Real-time measurement of cardiac vagal tone in conscious dogs

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1Department of Veterinary Clinical Studies, University of Glasgow Veterinary School, Bearsden, Glasgow G61 1QH; and 2Peripheral Nerve and Autonomic Unit, Southern General Hospital, Glasgow G51 4TF, United Kingdom

Little, C. J. L., P. O. O. Julu, S. Hansen, and S. W. J. Reid. Real-time measurement of cardiac vagal tone in conscious dogs. Am. J. Physiol. 276 (Heart Circ. Physiol. 45): H758–H765, 1999.—Rapid changes in heart rate are caused by changes in parasympathetic tone. The NeuroScope is an electronic device designed to offer an objective real-time measure of instantaneous cardiac vagal tone by phase demodulation of a high-resolution time domain of R-R wave intervals. Data are displayed against an arbitrary but linear scale, the cardiac index of parasympathetic activity (CIPA). To validate this method, 10 conscious healthy dogs were each given six incremental doses of atropine (0.01 mg/kg) to a total dose of 0.06 mg/kg or equal volumes of saline. A dose-response curve was constructed. At the maximum dose of atropine, CIPA values fell to 1.3 ± 0.7% (SD) of baseline, whereas R-R intervals fell to 51.5 ± 11.5% of baseline, and standard deviation of the R-R wave interval fell to 10.6 ± 6.5% of baseline. These findings show that the NeuroScope can provide a specific real-time index of cardiac vagal tone in dogs without need for recourse to atropine.

NeuroScope; parasympathetic tone; heart rate variability; autonomic nervous system; atropine

Cardiac vagal tone is reflexly generated through baroreceptor stimulation (23). These baroreflexes are mainly responsible for resting cardiac vagal tone in the conscious breathing animal, although chemoreceptor afferents and other reflex inputs associated with breathing, for instance, may modify vagal outflow (8, 17). Baroreflex modulation of SA node function is strongly influenced by the timing as well as the intensity of the stimulus (6, 10), but the usual physiological stimulus is the rapid rise in blood pressure during the ejection period of ventricular systole. The latency of this reflex is constant and short compared with the length of the cardiac cycle, averaging ~160–180 ms in dogs and 240 ms in humans (10, 22). It follows that the effect of brief arterial baroreceptor stimulation arrives at the SA node in the period of the cardiac cycle after the stimulus and is synchronized to the preceding pulse as a result of the fixed latency. Moreover, at normal heart rates, this volley of vagal impulses arrives at the SA node at the stage of the cardiac cycle during which it seems to be particularly sensitive to this stimulus (10). Thus each ventricular systole causes baroreceptor stimuli that act via cardiac vagal tone to delay the subsequent cardiac cycle or cycles (9). The delay in the cycle(s) induced by baroreceptor stimulation is proportional to the baroreceptor stimulus strength and duration (10). Volleys of vagal nerve impulses liberate ACh from synaptic vesicles, which is added to the ACh remaining at the SA node at the end of the previous heart period, but the concentration of this neurotransmitter also declines exponentially because of degradation by acetylcholinesterase (29). In effect, the vagally mediated prolongation of heart period is proportional to the concentration of ACh at the SA node (22). Thus the SA node actually integrates (or adds up) the vagal nerve impulses.

Rapid changes in heart rate are caused by changes in the level of parasympathetic tone. Sympathetic influences affect heart rate directly and reflexly via changes in peripheral resistance, but these controls act slowly in comparison to cardiac vagal tone (14). Baroreceptor control of heart rate can be modeled as a closed-loop feedback system with a delay in the sympathetic nervous system that makes it resonate at ~0.1 Hz (9). In a classical study of the firing rate of cardiac vagal efferent fibers on the heart period performed in dogs, Katona and others (22) showed that it was possible to
predict the heart period from cardiac vagal efferent activity, even while the sympathetic nerves were intact. These authors further demonstrated that a simple electronic model successfully described moderate changes in the heart period caused by changes in vagal nerve activity. Therefore, it follows that it should be possible to deduce the immediate effect of parasympathetic activity on the heart by appropriate analysis of the pulse-synchronized variation in heart period.

The NeuroScope is a novel electronic device that has been designed to offer an objective real-time measure of instantaneous cardiac vagal tone on the basis of these physiological principles. Cardiac vagal tone introduces delays in the onset of cardiac cycles (10) that can be detected with the aid of a template similar to that of Katona and others (22). The instrument detects pulse-synchronized delays in the onset of successive cardiac cycles as phase shifts, quantifies these delays in milliseconds, and converts them to measures of cardiac parasympathetic activity. It follows from the arguments set out previously that the NeuroScope is only of use for the evaluation CIPA when the heart is in sinus rhythm.

Here we compare the NeuroScope technique with other conventional and commonly used methods of measuring cardiac parasympathetic activity in dogs given graded doses of atropine. The aim was to evaluate the specificity of CIPA as an index of cardiac parasympathetic activity.

MATERIALS AND METHODS

Ten healthy mature male beagle dogs exhibiting sinus heart rhythm were used in the study. All procedures were performed in fully conscious animals between 10 AM and 4 PM after an overnight fast. Each dog had been trained to stand in a semisupine standing position. They were familiar with the laboratory environment and the investigators. Adhesive silver-silver chloride electrocardiogram (ECG) electrodes were fastened to the limbs with tape, and a 20-gauge intravenous catheter was placed in the cephalic vein of a forelimb for drug administration.

Measurement of the CIPA. Beat-by-beat R-R wave intervals were recorded by downloading the analog ECG signal to the NeuroScope and using an R wave recognition template. The detection procedure for the CIPA involves accurate sampling of the ECG to obtain a high-resolution time domain of the R-R wave intervals. Pulse-synchronized delays that are specifically caused by cardiac vagal tone are obtained through phase demodulation of the time domain. A patented system of phase locks filters out slow responses originating from the sympathetic nervous system and other sources, thereby allowing the pulse-synchronized parasympathetic responses to be measured (19a). Results are displayed on a computer screen in real time against an arbitrary but linear scale, the CIPA, which was derived from human volunteers through atropinization (18). The parasympathetic control of the heart measurable by any index can be defined as the difference in magnitudes of that index before and after the elimination of all vagal effects on the heart, i.e., the response to full atropinization (18).

Mean basal CIPA in a sample of supine resting human volunteers breathing quietly was arbitrarily set at 10 units. The units were derived by dividing the measurable effects of cardiac vagal activity into 10 equal parts, thus giving a linear scale with an absolute zero reference point. Zero on the CIPA scale represents no measurable parasympathetic influence on heart rate, as observed during full atropinization. All data were downloaded in real time to a laptop computer, where these data were stored automatically. (Further details concerning the operation of NeuroScope are supplied in the APPENDIX.)

After instrumentation, each dog was allowed to rest for 2,000 heartbeats. Then, as a test of intact cardiac vagal tone, the oculocardiac reflex was induced by pressing on the closed eyelids for >20 s (13). A further rest period of >800 heartbeats was allowed before the study continued, and the last 500 beats of this rest period were designated baseline. Six aliquots of atropine (0.01 mg/kg iv) were given to each dog at 10-min intervals to a cumulative dose of 0.06 mg/kg. Each injection was followed by a 1.5-ml flush of saline. Data were collected continuously during the study and for 10 min after the final dose. At the end of the study period, digital pressure was applied to the eyes again to induce evidence of residual cardiac vagal tone. On a separate occasion the same dogs were prepared and studied in exactly the same way, but the atropine injection was replaced with a similar volume of normal saline solution. All studies were performed in random order according to a predetermined schedule.

All data were examined visually during data collection throughout the recording to ensure that the dogs were exhibiting a sinus rhythm. For the purposes of data analysis, beats that were not of sinus origin were excluded. The 50 heartbeats before each injection and the subsequent 50–100 beats were also excluded from data analysis to avoid effects from handling of the dogs and to allow for equilibration as observed during the continuous monitoring. For each dose aliquot in each dog, all the remaining heartbeats were subject to further analysis.

Statistical methods. To assess whether there was a significant difference between the responses of the animals between the two treatments, the resting and experimental data were analyzed using ANOVA techniques with repeated measures. The factors were dog, treatment, and dose, where treatment and dose were regarded as fixed effects and dog as a random effect. For the resting data the analysis was performed on the mean CIPA values for each segment of 250 heartbeats for each dog and for each treatment. For the experimental data, because the heart rate changed and episodes of second-degree heart block were sometimes observed during administration of atropine, the analysis was performed on the mean of the CIPA values for each animal at each dose for each treatment. The analysis of the experimental data was also performed on normalized data, where the CIPA values were expressed as a percentage of the baseline value at dose 0 (i.e., the 500-beat time frame immediately preceding the injections of atropine or saline). Finally, ANOVA was applied to the treated (atropine) group alone, and Tukey’s pairwise comparison of means was used to identify at which dose the CIPA values were significantly different. Significance was set at 5% for all analyses.

RESULTS

Resting and baseline data. All dogs exhibited sinus rhythm during the resting and baseline recordings, and only nine nonsinus beats were identified in three dogs during this period. The CIPA values recorded from the dogs in this study were generally much higher than those recorded from healthy human subjects. The mean of the mean CIPA values over 500 heartbeats for the 10 dogs at baseline, before the injection of saline and atropine, were 38.9 ± 16.8 and 43.8 ± 19.1 (SD) units,
respectively. Table 1 provides a summary of these measurements for each dog at baseline before the injection of saline or atropine. During these recordings, CIPA values and R-R interval length in each dog varied over a short time frame. In some, but not all, animals, respiratory modulation of the R-R interval length was marked.

CIPA values generally rose as R-R interval length increased and as variability in the R-R wave intervals rose. Over segments of 250 cardiac cycles in the resting period before injection of saline or atropine, the average CIPA value for each dog tended to be relatively stable and characteristic for that dog, although dog-to-dog differences were not explored statistically. Typical examples of these resting recordings are shown in Fig. 1, where each example consisted of a time series of 500 heartbeats.

Figure 2 illustrates the induction of the oculocardiac reflex in one dog by the application of pressure to the eyes; in all dogs, induction of this reflex was associated with lengthening of the R-R interval and increase in R-R wave variability; beat-by-beat CIPA values rose steeply to reach a peak within 15–20 s in all dogs.

Statistical analysis of the resting data in the 10 dogs showed no systematic or significant differences between the measurements preceding atropine or saline injection. Moreover, systematic and statistically significant differences were also absent among the eight individuals, and characteristic for that dog, although dog-to-dog differences were not explored statistically. Typical examples of these resting recordings are shown in Fig. 1, where each example consisted of a time series of 500 heartbeats.

Table 1. CIPA in conscious dogs at baseline before administration of atropine or saline

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>CIPA Before atropine (ms)</th>
<th>CIPA Before saline (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>49.8 ± 11.7</td>
<td>58.9 ± 14.1</td>
</tr>
<tr>
<td>2</td>
<td>21.5 ± 9.7</td>
<td>45.5 ± 9.6</td>
</tr>
<tr>
<td>3</td>
<td>32.3 ± 10.3</td>
<td>25.3 ± 6.8</td>
</tr>
<tr>
<td>4</td>
<td>50.4 ± 14.7</td>
<td>68.7 ± 21.7</td>
</tr>
<tr>
<td>5</td>
<td>63.5 ± 14.2</td>
<td>47.3 ± 15.8</td>
</tr>
<tr>
<td>6</td>
<td>21.5 ± 9.6</td>
<td>12.3 ± 7.1</td>
</tr>
<tr>
<td>7</td>
<td>53.0 ± 19.7</td>
<td>33.5 ± 12.2</td>
</tr>
<tr>
<td>8</td>
<td>24.3 ± 24.0</td>
<td>23.1 ± 14.3</td>
</tr>
<tr>
<td>9</td>
<td>42.4 ± 13.7</td>
<td>41.8 ± 18.1</td>
</tr>
<tr>
<td>10</td>
<td>78.9 ± 14.2</td>
<td>38.0 ± 8.7</td>
</tr>
</tbody>
</table>

Values are means ± SD of 500 heartbeats. Dogs have long been considered to have high cardiac vagal tone compared with human subjects. Mean cardiac index of parasympathetic activity (CIPA) for a healthy adult man in the supine position and breathing quietly is 10 (18).
Atropine is a competitive muscarinic receptor antagonist that prevents the action of ACh on the SA node of the heart. Physiological responses to parasympathetic nerve impulses are thereby attenuated or abolished. Because the peripheral vasculature is virtually devoid of muscarinic receptors, the effects of atropine on heart rate account for all the important direct effects of this drug on the cardiovascular system. In veterinary clinical use, atropine is generally used in the dose range 0.01–0.04 mg/kg in dogs (5). However, numerous experimental studies have been performed using this drug in conscious and anesthetized dogs in doses ranging from 0.0003 to 1.5 mg/kg (7, 15, 21, 25, 38). These studies have established that very small doses of atropine (<0.01 mg/kg) slow the heart, possibly because of a central vagal-stimulating action, although the mechanism is controversial (19, 21, 25). In conscious dogs, vagal effects on the heart are reported to be abolished at doses of ~0.04 mg/kg, but certain anesthetic agents such as morphine and α-chloralose seem to enhance cardiac vagal tone. Very high doses of atropine (>0.2 mg/kg) appear to cause excess tachycardia, an effect that may be due to ganglionic blockade (7, 40).

In the present study, cumulative intravenous atropine injections caused a dose-dependent reduction in the CIPA values recorded from conscious dogs, and at the maximum dose utilized, this index was almost abolished in all cases to a mean value of only 1.3% of the resting value. This observation indicates that CIPA is a highly selective and dependable index of cardiac vagal tone. In keeping with this observation, in unmedicated dogs the CIPA values generated on a beat-by-beat basis rose steeply when cardiac vagal tone was increased by the application of digital pressure to the eyes, a well-established vagal maneuver. This phenomenon was abolished by atropinization. At rest, CIPA values recorded from these dogs varied widely over a short time scale, which is consistent with known physiological concepts of the beat-by-beat control of the cardiovascular system (9).

As atropinization proceeded in these dogs, the mean R-R wave interval fell. This observation was expected but was of only limited value as an index of cardiac vagal tone, because the initial and final mean R-R wave intervals varied quite substantially between dogs. The final value varied from 30.3 to 67.3% of that recorded at baseline [51.5 ± 11.5% (SD)]. A dose-dependent reduction in the standard deviation of the R-R wave interval with cumulative atropinization was also observed in this experiment. However, at the maximum dose of atropine this index was not completely abolished, with the mean value recorded from the 10 dogs 10.6% of the baseline value. Moreover, very substantial differences were observed between dogs. This observation was to be expected, because although the main contributor to the variability of the R-R wave interval is cardiac vagal tone, cardiac sympathetic tone will also affect this, particularly because this index was measured over a period of several minutes. The heterogeneity of responses of the R-R wave intervals and its standard deviations between dogs can be explained by differences in the relative dominance of sympathetic and parasympathetic autonomic tone between individuals.
since these two indexes are not very specific for cardiac vagal tone.

Various methods for the evaluation of vagal effects on heart rate have been described previously. Respiratory modulation of heart rate, respiratory sinus arrhythmia (RSA), occurs by modulation of cardiac vagal tone (8, 16, 20, 34, 39). Whether RSA is secondary to respiratory blood pressure variability or vice versa is controversial, but because these components are connected through a closed-feedback loop, either variable could lead the other (2, 4, 8, 9, 28, 34). In dogs and humans under controlled conditions, RSA has been shown to be closely correlated with parasympathetic control (11, 12, 20). On the other hand, it has long been known that, over a range of breathing frequencies, fluctuations in heart rate are not necessarily in phase with respiration (3, 28). RSA in cooperative human patients can be maximized by asking them to breathe deeply at a slow rate, often with the aid of a timing device such as a metronome. By contrast, in conscious and freely behaving animals, rate and depth of breathing can vary substantially from moment to moment, particularly in dogs, which tend to pant rapidly when they are warm, stressed, or excited. Moreover, because breathing rate and depth differ, it is difficult to make meaningful comparisons between individuals or over a given time period.

Another approach to the issue of noninvasive assessment of cardiac vagal tone is to study heart rate variability (HRV). This latter approach has generated...
Fig. 4. Second-degree heart block during intravenous atropinization (dog 6 in Table 1). Consecutive R-R wave intervals are shown while 2nd aliquot of atropine was administered (vertical line). Second-degree heart block, peaks approximately twice as long as predominant R-R wave intervals, occurred frequently during this period. Longest R-R interval corresponded to 2 unconduted P waves on electrocardiogram recording (not shown). Because NeuroScope can only be used for evaluation of cardiac vagal tone when heart is in sinus rhythm, all episodes of 2nd-degree heart block were excluded from our analyses.

numerous publications, but interpretation of the results vis-à-vis cardiac vagal tone is difficult. There is agreement that high-frequency variation in heart periods is solely associated with parasympathetic tone (2, 35). Lower-frequency HRV seems to be associated with changes in sympathetic and parasympathetic tone interacting with each other; additional mechanisms such as thermoregulation and the renin-angiotensin-aldosterone system have also been implicated, particularly for rhythms with frequencies <0.1 Hz (2, 35, 38). Evaluation of HRV to elucidate the components of autonomic control is crucially dependent on stationary conditions (1), which are difficult to achieve in freely behaving animal subjects. The necessity for stationary conditions prevents the use of HRV for studies of reflex modulation of heart rate, such as in the oculocardiac reflex or exercise tests. It is for such dynamic autonomic states that CIPA would appear to be most useful.

In contrast to measures of RSA and HRV as indexes of cardiac vagal tone, the CIPA reported in this study is generated in real-time without the active cooperation of the subject. The measurement appears to be a specific and a sensitive indicator of beat-by-beat cardiac vagal tone that varies appropriately in response to well-established vagal maneuvers. Moreover, because the index is displayed in units of a linear scale with an absolute zero reference point, comparisons of cardiac vagal tone between individuals and over a given period of time are greatly facilitated.

An interesting observation from the present study was that the dose-response curves for the indexes of variability (CIPA and standard deviation of the R-R wave interval) closely resemble each other but differ from the curve for the effect of atropine on mean R-R interval. We are uncertain as to the explanation for this. It is conceivable that, at a submaximal dose, atropine has a more marked effect on the beat-by-beat modulation of cardiac cycle length than on absolute cycle length. It is also possible that, during the course of this experiment, blood pressure, cardiac sympathetic tone, afterload, or other variables such as circulating neurohormone concentrations were changing in response to the alterations in cardiac cycle length caused by the administration of atropine. Because we are uncertain of the explanation for this observation, we have based our conclusions on the absolute change in each index caused by full atropinization rather than on the shape of the dose-response curves.

Disadvantages of the CIPA. The NeuroScope is very sensitive to galvanic noises from any source, particularly electromyographic noises, and therefore elaborate algorithms for the recognition of the QRS complexes are necessary. It also requires a very-high-resolution digitized ECG signal, which is not usually provided by medical equipment currently available in the market. The method depends solely on the characteristics of cardiac cycles generated by the SA node under the influences of vagal nerve impulses and, therefore, cannot assess CIPA by using ectopic beats. Most importantly, the precise relationship between the actual neural signals traveling in the cardiac vagal nerve to the SA node and the CIPA has not been defined.

Limitations of the study. In designing this experiment, we deliberately strove to avoid obliterating normal reflex behavior of the cardiovascular system, because the aim was to validate CIPA in an intact and freely behaving animal. These dogs were alert and received no drugs except atropine. We did not measure blood pressure, afterload, or the circulating levels of norepinephrine. No attempt was made to assess sympathetic nervous activity in the peripheral vasculature. All these deliberate omissions mean that although we can speculate as to why the dose-response curve of the CIPA has the shape that it does, it is difficult to explain that shape with confidence. Nevertheless, it is clear that this index is obliterated in all these animals when a sufficient dose of atropine is administered, and this observation contrasts with the behavior of the alternative indexes that we have assessed; for that reason we can be confident that CIPA is a more specific measure of cardiac parasympathetic tone than these alternative measures.

There is great interest in the measurement of cardiac vagal tone in clinical practice to facilitate the recognition of cardiac failure and the identification of patients at risk of early mortality after myocardial infarction (24, 27, 30, 37). It is also increasingly recognized that many cardioactive drugs such as the angiotensin-converting enzyme inhibitors and digitalis glycosides affect the autonomic nervous system and that these effects may be crucial to the overall therapeutic utility of these drugs (26, 31). Noninvasive real-time monitoring of cardiac vagal tone with use of the technique reported here is rapidly and easily performed. It promises to offer an important new method for the evaluation of the cardiovascular system in humans and animals and is of utility to the physiologist or physician who wishes to study dynamic autonomic states.
APPENDIX

Further details of the generation of the CIPA by the NeuroScope. The apparatus and methods were developed by P. O. O. J ulu and are described fully in International Patent Application PCT GB97 03202 (19a). Briefly, a high-resolution time domain of the R-R intervals is integrated using a time constant of 2 s. The output from the integrator is fed in parallel into a high-pass filter with a cutoff frequency of 0.1 Hz and a low-pass filter of the same cutoff frequency. The output of the low-pass filter drives a voltage-controlled oscillator (VCO) with a linear negative gradient; the output of the high-pass filter is further integrated and then allowed to drive a second VCO with a positive linear gradient. The outputs of the two VCOs are fed into a phase detector, which produces voltages proportional to the phase differences in its inputs.

The system filters out slowly varying or nonvarying heart periods as follows: a very low nonvarying heart rate, e.g., 30 beats/min (R-R interval = 2,000 ms), will generate nearly equal outputs, ~0.6 V in the high- and low-pass filters. This will cause the two VCOs to generate output pulses at the same frequency, and the output of the phase detector will be zero, indicating no cardiac parasympathetic tone. A very high nonvarying heart rate, e.g., 200 beats/min (R-R interval = 300 ms), if integrated using the time constant of 2 s, will generate a high-voltage output in the low-pass filter, close to the maximum of 1 V, and a very-low-voltage output in the high-pass filter, close to 0 V. Because the low-pass filter drives a VCO with a negative gradient, the high voltage will cause it to generate low-frequency pulses that are fed into the phase detector. Likewise, because the high-pass filter drives a VCO with a positive gradient, the low voltage will cause it to generate low-frequency pulses similar to those from the other VCO. The output of the phase detector will be zero, indicating no cardiac parasympathetic tone. This system is therefore independent of the heart rate for the detection of cardiac parasympathetic tone.

Pulse-synchronized phase shifts in the heart periods will generate high-voltage outputs from the high-pass filter and a varying output from the low-pass filter. These in turn will generate variable frequencies in the two VCOs. Therefore, the output of the phase detector will be a time-dependent voltage determined by the phase differences in the outputs of the VCOs at the end of each heartbeat to indicate the detectable effects of cardiac parasympathetic (vagal) tone on the heart. Pulse-synchronized phase shifts in the heart periods are independent of respiration. In all, the arrangement is independent of heart rate and respiration for the detection of cardiac vagal tone.

The CIPA is measured using an arbitrary scale. The parasympathetic control of the heart measurable by any index can be defined as the difference in magnitudes of that index before and after the elimination of all vagal effects on the heart, i.e., the response to full atropinization (18). Mean basal CIPA in a sample of supine resting human volunteers was arbitrarily set at 10 units. The units were derived by dividing the measurable effects of cardiac vagal activity into 10 equal parts, thus giving a linear scale with an absolute zero reference point. It is not implied that the CIPA units are linearly related to the cardiovagal nervous discharges.

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