Mutual interactions of respiratory sinus arrhythmia and the carotid baroreceptor–heart rate reflex

S. J. CROSS*, M. R. COWIE† and J. M. RAWLEST
*Cardiac Department, Aberdeen Royal Infirmary, Aberdeen, U.K., and †Department of Medicine and Therapeutics, University of Aberdeen, Aberdeen, U.K.

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1. In six healthy subjects the amplitude and phase of respiratory sinus arrhythmia were determined at five different respiratory cycle lengths ranging from 3 to 9.5 s.

2. At each respiratory cycle length the carotid baroreceptor–heart rate reflex response was determined by cyclical neck suction at −40 mmHg at five different cycle lengths covering the same range of 3–9.5 s.

3. The application of cyclical neck suction increased the amplitude of respiratory sinus arrhythmia in all but the longest respiratory cycle lengths.

4. With increasing respiratory cycle length the amplitude of sinus arrhythmia increased, and R–R intervals were at their longest at an earlier phase of the respiratory cycle.

5. The cardiac interval responses to respiration and to neck suction at different frequencies were independent of each other, the heart rate at any moment resulting from the algebraic summation of the two responses.

INTRODUCTION

Respiratory sinus arrhythmia and the carotid baroreceptor–heart rate reflex are both mediated principally by the vagus nerve acting on the sinus node. Brief stimulation of the carotid baroreceptors, electrically or by an acute rise in arterial or transmural pressure, evokes bradycardia during expiration, but little or no response when delivered during the inspiratory phase of respiration [1–4]. This had led to the suggestion that the afferent limb of the baroreflex is gated, stimuli from the baroreceptors being prevented from reaching the nucleus ambiguus of the vagus nerve during inspiration [5]. The all-or-none interaction implied by a gating mechanism has been discredited [6], but the interaction has not been precisely quantified. Mostly, the baroreflex in man has been studied in isolation, the confounding interaction with respiration being circumvented by only analysing data collected during expiration, which may be voluntarily prolonged in human experiments [7].

We have previously described separate methods for quantifying respiratory sinus arrhythmia [8] and the baroreflex [9]. Both methods permit the acquisition of data over an indefinite time period and assume a sinusoidal cardiac interval response to the cyclical stimulus of respiration and neck suction, respectively. In this study we combine the previous methods to enable the separate cardiac interval responses to respiration and neck suction at different frequencies to be determined from simultaneous recordings. In this way the extent of the mutual interactions of respiratory sinus arrhythmia and the baroreflex may be demonstrated.

EXPERIMENTAL

Subjects

The subjects studied were six healthy volunteers, two females and four males, aged 22–51 years. None had evidence of autonomic neuropathy or was taking drugs known to affect the heart rate or to interfere with autonomic function.

Methods

Each subject lay supine throughout the experiments, which were completed in one session. Suction at 40 mmHg was applied cyclically to a rubber-edged lead collar moulded to the subject's neck to provide an air-tight seal. During each suction cycle the periods of suction and no suction, when the pressure in the neck chamber rapidly reverted to atmospheric, were of equal duration. Respiration was voluntarily synchronized with a sine wave displayed on a monitor. Five different cycle lengths were used for both respiration and for neck suction: 3.0, 4.0, 5.3, 7.1 and 9.5 s. Each cycle length is one-third longer than the one below, and none is a multiple of another. When the nominal lengths of respiratory and suction cycles were due to be the same, the suction cycle length was increased slightly so that there were two fewer suction than respiratory cycles in a 2 min run.

The subject's respiratory movements were observed and an event marker was depressed at the end of each

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Correspondence: Dr. J. M. Rawles, Department of Medicine and Therapeutics, University of Aberdeen, Foresterhill, Aberdeen AB9 2ZD, U.K.
expiration. This mark, together with the sine wave with which respiration was synchronized, the pressure in the neck chamber and the subject's ECG, were recorded on a multi-channel recorder. An example of the paper record is given in Fig. 1.

**Procedure**

Starting with the shortest respiratory and suction cycles, and lengthening them progressively, two 2 min runs were performed at each of the 25 combinations of respiratory and suction cycle lengths. In the first run of each pair, suction was diverted away from the neck chamber although still connected to the manometer. This constituted a control for the suction run which came next.

**Digitizing the ECG**

From the paper record of each of the 50 2 min runs consecutive R–R intervals were measured together with their relations with the onset of suction and respiratory cycles. The digitizing pad used had a resolution of 0.1 mm, equivalent to 4 ms; the results were stored and analysed on a microcomputer.

**Cosinor analysis**

In a control run, when no suction is applied to the neck chamber, a plot of instantaneous heart rate against time shows a sinusoidal variation in phase with respiration. When suction is applied cyclically the response to respiration is modulated by a sinusoidal response to suction. Provided that the respiratory and suction cycles lengths are not multiples of each other, respiration will move in and out of phase with the suction cycle and the separate contributions of respiration and neck suction to variation of R–R intervals may be computed by cosinor analysis. In this technique, the best fit of two cosine function curves to the variation of R–R intervals is found by multiple regression analysis. One cosine function curve has the same angular position (r degrees) in the respiratory cycle in which it occurs. In a similar way the position of each R-wave in its respective suction cycle is calculated (s degrees). The duration of the associated R–R interval is then regressed against cos (r degrees), sin (r degrees), cos (s degrees) and sin (s degrees), to give the amplitude, phase angle and statistical significance of the variation of R–R intervals induced by respiration and suction.

The regression equation is:

$$R-R = a + b \cos(r) + c \sin(r) + d \cos(s) + e \sin(s)$$

where a–e are constants.

Cosinor analysis for a single cosine function has been described by Halberg et al. [10], and has been applied to respiratory sinus arrhythmia and baroreflex measurement [8, 9].

**Example.** Fig. 2 shows a plot of instantaneous heart rates (with which readers may be more familiar than R–R intervals) during a 2 min run in which the suction and respiratory cycle lengths were 9.5 and 7.1 s, respectively. The continuous line shows the predicted instantaneous heart rates derived from the regression of R–R intervals on respiration alone, the regression equation being:

$$R-R = a + b \cos(r) + c \sin(r)$$

The multiple correlation coefficient is 0.49, indicating that 24% of the variance of R–R intervals may be explained in terms of respiration, the amplitude of the respiratory variation of R–R intervals being 5.7%.

Fig. 3 shows the same data with the predicted instantaneous heart rates derived from the regression equation:

$$R-R = a + d \cos(s) + e \sin(s)$$

The multiple correlation coefficient is 0.73, indicating that 53% of the variance of R–R intervals may be explained in terms of neck suction working through the baroreflex; the amplitude of the response is 9.0%.

Fig. 4 shows the same data; this time the continuous line indicates the predicted instantaneous heart rates derived from the equation:

$$R-R = a + b \cos(r) + c \sin(r) + d \cos(s) + e \sin(s)$$

The multiple correlation coefficient is 0.87, indicating that 77% of the variance of R–R intervals is now accounted for, partly by sinus arrhythmia (24%) and partly by the baroreflex (53%). The amplitude of the beat-to-beat changes in heart rate varies markedly as respiratory and suction cycles move in and out of phase with each other.

**Calculation of amplitude and phase.** The technique of cosinor analysis averages multiple cycles, and determines the average cardiac interval response to respiration and suction, respectively. The regression equation obtained for each run predicts two additive sinusoidal variations of R–R intervals above and below their mean value, with periods corresponding respectively to respiratory and suction cycle lengths. The predicted maximum increase of
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R–R intervals above their mean value in response to respiration or neck suction is calculated from the coefficients of the regression equation. The amplitude of the R–R interval response is defined as the percentage increase of the maximum above the mean R–R interval for a 2 min run, and is calculated separately for respiration and neck suction.

The commencement of a respiratory cycle is taken as the end-expiratory point; the start of a suction cycle is taken as the onset of suction. The phase of the cardiac interval response is defined as the angular position in the average respiratory or suction cycle when R–R intervals are longest. Thus the phase of the cardiac interval response to respiration is generally close to 0 degrees or 360 degrees, indicating that R–R intervals are longest (heart rate is slowest) at about the time of end-expiration.

Phase angles are averaged trigonometrically; comparisons are made by Student's paired t-test, and analysis of variance; statistical significance is taken as \( P < 0.05 \).

RESULTS

Respiratory sinus arrhythmia

Table 1 shows the mean amplitudes of respiratory sinus arrhythmia at different respiratory cycle lengths for control runs and for suction runs from six subjects. Overall, the amplitude of respiratory sinus arrhythmia is greater during the application of neck suction than during sham suction (\( P < 0.001 \)); this is true for all combinations of respiratory and suction cycle lengths except the longest respiratory cycles (Fig. 5).

Average phase angles for respiratory sinus arrhythmia are also shown in Table 1; phase angles are similar whether or not suction is applied (Fig. 6).

During both sham suction and suction runs, the amplitude of respiratory sinus arrhythmia increased with increasing respiratory cycle length (Fig. 5). The phase angle of the cardiac interval response to respiration decreased with increasing respiratory cycle length (Fig. 6).

Effect of neck suction

The average amplitude of the cardiac interval response to neck suction increased, and its phase angle decreased, with increasing suction cycle length (Table 2, Figs. 5 and 6). The cardiac interval response to sham suction was of very low amplitude with a seemingly random distribution of phase angles.

Interactions

Fig. 7(a) shows the amplitude of respiratory sinus arrhythmia for all combinations of respiratory and suction cycle lengths. Two-way analysis of variance (Table 3), while confirming the effect of respiratory cycle length on amplitude, shows no significant effect of suction cycle length on the amplitude of the respiratory response; the interaction term is not significant. There is no significant effect of neck suction on the phase of respiratory sinus arrhythmia.

Fig. 7(b) shows the amplitude of the response to neck suction for all combinations of respiratory and suction cycle lengths. Two-way analysis of variance (Table 4), while confirming the effect of suction cycle length on amplitude, shows no significant effect of respiratory cycle length on the amplitude of the response to suction; the
Table 1. Mean amplitudes and phase angles of respiratory sinus arrhythmia at various respiratory and suction cycle lengths in six subjects, with neck suction applied (Suction) or diverted (Sham)

<table>
<thead>
<tr>
<th>Respiratory cycle length (s)</th>
<th>3</th>
<th>4</th>
<th>5.3</th>
<th>7.1</th>
<th>9.5</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suction cycle length (s)</td>
<td>3</td>
<td>4</td>
<td>5.3</td>
<td>7.1</td>
<td>9.5</td>
<td></td>
</tr>
<tr>
<td>Amplitude (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phase angle (degrees)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

interaction term is not significant. Neither is there any significant effect of respiration on the phase of the response to neck suction.

**Goodness-of-fit of the mathematical model adopted**

In all of the 150 suction runs, either respiratory, suction or both groups of predictor variables were statistically significant, and in every case the multiple regression was significant overall. The multiple correlation coefficients ranged from 0.36 to 0.92 (mean 0.73). With short cycle lengths, response amplitudes were small (1–2%) in relation to background variance, and correlation coefficients tended to be smaller.

Various mathematical models were tried, but none performed consistently better than the one adopted and shown above.

**DISCUSSION**

The forced response of a linear system to a sinusoidal input is itself sinusoidal, and is characterized by its ampli-
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Table 2. Mean amplitudes and phase angles of cardiac interval response to neck suction at various respiratory and suction cycle lengths in six subjects, with neck suction applied (Suction) or diverted (Sham)

<table>
<thead>
<tr>
<th>Respiratory cycle length (s)</th>
<th>3</th>
<th>4</th>
<th>5.3</th>
<th>7.1</th>
<th>9.5</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suction cycle length (s)</td>
<td>Sham</td>
<td>Suction</td>
<td>Sham</td>
<td>Suction</td>
<td>Sham</td>
<td>Suction</td>
</tr>
<tr>
<td>Amplitude (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.89</td>
<td>4.81</td>
<td>0.90</td>
<td>4.89</td>
<td>0.34</td>
<td>5.27</td>
</tr>
<tr>
<td>Phase angle (degrees)</td>
<td>242</td>
<td>249</td>
<td>61</td>
<td>251</td>
<td>50</td>
<td>238</td>
</tr>
<tr>
<td>4</td>
<td>0.56</td>
<td>7.00</td>
<td>0.71</td>
<td>6.64</td>
<td>0.94</td>
<td>6.47</td>
</tr>
<tr>
<td>Phase angle (degrees)</td>
<td>255</td>
<td>225</td>
<td>286</td>
<td>222</td>
<td>154</td>
<td>222</td>
</tr>
<tr>
<td>5.3</td>
<td>0.66</td>
<td>8.34</td>
<td>0.65</td>
<td>8.99</td>
<td>0.94</td>
<td>7.19</td>
</tr>
<tr>
<td>Phase angle (degrees)</td>
<td>13</td>
<td>196</td>
<td>41</td>
<td>192</td>
<td>89</td>
<td>185</td>
</tr>
<tr>
<td>7.1</td>
<td>0.80</td>
<td>10.66</td>
<td>0.63</td>
<td>11.01</td>
<td>0.85</td>
<td>9.78</td>
</tr>
<tr>
<td>Phase angle (degrees)</td>
<td>289</td>
<td>171</td>
<td>223</td>
<td>173</td>
<td>9</td>
<td>173</td>
</tr>
<tr>
<td>9.5</td>
<td>0.66</td>
<td>14.75</td>
<td>1.23</td>
<td>13.61</td>
<td>1.57</td>
<td>12.41</td>
</tr>
<tr>
<td>Phase angle (degrees)</td>
<td>263</td>
<td>148</td>
<td>244</td>
<td>151</td>
<td>214</td>
<td>151</td>
</tr>
<tr>
<td>Mean</td>
<td>0.71</td>
<td>9.11</td>
<td>0.82</td>
<td>9.03</td>
<td>0.93</td>
<td>8.22</td>
</tr>
<tr>
<td>Amplitude (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.77</td>
<td>9.18</td>
<td>0.82</td>
<td>9.03</td>
<td>0.93</td>
<td>8.22</td>
</tr>
<tr>
<td>Phase angle (degrees)</td>
<td>277</td>
<td>198</td>
<td>289</td>
<td>197</td>
<td>93</td>
<td>194</td>
</tr>
</tbody>
</table>

Fig. 7. Average amplitudes in six subjects of the cardiac interval response to neck suction (a) and respiration (b) for combinations of five respiratory and five suction cycle lengths. The result of two-way analysis of variance is shown. Abbreviations: Resp., respiratory; CL, cycle length; VR, variance ratio; NS, not significant.

tude and phase relationship to the stimulus. The response of such a system to two inputs applied simultaneously is the same as the sum of the two responses to each of the inputs applied individually [11]. We have shown in previous studies that both respiration and the cyclical application of neck suction may be considered as sinusoidal inputs to the heart rate control system, each resulting in sinusoidal outputs. We now show that the two
inputs applied simultaneously produce the same result as the sum of the responses to each of the inputs applied separately. Our results indicate that the response of the heart rate control system is substantially linear over the range of stimuli that we have used. The cardiac interval responses to respiration and to neck suction at different frequencies were independent of each other, the heart rate range of stimuli that we have used. The cardiac interval response to respiration and to neck suction at different frequencies in six subjects. In 150 runs with neck suction there was no evidence of interaction between respiratory sinus arrhythmia and the heart rate response to neck suction, but the combined effect was described by a regression equation with additive terms representing sinus node responses to inspiration and baroreceptor stimulation. However, in similar experiments utilizing brief carotid sinus nerve stimulation, Borst & Karemaker [6] concluded that net heart interval prolongation evoked by nerve stimulation was similar in both phases of respiration. In these two studies, stimuli were delivered during inspiration or expiration, but no attempt was made to relate the heart interval responses more precisely to the phase of respiration in which they occurred. Moreover, in neither study was respiratory rate controlled, even though the magnitude and phase of the response depends on the frequency of respiration.

In our study, each beat is accurately located in its respective respiratory and suction cycle, and the duration of each R–R interval is expressed as a function of its timing in both cycles. The analyses were performed at 25 different combinations of respiratory and suction frequencies in six subjects. In 150 runs with neck suction there was no evidence of interaction between respiratory sinus arrhythmia and the heart rate response to neck suction, but the combined effect was described by a regression equation with additive terms representing respiration and neck suction. The regression equation was statistically significant on every occasion, and accounted for a majority of the variance of heart rate.

We conclude that the heart rate at any moment results from the algebraic summation of the independent responses to respiration and the baroreflex.

Table 3. Analysis of variance of the amplitude of the cardiac interval response to neck suction. Abbreviations: SSq, sum of squares; DF, degrees of freedom; MSq, mean of squares; VR, variance ratio; NS, not significant.

<table>
<thead>
<tr>
<th></th>
<th>SSq</th>
<th>DF</th>
<th>MSq</th>
<th>VR</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suction cycle length</td>
<td>1267.7</td>
<td>4</td>
<td>316.9</td>
<td>7.55</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Respiratory cycle length</td>
<td>16.9</td>
<td>4</td>
<td>4.2</td>
<td>0.10</td>
<td>NS</td>
</tr>
<tr>
<td>Interaction</td>
<td>41.8</td>
<td>16</td>
<td>2.6</td>
<td>0.06</td>
<td>NS</td>
</tr>
<tr>
<td>Residual</td>
<td>5240.2</td>
<td>125</td>
<td>42.0</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>6574.7</td>
<td>149</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4. Analysis of variance of the amplitude of the cardiac interval response to respiration. Abbreviations: SSq, sum of squares; DF, degrees of freedom; MSq, mean of squares; VR, variance ratio; NS, not significant.

<table>
<thead>
<tr>
<th></th>
<th>SSq</th>
<th>DF</th>
<th>MSq</th>
<th>VR</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiratory cycle length</td>
<td>239.0</td>
<td>4</td>
<td>59.7</td>
<td>4.34</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Suction cycle length</td>
<td>9.0</td>
<td>4</td>
<td>2.2</td>
<td>0.16</td>
<td>NS</td>
</tr>
<tr>
<td>Interaction</td>
<td>51.4</td>
<td>16</td>
<td>3.2</td>
<td>0.23</td>
<td>NS</td>
</tr>
<tr>
<td>Residual</td>
<td>1721.9</td>
<td>125</td>
<td>13.8</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>2021.3</td>
<td>149</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Eckberg [12] has reported non-linearities of the human carotid baroreceptor–cardiac reflex. He showed that the sinus node response to brief baroceptor stimulation is sigmoidal, with threshold, linear and saturation zones. The threshold is the carotid distending pressure at the foot of the steep linear portion of the stimulus–response curve. The systolic blood pressure in young healthy adults is commonly just above threshold, which is about 100 mmHg. The steep linear portion extends over about 60 mmHg, so that the saturation zone is about 160 mmHg [13]. Carotid distending pressures in excess of saturation do not evoke any greater response than pressure at saturation level. The threshold pressure appears to be determined centrally rather than by the properties of the baroceptors themselves, and it is this central setting of the operating point of the reflex close to threshold that determines the asymmetry of response, brief neck suction eliciting greater cardiac interval changes than brief neck pressure [12]. In our study we used neck suction and not neck compression, thus operating on the linear section of the stimulus–response curve, and, by limiting suction to 40 mmHg, we avoided the saturation zone. The cardiac interval response to neck suction was therefore expected to be linear.

We have shown that the amplitude of the cardiac interval response to cyclical neck suction is positively correlated with cycle length, confirming our previous findings [9]. The slope of this relationship is similar to that between respiratory cycle length and the amplitude of sinus arrhythmia, an effect which has also been described previously [8, 14].

The heart rate response that follows stimulation of the carotid baroceptors occurs after a fixed delay that is determined by the properties of the baroreflex. When the stimulus is applied repeatedly, and with variable repetition frequencies, the maximum response occurs relatively earlier in the stimulation cycle as the stimulation cycle lengthens; this is indicated by a falling phase angle (Fig. 6). The decreasing phase angle of sinus arrhythmia with increasing respiratory cycle length has a similar explanation.

Eckberg & Orshan [3] have investigated the interaction between respiration and the baroreflex. Brief neck suction was applied at various points of the cardiac cycle and in inspiration and expiration; the post-stimulus interval was compared with the pre-stimulus interval. Sham suction was also applied in order to determine the extent of the heart interval changes due to respiration alone. Heart interval prolongation was less in inspiration than expiration, even when the responses to sham suction were subtracted to give the net response to suction alone. The authors concluded from this evidence that inhibition of the baroreflex response during inspiration was unlikely to be due to an algebraic summation of the opposing sinus node responses to inspiration and baroreceptor stimulation. However, in similar experiments utilizing brief carotid sinus nerve stimulation, Borst & Karemaker [6] concluded that net heart interval prolongation evoked by nerve stimulation was similar in both phases of respiration. In these two studies, stimuli were delivered during inspiration or expiration, but no attempt was made to relate the heart interval responses more precisely to the phase of respiration in which they occurred. Moreover, in neither study was respiratory rate controlled, even though the magnitude and phase of the response depends on the frequency of respiration.
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REFERENCES


