β Adrenoreceptor subtype cross regulation in the human heart

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Abstract

Objectives—To find out in a prospective study whether β1 blockers treatment causes selective β1 adrenoreceptor sensitisation, and to find whether such sensitisation is confined to the heart.

Design—A placebo controlled cross over study of two weeks of selective β1 blocker treatment with 10 mg of bisoprolol daily.

Subjects—Six healthy volunteers.

Outcome measures—Three days after stopping the 10 mg of bisoprolol or placebo, subjects underwent treadmill exercise (to measure cardiac β1 receptor responsiveness) and were given salbutamol injections (to measure cardiac β2 receptor responsiveness). Secondary end points were the responses of serum potassium, glucose, and insulin to β1 stimulation.

Results—There was no difference in exercise induced increases in heart rate, but after treatment with bisoprolol the dose of salbutamol required to increase heart rate by 40 beats/min was 1·9 μg/kg compared with 2·9 μg/kg after placebo (p < 0.005). The fall in diastolic blood pressure was not significantly different on the two occasions. Hypokalaemia induced by salbutamol, but not hyperglycaemia or hyperinsulinaemia, was enhanced after bisoprolol.

Conclusion—This study shows that treatment with a β1 blocker in vivo leads to sensitisation of cardiac β1, adrenoreceptors but not cardiac β2, adrenoreceptors or vascular β receptors. This previously unrecognised form of receptor cross sensitisation in the heart may noticeably diminish the efficacy of selective β1 blockade in preventing arrhythmias in patients with ischaemic heart disease. These findings reopen the question of which type of β blocker is more appropriate for such patients.

The β blockers are among the most widely used drugs in clinical practice. Both β1 selective and non-selective blockers seem to be equally effective as antihypertensive and antianginal agents, but this has masked our ignorance about which type of β blocker is to be preferred for achieving the long-term objective in both groups of patients, namely a reduction in the incidence of myocardial infarction and its complications. The possibility that so called cardioselective β blockers offer less cardioprotection than non-selective β blockers has existed since the human heart was found to differ from other species in not, after all, being itself β1 selective.1 About 30% of the β receptors are of the β1 subtype1 and selective β1 receptor stimulation causes tachycardia.3

Chronic β blockade may paradoxically increase the sensitivity of patients to sympathetic stimulation, although in the past this has been considered important only during the immediate withdrawal period.4 Recent in vitro studies, however, on strips of atrial myocardium from patients still receiving β blockers have shown a previously unrecognised cross regulatory effect of chronic β1 blockade on other receptors coupled to adenyl cyclase, including a selective 10 fold increase in sensitivity to β2 stimulation by adrenaline, with no change in sensitivity to β1 stimulation.5-7 Therefore, such patients may be at enhanced risk of tachycardia induced by adrenaline, and have indeed been found to be 4 times more sensitive to β2 receptor stimulation by intracoronary salbutamol than patients who are not β blocked.6 These previous studies were performed on patients, or on tissue from patients, whose drug treatment had been chosen by referring physicians long before the time of study. Therefore to establish that the β1 hypersensitivity seen in these studies is being induced by β blocker treatment, rather than being a consequence of patient selection criteria, we undertook the present prospective investigation. This was a cross over study of β1 blocker treatment in normal volunteers to find whether selective β1 receptor sensitisation can be induced by β1 blockade. To find whether any sensitisation occurs in the heart only, we gave salbutamol and measured the dose related changes in diastolic blood pressure and the metabolic responses to salbutamol as indicators of extracardiac β2 receptor stimulation.

Patients and methods

SUBJECTS

Six healthy men aged 24–35 took part in the study. The subjects were non-smokers with no contraindications to β blockade on history, examination, or electrocardiogram.

PROCEDURE

Before starting drug treatment the subjects

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exercised on a treadmill to familiarize themselves with the equipment and protocol. A level of exercise was found for each subject that produced a stable heart rate of about 150 beats/min. The subjects then took a 14 day course of treatment with bisoprolol (10 mg) or matched placebo once daily. The order of treatment was double blinded and randomised according to a latin square design. Bisoprolol was used because its $\beta_1$ selectivity (about 75-fold) is higher than that of atenolol or metoprolol.9

Three days after stopping each course of treatment the subjects underwent treadmill exercise followed by an intravenous salbutamol dose response study. A similar light breakfast was permitted before the two visits, excluding all caffeine containing drinks. Two intravenous cannulae were inserted in forearm veins. The subjects rested for 30 minutes and the resting heart rate was noted. They then walked on the treadmill at the previously determined level of exercise for two minutes and the heart rate was measured. Blood samples were taken to measure plasma potassium before exercise, at peak exercise, and at five minutes after exercise.

After exercise the subjects rested for 15 minutes and were then given salbutamol by intravenous injections. The bolus injections were given over two minutes, and after a further three minutes the next dose was given. Injections started at 0.5 $\mu$g/kg and were increased by doubling increments up to a final dose of 4 $\mu$g/kg. Heart rate and blood pressure were recorded at one minute and three minutes after the end of each injection. Baseline measurements were taken in duplicate. During the dose response curve heart rate and blood pressure were determined by single measurements. Peak responses to each injection were seen at the one minute recording and these readings were used for further analysis. Blood samples were taken for measurement of potassium, glucose, and insulin concentrations before the start of the salbutamol injections and at five minute intervals for 40 minutes.

METHODS OF ASSESSMENT
Heart rate was calculated from the RR intervals averaged over 15 seconds from a continuously monitored electrocardiogram. Blood pressure was measured with an automated sphygmomanometer (Datascope, USA). Potassium was measured with an ion sensitive electrode, glucose by the glucose oxidase method, and insulin by a double antibody radioimmunoassay.10

The study was approved by the district medical ethics committee and was in accord with the Helsinki Convention (Tokyo and Venice amendments). Subjects gave written informed consent.

STATISTICS
Changes in heart rate induced by exercise and plasma potassium concentration after bisoprolol treatment were compared with those after placebo by a paired Student’s $t$ test. Changes in systolic blood pressure induced by salbutamol after bisoprolol treatment were compared with those after placebo by repeated measures analysis of variance. The dose of salbutamol needed to decrease diastolic blood pressure by 10 mm Hg (vasodilator dose 10 (VD10)) was found for each dose response curve from a fitted linear equation. VD10s (log doses) were compared after bisoprolol treatment and after placebo by a paired Student’s $t$ test. The dose of salbutamol to increase heart rate by 40 beats/min (chronotropic dose 40 (CD40)) was found for each individual dose response curve from a fitted quadratic equation, and CD40s (log doses) were compared by a paired Student’s $t$ test.11 For changes in the metabolic variables, serum potassium, insulin, and glucose concentrations, responses after bisoprolol treatment were compared with those after placebo by repeated measures analysis of variance. A $p$ value of $<0.05$ was considered significant. All results are expressed as mean (SEM).

Results
There was no significant difference in the basal heart rate or blood pressure three days after treatment with bisoprolol or placebo.

The increases in heart rate in response to exercise (peak exercise rate—resting rate) after treatment with bisoprolol (66.5 (3.0) beats/min) were not significantly different from those after placebo (57.3 (4.0) beats/min) (table 1). The study had a 90% power to detect a $>15\%$ difference in exercise tachycardia at the 5% level.

By contrast, the increases in heart rate in response to salbutamol injection were significantly greater after bisoprolol treatment than placebo. The maximum increases (peak rate after 4 $\mu$g/kg—preinjection heart rate) were 66.3 (6.1) beats/min after bisoprolol and 51.3 (5.9) beats/min after placebo, with CD40s of 1.9 $\mu$g/kg and 2.9 $\mu$g/kg, ($p<0.002$; fig 1 and table 2). Salbutamol caused a fall in diastolic and no change in systolic blood pressure (fig 1): the dose of salbutamol required to decrease diastolic blood pressure by 10 mm Hg (VD10) was similar on the two occa-

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Cardiovascular and metabolic responses to two minutes sub-maximal exercise</th>
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<tbody>
<tr>
<td></td>
<td>Placebo Before</td>
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<tr>
<td>Heart rate (beats/min)</td>
<td>61·5 (2·6)</td>
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<tr>
<td>Plasma potassium (mmol/l)</td>
<td>4·17 (0·08)</td>
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Values are mean (SEM); differences were non-significant.
Figure 1  Cardiovascular responses to salbutamol infusion. (A) Heart rate (HR) was measured in response to incremental doses of salbutamol injected three days after bisoprolol or placebo. Bars = SEM. The sensitivity to salbutamol, measured as the dose required to raise HR by 40 beats/min, was significantly greater after bisoprolol than after placebo (p < 0.005). (B) Systolic and diastolic blood pressures (BP) were measured one minute after the end of each injection of salbutamol. Bars = (SEM). Differences were non-significant.

<table>
<thead>
<tr>
<th>Subject</th>
<th>CD40</th>
<th>VD10</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>After</td>
<td>After</td>
</tr>
<tr>
<td></td>
<td>Bisoprol</td>
<td>Placebo</td>
</tr>
<tr>
<td>1</td>
<td>1.09</td>
<td>4.11</td>
</tr>
<tr>
<td>2</td>
<td>1.62</td>
<td>2.22</td>
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<tr>
<td>3</td>
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<td>2.34</td>
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<tr>
<td>5</td>
<td>1.74</td>
<td>2.20</td>
</tr>
<tr>
<td>6</td>
<td>1.86</td>
<td>2.32</td>
</tr>
<tr>
<td>Mean (SEM)</td>
<td>1.89 (0.10)</td>
<td>2.93 (0.33)</td>
</tr>
</tbody>
</table>

CD40 the dose of salbutamol (µg/kg) to increase heart rate by 40 beats/min calculated from a fitted quadratic equation for each individual log dose response curve; VD10 the dose of salbutamol (µg/kg) to decrease diastolic blood pressure by 10 mm Hg calculated from a fitted linear equation for each individual log dose response curve. The log CD40s were significantly lower after placebo than after bisoprolol (p < 0.005). The difference between VD10s was not significant.

Figure 2  Metabolic responses to salbutamol infusion. (A) Plasma potassium (K+); (B) plasma glucose, and (C) plasma insulin were measured every five minutes after the start of five minute incremental doses of salbutamol. Subjects were studied three days after stopping treatment with bisoprolol or placebo. Bars = (SEM). The fall in plasma K+ was greater after bisoprolol treatment (p < 0.004), but the increases in glucose and insulin were non-significant.
Discussion

The principal finding was that in each of these normal subjects, two weeks of treatment with a β₁ selective blocker led to an increased tachycardia in response to a β₂ agonist, with a significant increase in the chronotropic potency of salbutamol. There was, however, no increase in the β₂ mediated tachycardia on exercise and no enhancement of the peripheral vascular β₁ receptor mediated fall in diastolic blood pressure in response to salbutamol. Does the study therefore confirm, in vivo, that β₂ blockade causes selective cross sensitisation of β₁ receptor responses in the human heart?

Our previous studies with intracoronary salbutamol gave doses without systemic effects to show that the tachycardia was solely due to direct stimulation of cardiac β₂ receptors (rather than vagal withdrawal after peripheral vasodilatation). By giving intravenous salbutamol in the present study, we found no effect of previous β₁ blockade on the fall in diastolic blood pressure, again suggesting that the enhanced tachycardia is mediated directly through increased cardiac β₂ receptor sensitivity rather than indirectly through peripheral vascular β₁ receptors causing vasodilatation. This conclusion is also consistent with recent organ bath experiments on mammary artery and saphenous vein strips, which show no difference in β₂ mediated vasodilatation between β₂ blocked and untreated patients.

In the absence of an appropriate β₁ agonist for intravenous use, we used exercise to produce tachycardia mediated by β₂ receptors. Several previous studies have concluded that tachycardia induced by exercise is due to a combination of vagal withdrawal and β₁ receptor stimulation with no evidence of a component due to β₂ receptor stimulation. Tachycardia induced by exercise was unaltered after bisoprolol treatment. Therefore our finding of increased tachycardia induced by salbutamol and no difference in tachycardia induced by exercise after treatment with a β₁ blocker is consistent with our organ bath findings in atrial strips. No change was found in responsiveness of β₁ receptors in atrial strips from β₁ blocked patients despite a 10-fold increase in the responsiveness to stimulation of β₂ receptors. Also, our results are consistent with a previous study of withdraw-
al from atenolol in which increased sensitivity to isoprenaline (β₁ + β₂) was accompanied by no increase in exercise tachycardia (β₂). Our in vivo and in vitro findings were surprising as previous radioligand binding studies had shown that treatment of atrial strips with a β₁ blocker leads to an increase in the density of β₂ receptors and not β₁ receptors; these density changes (upregulation) do not therefore seem to have functional consequences. The finding of less sensitisation of cardiac β₂ receptors in our present study could be due to the shorter duration of β₁ blocker treatment (two weeks vs several months) and also due to the need to study the subjects after three days of withdrawal from bisoprolol to compare the exercise induced (β₁ mediated) tachycardia on the two occasions.

From our in vitro studies it was apparent that the process of sensitisation was a new phenomenon of receptor cross reaction unrelated to simple upregulation of the number of receptors. Taken with our present findings it seems that the cross sensitisation is a cellular event peculiar to the heart. It is possible that the prerequisite for cross sensitisation is a mixed population of β₁ and β₂ receptor subtypes, and a high basal stimulation of β₁ receptors.

Stimulation of β₂ receptors, unlike β₁ receptors, has additional important effects on metabolism. As a secondary objective of the study therefore, we included measurements of metabolic variables under β₂ receptor control. This was partly to find whether there might be clinically important metabolic effects of β₂ receptor sensitisation and partly to pursue further the mechanistic question of whether β₂ receptor sensitisation can occur only in cell types where both β₂ receptor subtypes are present. The metabolic responses to stimulation of β₂ receptors take longer than the haemodynamic changes to reach equilibrium and we therefore compared their response over a time period to the total dose of salbutamol given. Stimulation of β₂ receptors that occurs when the concentration of the endogenous β₂ receptor agonist adrenaline is high, has been shown repeatedly to cause hypokalaemia. The failure of selective β₂ receptor blockers to reverse this has been one of the main arguments in favour of non-selective β₁ blockade. From the clinical standpoint, therefore, this concern may be enhanced by our finding that β₁ blockade actually potentiates hypokalaemia mediated by β₂ receptors. Hypokalaemia in response to β₂ receptor stimulation is predominantly due to skeletal muscle uptake of potassium through stimulation of sodium-potassium ATPase. As skeletal muscle is not known to contain β₁ receptors we had not anticipated this to be potentiated after β₁ blockade. This finding therefore seems to contradict our hypothesis that a mixed population of β₂ receptors would allow cross sensitisation. This contradiction could, however, be reconciled if there was a component of the hypokalaemia due to stimulation of β₁ receptors in cells, other than skeletal muscle, which contain both β₁ and β₂ receptors—for example, red blood cells. Involvement of β₁ receptor bearing cells in the hypokalaemic response to β₂ agonists has been suggested by the finding that β₁ selective agonists can lower serum potassium and that β₁ selective antagonists can antagonise the hypokalaemic response to adrenaline and isoprenaline, but these findings may only reflect the poor

(0.16 mM, p = 0.04). The rise in plasma glucose in response to salbutamol, however, was similar after either treatment (fig 2). Basal plasma insulin concentration was unaltered by bisoprolol treatment and rose equally in response to salbutamol injection (fig 2).

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selectivity of the agents used. An alternative explanation of our increased hypokalaemia in response to salbutamol after treatment with bisoprolol may be that hypokalaemia is greater because the greater increase in cardiac output augments the potassium load delivered to the predominant site of potassium uptake—namely, skeletal muscle.

By contrast there was no increase after β1 blockade of salbutamol induced release of glucose or insulin consistent with a lack of known β1 receptors on liver or pancreatic cells. We found, however, that basal plasma glucose (before salbutamol infusion) was increased after bisoprolol treatment, with no difference in basal plasma insulin. The implied insulin resistance has been previously noted with β-blockade. We would not attach too much importance to our own findings until they are repeated with measurements before and after a standard glucose load, but they may shed indirect light on the mechanism of cross sensitisation.

Previous experiments have pointed away from changes in the β receptor itself being responsible for sensitisation and have indicated that the coupling of the receptor to its adeny1 cyclase through G proteins, is a more likely site for regulation of sensitivity. The mitral valve prolapse syndrome β receptor sensitisation is found due to a change in the stimulatory G protein (Gs). On the other hand, altered β receptor sensitivity could be due to changes in the inhibitory G protein (Gi). Conditions in which there is a chronic increase in β receptor stimulation, such as cardiac failure, have been associated with increased expression of Gi. Consequently one of our hypotheses is that β blockade leads to the reverse of chronic β receptor stimulation—namely, a reduced concentration of Gi. If this hypothesis proves to be correct then the hint that β blockade induces insulin resistance is of mechanistic as well as clinical interest as some of the actions of insulin are Gi dependent and reduced expression of this protein has been found in some experimental models of insulin resistance.

The possible clinical implications are perhaps of greater relevance to the present study. To find sensitisation of β1 receptors might provide the basis for explaining the clinical syndrome of β blocker withdrawal. Also a major clinical concern is whether sensitisation of, and failure to block, cardiac β receptors in response to salbutamol with ischaemic heart disease might paradoxically increase the risk of arrhythmias. There have been no prospective comparisons of β1 selective and non-selective antagonists in such patients, but we have commented previously on the generally better performance of non-selective antagonists in the secondary prevention trials of β blockade after myocardial infarction and the perhaps surprising failure of the β1 selective atenolol to prevent arrhythmia when given acutely to such patients. These speculations on the clinical implications will now need to be tested in groups of patients with ischaemic heart disease or hypertension.
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31 Green A. Adenosine receptor downregulation and insulin resistance follows prolonged incubation of adipocytes with an \( A_1 \) adenosine receptor agonist. *J Biol Chem* 1987;262:15702-7.