Cardiac vagal efferent activity and heart period in the carotid sinus reflex

PETER G. KATONA, JAMES W. POITRAS, G. OCTO BARNETT, AND BRIAN S. TERRY

Laboratory of Computer Science, Massachusetts General Hospital, Boston 02114, and Research Laboratory of Electronics, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139


—The activity of single and multiple cardiac vagal efferent (CVE) fibers was recorded in anesthetized dogs under a variety of conditions. During the inspiratory phase of natural respiration the activity of CVE fibers always stopped or greatly decreased regardless of the value of blood pressure. When spontaneous respiratory activity was suppressed by producing a slight hyperventilation during artificial respiration, the following attributes of variation of CVE activity with blood pressure were observed: a) an increase in blood pressure increased vagal activity, whereas a decrease in pressure caused the vagal activity to decrease, b) variations in blood pressure affected CVE firing with a delay that depended on the blood pressure, c) sudden changes in baroreceptor activity were reflected as more gradual changes in CVE firing frequency, and d) under certain experimental conditions CVE fibers were inactive even though the baroreceptors were firing. A simple dynamic model, consisting of an ideal delay and an integrator with a single time constant, successfully described moderate changes in heart period due to changes in vagal activity. This model should aid the quantitative characterization of the dynamics of the cardiovascular control system. Techniques similar to those that were successfully used to record CVE activity in the dog seldom yielded positive results in the cat.

Although it has been known for several decades that electrical stimulation of the vagus nerve decreases heart rate, there have been only a few results published which describe the activity of single cardiac vagal efferent (CVE) fibers. Jewett (6) recorded from a large number of single efferent fibers in the cervical vagus of the dog, and he grouped the patterns of activity into seven categories. Since one of these groups contained fibers that responded to respiration and blood pressure changes in a manner similar to fibers in branches of the cardiac vagus nerve, and since there was also a strong correlation between the neural firing frequency and the duration of heart beats, he suggested that these fibers were cardioinhibitory. Iriuchijima and Kumada (4, 5) demonstrated that stimulation of the carotid sinus nerve of the dog could induce impulses in the cardiac vagal branches after a delay of approximately 60 msec. They attributed the magnitude and variability of this delay to a synaptic pathway for the reflex. They then used carotid sinus nerve stimulation to identify fibers in the cervical vagus, and the nerves so identified as CVE fibers had similar characteristics to those identified as cardioinhibitory by Jewett. Calaresu and Pearce (1), however, failed in their attempt to consistently record CVE activity in the vagal trunk of the cat, and they attributed the apparent paucity of CVE fibers to a species difference between the cat and the dog.

This study was undertaken to confirm and extend the findings relating to CVE activity in these reports. Particular emphasis was placed on the description of changes in CVE firing during changes in blood pressure and on the quantitative characterization of changes in heart rate due to changes in vagal activity. Such a characterization is a necessary step toward a quantitative understanding of the cardiovascular control mechanism.

METHODS

Mongrel dogs of both sexes weighing approximately 10 kg were preanesthetized with phencyclidine hydrochloride (Sernylan, 0.1 mg/kg). Surgical anesthesia was obtained by injecting alpha-chloralose (100 mg/kg) through a lateral saphenous venous catheter. Subsequent doses of chloralose were given to maintain the level of anesthesia desired. A balloon-tipped catheter was introduced into the abdominal aorta through one of the femoral arteries, and highly reproducible transient blood pressure disturbances were produced by electronically controlling the times within a cardiac cycle when the balloon was inflated and deflated. Occasionally levarenol bitartrate (Levophed) or trimethaphan camphorsulfonate (Arfonad) was used to produce more gradual changes in blood pressure. Arterial pressure was recorded from the brachial artery by a catheter connected to a Statham P23Db strain-gauge manometer. The trachea was cannulated and during parts of each experiment the animal was artificially ventilated at about 25 strokes/min. Respiration was monitored by recording the temperature changes of a thermistor placed in the tracheal cannula.

A 3- to 6-cm length of the common carotid artery and right cervical vagus was exposed following a mid-line incision along the neck. In order to avoid desiccation of the nerve, warm mineral oil was poured into the pool formed by the trachea and the flap of the skin on the right side of the incision. A dental mirror with a black glass insert was slipped under the nerve trunk and secured to the table. The de-sheathing and dissection of the nerve were performed on this
stable platform. The vagus was removed from the trunk by making a longitudinal cut through the sheath and then gently lifting or rolling the nerve out of the sheathing. It was unnecessary to separate the vagal trunk from the common carotid artery. Care had to be exercised to cut the innermost layer of sheath without damaging the nerve bundle. It was essential that bleeding caused by the desheathing be completely stopped by ligation or cauterization.

Initially, locating cardioinhibitory fibers in the nerve trunk was a long and tedious procedure. Our early experiments confirmed the observations of Jewett (6) that the CVE fibers were usually confined to a small cross-sectional area of the vagus and that they were likely to be found in the vicinity of fibers which fired with large action potentials during inspiration and showed no activity at other times (type III of Jewett). In order to utilize these findings, spontaneous respiration was maintained throughout the dissection procedure, and the anesthesia was kept at such a level that the average heart rate was less than 2 beats/sec and a marked respiratory sinus arrhythmia was present. Occasionally small doses of morphine were given to insure at least moderate vagal tone.

To facilitate locating a portion of the vagus that contained CVE fibers, one-fifth of the vagus was separated from the rest of the nerve trunk and cut. If there was little or no change in heart rate and there was no decrease in sinus arrhythmia, another fifth of the vagus was separated and cut. If, on cutting a portion of the nerve, a change in heart rate and/or respiratory arrhythmia was observed, it was tentatively concluded that this fifth contained CVE fibers, and it was then further dissected into three to five smaller bundles. One at a time, these bundles were placed on the electrodes to observe if either the decreased firing during inspiration typical of CVE fibers or the more easily seen large amplitude fibers that fire during inspiration were present. If there was a sign of either of these two types of activity, the particular bundle was further subdivided and the procedure repeated until it was possible to identify a small portion of the vagus which definitely showed CVE activity. At this point single-fiber preparations were obtained either by further subdivision or by peeling off small filaments from the surface of the bundle.

Before a vagal efferent fiber was considered to be cardioinhibitory, a careful examination of its properties was carried out. In agreement with Jewett (6), the following criteria were used to identify CVE fibers: a) conspicuous decrease in firing frequency during spontaneous inspiration, b) increase in activity when the blood pressure rose and decrease in activity when the pressure fell, and c) strong correlation between heart period and vagal activity. This last criterion is based on the assumption that enough CVE fibers, including the entire left vagus, were left intact to affect heart rate.

The nerve signal was recorded from the dissected nerve bundles using a pair of platinum-iridium electrodes and a Tektronix 122 preamplifier. The signal was further amplified and then band-pass filtered with a low-frequency cutoff around 30 cycles/sec and a high-frequency cutoff of 3,500–6,000 cycles/sec. It was often possible to record from a nerve for more than an hour, but extreme care had to be exercised to keep the electrodes and the surrounding oil free of blood.

The nerve signal, blood pressure, respiration, and electrocardiogram were recorded on magnetic tape for further analysis with the aid of a digital computer.

A procedure similar to the one described above was also used in eight cats in an attempt to record CVE activity. These animals usually exhibited a weak vagal tone as indicated by the small changes in heart rate when cutting the vagus nerve, the lack of conspicuous respiratory heart rate variations, and the smaller, slower heart rate responses to transient pressure increases than found in the dog. Thus in the experiments involving cats, it was usually necessary to dissect the entire vagus nerve without a reliable preliminary indication as to the location of CVE fibers within the bundle. Since the low vagal tone also indicates that few active CVE fibers were present, recording of CVE activity in the cat was tedious and difficult.

RESULTS

In 18 dogs CVE activity was recorded from a total of 17 multifiber preparations and 21 single fibers. Possible CVE activity was recorded only from two cats in a total of three nerve preparations. Sections A through D describe findings in the dog, whereas section E gives an account of observations made in the cat.

A) Effect of respiration. Natural respiration had a consistent and profound effect on cardiac efferent activity. In all experiments CVE firing greatly diminished or completely stopped during natural inspiration, and the conspicuous decrease in firing was present even when the inspiration coincided with a sharp increase in blood pressure. Figure 1 illustrates the markedly different vagal activity during two brief transient increases in pressure that were generated during different phases of the respiratory cycle.

When the lungs were artificially ventilated so that there was no spontaneous respiratory activity, the conspicuous modulation of vagal activity by respiration was absent. Small periodic changes in nerve firing that were still present under these conditions could be accounted for by the fluctuations in blood pressure that were caused by chest movements. However, any attempt by the animal to breathe spontaneously was accompanied by an abrupt decrease in CVE activity. The relationship between respiration and the disappearance of CVE firing could be especially well observed in a preparation in which CVE activity was simultaneously recorded with the activity of a single respiratory fiber. As shown in Fig. 2, the cardiac vagal fiber ceased firing 0.5 sec before the onset of activity of the respiratory fiber, and it resumed its activity just before the inspiratory fiber stopped firing.

Unless otherwise specified, the animals were artificially ventilated at a high rate and low volume to produce a slight hyperventilation. This prevented any attempts at spontaneous respiration, and thus also prevented the respiratory modulation from masking changes in CVE activity caused by blood pressure variations.

B) Firing pattern at steady pressure levels. In all of the experiments, the firing frequency of active CVE fibers increased as the pressure level was raised, and decreased as the pressure
Figure 3 displays the firing pattern of a CVE fiber for two different mean pressure levels and illustrates that large cycle-to-cycle variations existed in CVE firing times even though the pressure was periodic with little cycle-to-cycle variations. In order to determine the average distribution of firing times over several cardiac cycles, each cycle was divided into successive short (typically 8–16 msec) intervals, and the total number of firings that occurred in each of these intervals was counted with the aid of a digital computer. Synchronization of the cardiac cycles was derived from the QRS complex of the electrocardiogram, and care was exercised to choose stretches of data in which the heart rate was relatively constant. (The animals were artificially respirated throughout this procedure.) The histograms that were thus obtained give a measure of the probability that the CVE fiber fires in a particular portion of the cardiac cycle.

Such histograms generally exhibited a dominant peak in a time interval starting 60–80 msec after the beginning of the systolic pressure rise, but delay times as low as 55 msec and as high as 140 msec were also observed. In half of the records a secondary peak was also discernible, which started 80–200 msec after the dicrotic pressure rise in those cases where the peak was conspicuous enough for this delay to be measured. Figure 4 shows the histograms obtained for the data of Fig. 3, and it illustrates the case in which both peaks were clearly visible.

In an attempt to characterize the distribution of single-fiber nerve firings more completely, histograms were also obtained which selectively display the occurrence of the first, second, third, or any other firing of the nerve in each heart beat. Such a group of histograms is shown in Fig. 5 for the first experiment illustrated in Figs. 3 and 4. These histo-
grams show that although the maximum number of firings in any cycle is four, the first peak contains a burst of three firings only rarely, and in most cases it is made up of first and second firings. The secondary peak mainly consists of second and third firings. Similar histograms for the second experiment illustrated in Figs. 3 and 4 show that normally there are at least three firings in the dominant peak. However, in experiments in which the firing is sparse, the first and usually only firing in a cycle may occur in the latter half of the cardiac cycle. These observations confirm that although vagal activity has a tendency to concentrate in portions of the cardiac cycle, the details of the firing vary considerably from one cycle to the next.

C) Vagal firing during fast pressure changes. Regardless of whether the blood pressure was changed by drugs or by inflating and deflating a balloon in the abdominal aorta, vagal firing frequency varied directly with the pressure. Figure 6 shows the firing pattern of a typical CVE fiber during a sudden rise and fall of blood pressure which was introduced by the inflation and subsequent deflation of the balloon. The response to the rise in pressure level following the inflation of the balloon was evident within 100 msec, but there was a delay of approximately 300 msec before the onset of the response to the rapidly falling pressure after the deflation of the balloon. Firing continued for almost a full beat after the precipitous drop in the pressure. In a few animals, vagal fibers had such a high threshold under the conditions of the experiment that they fired only when the pressure was elevated.

D) Cardiac vagal efferents and heart period. The heart period was closely related to the activity of CVE fibers, and as long as only moderate and relatively rapid fluctuations in the pressure were considered, the heart period could usually be predicted from CVE activity even while the sympathetic nerves were intact. To obtain the predicted heart period throughout the experiment, a Schmitt trigger, activated by the CVE nerve spikes, was used to generate a train of uniform pulses which were then fed into an RC integrator with a time constant of 1.0–2.5 sec. The duration of the pulses was manually adjusted to be as wide as possible without causing an overlap of successive pulses. The sum of a d-c voltage and the suitable scaled output of the RC integrator was the simulated heart period. Figure 7 shows the actual and simulated heart periods for the portion of an experiment where the variations in heart rate were caused both by respiratory arrhythmia and balloon inflation in a spontaneously breathing dog.

In six animals sympathetic effects on heart period were blocked by using propranolol as described by Ledsome et al. (13). Complete sympathetic blockage was demonstrated by noting the lack of any heart rate response to large changes in blood pressure following bilateral vagotomy in three of these experiments. In order to evaluate the relationship between vagal activity and heart period both before and after propranolol, a digital computer was used to optimize the model parameters. In the digital simulation a delay on the order of 150 msec was also introduced to account for the time necessary for the vagal impulses to influence heart rate. The resulting model is shown in Fig. 8.

In five of these six animals the heart period response remained similar to the response prior to the injection of propranolol, and the decrease in the steady heart rate was accompanied only by a change in the general scale factor of the system. In four cases the effectiveness with which a change in vagal firing frequency influenced heart period was reduced by an average of 20% (ranging from 17 to 23% between animals), whereas in only one animal did this

![Figure 5](http://ajplegacy.physiology.org/)

**Fig. 5.** Histograms showing distribution of first through 5th CVE nerve impulses in 100 consecutive cardiac cycles for experiment illustrated in left half of Figs. 3 and 4. For example, second graph from bottom indicates that in 88 of 100 cycles there were at least 2 nerve firings, that second firing occurred in first and last thirds of cardiac cycle with approximately equal probability, and that probability of a second firing occurring in middle third of cycle was small.

![Figure 6](http://ajplegacy.physiology.org/)

**Fig. 6.** CVE nerve firing during a sudden rise and subsequent fall of blood pressure. Upper trace: blood pressure; lower trace: single-fiber CVE activity. Nerve firing increased almost immediately when pressure was raised, but it decreased more slowly when pressure dropped.
effectiveness appear to increase. In this animal the effect of propranolol was the smallest observed (the steady heart period increased by only 46 msec compared with an average of 118 msec for the other four animals), and the appearance of intermittent A-V conduction blocks prevented reliable estimation of the increase in the scale factor. In one of the four typical experiments in which the scale factor decreased, Fig. 9 shows the actual and predicted heart periods for two sets of experiments both before and after propranolol. Regardless of the type of heart period disturbance, the model of Fig. 8 described the experimentally measured heart period changes.

The results of the experiment in which disabling the sympathetic nerves significantly changed the heart period response is illustrated in Fig. 10. It shows that before propranolol there was a substantial drop of heart period below its control level following the deflation of the balloon, whereas this undershoot disappeared after propranolol. Since the steady level of vagal firing rate was essentially zero and it could not decrease below this value, the model of Fig. 8 was not suitable to characterize the system before abolishing the heart period changes caused by the sympathetic nerves.

If the cardiac cycle was much longer than its control value, the model tended to underestimate the actual heart period. Under these conditions small increases in pressure occasionally resulted in disproportionate large increases in the heart period, causing sudden changes in the duration of heart beats. In most experiments in which the vagal activity was recorded (and thus one vagus was at least partially cut) the sudden appearance of abnormally long beats was due to atrioventricular conduction block, while experiments in which both vagi were intact more often showed skipped beats of sinoatrial origin. In many experiments there was a certain position of the balloon in the aorta such that in successive identical tests a single skipped beat developed only occasionally when the balloon was inflated. When larger pressure rises during inflation were produced by moving the balloon further up the aorta, sinoatrial suppression developed consistently, but it disappeared when the balloon was moved downstream from its original position. Skipped beats could also be generated by increasing the blood pressure by Levophed.

In order to compare the simple model of Fig. 8 with the nonlinear model proposed by Warner and Cox (14), the latter model was also simulated on the digital computer. Although in its original form the Warner-Cox model did not include the pure delay shown in Fig. 8, we incorporated this refinement into that model to facilitate the comparison. As described in the APPENDIX, best results with the nonlinear model were achieved when the model parameters were adjusted to make the nonlinearity negligible. With this choice of parameters the two models became identical.

**E) Cardiac vagal efferent activity in the cat.** Although vagal efferent activity was recorded from several dozens of single and multifiber preparations in the cat, only three of these nerve preparations were possibly cardioinhibitory. Two multifiber preparations showed activity similar to those of CVE fibers in the dog, but the activity in a single-fiber preparation had an unusually strong concentration of one or two
substantial evidence indicates that the activity studied on dogs similar to those used by Jewett (6) and by Iriuchijima and Kumada (CVE fibers). The criteria to identify CVE fibers were region and from subsequent rise in blood pressure resulted in a decreased heart period while the recordings were made. Consequently, the cardioinhibitory nature of this unusual fiber could not be demonstrated.

**DISCUSSION**

Although there is no direct and practical way of verifying whether or not a particular afferent fiber in the cervical vagus is involved in the control of heart rate, strong circumstantial evidence indicates that the activity studied on dogs in these experiments is characteristic of cardiac vagal efferent (CVE) fibers. The criteria to identify CVE fibers were similar to those used by Jewett (6) and by Iriuchijima and Kumada (4, 5), who recorded activity both from the cervical region and from the cardiac branches of the vagus nerve of the dog. Jewett classified those fibers in the cervical vagus that he considered to be cardioinhibitory as type I, while another group of fibers, showing a lower firing frequency but otherwise having similar characteristics to those in type I, was classified as type II. Since the degree of activity of potential CVE fibers can be easily changed by changing the blood pressure and/or level of anesthesia, we considered both of these types to reflect cardioinhibitory activity.

The properties of these fibers are in agreement with known physiologic data involving vagal inhibition of heart rate. Most significant is that, for moderate changes in heart rate, the simple dynamic model of Fig. 6 can describe the effects of vagal firing on heart period whether they are caused by spontaneous respiration or by blood pressure changes in the artificially ventilated animal. The finding that the activity of a cut single fiber can account for the dynamics of heart rate changes mediated by the remaining intact fibers indicates that the recorded activity is typical of cardiac vagal efferents. The model used for the simulation is identical to one that has been used to describe changes of heart period caused by the stimulation of the peripheral end of the cut vagus nerve by electrical impulses (8).

Warner and Cox (14) presented a nonlinear mathematical model to describe heart period from the frequency of electrical impulses applied to the cut cervical vagus of the dog anesthetized with pentobarbital. They tested their model over a wide range of heart period, and the results obtained for heart periods between 1 and 2 sec were critical to their justification of the model. These heart periods are longer than those normally encountered in the intact chloralose-anesthetized dog; in our experiments such periods were usually observed only when sinoatrial block (or sinus suppression) or A-V block developed. Under such conditions neither the Warner-Cox nonlinear model nor the presented linear model could describe the system.

When artificial vagal stimulation caused moderate changes in heart period, the model of Warner and Cox gave acceptable results only when the nonlinearity was neglected (8); this same conclusion has now been obtained for natural vagal impulses. This result implies, in terms of the assumptions of the nonlinear model, that acetylcholine at the nerve endings is liberated from an essentially infinite supply and that the only rate process involved is the hydrolysis of the free acetylcholine. The resulting description of the system is identical to the simple linear model presented here. The possibility of characterizing the heart period response to vagal stimulation by a single time constant has been considered by Clynes (2). He raised this possibility in a description of respiratory arrhythmia in human subjects, where the experiment did not involve recording from or stimulating the vagus nerve.

Although for moderate heart periods the relationship between changes in vagal firing and changes in heart period could be reasonably well described by a linear dynamic model, the dependence of vagal activity on blood pressure was more complicated. In general, an increase in blood pressure increased vagal firing, and a decrease in pressure decreased vagal activity. During the inspiratory phase of spontaneous respiration, however, the vagal firing disappeared or greatly diminished regardless of the level of blood pressure. This finding is in agreement with the observations of Koepchen et al. (11), who reported that single electric impulses applied to the carotid sinus nerve of the dog reduced heart rate only when the stimulation did not coincide with inspiration, and Iriuchijima and Kumada (5), who showed that stimulation of the carotid sinus nerve did not cause CVE firing during inspiration. Since in all our experiments cardiac vagal efferent activity accounted for most of the variations in heart rate, respiratory arrhythmia was moderated predominantly by the vagus nerves.

Another reason why vagal activity did not always vary directly with blood pressure was the lack of vagal firing under some experimental conditions. CVE activity was
occasionally absent even at normal blood pressures, whereas at lower pressures it almost always disappeared. When the blood pressure was high and there existed a considerable vagal tone, the heart period was controlled predominantly by the vagus nerves. This accounts for the lack of significant difference in accuracy with which the model of Fig. 8 described rapid changes in heart period before and after propranolol as long as vagal activity was present. When, as in Fig. 10, there was no vagal activity at the control level of heart period, a decrease in heart period below this level was caused by sympathetic activity which was abolished by propranolol. These results are in essential agreement with those reported by Glick and Braunwald (3) on the basis of selectively blocking the sympathetic and parasympathetic systems by drugs, but it appears that the threshold of heart rate which separates the regions in which either sympathetic or parasympathetic effects dominate does not necessarily coincide with the control level of heart rate. The cessation of vagal activity at low or rapidly falling pressure levels is a factor which can cause the reflex change of heart period to be slower when the pressure falls than when it rises (7).

The first and dominant peak in the histogram describing the average distribution of CVE firing within the cardiac cycle can be attributed to the burst of activity of baroreceptors during the systolic rise of blood pressure (6). Since the dicrotic pressure rise is smaller and slower than the pressure increase during systole, the often observed secondary peak in the histogram can possibly be attributed to increased baroreceptor activity during the dicrotic pressure rise in spite of the finding that the delay between the beginning of the dicrotic pressure rise and the diastolic peak in the histogram is usually longer than the delay between the beginning of systolic pressure rise and the dominant peak in the histogram. By introducing sharp pressure pulses in the isolated carotid sinus of the dog, Koepchen et al. (10) showed that the faster the pressure pulse increased, the sooner its slowing effect on the heart could be observed. He also noticed that known characteristics (12) of baroreceptors could at least partially account for this effect.

The delay with which the effect of a single electric impulse applied to the carotid sinus nerve appears in the cardioinhibitory fibers of the cervical vagus was directly measured to be between 50 and 100 msec by Iriuchijima and Kumada (5). This result is in general agreement with our data showing a delay of 55–140 msec between the beginning of the systolic pressure rise and the onset of increased firing in the cervical vagus.

Although our preparation, using disturbances in the natural pressure waveform as input, is not suitable for the accurate determination of the delay before a sudden drop in blood pressure decreases CVE activity, the obtained data reveals an asymmetrical response of CVE activity to rising and falling blood pressures. It often appears that an increasing pressure level significantly increases CVE firing sooner than a similar drop in the level of pressure can initiate a significant decrease in CVE activity. The vagal firing that is occasionally sustained for a few seconds after a sudden fall in pressure level must be due to the characteristics of the central portion of the reflex, since baroreceptors respond both to the level and rate of change of the pressure, and thus their minimum rate of firing occurs immediately after the pressure drop. The central mechanism responsible for the sustaining of vagal activity after a pressure drop is probably the same as the one which causes the effect of the sharp bursts in baroreceptor firing to appear as delayed and widened peaks in CVE activity.

The times at which individual CVE nerve firings occur show considerable variability. Since it was shown (9) that there is little random variation in the firing of baroreceptors from one cycle to the next as long as the blood pressure is periodic, the large fluctuations that were observed in CVE firing times must originate in the vasomotor area of the medulla. Neuronal inputs from other brain structures and fromafferent nerves other than baroreceptors may also contribute to the lack of reproducibility of CVE firing patterns.

Experiments that we performed on cats using techniques similar to those that were successful when applied to dogs indicate that the failure of Calaresu and Pearce (1) to consistently obtain recordings of CVE activity in the cat was indeed due to a species difference between the cat and the dog. Vagal influence on the heart rate of the cat is considerably weaker than it is in the dog, and this makes the locating and identifying of cardioinhibitory fibers extremely difficult. Contrary to the findings of Calaresu and Pearce, however, is our observation that the activity characteristic of CVE fibers showed cardiac synchronization in the cat. This synchronization was not always readily apparent, and its presence had to be verified by averaging techniques.

**APPENDIX**

Warner and Cox (14) described the effect of artificially generated vagal impulses on the heart period by the following set of equations:

\[
\begin{align*}
\frac{dN(t)}{dt} &= k_1 [N_m - N(t)] - k_s N(t)V(t) & (1a) \\
\frac{dC(t)}{dt} &= k_2 N(t)V(t) - k_5 C(t) & (1b) \\
Y(t) &= \begin{cases} 
T_a + k_3 C(t) & \text{for } C(t) \leq C_a \\
\infty & \text{for } C(t) > C_a 
\end{cases} & (1c)
\end{align*}
\]

where \(V(t)\) is the frequency of vagal stimulation; \(N(t)\) is the number of acetylcholine-filled vesicles at the vagal nerve endings; \(C(t)\) is the concentration of ACh at the active site at the pacemaker; \(Y(t)\) is the heart period; \(N_m\) is the maximum number of charged vesicles at the nerve endings; \(T_a\) is the heart period without vagal stimulation; and \(k_1, k_2, k_3, k_4, k_5, C_a, C\) are constants. In essence, this model assumes that the acetylcholine is liberated at a rate proportional both to the vagal firing frequency and to the available supply of the transmitter, that the supply of acetylcholine is replenished at a rate determined by the degree of its depletion, and that the free acetylcholine is hydrolyzed at a rate proportional to its concentration.

In a detailed study of these equations, we treated each CVE action potential individually, and considered the firing frequency to be a train of unit impulses

\[
V(t) = \sum_{k=1}^{k} u_k(t - t_k - T_d) \quad k = 1, 2, \ldots
\]

where \(t_k\) are the times at which the observed nerve firings occurred, \(T_d\) is the time delay necessary for the cervical vagal impulses to affect the pacemaker area, and \(u_k(t)\) denotes the unit impulse at \(t = 0\). This is equivalent to stating that the action of each nerve impulse is determined by the physical process underlying equations 1a, 1b, and 1c. When the firing frequency was determined by counting the number of CVE impulses in consecutive time intervals, the obtained results were similar to those obtained when the nerve impulses were considered individually.
Using the above $V(t)$ for firing frequency, the set of equations 1a, 1b, and 1c were solved analytically. Assuming the knowledge of $N(t)$ and $C(t)$ just before the first nerve action potential, expressions were obtained for the values of these functions immediately after the nerve firing, and from these quantities $N(t)$ and $C(t)$ were computed at the instant just before the second nerve firing. Repeating the procedure for each nerve impulse, $N(t)$ and $C(t)$ were computed for the duration of the entire experimental record. The initial values of $N(t)$ and $C(t)$ were estimated from the model parameters and from the average time interval between CVE firings during steady-state conditions.

In order to carry out the numeric computation, it is advantageous to reduce the number of parameters to a minimum, and to choose these parameters in such a manner that at least their order of magnitude can be easily estimated on the basis of physical principles or experimental data. For this purpose, $N_m$, $k_1$, and $k_4$ were replaced by an overall scale factor, and $k_3$ was estimated by first assuming the quantity $R = N_m/N$. Since this is the ratio of the number of ACh-filled vesicles in steady state ($N_m$) to the total number of vesicles ($N$), its value is always between zero and one. The model parameters $k_1$ and $k_4$ are rate constants (the reciprocal of which are time constants) so that the range of their possible values could be relatively easily estimated.

A computer program was written to simulate the above model and to determine the parameters giving the best possible correspondence between the experimental and simulated heart periods. The error was measured by summing the squared errors between the actual and simulated heart periods for each heart beat in an experimental run, typically lasting for about 100 heart beats. It was found that under all studied experimental conditions the model gives the best results when $R = 1$. This implies that $N_m = N$, and that in the steady state the nerve impulses liberate ACh from an essentially undepleted supply of the transmitter. For moderate changes in vagal activity $N(t)$ then stays at approximately $N_m$, and equation 1b becomes

$$\frac{dC(t)}{dt} = k_N N(t) V(t) - k_C(t)$$

This is a first-order differential equation with constant coefficients, and the system it describes is identical to the one shown in the second block of Fig. 8 with $k_4 = 1/r$ and with a properly chosen scale factor.

This work was supported, in part, by the Joint Services Electronics Program under Contract DA29-049-AMC-02536 (E), the National Institutes of Health Research Grant HE 10359-03, and the American Heart Association Research Grant 62 F40 EG.

Present address of P. G. Katona: Biomedical Engineering Department, Wickenden Building, Case Western Reserve University, Cleveland, Ohio 44106.

Received for publication 9 June 1969.

REFERENCES