, consistent with β_2 antagonism: potassium 0·80 (0·54 to 1·06) mmol/l, heart rate −18 (−29 to −7) beats/min, tremor −0·85 (−1·66 to −0·04) log units (fig 2). Salmeterol and formoterol also significantly (p<0·05) attenuated the hypokalaemic and tremor response to fenoterol compared with placebo: potassium: salmeterol 0·18 (0·0 to 0·36) mmol/l, formoterol 0·17 (−0·03 to 0·37) mmol/l; tremor: salmeterol −0·69 (−1·26 to −0·12) log units, formoterol −0·71 (−0·53 to 0·11) log units. In addition, formoterol also significantly (p<0·05) attenuated the heart rate response to fenoterol compared with placebo, with salmeterol showing a similar trend (p = 0·06): formoterol − 10 (−19 to −1) beats/min, salmeterol −6 (−13 to −1) beats/min. Propranolol produced significantly greater blunting of fenoterol induced delta heart rate and delta potassium responses (but not tremor responses) than either salmeterol or formoterol. There were no significant differences in the absolute values for serum potassium, finger tremor, or heart rate after fenoterol following treatment with salmeterol or formoterol compared with placebo. The absolute values for heart rate and finger tremor after fenoterol were significantly lower (p<0·05) after treatment with propranolol than
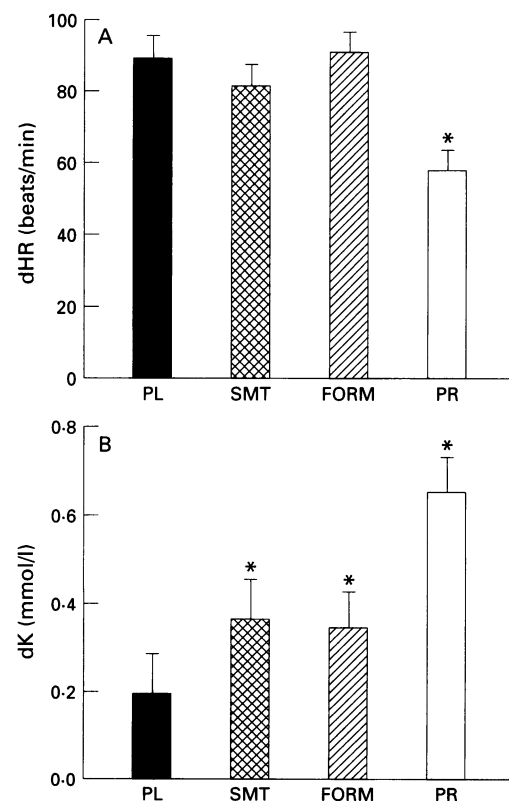

Figure 1 β_1/β_2 mediated effects on peak exercise. A comparison of the effect of treatment with single doses of placebo (PL), salmeterol 300 μg (SMT), formoterol 72 μg (FORM), and propranolol 80 mg (PR) on (A) delta heart rate (β_1) and (B) delta serum potassium (β_2). Values are given as means and pooled SE. *p<0·05 versus placebo.
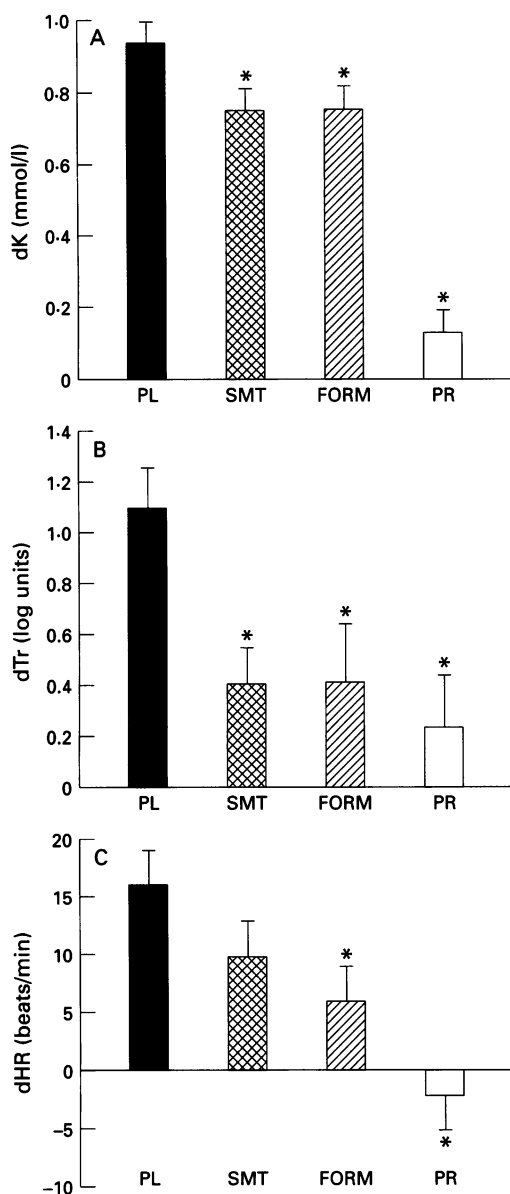
Figure 2 β_2 mediated responses to fenoterol 2·4 mg. A comparison of the effect of treatment with single doses of placebo (PL), salmeterol 300 µg (SMT), formoterol 72 µg (FORM), and propranolol 80 mg (PR) on (A) delta serum potassium, (B) delta finger tremor, and (C) delta heart rate. Values are given as means and pooled SE. *p<0·05 versus placebo.

with placebo, whilst the serum potassium level was significantly (p<0·05) higher (table).

Discussion

The results of the present study show that, in contrast to their β_2 agonist effects at rest, in the presence of endogenous adrenaline or exogenous fenoterol, salmeterol and formoterol behave as β_2 receptor antagonists, as evidenced by the potentiation of exercise induced hyperkalaemia and the attenuation of hypokalaemic, tremor, and heart rate responses to fenoterol.

During physical exercise potassium leaks out of skeletal muscle cells by an active process causing a transient rise in the serum potassium level. Normally this is rapidly corrected by a membrane bound β_2 adrenoceptor linked

sodium/potassium ATPase driving potassium back into the cells. β_2 receptor antagonists such as propranolol have therefore been shown to augment the rise in potassium levels on exercise.[10] Furthermore, digitalis which directly inhibits the sodium/potassium ATPase has also been found to augment exercise-induced hyperkalaemia.[11] Thus, in the present study the potentiation of the potassium response to exercise by salmeterol and formoterol, like propranolol, was consistent with β_2 receptor antagonism. The heart rate response to exercise is mediated via β_1 adrenoceptors, as indicated by the lack of effect of the selective β_2 blocker ICI 118 551 on this response.[12] In this study propranolol significantly attenuated the heart rate response to exercise, consistent with its β_1 blocking properties. Salmeterol and formoterol had no effect, thus showing that the β receptor antagonism of these drugs, as one might predict, is β_2 receptor selective. In contrast, the heart rate response to drugs such as fenoterol is predominantly β_2 receptor mediated.[6 13 14] Thus, as salmeterol and formoterol, like propranolol, attenuated this response, this is again in keeping with β_2 blockade.[15]

It is important to consider the possible confounding influence of pre-exercise (t_1) and pre-fenoterol (t_3) absolute values on the delta responses to both exercise and fenoterol. Salmeterol and formoterol significantly lowered the serum potassium level compared with placebo before exercise (at t_1). It is therefore possible that changes in the delta potassium response to exercise could have been due to functional antagonism. If this were the case, the values after exercise (t_2) would also be expected to be lowered to the same degree. However, this was clearly not the case, there being, in fact, no significant difference in serum potassium levels after exercise following treatment with salmeterol, formoterol, or placebo. Furthermore, propranolol, which had no effect on pre-exercise potassium levels, produced a marked increase in the response to exercise, thus suggesting that the pre-exercise potassium concentration did not confound the delta potassium response to exercise.

Similar arguments can be applied to the responses to fenoterol. Whilst pre-fenoterol (at t_3) heart rate and finger tremor were increased after treatment with salmeterol and formoterol compared with placebo, the post-fenoterol values (at t_4) were not significantly different from placebo. Likewise with the hypokalaemic response, although the pre-fenoterol serum potassium level was lower after salmeterol and formoterol than with placebo, there were no significant differences in the post-fenoterol values.

In this study the extrapulmonary β_2 receptor mediated responses at rest produced by salmeterol 300 µg and formoterol 72 µg were equivalent - that is, formoterol 6 µg is equivalent to salmeterol 25 µg. This finding is consistent with previous studies determining the potency of these agonists in relation to salbutamol.[16] Our in vivo results confirm the findings of previous in vitro studies suggesting salmeterol and formoterol have a lower intrinsic activity

than a full agonist such as isoprenaline.[1] Formoterol was said to have an efficacy of 0·96 compared with isoprenaline (given a nominal efficacy value of 1·00) in terms of their ability to maximally relax human bronchi, while salmeterol has an efficacy value of 0·71. Similar studies have shown that formoterol does, indeed, exhibit greater intrisic activity than salmeterol.[17 18] In vitro studies with guinea pig airway have also shown that salmeterol can inhibit fenoterol and, to a lesser degree, salbutamol-induced relaxation.[4] In the present study we were unable to distinguish between the relative partial agonist activities of formoterol and salmeterol as they both produced comparable degrees of β_2 agonism at rest and comparable β_2 blockade with exercise and fenoterol responses. However, it was clearly evident that the β_2 blockade of formoterol and salmeterol was less than that of propranolol. It may, however, be possible to make such a distinction between salmeterol and formoterol if dose ranging studies were performed using lower doses of partial agonist.

This study was confined to evaluating extrapulmonary β_2 responses in normal individuals, and the clinical relevance in terms of modulation of bronchodilator response therefore remains unclear. Clearly salmeterol, a drug with relatively low efficacy, normally produces clinically significant degrees of bronchodilation. It is, however, worth noting that, in vitro, differences in intrinsic activity between agonists in terms of bronchodilator activity become more pronounced when bronchial tone is increased[1] as might occur in the setting of acute asthma. This may be compounded by the presence of raised endogenous adrenergic tone which might also result in the expression of antagonist effects by partial agonists such as salmeterol. Furthermore, it is possible that a β_2 agonist with lower intrinsic activity such as salmeterol might result in a reduced bronchodilator response to an exogenous agonist with higher intrinsic activity such as salbutamol.

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