β₂ Adrenergic receptors mediate important electrophysiological effects in human ventricular myocardium

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Abstract

Objective—To define the effects of β₂ adrenergic receptor stimulation on ventricular repolarisation in vivo.

Design—Prospective study.

Setting—Tertiary referral centre.

Patients—85 patients with coronary artery disease and 22 normal controls.

Interventions—Intravenous and intracoronary salbutamol (a β₂ adrenergic receptor selective agonist; 10–30 µg/min and 1–10 µg/min), and intravenous isoprenaline (a mixed β₁/β₂ adrenergic receptor agonist; 1–5 µg/min), infused during fixed atrial pacing.

Main outcome measures—QT intervals, QT dispersion, monophasic action potential duration.

Results—In patients with coronary artery disease, salbutamol decreased QT₉₀ and QT₃₀ but increased QT₁₀₀ duration; QT₁₀₀–QT₃₀ and QT₃₀–QT₉₀ intervals increased, resulting in T wave prolongation (mean (SEM): 201 (2) ms to 233 (2) ms; p < 0.01). There was a large increase in dispersion of QT₁₀₀, QT₃₀ and QT₉₀ which was more pronounced in patients with coronary artery disease—for example, QT₉₀ dispersion: 50 (2) ms baseline v 98 (4) ms salbutamol (controls), and 70 (1) ms baseline v 108 (3) ms salbutamol (coronary artery disease); p < 0.001. Similar responses were obtained with isoprenaline. Monophasic action potential duration at 90% repolarisation shortened during intracoronary infusion of salbutamol, from 278 (4.1) ms to 237 (3.8) ms (p < 0.05).

Conclusions—β₂ adrenergic receptors mediate important electrophysiological effects in human ventricular myocardium. The increase in dispersion of repolarisation provides a mechanism whereby catecholamines acting through this receptor subtype may trigger ventricular arrhythmias.

(Heart 2001; 86:45–51)

Keywords: β₂ adrenergic receptors; ventricular repolarisation; QT dispersion; salbutamol; isoprenaline

Catecholamines acting predominantly through β₂ adrenergic receptors have a widespread influence on cardiac function, modulating both contraction and relaxation, and promoting arrhythmogenesis. Historically, cardiac β₂ adrenergic receptor responses were thought to be mediated exclusively through β₁ receptors, which promoted the development of selective β₂ receptor antagonists to reduce the extracardiac side effects of β₂ receptor blockade. More recently, however, this perspective has changed with the demonstration from binding studies that β₂ adrenergic receptors constitute 20–40% of the total number of β receptors in human heart, and that in the atrium β₂ receptors mediate increases in atrial contractility and sinoatrial activity. Ventricular responses are less well defined, but stimulation of β₂ receptors in vitro has been shown to be increase cardiac contractility, and β₂ receptor agonists mediate positive inotropic effects in vivo.

Characterisation of the electrophysiological responses to ventricular β₂ adrenergic receptor stimulation is required to define the role of cardiac β₂ receptors more fully under physiological and pathological conditions, and to refine the use of β₂ receptor antagonists in clinical practice. In vitro studies have shown model dependent changes with, for example, action potential lengthening in single cells, in contrast to the shortening seen in whole heart preparations. In human isolated atrial tissue, β₂ as well as β₁ adrenergic receptors mediate arrhythmic contractions, but β₂ receptor responses in ventricle have not been characterised in view of the practical difficulties in obtaining ventricular tissue for study.

Our aim in this study was to define the electrophysiological responses to β₂ adrenergic receptor stimulation in the human ventricle. Surface electrocardiographic indices of cardiac depolarisation and repolarisation have been analysed, and the effects of β₂ adrenergic receptor stimulation on the patterns of dispersion of repolarisation assessed, using previously described ECG parameters. β₂ Adrenergic receptor responses were determined following administration of the selective β₂ receptor agonist salbutamol, and compared with responses with isoprenaline (isoproterenol), an agonist widely used clinically and active at both β₁ and β₂ receptors. The repolarisation responses to β₂ receptor stimulation were confirmed directly by endocardial monophasic action potential recordings. In order to account for the systemic haemodynamic effects of β₂ receptor agonists, the responses to the predominantly arteriolar vasodilator hydralazine
Table 1 Characteristics of the patients enrolled

<table>
<thead>
<tr>
<th></th>
<th>Controls (n=22, 16 men)</th>
<th>CAD patients (n=85, 57 men)</th>
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<tr>
<td>Age (years) (mean (SEM))</td>
<td>54.8 (3.0)</td>
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<td>CCS score</td>
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<tr>
<td></td>
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<tr>
<td></td>
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<td>0</td>
</tr>
<tr>
<td></td>
<td>3 vessel</td>
<td>0</td>
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</tr>
<tr>
<td></td>
<td>Hypolipidaemics</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Aspirin</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>Others</td>
<td>7</td>
</tr>
</tbody>
</table>

Values are numbers of patients unless stated otherwise.

AR, adrenergic receptor; CCS, Canadian Cardiovascular Society.

and the predominantly venous dilator isosorbide dinitrate were also assessed in independent interventions. The possible confounding effect of a β1 receptor mediated fall in serum potassium was also addressed.

Methods

STUDY GROUP

Eighty five patients with coronary artery disease and 22 controls with normal coronary arteries were selected following routine coronary angiography (table 1). Significant coronary artery disease was defined as at least one coronary stenosis of >70%, assessed angiographically. All patients had normal (ejection fraction >50%) or mildly impaired left ventricular function (ejection fraction 40–50%), as assessed by planimetry, and were in New York Heart Association functional class II. Patients gave written informed consent before participating in the study, and approval was obtained from the local research ethics committee (Huntingdon Health Authority).

INFUSION PROTOCOLS

In each patient a 6 French bipolar pacing electrode (Bard UCSI, Billerica, Massachusetts, USA) was positioned in the right atrial appendage under fluoroscopic guidance. Salbutamol (Allen and Hanbury’s, Uxbridge, UK), 10–30 µg/min, and isoprenaline (Pharmax, Bexley, UK), 1–5 µg/min, were infused separately in each patient through the side arm of an introducer sheath into the right femoral vein. A dose–response curve for salbutamol and isoprenaline was obtained initially in each patient to establish individual heart rate responses to each agent (fig 1). To limit possible confounding effects from ischaemia, the heart rate was then maintained with atrial pacing (basic cycle length 500–600 ms) just above the intrinsic rate developed during the initial β adrenergic receptor agonist infusion, and simultaneous 12 lead ECG recordings at a paper speed of 50 mm/s and calibration 2 cm/mV were obtained during steady state at each incremental dose of either drug. A period of 10 minutes was allowed for stabilisation at each dose of salbutamol and isoprenaline, and 30 minutes between each infusion of β receptor agonist; salbutamol and isoprenaline were infused in random order in each patient. Haemodynamic monitoring was undertaken throughout each infusion, and serum electrolytes were sampled at each infusion rate. In 11 patients high dose salbutamol was infused from the outset (30 µg/min) with serial ECG recordings and serum potassium measurements performed at five minute intervals.

The effects of intravenous infusion with hydralazine (Ciba, Horsham, UK), 100–300 µg/min, and isosorbide dinitrate (Schwartz Pharma, Chesham, UK), 200–600 µg/min, on haemodynamic and ECG variables were each assessed in a further 10 patients during identical atrial pacing protocols.

HAEMODYNAMIC MONITORING

Intra-arterial pressure was monitored continuously in all patients. In addition, pulmonary capillary wedge pressure, pulmonary artery pressure, and right atrial pressure responses to salbutamol were obtained in a representative sample of 11 patients.

ECG MEASUREMENTS

Three QT parameters were measured: QT onset, the time from the start of the QRS complex to the beginning of the T wave; QT peak, the time from the start of the QRS complex to the peak of the T wave; and QT end, the time from the start of the QRS complex to the end of the T wave. The end of the T wave was defined as the point of return to the TP baseline. If the T wave was interrupted by a U wave before the return to baseline, the interval was measured as the nadir between T and U waves. The QRS interval was measured to determine the JT interval for each cycle. Each ECG parameter was measured manually on three consecutive complexes and the average value determined for each lead. Mean overall QT intervals were calculated using all leads suitable (n ≥ 10) for T wave measurements. The dispersion of repolarisation parameters (maximum minus minimum duration) was calculated using a standard method.17
MEASUREMENT OF MONOPHASIC ACTION POTENTIAL DURATION

Selected patients with a dominant right coronary artery with no significant stenoses (> 70%) along its course, underwent direct measurement of monophasic action potential duration (mAPD). A steerable 7 French mAPD catheter (EP technology, Mountain View, California, USA) was positioned against the endocardium of the right ventricular free wall, and a 6 French bipolar pacing electrode (Bard USCI) positioned in the right atrial appendage, under fluoroscopic guidance. mAPDs were amplified and filtered at a frequency of 0.05–500 Hz, and recorded on a chart recorder at a paper speed of 100 mm/s.

mAPDs were obtained in 12 patients during continuous haemodynamic monitoring. After a test dose of normal saline, salbutamol (1–10 µg) was infused into the right coronary artery, following a previously reported protocol, until the heart rate increased by (mean (SEM)) 30 (3) beats/min. The heart rate was fixed with atrial pacing with a basic cycle length 500–600 ms and mAPDs were recorded during repeat salbutamol infusion (fig 1). mAPD at 90% repolarisation (mAPD$_{90}$) was calculated using a standard method.

STATISTICS

All measurements are given as mean (SEM) unless stated otherwise. QT and mAPD variables were compared between patients using analysis of variance, and a probability value of p < 0.05 was considered significant. The intra- and interobserver error on a selection of ECGs was calculated.

Results

HAEMODYNAMIC RESPONSE

Heart rate increased to a maximum of 25.7 (1.3) and 29.7 (1.7) beats/min during the preliminary dose–response determinations with salbutamol and isoprenaline, respectively. Both drugs increased systolic and lowered diastolic blood pressure (fig 2). Mean blood pressure fell slightly (~10.1 (2.1) mm Hg with salbutamol, ~7.4 (1.9) mm Hg with isoprenaline). There were no significant changes in pulmonary capillary wedge pressure, mean pulmonary artery pressure, or mean right atrial pressure during salbutamol infusion.
PRIMARY ECG VARIABLES

QT_{onset} and QT_{peak} decreased in the controls and coronary artery disease patients during both salbutamol and isoprenaline infusion at fixed pacing rate (figs 3 and 4). QT_{end} increased in coronary artery disease patients but decreased in controls. The QT_{onset}–QT_{peak} and QT_{peak}–QT_{end} intervals increased; the greater increase in QT_{peak}–QT_{end} was largely responsible for the increased T wave duration seen. There was no significant change in QRS duration during infusion with either salbutamol or isoprenaline; hence the changes in JT_{onset}, JT_{peak}, and JT_{end} were similar (data not shown). The intra- and interobserver error in the measurement of QT intervals on 10 randomly selected ECGs was 10 (2.6) ms and 12.5 (3.8) ms, respectively.

DERIVED ECG VARIABLES

Baseline QT dispersion was greater in coronary artery disease patients for each variable measured (coronary artery disease v controls; QT_{onset} 85.2 ms v 68.5 ms; QT_{peak} 80 ms v 48.5 ms; and QT_{end} 70.3 ms v 49.4 ms; SEM < 5 ms for each variable; p < 0.01). All dispersion indices increased during infusion with salbutamol and isoprenaline; in particular there was a large increase in QT_{end} dispersion in both coronary artery disease and control patients (fig 5). The changes in QT intervals and dispersion parameters in the subset of patients who had initial high dose salbutamol infusion were similar to the above (for example, QT_{end} dispersion at baseline, 58.4 (4.3) ms; with salbutamol 30 µg/min, 104.3 (7.8) ms, p < 0.001) and occurred before any change in serum potassium (fig 6). In patients chronically treated with the β_{1} adrenergic receptor selective antagonist atenolol, mean resting heart rate was lower, at 55 (2.4) beats/min. The haemodynamic and ECG responses to infused salbutamol were similar to those in patients not receiving atenolol (heart rate increase, 24.8
beats/min; mean blood pressure reduction, 9.8 (2.8) mm Hg; QTend dispersion peak, 105 (4.7) ms; all p < 0.01). In contrast, in atenolol treated patients isoprenaline responses were reduced (heart rate increase, 21.2 (4.3) beats/min; mean blood pressure reduction, 7.4 (2.7) mm Hg; QTend dispersion peak, 98.3 (4.5) ms). Hydralazine and isosorbide dinitrate each increased heart rate (by 7.1 (2.2) and 5.4 (2.1) beats/min, respectively) and lowered mean blood pressure (by 7.8 (2.1) and 5.2 (1.4) mm Hg) during the preliminary dose–response study. At fixed heart rate with atrial pacing there was no significant change in QT interval and dispersion parameters during infusion with either drug (fig 7).

**MONOPHASIC ACTION POTENTIAL DURATION**

Monophasic action potential duration at 90% repolarisation during intracoronary infusion of salbutamol (mean dose 9.1 (1.2) µg) was significantly reduced (mAPD₉₀, 278 (4.1) ms to 257 (3.8) ms; n = 12; p < 0.05).

**Discussion**

Both salbutamol and isoprenaline caused significant, dose dependent changes in QT interval parameters, with increases in QT₀, QTₚₑₑₚ and QTₚₑₑₚₚ dispersion. The results obtained with salbutamol support a direct effect on ventricular β₂ adrenergic receptors. Salbutamol, a selective β₂ agonist,⁴⁵ would not be expected to stimulate β₁ adrenergic receptors under the conditions applied. An effect on presynaptic β₂ adrenergic receptors with potentiation of noradrenaline release and indirect stimulation of β₁ adrenergic receptors is also unlikely, as there was no blunting of the effects of salbutamol in patients taking the β₁ selective adrenergic receptor antagonist atenolol. Reflex changes in preload and afterload secondary to β₂ adrenergic receptor mediated vasodilatation are not likely to be contributory factors, as hydralazine and isosorbide dinitrate—agents that have their principal haemodynamic effects on arterial and venous vessels, respectively—had no significant effect on the dispersion indices assessed. Although a contribution from ischaemia to the effects seen with salbutamol and isoprenaline cannot be completely excluded, the pronounced increase in QTₚₑₑₚ dispersion was seen both in controls and in coronary artery disease patients. The increase in QT dispersion seen with initial high dose salbutamol occurred before the onset of hypokalaemia, excluding this as a cause for the changes seen.

The decrease in mAPD with intracoronary salbutamol was also consistent with the direct stimulation of ventricular β₂ adrenergic receptors. There were no significant changes in mAPD with intracoronary saline, indicating the absence of an important volume effect, and no patient developed chest pain or ischaemic ECG changes during salbutamol infusion, consistent with at worse a small contribution from ischaemia to mAPD₉₀ shortening. Furthermore, the mean intracoronary dose of salbutamol used (9.1 µg) has previously been shown to produce no change in mean arterial
pressure, pulmonary capillary wedge pressure, cardiac output, or systemic vascular resistance when infused into the right coronary artery, thus excluding indirect effects mediated by vascular β, adrenergic receptors.

Systemic isoprenaline, a mixed β/β agonist, produced similar changes to salbutamol in the ECG variables measured. The reduction in responses seen in atenolol treated patients is consistent with a β, adrenergic receptor component of the effect of isoprenaline. Previous studies with isoprenaline have shown either biphasic responses, with an initial lengthening and subsequent shortening of the QT\textsubscript{end} interval (corrected for heart rate (QT\textsubscript{c}))\textsuperscript{20} or a shortening in absolute QT\textsubscript{end} interval and an increase in QT\textsubscript{c}.\textsuperscript{20} In addition, isoprenaline infusion has been shown to modulate T wave amplitude and polarity.\textsuperscript{19,20}

The QT\textsubscript{end} interval represents the total duration of ventricular depolarisation and repolarisation. Accordingly, as there was no significant effect on QRS interval duration seen during either salbutamol or isoprenaline infusions, the observed changes in QT duration result entirely from altered repolarisation. To refine the analysis of repolarisation, the individual variables QT\textsubscript{onset} and QT\textsubscript{peak} were studied along with the QT\textsubscript{end} instead of the QT\textsubscript{peak}, QT\textsubscript{end} intervals, representing, respectively, the early and late contributions from the T wave. These analyses show that under β adrenergic receptor stimulation the total T wave duration increases, and that this is mainly secondary to an increase in the late T wave phase. These changes in T wave morphology are likely to reflect selective coupling of β adrenergic receptors to individual ion channels. Recent reports of specific T wave appearances corresponding to individual channel mutations in the long QT syndrome\textsuperscript{14} are consistent with such an observation.

Dispersion of the QT\textsubscript{end} interval duration between ECG leads has been shown to reflect regional variation in ventricular repolarisation and an overall increase in repolarisation heterogeneity.\textsuperscript{17,21} In studies this has been correlated with arrhythmia risk,\textsuperscript{17,22} sudden cardiac death,\textsuperscript{23} and total mortality,\textsuperscript{23} but this has not been a universal finding.\textsuperscript{23} The pronounced effects of salbutamol and isoprenaline on QT\textsubscript{end} dispersion suggest that heterogeneity of repolarisation is strongly influenced by adrenergic stimulation.

RELATION TO VENTRICULAR ARRHYTHMIAS

Adrenergic stimulation is known to be important in the genesis of ventricular arrhythmias\textsuperscript{24} through the mechanisms of increased automaticity, induction of early afterdepolarisations, and a reduction in fibrillation threshold. Adrenergic activation also leads to a shortening of ventricular refractoriness both in dogs and humans.\textsuperscript{26} It is known that stimulation of cardiac sympathetic nerves leads to an increase in the dispersion of repolarisation and refractoriness,\textsuperscript{27,28} thereby enhancing the conditions for cardiac reentry.\textsuperscript{30,31} The individual contributions of β and β, adrenergic receptors to these effects are not known, but the results seen here provide a mechanism whereby circulating adrenaline, acting through β, receptors, may increase dispersion of repolarisation and contribute to the triggering of arrhythmias in susceptible patients. This mechanism may be especially important when circulating plasma concentrations of adrenaline are high—for example, around the time of myocardial infarction\textsuperscript{32} and during heart failure.\textsuperscript{33} Indeed salbutamol is known to increase the incidence of ventricular arrhythmias in patients with heart failure,\textsuperscript{14} and experimentally β, receptor antagonists have been shown to protect against ventricular fibrillation.\textsuperscript{35}

β BLOCKERS DECREASE RISK

The finding that β, adrenergic receptors mediate significant electrophysiological effects has implications for the use of β, receptor blocking drugs. While these drugs may reduce the unwanted peripheral side effects associated with β blocking treatment, they will not block electrophysiological responses mediated through β, receptors. This may explain the significant body of data suggesting that non-selective β blockers have a greater antiarrhythmic effect than β, selective blockers after acute myocardial infarction.\textsuperscript{36} In heart failure patients, although β, receptor selective antagonists have been found to reduce mortality,\textsuperscript{37} a recent meta-analysis has shown that this is greater for non-selective β blockers than for β, receptor selective antagonists.\textsuperscript{38}

LIMITATIONS OF THE STUDY

Although we used a β, adrenergic receptor selective agent—salbutamol—and showed no reduction in responses in β, receptor blocked patients, we cannot completely exclude effects mediated through β, receptors. There are no selective β, receptor antagonists available for use in vivo, and hence the selectivity of the responses to salbutamol could not be confirmed with β, receptor blockade. In addition extracardiac effects of salbutamol and isoprenaline may have contributed to the effects seen. However, reflex responses through arteriolar and venular dilatation alone would not account for the ECG changes seen (as demonstrated by the lack of effect seen with hydralazine and isosorbide dinitrate), and the reduction in mAPD\textsubscript{90} with intracoronary salbutamol could not be mediated through an alteration of central adrenergic tone. The paced heart rates during salbutamol and isoprenaline infusion were chosen to be as low as possible in order to avoid inducing ischaemia, and the changes in QT indices and dispersion seen occurred in both coronary artery disease and control patients, showing that ischaemia was not solely responsible for the changes found. A contributory effect of ischaemia during high dose catecholamine infusion cannot, however, be excluded.

CONCLUSIONS

This study shows the important electrophysiological responses mediated through β, adrenergic receptors in human ventricular myocardium, with in particular the pronounced effects
on temporal dispersion of cardiac repolarisation. This provides further insights into the mechanisms whereby sympathetic activity increases arrhythmia risk, and should help to rationalise the use of β blockers in clinical practice.

We thank all the staff in the cardiac catheterisation laboratory at Papworth Hospital for their help with this work. Dr Peter Ladman assisted with the mAPD studies, and Mr Stuart Newell provided technical expertise for mAPD and ECG recordings. We acknowledge the support of the Grimsby-Parkinson Trust and British Heart Foundation. AAG is a British Heart Foundation Senior Research Fellow. The work described was presented at the North American Society for Pacing and Electrophysiology 20th Annual Scientific Sessions, Toronto, May 1999, for which MDL was awarded the Young Investigator Award (Clinical).

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