A central \( \gamma \)-aminobutyric acid mechanism in cardiac vagal control in man revealed by studies with intravenous midazolam

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ABSTRACT

1. Animal studies show that cardiac vagal tone can be modified by \( \gamma \)-aminobutyric acid neurons acting at several sites in the central nervous system. The present study has attempted to determine whether similar control exists in humans by using midazolam, a benzodiazepine. Benzodiazepines exert their main actions on the central nervous system by interacting cooperatively at the \( \gamma \)-aminobutyric acid receptor.

2. Twenty patients took part in the study before undergoing cardiac catheterization. After resting for 20 min in a semi-supine position on a couch, ECG, blood pressure and respiration were recorded for 5-min periods with either controlled (fixed) or free respiration. During this time a baroreceptor sensitivity test was conducted.

3. Doses of 1 mg and 5 mg of midazolam were administered intravenously.

4. Five-minute segments of data, before and after midazolam, were subjected to power spectral and time-domain analysis.

5. Midazolam caused a decrease in the high-frequency and an increase in the low-frequency components of the power spectral density plot, and in addition reduced the mean R–R interval and R–R variability expressed as the interquartile difference, and pNN50. There were no significant changes in the sensitivity of the baroreflex or in the systolic, diastolic and average blood pressures.

6. This decrease in variability of heart period, particularly at a controlled respiratory frequency, strongly suggests that cardiac vagal tone in man can be regulated by \( \gamma \)-aminobutyric acid neurons.

INTRODUCTION

Animal studies have shown that the inhibitory neurotransmitter \( \gamma \)-aminobutyric acid (GABA) has a profound influence on the central pathways involved in parasympathetic control of the heart. Antonaccio and Taylor [1] studied the effect of GABA, glycine and the potent GABA\(_A\)-agonist muscimol when administered intracerebroventricularly to adult cats. Both GABA and glycine elicited dose-dependent reductions in blood pressure and heart rate, GABA producing a more pronounced effect. Muscimol produced a response similar to that of GABA, but the response was elicited at much smaller doses. In an attempt to identify the site of
action of GABA, DiMicco et al. [2] injected the GABA_\text{R}^\alpha_\text{ antagonist bicuculline into several nuclei including the nucleus ambiguous. Injections into the nucleus ambiguous produced a slowing of the heart rate that was greater than at any other site. From this they hypothesized that there are GABAergic inhibitory synapses on cardiac vagal motoneurons within the nucleus ambiguous, and in the anesthetized animal these are tonically active. In support of this, Bennett et al. [3] found that baroreflex-mediated bradycardia in cats was potentiated by bicuculline. Gilbey et al. [4] examined the activity of cardiac vagal motoneurons in anesthetized cats and found that the direct application of GABA silenced these neurons. This effect of GABA was blocked by bicuculline, and bicuculline applied alone caused an increase in the activity of these neurons. These studies on anesthetized animals imply that there are tonically active central nervous GABAergic mechanisms involved in cardiac vagal control of heart rate. However, whether such a mechanism is active in humans is unclear, although descriptions of the effect of GABA-mimicking drugs such as benzodiazepines suggest this is possible.

The effect of benzodiazepines on heart rate and blood pressure in man has been investigated by several groups [5–9], but these studies were not designed to determine the role of GABA synapses in cardiac vagal pathways. Marty et al. [5] examined the anesthetic effects of diazepam and midazolam on baroreflex control of heart rate, showing that large doses of these drugs depress the sensitivity of the reflex. Ikeda et al. [8] looked at the effects of benzodiazepines on the increase in heart rate associated with entering the operating room in young and elderly patients. For this purpose they measured the heart rate variability using power spectral analysis of the R–R interval. Although this method is not reliable, it was used to determine changes in cardiac autonomic activity, which is a commonly used technique [10–12]. With this method the high-frequency band is a measure of parasympathetic influences on the heart whereas the low frequency measures a mixture of cardiac sympathetic and parasympathetic influences. The high-frequency band occurs at the frequency of breathing and as such is markedly affected by changes in breathing rate and tidal volume. Ikeda et al. [8] found that midazolam and diazepam were effective in reducing the increase in sympathetic activity associated with entering the operating room. They also noted that diazepam caused an increase in the total power of heart rate variability. However, they did not control breathing pattern and their data may not provide a precise indication of changes in cardiac vagal tone. McLeod et al. [13] examined the effects of alprazolam in patients with generalized anxiety disorder and found no change in heart rate or respiratory sinus arrhythmia, but there was a drop in systolic blood pressure. The change in blood pressure combined with a respiratory rate and depth that was not fixed prevents any definite conclusion about potential GABAergic effects upon cardiac vagal control to be drawn. Tulen et al. [14] observed the cardiovascular effects of lorazepam: this decreased heart rate and was associated with an increase in variability of the R–R interval fluctuations at both low and high frequency in spectral density plots. It is therefore unclear whether the changes were due to decreased sympathetic activity or increased vagal activity. Furthermore, the respiratory rate was unfixed in this study, so a totally accurate estimation of vagal tone could not be made. Adinoff et al. [15] investigated the effects of intravenous diazepam on the amplitude of respiratory sinus arrhythmia, and found an increased heart rate accompanied by an attenuation of the changes, an effect which could be due to an increase in sympathetic activity. Therefore none of these studies in man enable us to conclude that GABA is specifically involved in the regulation of cardiac vagal neurons.

Unlike the previous studies, the specific aim of the present study was to determine the importance of GABAergic synaptic transmission in the cardiac vagal pathway in man. For this purpose, as with the previous studies, we have taken advantage of the property of the benzodiazepine drugs which mimic the action of GABA by associating with a binding site on the GABA receptor. In an attempt to specifically determine the effect of the benzodiazepine midazolam on cardiac vagal tone in man, we have controlled respiration and measured heart rate variability in the time- and frequency-domain while also measuring baroreflex sensitivity.

**METHODS**

With the approval of the local research ethics committee, 20 patients (mean age 54.2 years) were asked to give their written and informed consent to take part in this study. All patients were attending the Queen Elizabeth Hospital in Birmingham as day patients to undergo cardiac catheterization. As part of this procedure it is routine practice to administer light sedation with benzodiazepine drugs such as midazolam (Roche, Welwyn Garden City, U.K.). At no point was the clinically appropriate dose of midazolam exceeded or withheld. Midazolam is a member of the benzodiazepine group of compounds, which have a GABA-mimetic property caused by the close association on the GABA receptor protein of both a GABA binding site and a benzodiazepine binding site, as reviewed by Kostowski [16]. It appears to have no antimuscarinic effects. All patients had an intravenous cannula sited and had been resting in bed for at least 1 h before the study. None of the patients had eaten or taken any fluid for at least 8 h. Before commencement of the study patients were asked to lie semi-supine on a couch for 20 min of rest. An ECG was obtained and the signal amplified (Grass Polygraph), digitized, fed into a microcomputer (Apple Macintosh, PowerMac 8100/100) at
165 samples per second and displayed on-line using a custom-written suite of programs within the LabVIEW programming application (National Instruments Ltd, Austin, TX, U.S.A.), as described previously [17]. A possible limitation of setting the sampling rate at 165 Hz is that it provides accuracy of the R–R intervals only up to 6.1 ms. Using the method of Merri et al. [18] we have calculated that this would introduce a small error of less than 10% in the spectral density. Since this would be similar for spectral density peaks at controlled frequency bands both before and after treatment, the sampling frequency would not have significantly affected the interpretation of our data. Blood pressure was monitored using a FINAPRES (Ohmeda 2300, Ohmeda, Louisville, CO, U.S.A.) and respiration monitored using a respiratory strap consisting of a strain gauge attached to a chest band. These two signals were also digitized, fed into the computer at 165 samples per second, and visualized on-line within the LabVIEW application.

Patients were then asked to lie semi-supine without speech or movement and breathing at their natural, uncontrolled rate while a 5-min segment of data was collected (at least 260 beats). After this the patients were asked to time their rate of respiration to a metronome when the patients breathed freely at their own but regular rate. After this, measurement of the baroreceptor–heart rate reflex sensitivity was repeated. This figure for baroreceptor reflex sensitivity was found most comfortable. Baroreceptor–heart rate reflex sensitivity was then measured using the ‘Oxford’ bolus phenylephrine technique [19]. The dose of phenylephrine was incremented in steps from 30 µg up to 100 µg in order to obtain an appropriate series of increasing blood pressure values. A 1-mg intravenous dose of midazolam was administered to all patients. A 5-min period elapsed to allow for circulation of the midazolam before the patient was asked to resume breathing with the metronome and a 5-min segment of data collected. If patients began to deviate from the sound of the metronome as a result of the drowsiness caused by the sedative action of the drug, they were given verbal instruction to ensure they kept to the required frequency. After this recording was made, the metronome was turned off and a further dose of midazolam administered, in order to increase the dose up to the clinically appropriate sedative dose, as stated by the attending medical practitioner. The appropriate dose for all of the patients studied was between 4 and 6 mg of intravenous midazolam. After another 5-min period to allow for circulation of the drug, data were collected while the patients breathed freely at their own but regular rate. After this, measurement of the baroreceptor–heart rate reflex sensitivity was repeated.

All 5-min segments of data were analysed using a custom-written suite of programs within the LabVIEW programming application. The ECG data were first converted to R–R intervals and the data visually inspected by the investigators. Any ectopic or aberrant beats were removed and the values interpolated from the preceding beats, in accordance with the guidelines of the report of the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology [20]. Any segments with more than two ectopic beats, or any R–R interval deviating from its predecessor by more than 200 ms, were excluded from the study. These R–R data were then subjected to power spectral analysis using an autoregressive model, to produce a power spectral density plot where two major peaks were identified. A low-frequency peak between 0.01 and 0.15 Hz represented both vagal and sympathetic nervous activity, and a high-frequency peak between 0.15 and 0.5 Hz, which corresponded directly with the respiratory frequency of the patient, represented the purely vagal component caused by respiratory sinus arrhythmia.

From these plots, values for the high-frequency peak (HF) and the low-frequency peak (LF) were obtained as an absolute power (ms²), as normalized units, and as the common coefficient of variance, as previously described [21]. The ratio of LF/HF was also calculated, with LF and HF calculated as absolute power measurements. The segments of R–R data also enabled time-domain measurements of heart rate variability to be made. These included the mean R–R interval, the standard deviation of the R–R intervals (SDNN), the square root of the mean of the sum of the squares of differences between adjacent R–R intervals (RMSSD), the percentage of R–R intervals that deviate from the preceding R–R interval by more than 50 ms (pNN50), and the interquartile difference (75th to 25th percentile) of R–R intervals (RRIQD) and R–R interval differences (RRDiffIQD) [20].

The heart rate and blood pressure data collected during the baroreceptor reflex were analysed by another custom-written program within LabVIEW. This application allowed both the heart rate and blood pressure data to be visualized upon the same graph on- and off-line. On-line the investigator verified that a ramp in the blood pressure, of an appropriate size (about 10–20 mmHg, and a duration of over eight beats), had occurred. Off-line this ramp was selected by the operator and from this a regression plot of blood pressure (mmHg) against heart rate (beats/min) was generated. The slope of the line was used as a measure of the sensitivity of the baroreceptor reflex. This figure for baroreceptor reflex sensitivity was only accepted if the correlation coefficient (r) was greater than 0.8.

All results were analysed using paired t-tests and differences in the results were considered significant if \( P < 0.05 \).

**RESULTS**

The values obtained for systolic, diastolic and average blood pressures were not significantly different before and after midazolam at either dose level (see Table 1).
Table 1  Systolic, diastolic and average blood pressure measurements for all subjects (n = 20) before and after midazolam
All values are means ± S.D. BP, blood pressure.

<table>
<thead>
<tr>
<th></th>
<th>Systolic BP (mmHg)</th>
<th>Diastolic BP (mmHg)</th>
<th>Average BP (mmHg)</th>
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</thead>
<tbody>
<tr>
<td><strong>Fixed respiration</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-midazolam</td>
<td>141 ± 22</td>
<td>85 ± 16</td>
<td>103 ± 12</td>
</tr>
<tr>
<td>Post-midazolam (1 mg)</td>
<td>137 ± 23</td>
<td>82 ± 16</td>
<td>99 ± 17</td>
</tr>
<tr>
<td>P-value</td>
<td>0.272</td>
<td>0.374</td>
<td>0.169</td>
</tr>
<tr>
<td><strong>Free respiration</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-midazolam</td>
<td>140 ± 15</td>
<td>86 ± 13</td>
<td>103 ± 14</td>
</tr>
<tr>
<td>Post-midazolam (5 mg)</td>
<td>133 ± 19</td>
<td>84 ± 13</td>
<td>100 ± 14</td>
</tr>
<tr>
<td>P-value</td>
<td>0.168</td>
<td>0.58</td>
<td>0.33</td>
</tr>
</tbody>
</table>

Figure 1  A typical heart rate trace from one subject demonstrating the rise in response to 5 mg of intravenous midazolam
The beat number is given on the x-axis, and the corresponding heart rate on the y-axis. The 4-mg supplementary dose was injected at around beat 80, and a sustained increase in heart rate can be seen from beat 210.

Although blood pressure remained stable, heart rate increased significantly, from a mean of 55.5 to 62.2 beats/min, after 5 mg of midazolam. A typical example is shown in Figure 1. The increase in heart rate was also demonstrated by the decrease in the mean R–R interval (see Table 2). However, there were no significant changes in heart rate after 1 mg of midazolam. In addition to the decrease in R–R interval, significant decreases in heart rate variability occurred—the heart rate trace becomes much smoother after midazolam (see Figure 1), and there was a leftward shift of the frequency distributions of R–R intervals and a narrowing of the curve (Figure 2). Quantitatively this effect was expressed by a decrease in the pNN50 value (Table 2) and by a decrease in the RRDiffIQD. There were no significant differences in the remaining time-domain indices (Table 2).

Power spectral analysis revealed several significant changes after the administration of midazolam, and a typical example is shown in Figure 3. The HF component of the power spectral density plot, when expressed as normalized units, was seen to decrease significantly after doses of 1 mg (Figure 4) and 5 mg (Figure 5) of midazolam (P < 0.01, see Table 2). When expressed as an absolute power or as common coefficient of variance, the large decrease in the HF component was not significant after 1 mg but was significantly lower after 5 mg of midazolam (Table 2). After 1 mg of midazolam there was no significant change in the LF component of the power spectral density plot, when expressed as normalized units (Figure 4), absolute power or common coefficient of variance. However, after 5 mg the LF component increased significantly when expressed as normalized units (Figure 5).

The sensitivity of the baroreflex was not significantly affected by the administration of intravenous midazolam, the group data showing it to be 11.05 ± 6.6 beats·min⁻¹·mmHg⁻¹ at baseline compared with 10.91 ± 7.4 beats·min⁻¹·mmHg⁻¹ after 5 mg of midazolam.

DISCUSSION

This study has provided strong evidence for a role of GABA in the control of cardiac vagal tone in man. Midazolam, a benzodiazepine with GABA-mimetic properties, caused an increase in heart rate and a decrease in indices of high-frequency heart rate variability including the HF component of power spectral density plots under conditions where respiration had been controlled. We are unaware of any evidence that midazolam interferes significantly at the muscarinic receptor, so it is unlikely to have been affecting transmission at the ganglion or at the heart pacemaker. The results therefore strongly suggest that midazolam causes a reduction in cardiac vagal activity. This is emphasized by the effect of 1 mg of midazolam which did not significantly change heart rate but reduced the variability as well as markedly reducing the HF component while respiration was fixed. Much evidence exists showing that HF heart rate variability, however it is measured, is a measure of cardiac vagal tone. This includes the basic physiological observation that these respiratory-generated effects are seen in the next cardiac cycle and therefore cannot possibly be a manifestation of sym-
γ-Aminobutyric acid and cardiac vagal control in man

Table 2  Time-domain and power spectral data obtained for all subjects before and after 1 mg and 5 mg of midazolam during fixed and free respiration respectively

All values are means ± S.D. *P < 0.01. Abbreviations: RRIQD, interquartile difference (75th to 25th percentile) of R–R intervals; RRDiffIQD, R–R interval differences; SDNN, standard deviation of the R–R intervals; RMSSD, square root of the mean of the sum of the squares of differences between adjacent R–R intervals; HF, high-frequency peak; LF, low-frequency peak; NU, normalized units; CCV, common coefficient of variance.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Fixed respiration</th>
<th>Free respiration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>1 mg midazolam</td>
</tr>
<tr>
<td>Time-domain</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean R–R (ms)</td>
<td>1016 ± 209</td>
<td>1013 ± 185</td>
</tr>
<tr>
<td>RRIQD (ms)</td>
<td>56.8 ± 27.9</td>
<td>52.9 ± 26.1</td>
</tr>
<tr>
<td>RRDiffIQD (ms)</td>
<td>52.5 ± 35.9</td>
<td>43.0 ± 23.6</td>
</tr>
<tr>
<td>SDNN (ms)</td>
<td>43 ± 21</td>
<td>49 ± 31</td>
</tr>
<tr>
<td>RMSSD (ms)</td>
<td>40 ± 2</td>
<td>42 ± 3</td>
</tr>
<tr>
<td>SDS (ms)</td>
<td>39 ± 2</td>
<td>42 ± 34</td>
</tr>
<tr>
<td>pNN50 (%)</td>
<td>17.9 ± 20.9</td>
<td>14 ± 14</td>
</tr>
<tr>
<td>Power spectral analysis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HF (NU)</td>
<td>54.4 ± 26.5</td>
<td>38.7 ± 22.4*</td>
</tr>
<tr>
<td>HF (CCV)</td>
<td>0.0375 ± 0.07</td>
<td>0.0176 ± 0.01</td>
</tr>
<tr>
<td>HF absolute power (ms²)</td>
<td>742.4 ± 782.5</td>
<td>468.3 ± 582.2</td>
</tr>
<tr>
<td>LF (NU)</td>
<td>29.9 ± 21.1</td>
<td>37.3 ± 20.9</td>
</tr>
<tr>
<td>LF (CCV)</td>
<td>0.0187 ± 0.02</td>
<td>0.0174 ± 0.05</td>
</tr>
<tr>
<td>LF absolute power (ms²)</td>
<td>604.8 ± 1128.9</td>
<td>428.3 ± 409.2</td>
</tr>
<tr>
<td>LF/HF (absolute power)</td>
<td>1.080 ± 1.918</td>
<td>2.978 ± 5.855</td>
</tr>
</tbody>
</table>

Figure 2  An example of the frequency distribution of R–R intervals before and after 5 mg of midazolam in one subject

pathetic activity, and that cooling of the vagi decreases the average heart period and eliminates respiratory sinus arrhythmia [22]. In addition, it has been seen that the HF component of heart rate variability is eliminated by atropine, a classic blocker of parasympathetic activity [23], and is increased during trigeminal stimulation in man [12,24]. These effects have also been demonstrated in animals, where vagal blockade with atropine was seen to abolish the respiratory fluctuations in the heart rate when given intravenously in rats [25].

These effects were unlikely to be manifestations of unconsciousness, because similar effects were observed in all patients at low dose, where the subjects were fully conscious and able to control the breathing rate and depth. Furthermore, sedation would most probably lead to an increase in vagal tone as occurs during sleep [26]. It is this action of the benzodiazepine lorazepam which could be manifested in the study by Tulen et al. [14] which surprisingly reports decreased heart rate and increases in the HF component of the power spectral density plots of R–R interval. Reves et al. [27] explained the increase in heart rate observed by other authors in response to benzodiazepines as being mediated via the baroreceptor–heart rate reflex loop, due to a fall in blood pressure. This has been reported in previous studies [28,29]. However, Boralessa et al. [29] found that 0.3 mg/kg intravenous midazolam (some four times the present dose) caused a major reduction in the rise in blood pressure normally associated with intubation in man, which is not the same as a decrease in blood pressure in a resting semi-supine patient. Similarly, Lindqvist et al. [9] observed that the blood pressure change, notably a fall in arterial blood pressure as a response to head-up tilt, was greater than normal after the administration of a
Figure 3 Typical power spectral density plots obtained from one subject before (A) and after (B) a 1-mg intravenous dose of midazolam during fixed respiration.

The continuous line represents the power spectral density plot and the dashed line represents the HF component, calculated by removal of the LF and direct current components.

Figure 4 Mean HF and LF components of the power spectral density plots for all subjects (n = 20) before and after 1 mg of intravenous midazolam during a fixed respiratory rate.

*P < 0.01. Error bars denote S.E.M.
applications and it would thus seem likely that at this dose, ‘classic’ benzodiazepine respiratory depression would occur. Forster et al. [31] found that midazolam also decreased the mean ventilatory response to carbon dioxide, but again larger doses (0.15 mg/kg, which is equivalent to 10.5 mg in a 70-kg individual) than those used in the present study were employed. Central respiratory depression does not appear to be a problem with lower doses of midazolam similar to those used by us. Power et al. [32] showed that doses of 0.075 mg/kg intravenous midazolam (equivalent to 5.25 mg in a 70-kg man) had no effect upon the ventilatory response to carbon dioxide. We are therefore confident that the decrease in cardiac vagal tone observed by us is not an indirect effect of the benzodiazepine on respiratory neurons. This is supported by a recent investigation showing that heart rate–respiratory synchronization can occur after midazolam, an effect which can be reversed by the antagonist flumazenil [33]. This could hardly have occurred if respiratory neurons were suppressed.

The cardiovascular effects of other benzodiazepines have been investigated but these provide equivocal results. McLeod et al. [13] examined the longer term (6 weeks) autonomic effects of medication with alprazolam in a group of patients with anxiety disorder. Power spectral analysis of ECG data did not reveal any change in the spectral power at respiratory frequency suggesting there was no change in cardiac vagal tone after treatment. These studies only used doses of alprazolam in the therapeutic range and did not control respiration although this was noted to have a similar periodicity at the beginning and end of the 6 weeks. Therefore these valuable clinical data do little to inform the debate on whether GABA neurons exert an influence on brainstem circuits controlling heart rate. Tulen et al. [14,34] reported the effects of cumulative intravenous doses of lorazepam, and surprisingly observed a decrease in heart rate which was associated with an increased HF component in the spectral density plots. Thus it would appear that these doses of lorazepam increased cardiac vagal tone. This is a puzzling result which is difficult to explain with our current knowledge, based on animal models, of the central nervous GABA influences on cardiovascular control mechanisms. It could, however, be a consequence of sedation, as referred to earlier.

**CONCLUSION**

This study has shown that midazolam, a benzodiazepine given intravenously at relatively low doses, caused an increase in heart rate, a decrease in variability of pulse interval, and a decrease in the HF component in the power spectral density plots of R–R interval. At the same time there was no change in blood pressure, baroreceptor–heart rate reflex sensitivity or respiration, suggesting the action of midazolam was directly affecting central cardiac neuronal networks. Since the benzodiazepine acts at GABA_\_ receptors [16], this study provides strong evidence for regulation of cardiac vagal tone by GABA in man, acting via a GABA_\_ receptor site.

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**REFERENCES**


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