Use of opposing reflex stimuli and heart rate variability to examine the effects of lipophilic and hydrophilic β-blockers on human cardiac vagal control

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ABSTRACT

Evidence from animal studies suggests that β-blockers can act within the central nervous system to increase cardiac vagal motoneuron activity. We have attempted to determine whether such an effect is evident in healthy humans, by examining the effects of lipophilic and hydrophilic agents on heart rate variability and cardiac vagal reflexes. A total of 20 healthy volunteers took part in the study. Autonomic studies were performed after 72 h of treatment with placebo, atenolol or metoprolol in a blinded cross-over design. ECG recordings were taken at rest and during mental and orthostatic stress. Heart rate variability was measured in the time and frequency domains. The effects on heart rate of two opposing cardiac vagal reflexes were examined. Trigeminal stimulation causing vagal stimulation, and isometric forearm muscle contraction ('muscle heart reflex') causing vagal inhibition, were performed alone and simultaneously. At rest, during mental stress and during trigeminal stimulation, β-blocker therapy was associated with significantly increased high-frequency beat-to-beat heart rate variability when compared with placebo. There were no significant differences in effects on heart rate or heart rate variability between atenolol and metoprolol. Analysis of the muscle heart reflex, alone and with simultaneous trigeminal stimulation, showed that the magnitude of the R–R interval response was significantly greater after β-blocker therapy compared with placebo, but the effects of atenolol and metoprolol were equivalent. β-Blocker therapy increased cardiac vagal activity, as shown by measures of high-frequency heart rate variability and reflex studies. Lipophilic and hydrophilic β-blockers appeared to be equally efficacious in increasing the cardiac vagal modulation of heart rate.

INTRODUCTION

β-Blockers reduce mortality in survivors of myocardial infarction [1] and in patients with heart failure [2]. A reduction in sudden cardiac death explains much of the efficacy of this class of drugs, but the reason for this action is unclear. A conventional anti-arrhythmic action appears unlikely, as β-blockers at standard therapeutic doses are relatively ineffective in suppressing ventricular arrhythmias [3]. Furthermore, the failure of other anti-

Keywords: Autonomic, β-adrenergic receptor antagonist, heart rate variability, parasympathetic nervous system, reflex.
Abbreviations: CCV, coefficient of component variance (heart rate adjusted units of spectral power); HF, high-frequency; IQDNN, interquartile differences (75th–25th percentile) of the frequency distributions of the total number of R–R intervals; IQDSD, interquartile differences (75th–25th percentile) of the frequency distributions of the total number of successive R–R interval differences; LF, low-frequency; n.u., normalized units of spectral power; pNN50, percentage of successive R–R interval differences exceeding 50 ms; RMSSD, root-mean-square of successive R–R interval differences; SDNN, standard deviation of R–R interval values.
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arrhythmic drugs to reduce mortality in these clinical settings [4] suggests that \( \beta \)-blockers differ fundamentally from these agents in their mechanism of action.

Evidence from animal studies suggests that \( \beta \)-blockers may act within the central nervous system to prevent ventricular fibrillation. Intracerebral injection of propranolol, at doses causing negligible peripheral anti-adrenergic effects, prevented ventricular fibrillation after coronary occlusion in conscious pigs [5]. This effect has been explained by a centrally mediated increase in cardiac vagal activity [6]. Cardiac autonomic activity exerts powerful effects on the susceptibility of ischaemic myocardium to arrhythmias and ventricular fibrillation. High levels of sympathetic activity reduce the fibrillatory threshold of ischaemic myocardium [7,8]; conversely, vagal activity exerts a protective, anti-arrhythmic effect [7,9]. Studies in the rabbit have confirmed that the central vagotonic effect of \( \beta \)-blockade is accompanied by a reduction in the incidence of ventricular fibrillation after coronary occlusion [10].

Lipophilic \( \beta \)-blockers are able to penetrate the blood–brain barrier and achieve higher concentrations within the central nervous system than are hydrophilic agents [11]. Clinical trials of long-term secondary preventive \( \beta \)-blocker therapy after myocardial infarction have shown that lipophilic agents reduce the incidence of sudden cardiac death [1,12–14], while no such effect has been demonstrated with hydrophilic agents [15]. We and others have suggested that this may be explained by different effects on central cardiac vagal control [6,16–18].

The present study was undertaken in order to compare the effects of lipophilic and hydrophilic \( \beta \)-blockade on parasympathetic activity in healthy subjects, at rest and under conditions of stress. To assess cardiac vagal activity, we have used heart rate variability and quantitative analysis of the early, vagally mediated, responses to the muscle heart reflex and trigeminal stimulation. We have also measured the net heart rate response to these opposing ‘vagal’ reflexes, which, by virtue of their interaction within the central nervous system, may reflect central cardiac vagal nerve activity.

**METHODS**

**Subjects**

The protocol involved 20 healthy male volunteers, taking no medication. All subjects were in good health, with no history of cardiovascular or other disease, and all were normotensive (casual blood pressure < 140/90 mmHg). Each volunteer provided written consent, the protocol was approved by the research ethics committee of South Birmingham Health Authority, and the study was carried out in accordance with the Declaration of Helsinki (1989).

**Study design**

After an initial screening and acclimatization visit, subjects were assigned to 72 h periods of treatment with placebo, atenolol or metoprolol in a cross-over study design. A washout period of at least 7 days was allowed between each treatment period. Each subject was treated single-blind with placebo on visit 1, and then received double-blind atenolol (50 mg once per day plus dummy once per day) or metoprolol (50 mg twice per day) in a random order on visits 2 and 3. This enabled us to ensure bioequivalence of \( \beta \)-blockade on visits 2 and 3, using submaximal bicycle exercise testing. On visit 1, an ergometric bicycle exercise test was performed to determine the workload required to elicit a heart rate of 80% of the maximum predicted. The heart rate was measured with the same workload on visits 2 and 3. If the exercise heart rates on visits 2 and 3 differed by more than 10%, the dose of \( \beta \)-blocker associated with the higher rate was increased (double-blind) and the study visit was repeated after 48 h.

**Experimental protocol**

Subjects attended for experiments on the final day of each 72 h treatment period, 2 h after the final oral dose of trial medication. They were instructed not to eat or drink for at least 4 h before attending, and to abstain from caffeine, alcohol and tobacco in the preceding 24 h. All experiments were carried out in a quiet room with the temperature maintained at 21–24 °C. Subjects lay near-supine on a couch, and before the start of each test a 20 min rest period was allowed to enable blood pressure, heart rate and respiration to stabilize. The electrocardiographic signal was amplified, processed and digitized at 165 Hz for each channel using a National Instruments NB/M10/16XH/18 analogue-to-digital converter board (National Instruments Corp., Austin, TX, U.S.A.). R-waves were detected by individually adjusted thresholds and a maximum-to-minimum voltage difference within five samples (0.04 s) of > 0.5 V. The signal was displayed on the screen of a personal computer (Power Macintosh 7100/80) running Lab View 3.1 software (National Instruments Corp.), and selected periods were stored to disk. A respiratory signal was obtained, displayed and recorded from two electrodes attached to the chest wall to monitor changes in impedance with respiration.

Steady-state recordings of at least 160 beats were acquired under the following conditions. (a) During unrestricted breathing at rest. (b) During verbal mental arithmetic testing (serial subtraction, with the difficulty level titrated according to each subject). (c) During breathing synchronized to a metronome, at a respiratory frequency adjusted to suit each individual. During the initial visit subjects were asked to choose a respiratory frequency between 0.2 and 0.3 Hz with which they were comfortable; respiration was then maintained constant in
each arm of the study by asking subjects to synchronize their respiration to an audio signal generator set to this frequency. (d) During a 70° head-up tilt test, with breathing synchronized to a metronome at the predetermined rate. Recording began on stabilization of the heart rate, 5 min after assuming the head-up position.

**Muscle heart reflex and trigeminal stimulation**

Isometric voluntary contractions of the flexors of the upper arm were performed, with the arm fixed at the elbow and wrist by a frame attached to a couch on which the subject reclined in a semi-supine position. Isometric force was measured by a strain gauge attached to a wrist clamp. The tension signal was amplified and digitized at 165 samples/s and displayed on a computer screen. Each subject was asked to perform a maximum isometric contraction of the upper arm flexors (maximum voluntary contraction), and from this a value for 60% of maximum voluntary contraction was determined and indicated on the screen. During synchronized breathing, the subject was instructed to perform a contraction (60% of maximum voluntary contraction) when they heard a sound initiated by a signal from the computer at the beginning of the expiratory phase, and the contraction was maintained for at least one respiratory cycle. With practice, this was achieved in all subjects without interrupting the breathing pattern and without causing a Valsalva manoeuvre.

To simulate the diving response, ice mats (0 °C; 10 cm × 15 cm) were placed on both sides of the face over the areas innervated by the maxillary division of the trigeminal nerve. This is referred to as trigeminal stimulation. The ice mats remained completely flexible when frozen and adapted easily to facial contours, thus providing good contact with the skin.

Data were collected 30 s before and during each of three muscle contraction tests (60% of maximum voluntary contraction), with a recovery period between tests of at least 2 min. Also, during synchronized breathing, data were collected over 2 min while trigeminal stimulation was performed. The subject then rested for 10 min to allow face temperature and heart rate to recover to the control level. Finally, the interaction of the two responses was determined by performing a muscle contraction test (60% of maximum voluntary contraction) during trigeminal stimulation. Muscle contraction was initiated at the beginning of the expiratory phase, 30 s after the application of ice mats to the face. This was performed three times, with a recovery period between tests of 10 min.

**Data analysis**

**Analysis of heart rate variability**

All ECG series were reviewed, and if necessary edited before analysis to exclude ectopic and artefact signals. Signals containing more than 1% ectopic beats were not accepted for analysis; less frequent ectopics were deleted and the R–R interval was replaced with a running mean. Heart rate variability data were analysed off-line using Lab View 3.1 software (National Instruments Corp.) and Statview (Abacus Concepts Inc., Berkeley, CA, U.S.A.). The following time domain measures of heart rate variability were determined: SDNN (standard deviation of R–R interval values), RMSSD (root-mean-square of successive R–R interval differences) and pNN50 (percentage of successive R–R interval differences exceeding 50 ms). In addition, we determined the interquartile differences (75th–25th percentile) of the frequency distributions of the total number of R–R intervals (IQDNN) and of successive R–R interval differences (IQQSD) [19,20].

Frequency domain analysis was performed using autoregressive modelling, using the Burg algorithm [21] with a model order between 8 and 12 [22]. This enabled the determination of low-frequency (LF) power at ~0.1 Hz (reflecting both sympathetic and vagal activity [23]), and high-frequency (HF) power at the measured respiratory frequency (providing an index of cardiac vagal tone [24]). Power was determined in absolute units, normalized units (n.u.) and heart rate adjusted values, using the coefficient of component variance (CCV). The power in n.u. was calculated by dividing the absolute power of a given component (area under the component curve) by the total power minus the 0–0.04 Hz component. Heart rate adjusted values (CCV) were calculated by dividing the square root of the absolute power by the mean R–R interval [25]. Values of power in n.u. and CCV were expressed as percentages after multiplying by 100.

**Analysis of muscle heart reflex and trigeminal stimulation**

Custom-written software (using Lab-View 3.1) was used to measure the R–R interval responses to the muscle heart reflex and trigeminal stimulation, while controlling for the effects of ventilation. For the purposes of analysis, each respiratory cycle during a test recording was divided into four phases of equal duration from the beginning of expiration to the end of inspiration. The duration of each R–R interval occurring in any recording was measured and that value consigned to the respiratory phase in which the R–R interval began. During control recordings before muscle contraction (synchronized metronomic breathing, with and without trigeminal stimulation), this procedure was repeated for all respiratory cycles, and the mean R–R interval in each arbitrary phase was calculated. For the purposes of muscle heart reflex analysis, we considered only the R–R intervals within the first respiratory cycle after initiation of a muscle contraction. We measured the difference between the R–R interval in phase 2 of the first respiratory cycle after muscle contraction and the mean ‘phase 2’ R–R interval derived...
from the control recording before contraction. We
concentrated on phase 2 (late expiration), since we have
shown previously that analysis of this phase reveals the
greatest magnitude of differences in R–R interval, which
are still attributable to changes in cardiac vagal activity
[26].

Statistical analysis
R–R interval and R–R interval variability were tested for
normality of distribution. Frequency domain data were
skewed towards high values, and were therefore log-
arithmically transformed before statistical analysis. The
significance of the differences between groups was
determined using analysis of variance and Student’s t test
for normally distributed data; otherwise, Friedman’s test
and the Wilcoxon signed rank test were used.

RESULTS

Study subjects, and bioequivalence of
β-blockage
A total of 20 subjects were enrolled in the study (all male,
average age 20±2 years, range 18–24 years). No side
effects of study treatment were reported. Of these, 17
subjects completed the study (three subjects failed to
attend for the second and/or third study visit). In two
subjects dose adjustment of β-blocker was required, with
a doubling of the dose of metoprolol in one case and of
that of atenolol in another. Allowing for this adjustment,
the exercise heart rates during atenolol and metoprolol
therapy remained within 10% of each other for all
subjects, and mean exercise heart rates were equivalent.

Heart rate and heart rate variability
The ECG recordings from 15 subjects were accepted for
analysis (in two subjects there were frequent ectopic
beats, accounting for more than 1% of all beats). For each
of the experimental conditions, mean values of R–R
interval and R–R interval variability data were derived
from 160 consecutive R–R interval values, and results are
displayed in Tables 1–3.

Resting values, during metronome-controlled breathing
The mean respiratory frequency was 0.26±0.02 Hz (range
0.23–0.29 Hz). Mean values of R–R interval and R–R
interval variability during metronomic breathing are
shown in Table 1. The mean R–R interval was signifi-
cantly greater during β-blocker therapy than with pla-

Table 1 R–R interval and R–R interval variability at rest during synchronized metronomic breathing

<table>
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<tr>
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<th>Placebo</th>
<th>Atenolol</th>
<th>Metoprolol</th>
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<tbody>
<tr>
<td>R–R interval (ms)</td>
<td>1007 ±31</td>
<td>1167 ±34***</td>
<td>1126 ±35**</td>
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<tr>
<td>SDNN (ms)</td>
<td>67.6 ±8.6</td>
<td>91.2 ±8.3*</td>
<td>88.8 ±10.5**</td>
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<td>IQDNN (ms)</td>
<td>90.9 ±14.2</td>
<td>116.9 ±11.2</td>
<td>124.9 ±16.9*</td>
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<tr>
<td>IQDSD (ms)</td>
<td>98.5 ±16.6</td>
<td>151.8 ±14.5**</td>
<td>147.1 ±19.7**</td>
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<tr>
<td>RMSSD (ms)</td>
<td>69.8 ±12.4</td>
<td>114.3 ±13.1**</td>
<td>107.3 ±15.2**</td>
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<tr>
<td>pNN50 (%)</td>
<td>39.2 ±6.1</td>
<td>59.4 ±4.8**</td>
<td>55.8 ±6.4***</td>
</tr>
<tr>
<td>Total power (ms²)</td>
<td>5518 ±1514</td>
<td>9030 ±1521**</td>
<td>9648 ±2045*</td>
</tr>
<tr>
<td>LF power (absolute) (ms²)</td>
<td>994 ±260</td>
<td>1727 ±497*</td>
<td>1590 ±414</td>
</tr>
<tr>
<td>LF power CCV (%)</td>
<td>2.77 ±0.39</td>
<td>3.33 ±0.49</td>
<td>3.06 ±0.36</td>
</tr>
<tr>
<td>LF power (n.u. %)</td>
<td>35.4 ±5.1</td>
<td>25.9 ±3.5</td>
<td>27.1 ±3.7</td>
</tr>
<tr>
<td>HF power (absolute) (ms²)</td>
<td>2110 ±894</td>
<td>4385 ±881***</td>
<td>4226 ±1042**</td>
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<tr>
<td>HF power CCV (%)</td>
<td>3.67 ±0.50</td>
<td>5.38 ±0.60*</td>
<td>5.00 ±0.62*</td>
</tr>
<tr>
<td>HF power (n.u. %)</td>
<td>52.4 ±5.8</td>
<td>66.5 ±3.5***</td>
<td>63.3 ±4.3**</td>
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<tr>
<td>LF/HF ratio</td>
<td>1.10 ±0.44</td>
<td>0.46 ±0.11</td>
<td>0.54 ±0.16</td>
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mental stress were 13.3 ± 8.6% (placebo), 8.0 ± 7.1% (atenolol) and 7.3 ± 7.4% (metoprolol). The reduction in mean R–R interval during mental stress was significantly (P < 0.01) attenuated by both atenolol and metoprolol; during mental stress, β-blocker therapy was associated with significantly greater values of mean R–R interval, time domain indices of short-term beat-to-beat variability and HF power, compared with placebo. There were no differences in mean R–R interval or any measure of heart rate variability in the time and frequency domains between atenolol and metoprolol treatments during mental stress.

Mean percentage reductions in R–R interval during orthostatic stress were 27.9 ± 7.0% (placebo), 21.3 ± 8.4% (atenolol) and 23.5 ± 7.8% (metoprolol). The reduction in R–R interval during orthostatic stress was significantly (P < 0.01) attenuated by both atenolol and metoprolol. During orthostatic stress mean values of R–R interval, total power and absolute HF power were significantly greater during β-blocker therapy compared with placebo; time domain indices of variability tended to be greater during β-blocker therapy, but the differences were not significant (with the exception of SDNN during metoprolol treatment). During orthostatic stress, the mean R–R interval was significantly greater with atenolol treatment than with metoprolol treatment, but there were no differences between the drugs in any measure of heart rate variability in the time or frequency domains.
Table 4  R–R interval and R–R interval variability during trigeminal stimulation

Values are means ± S.E.M. Significance of differences compared with placebo: * P < 0.05; ** P < 0.01; *** P < 0.001. Significance of differences compared with resting values: † P < 0.05; †† P < 0.01; ††† P < 0.001. There were no significant differences between the effects of atenolol and metoprolol.

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<th>Placebo</th>
<th>Atenolol</th>
<th>Metoprolol</th>
</tr>
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<tbody>
<tr>
<td>R–R interval (ms)</td>
<td>1047 ± 36†</td>
<td>1278 ± 32***†††</td>
<td>1260 ± 41***†††</td>
</tr>
<tr>
<td>SDNN (ms)</td>
<td>114 ± 11††</td>
<td>170 ± 15***†††</td>
<td>163 ± 15***†††</td>
</tr>
<tr>
<td>IQRNN (ms)</td>
<td>159 ± 16†††</td>
<td>245 ± 30***†††</td>
<td>222 ± 31†††</td>
</tr>
<tr>
<td>IQRSD (ms)</td>
<td>161 ± 26†††</td>
<td>310 ± 48***†††</td>
<td>275 ± 32***†††</td>
</tr>
<tr>
<td>RMSSD (ms)</td>
<td>123 ± 30††</td>
<td>229 ± 27***†††</td>
<td>209 ± 21***†††</td>
</tr>
<tr>
<td>pNNS0 (%)</td>
<td>54.3 ± 4.7†††</td>
<td>79.0 ± 3.2***†††</td>
<td>74.6 ± 4.2†††</td>
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<tr>
<td>Total power (ms²)</td>
<td>14300 ± 3007†††</td>
<td>32380 ± 5862***†††</td>
<td>28400 ± 4491***†††</td>
</tr>
<tr>
<td>LF power (absolute) (ms²)</td>
<td>1289 ± 259†</td>
<td>4211 ± 1060*</td>
<td>4267 ± 991**†</td>
</tr>
<tr>
<td>HF power CCV (%)</td>
<td>3.21 ± 0.30</td>
<td>4.57 ± 0.62</td>
<td>4.63 ± 0.60†</td>
</tr>
<tr>
<td>HF power (n.u. %)</td>
<td>21.4 ± 3.0</td>
<td>20.2 ± 3.0</td>
<td>21.5 ± 3.5</td>
</tr>
<tr>
<td>HF power (absolute) (ms²)</td>
<td>6957 ± 2314†††</td>
<td>17130 ± 4170†††</td>
<td>13120 ± 2892†††</td>
</tr>
<tr>
<td>HF power CCV (%)</td>
<td>6.75 ± 1.09†††</td>
<td>9.32 ± 1.11***†††</td>
<td>8.43 ± 0.90***†††</td>
</tr>
<tr>
<td>HF power (n.u. %)</td>
<td>68.5 ± 32†</td>
<td>67.4 ± 3.4</td>
<td>65.6 ± 4.9</td>
</tr>
<tr>
<td>LF/HF ratio</td>
<td>0.34 ± 0.04†</td>
<td>0.34 ± 0.06</td>
<td>0.40 ± 0.09</td>
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**Muscle Heart Reflex without Trigeminal Stimulation**

**Muscle Heart Reflex during Trigeminal Stimulation**

The change in R–R interval was measured by subtracting the first R–R interval occurring during respiratory phase 2 after contraction from the mean value of all R–R intervals occurring during phase 2 in the 30 s period before contraction. Values are means ± S.E.M. Significance of differences compared with Δ R–R interval with placebo: * P < 0.01; ** P < 0.001.

Responses to trigeminal stimulation and muscle heart reflex

Mean values of R–R interval and R–R interval variability were derived from recordings before and after trigeminal stimulation, each consisting of 80 consecutive R–R intervals. The responses to trigeminal stimulation are shown in Table 4. Trigeminal stimulation resulted in significant increases in R–R interval, in all time domain measures of variability, in total power and in HF power. During trigeminal stimulation, β-blockade significantly increased all measures of time domain variability compared with placebo, but had no significant effect on frequency domain measures of variability. There were no significant differences between the effects of atenolol and metoprolol on mean R–R interval or heart rate variability data.

Isometric muscle contraction (60% of maximum voluntary contraction) was associated with a consistent and significant (P < 0.001) decrease in R–R interval, measured within phase 2 of the first respiratory cycle after initiation of contraction. The muscle heart reflex data are shown in Figure 1. The magnitude of the R–R interval response was significantly greater after β-blocker therapy compared with placebo, with equivalent effects of atenolol and metoprolol. When the muscle heart reflex was performed with simultaneous trigeminal stimulation, a similar pattern was seen.

**DISCUSSION**

**Effect of β-blocker therapy on heart rate variability**

The principal aim of this study was to determine the relative effects of lipid- and water-soluble β-blockers on cardiac vagal activity at rest and under conditions of mental and orthostatic stress. The vagus nerve is inaccessible to recording techniques in humans, but vagal
modulation of heart rate can be assessed indirectly using heart rate variability. HF (‘beat-to-beat’) variability is due almost entirely to respiratory sinus arrhythmia, which is dependent on intact vagal nerves. Evidence that HF variability reflects vagal cardiac control includes the following. (1) HF variability is proportional to efferent vagal activity in anaesthetized dogs [24]. Indeed, with its rapid onset and offset, this is the only neural mechanism capable of mediating variability at this frequency [27,28]. (2) HF variability is correlated strongly with heart rate acceleration in response to atropine in conscious humans [25,29]. (3) HF variability is virtually abolished by atropine, and is almost absent in heart transplant recipients [29–31].

Thus, although heart rate variability cannot provide an absolute measure of vagal activity, HF indices are widely held to reflect the degree of vagal modulation of heart rate in the conscious human [23,31,32]. We have found that both metoprolol and atenolol increased resting measures of HF heart rate variability, and that there were no differences between the two drugs in the magnitude of these effects. A major possible confounding factor when comparing heart rate variability before and after β-blockade is the large fall in heart rate that occurs with these drugs. It has been suggested that this can be corrected for by use of the CCV. Although the animal data remain unclear [33], in pigs the HF CCV has been shown to be linearly related to vagal activity and to be independent of sympathetic activity [34]. The significant increase in the HF CCV in the present study with β-blockade suggests a true increase in power at this frequency and an increase in resting cardiac vagal activity.

Acute psychological stress results in high levels of sympathetic nerve activity and acute withdrawal of vagal activity [35,36]. Evidence from animal studies suggests that the ability of β-blockers to preserve vagal activity during stress may be a more important aspect of their action in reducing the occurrence of sudden cardiac death than any effects on resting vagal tone [37]. We have shown that mental stress significantly reduced heart rate variability measures of cardiac vagal activity, and that both atenolol and metoprolol were associated with preservation of these measures compared with placebo. This finding is consistent with the results of a study in pigs in which lipophilic β-blockers caused a significant attenuation of the stress-induced reduction of vagal tone measured by power spectral analysis (although no such effect was demonstrable with a hydrophilic β-blocker) [37].

Measurement of opposing reflex stimuli

Stimulation of the trigeminal cutaneous receptors of the face is widely accepted as a powerful stimulus to cardiac vagal activity [38–40]. In keeping with this and our previous work [26], trigeminal stimulation resulted in a bradycardia which was sustained for a period of at least 80 beats, and analysis of R–R interval variability showed substantial increases in HF variability. The magnitude of the changes after trigeminal stimulation might be thought to be indicative of the difference between resting vagal tone and near-maximal vagal activity. However, even during trigeminal stimulation, β-blocker therapy was associated with significant increases in measures of time domain variability, providing evidence both that β-blockers increase cardiac vagal tone and that vagal activity can be further enhanced during trigeminal stimulation. When spectral analysis was used to measure heart rate variability during trigeminal stimulation, although total power tended to be greater with β-blockade compared with placebo, this difference was not significant. This may be due to difficulties with the use of spectral analysis for the short (80-beat) recordings taken during trigeminal stimulation.

In contrast with trigeminal stimulation, isometric muscle contraction inhibits vagal activity. Changes in R–R interval immediately (< 10 s) after isometric contraction are too fast to be mediated by cardiac sympathetic nerves [41], and can be obliterated by the administration of atropine [42–44]. These early (< 10 s) changes are therefore attributed entirely to changes in vagal activity. Because we ensured that the subjects continued to breathe normally during each reflex, we avoided the effect of breath holding, which itself produces a bradycardia [45]. The influence of ventilation, whereby inspiration decreases vagal tone, was further controlled by restricting our analysis of R–R interval responses to isometric contraction to a single phase of ventilation. We reasoned that the magnitude of the early changes in R–R interval after isometric muscle contraction could be used as an index of the level of vagal tone at rest. The magnitude of these changes was significantly greater after β-blocker therapy, again suggesting that such therapy results in an increase in cardiac vagal activity.

A central or peripheral effect of β-blockers: is lipophilicity important?

In 1984, Eckberg [16] advanced the theory that β-blockers may reduce the incidence of ventricular fibrillation after myocardial infarction by acting centrally to increase cardiac vagal motoneuron activity. Evidence was even then available to suggest that β-blockers are able to increase cardiac vagal activity via a central nervous action [46], and subsequent studies supported the concept that lipophilic β-blockers are more effective than hydrophilic agents in increasing cardiac vagal activity and fibrillatory thresholds in animals [5,10,37]. In an attempt to provide a measure of the central nervous modulation of cardiac vagal activity, we have examined the net effect on heart rate of the simultaneous application of the trigeminal and muscle heart reflexes. Because the afferent inputs from
the two reflexes are anatomically and functionally distinct [47], it can be argued that their combined effects are a consequence of an interaction within the central nervous system. The net effects of simultaneous isometric muscle contraction and trigeminal stimulation on heart rate were significantly greater after β-blocker therapy compared with placebo, indicating a central effect with both β-blockers. This was a surprising finding in view of the markedly lower central nervous system concentration of atenolol compared with metoprolol [11,48].

There are a number of possible explanations for the lack of a difference between the effects of the lipophilic and hydrophilic β-blockers in our study. It is possible that, at the doses used clinically and in this study, even water-soluble agents enter the central nervous system to a sufficient extent to exert their effects on cardiac vagal control. Although metoprolol achieves central nervous system concentrations of up to 20 times those of atenolol [11], the dose–response of a putative central β-adrenergic action on cardiac vagal control is unknown. At very low doses, differences might emerge between the effects of lipophilic and hydrophilic agents. An alternative possible explanation that we cannot exclude is that β-blockers exert their effects peripherally. Although the afferent pathways of the trigeminal and muscle heart reflexes are different, the final common pathway is the same, namely the cardiac vagal nerve endings. Sympathetic stimulation causes presynaptic inhibition of acetylcholine release [49] and reduces sino-atrial node responses to vagus nerve stimulation [33,50]. Thus, β-adrenergic blockade may enhance vagal responses via an effect on neurotransmitter release. Finally, it is also possible that the increase in HF variability may occur simply as a result of the increase in R–R interval caused by inhibition of β-receptor stimulation. The human sino-atrial node is not uniformly susceptible to vagal stimulation over time [51,52], and at lower heart rates acetylcholine may be arriving at more opportune times for lowering the rate of phase 4 depolarization. This would serve to increase HF power, despite our mathematical correction for R–R interval changes using the CCV. Both peripheral explanations appear plausible, and may have overshadowed any central effects.

In conclusion, we have found that β-blocker therapy significantly increased short-term heart rate variability in healthy subjects, and we interpret this as an increase in cardiac vagal activity. We have confirmed that this is an effect upon cardiac vagal control by testing reflexes that can only affect heart rate via the vagus; however, at the doses used, the effects of lipophilic and hydrophilic β-blockers on cardiac vagal activity were not significantly different.

Limitations

The effects of β-blocker compared with placebo may have been influenced by an order effect or by bias, since the initial study treatment was placebo in each case and was single-blind. This design was chosen because the main purpose of the study was a double-blind comparison of the effects of atenolol and metoprolol, and it enabled us to ensure bioequivalence of the two β-blockers by submaximal exercise testing. We believe that an order effect was unlikely to have influenced our results significantly, because each of our subjects attended for a baseline acclimatization visit, and in a similar previous study there was no difference between baseline values and those after placebo in any heart rate or heart rate variability parameter measured [53]. Bias was excluded because the measurement and analysis of heart rate and heart rate variability was performed by an observer blinded to the study protocol and treatments.

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