A Two-Compartment Model Describing the Release and Negative Inotropic Action of Acetylcholine on the Heart

By Max E. Valentinuzzi, Hebbel E. Hoff, and Leslie A. Geddes

ABSTRACT

Single-pulse stimulation of the vagus nerve in turtles (*Pseudymys floridana*) produced the well-known negative inotropic effect on the atria. After stimulation, the peak amplitudes of the atrial contractions exhibited an envelope which appeared to be proportional to the concentration of acetylcholine at the receptor sites. Postulating a quantized release of acetylcholine with each stimulus, the envelope was modeled by a two-equal-compartment configuration. In about 63% of the cases this envelope behaved as if it described a critically damped system, in 18% of the cases it behaved as if the system were slightly overdamped, and in the remaining 19% it corresponded to a lightly underdamped system. The average washout time constant per compartment calculated from the single-pulse response was $4.7 \pm 0.8$ seconds (SD). Repetitive stimulation was provisionally equated with a constant release of acetylcholine; its mathematical description was derived from the time integral of the single-pulse case, i.e., from a direct application of indicator-dilution theory. However, the average time constant obtained from this second type of fit was only $0.84 \pm 0.4$ seconds. This lower value was interpreted as a dynamic modification (perhaps nonlinear) due to accumulation of acetylcholine and probably associated with an increase in acetylcholine esterase activity.

KEY WORDS

*Pseudymys floridana* vagus nerve time constant single-pulse stimulation repetitive stimulation atrium force of contraction diffusion of cholinergic mediators appearance time build-up time

The atrial negative inotropic effect of vagal stimulation was probably first described by Gaskell (1) in experiments on the tortoise, and McWilliam (2) subsequently made a similar observation in the mammalian heart. This phenomenon was qualitatively and quantitatively studied by Gilson (3) in turtles following the application of a single electrical shock to the vagus nerve. De Geest et al. (4) showed negative inotropic effects of the vagus nerve on the canine ventricle, and Priola and Fulton (5) described positive and negative inotropic responses of both atria and ventricles to vagosympathetic stimulation in the isovolumic canine heart. In dog papillary muscle, Endoh et al. (6) described a negative inotropic response after the administration of small amounts of acetylcholine followed by a positive response. Other drugs have purely positive inotropic effects as demonstrated by Toda (7) with ouabain and norepinephrine in isolated rabbit atria. However, the relationship between the developed tension and the rate of contraction was affected differently by these two substances.

The present study attempted to analyze the negative inotropic response to vagal excitation by using a two-compartment model that assumed dilution of the chemical mediator. The mathematical nomenclature used throughout this paper is that recommended by a symposium on indicator-dilution techniques (8).

Methods

The atrial endings of the vagus nerve are not as clearly delineated as are other nerve terminals. Electron microscope studies (9) have indicated fairly well the discontinuity between the neural and the cardiac sides. Vagal action potentials release acetylcholine which, after traversing the cleft, hyperpolarizes the atrial membrane; this whole process is probably similar to that occurring at synapses or myoneural junctions (10, 11). Figure 1 depicts a simplified compartmental model in which an electrochemical transducer T1 accounts for the action of depolarization of the nerve endings on the...
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**Figure 1**

Simplified compartmental model of the vagal endings-atrial membrane system. VAP = vagal action potential, T1 = electrochemical transducer on nerve side, L-ACH = liberation of acetylcholine on nerve side, I = intracellular fluid (first compartment), P-ACH = passage of acetylcholine through nerve membrane, II = extracellular fluid in cleft (second compartment), C-ACH = concentration of acetylcholine in extracellular fluid facing the atrial membrane, T2 = chemomechanical transducer with coefficient \( k_t \) or \( K_t \) (see text), and MAC = modification of atrial contraction (reduced force).

Acetylcholine vesicles (release of substance). Compartment I corresponds to the intracellular fluid of the nerve side, compartment II represents the extracellular fluid of the cleft, and some chemomechanical transducer (T2) translates the effect of the chemical mediator on the atrial contraction. In this study, it was assumed that the two compartments were equal and that acetylcholine was diluted as it traversed the system from the site of release to the extracellular fluid facing the atrial membrane. The first assumption is reasonable as a first approximation and has the advantage of an easier mathematical analysis. In any event, a two unequal-compartment model can always be reduced to a two-equal-compartment equivalent.

**Experimental Technique**

Seven turtles (Pseudemys floridana) were used in these experiments. The turtles were pithed, and the two vagi were exposed in the neck. A suitable circular hole was made in the plastron to gain access to the heart. Left atrial and ventricular myograms were recorded with photoelectric transducers on a six-channel rectilinear Physiograph. The cardiac electrical signal was recorded because they helped in the identification and some cases, from the atria to obtain a clearer P wave. Derived either from a direct atrioventricular lead or, in some cases, from the atria to obtain a clearer P wave.

Although the electrogram and the ventricular myogram were not absolutely necessary for this study, they were recorded because they helped in the identification and the time correlation of the events. The atrial inotropic response was elicited either by a single shock or by repetitive vagal stimulation (left or right) using rectangular 2-msec pulses. The amplitude (and repetition frequency in the second case) were adjusted so that minimal or no chronotropic effect was elicited. A total of 48 single- and 16 repetitive-pulse responses were recorded. Table 1 gives the number of trials per turtle.

**Single-pulse Vagal Stimulation**

A single stimulus triggers a volley of impulses (12) which in turn produces the release of acetylcholine in accordance with the quantal concept of neural transmitters (13). One quantal release of acetylcholine might be a packet of \( 10^3-10^5 \) molecules (13). The transmitter substance appears diluted in the second compartment (Fig. 1) eliciting the atrial negative inotropic effect (1-3). The temporal decrease, \( f(t) \), in force of contraction of the atrium was considered in this model to be proportional to the temporal concentration, \( c(t) \), of acetylcholine facing the atrial membrane:

\[
f(t) = k_1 c(t),
\]

where \( k_1 \) is a constant. The results reported in this study tend to support the assumption of Eq. 1, which is also implicit in the work of Gilson (3). From the theory of indicator-dilution curves (14),

\[
c(t) = k_2 h(t),
\]

where \( k_2 \) is a constant and \( h(t) \) is a function which describes the temporal distribution of particles at the output of the system. Substituting Eq. 2 into Eq. 1 yields

\[
f(t) = k h(t),
\]

where \( k = k_1 k_2 \).

Accepting that there are \( N \) equal compartments in series between the site of release of acetylcholine and the atrial membrane (site of detection), the probability function \( h(t) \) becomes

\[
h(t) = \frac{1}{(N-1)!} \cdot \left( \frac{t}{\tau} \right)^{N-1} \exp \left( -\frac{t}{\tau} \right),
\]

where \( \tau \) is the time constant of each compartment (15, 16). When \( N = 2 \), Eq. 4 simplifies to

\[
h_2(t) = \frac{t}{\tau^2} \exp \left( -\frac{t}{\tau} \right).
\]

Therefore, the atrial negative inotropic effect can be described as

\[
f(t) = k \frac{t}{\tau^2} \exp \left( -\frac{t}{\tau} \right),
\]

where the constants \( k \) and \( \tau \) are treated as parameters to be determined experimentally.

**Table 1**

Average Parameters of the Atrial Negative Inotropic Response to Vagal Stimulation in Turtles

<table>
<thead>
<tr>
<th>Turtle</th>
<th>No. of trials</th>
<th>( t_s ) (sec)</th>
<th>( \overline{t} ) (sec)</th>
<th>No. of trials</th>
<th>( t_s ) (sec)</th>
<th>( \overline{t} ) (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>0.58</td>
<td>4.08</td>
<td>3</td>
<td>0.79</td>
<td>0.98</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>0.58</td>
<td>4.50</td>
<td>3</td>
<td>1.09</td>
<td>1.29</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>0.96</td>
<td>5.07</td>
<td>3</td>
<td>0.77</td>
<td>0.94</td>
</tr>
<tr>
<td>4</td>
<td>14</td>
<td>3.50</td>
<td>4.00</td>
<td>5</td>
<td>0.42</td>
<td>0.56</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>3.13</td>
<td>5.13</td>
<td>2</td>
<td>0.50</td>
<td>0.42</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>0.45</td>
<td>4.00</td>
<td>5</td>
<td>0.42</td>
<td>0.56</td>
</tr>
<tr>
<td>7</td>
<td>15</td>
<td>0.78</td>
<td>4.70</td>
<td>16</td>
<td>0.75</td>
<td>0.84</td>
</tr>
</tbody>
</table>

\( \overline{t}_s \) = average appearance time of the inotropic response, and \( \overline{t} \) = average time constant per compartment.
Numerical Procedure.—Eqs. 3 and 6 are valid for any value of \( t \). In particular, when \( t \) is equal to the build-up time \( (t_{ap}) \), i.e., the time elapsed between the onset of the curve and its peak, the maximum decrease \( (f_p) \) in force of contraction is given by \( f(t_{ap}) \) and the peak \( (h_p) \) of the probability function is, accordingly, \( h(t_{ap}) \), ignoring the existence of the appearance time, \( t_a \), or the latency (Fig. 2). Therefore, the constant \( k \) is

\[
k = f_p/h_p,
\]

where \( f_p \), in centimeters, corresponds to the maximum reduction in amplitude of the atrial myogram (Fig. 2). There is no need to calibrate the myogram in units of force. The build-up time, \( t_{ap} \), is read directly from the record by estimating the onset, \( t_a \), and the peak occurrence, \( t_p \), of the inotropic envelope and taking their difference, i.e., \( t_{ap} = t_p - t_a \). In other words, the build-up time is the distance (in time units) between \( t_a \) and \( t_p \) (Fig. 2). Since \( N \) has been assumed to be equal to 2, the time constant \( \tau \) can be calculated as

\[
\tau = t_{ap} (N - 1).
\]

In this case, \( \tau \) is always equal to \( t_{ap} \) (15). Knowing \( \tau \) and \( t_{ap} \), \( h_p \) is computed with Eq. 5 which in turn leads to the value for \( k \) with Eq. 7. Eq. 6 can then be plotted and compared with the envelope of the single-shock atrial negative inotropic response (Fig. 2, solid circles vs. peaks of atrial contractions in the atrial myogram). This procedure was applied to 48 experimental curves. Since there was some uncertainty in the position of \( t_a \), it was possible to adjust the build-up time, \( t_{ap} \), within certain limits; therefore, more than one fit was sometimes obtained. For this reason, the number of curve fits, 67, is greater than the number of experimental curves, 48.

REPETITIVE VAGAL STIMULATION

A train of stimuli elicits volleys of vagal action potentials which in turn produce a sequence of releases of acetylcholine. In this model, the releases of acetylcholine approximate a continuous outflow of transmitter substance. Thus, we may use the relationship between sudden and constant injections from the theory of indicator-dilution curves (14):

\[
C(t) = K_1 \int_0^t h(\theta) d\theta,
\]

where \( C(t) \) is the concentration of substance at the output of the system, \( K_1 \) is a system constant, \( h(t) \) is the probability function referred to earlier, and \( \theta \) is the integration variable (time). It is assumed, as in Eq. 1, that the decrease, \( F(t) \), in the force of atrial contraction is proportional to \( C(t) \):

\[
F(t) = K \cdot C(t),
\]

which, considering Eq. 9, becomes

\[
F(t) = K \int_0^t h(\theta) d\theta,
\]

where \( K = K_1 K_2 \). To avoid confusion, capital letters were used for the constant-infusion case, and lower case letters were used for the single-injection case.

Using Eq. 5, it is easily verified that

\[
F(t) = K \left[ 1 - \exp \left( -\frac{t}{\tau} \right) - \frac{t}{\tau} \exp \left( -\frac{t}{\tau} \right) \right].
\]

This equation is the function used to fit the envelope of the negative inotropic response to repetitive vagal stimuli (Figs. 3 and 4).

Numerical Procedure.—Eq. 12 can be rewritten as

\[
1 - \frac{F(t)}{K} = \exp \left( -\frac{t}{\tau} \right) \left( 1 + \frac{t}{\tau} \right),
\]

which becomes,

\[
\beta(t) = \exp (-x) \left( 1 + x \right),
\]

where \( \beta(t) = 1 - \frac{F(t)}{K} \) and \( x = t/\tau \). The constant

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Repetitive right vagal stimulation (RVS) produced a sustained, moderate negative inotropic effect on the left atrium (turtle 3). Appearance time, $t_a$, was 1.4 seconds. The values of the decrease in atrial contractions $F(1.3)$ and $F(2.7)$, measured in units of length, were used to calculate the time constants (1.18 and 1.13 seconds with an average of 1.15 seconds; see text). It took about 25 seconds for recovery after right vagal stimulation was turned off. Solid circles are points computed with Eq. 12 and the numerical procedure described in the text.

$K$ is read from the records (Figs. 3 and 4) directly in centimeters. As in the previous case, there is no need for calibration in units of force. For any value $t = t_0$ in the sigmoid portion of the curve, its corresponding $F_n$ is also read (Fig. 3). This procedure permits computation of $B(t_0) = 1 - F(t_0)/K$ which in turn leads to the values for $x_0$ and $\tau$. Knowing $K$ and $\tau$, Eq. 12 is plotted and compared with the experimental curve (Figs. 3 and 4, solid circles vs. peaks of atrial contractions). This procedure was applied in 16 curves recorded from our group of turtles.

Results

SINGLE-PULSE STIMULATION

The atrial inotropic responses were similar to those described by Caskell (1) and by Gilson (3). Application of Eq. 6 produced fits remarkably close to the experimental envelopes (Fig. 2); 12 of the 67 curve fits (18%) performed on the 48 experimental curves showed values consistently higher than the recorded ones at the end tail of the curves (Fig. 5), i.e., there was a persistency of the inotropic effect. Conversely, 13 of the 67 fits (19%) showed the opposite effect, i.e., there was a minor overshoot (Fig. 6). The average time constant $\tau$ for each of the two compartments (Fig. 1) was 4.7 seconds ($sd = 0.8$, $se = 0.1$), and the average appearance time $t_a$ (or latency) was 0.78 seconds ($sd = 0.56$, $se = 0.07$). Average values for each turtle are given in Table 1. Repeated trials in the same heart were quite similar, indicating that a single stimulus to the vagus nerve always triggers the same number of action potentials. Increasing the amplitude of the stimulus increased the response in all cases (Fig. 2).

REPETITIVE STIMULATION

The expected sigmoid curve predicted by the model of Eq. 12 was obtained in all 16 records (Figs. 3 and 4). The average time constant was 0.84 seconds ($sd = 0.37$, $se = 0.08$) from a total of 35 computations over the 16 experimental curves.
Persistent negative inotropic effect after single-pulse right vagal stimulation (RVS) (10 v, 2 msec; turtle 3). The peak amplitudes of the end tail of the envelope are consistently lower than the predicted values (solid circles). This finding is interpreted as indicating a slightly underdamped system (compare with Figs. 2 and 6). Appearance time, $t_a = 0.8$ seconds.

The average appearance time was 0.75 seconds ($SD = 0.41$, $SE = 0.1$). Average values for each turtle are shown in Table 1. Reproducibility of the response was always good, as it was with single-pulse stimulation. Some turtles, however, were more responsive than others.

**Discussion**

The model proposed in this paper is certainly not unique and, according to a statement by Rashevsky (17), "it should not be considered as being true or false, but as being acceptable or unacceptable." It appears to be acceptable, because the data fit it, resulting, therefore, in supporting evidence.

The compartmental hypothesis is implied in Gilson's rationale (3) and is expressed in the following paragraph quoted from his paper (p. 463). "Suppose the immediate peripheral effect of a single nerve fiber impulse to be the liberation of an inhibitory substance in concentration $n$. To produce its depressant effect this material must be diffused through a distance $x$. Moreover, since the recovery of the heart from depression is rather prompt, suppose the material to be destroyed at a rate proportional to its concentration." Gilson gave the following expressions to fit the inotropic response.

$$y = \frac{M}{t} \exp\left( -\frac{N}{t} \right),$$

and

$$y = m \left\{ \exp[-a(t - t_o)] - \exp[-b(t - t_o)] \right\},$$

where $y$ represents the ordinates of the peak envelope, $t$ is time, and $M$, $N$, $m$, and $t_o$ are parameters. The double exponential model can be derived more logically by applying Blair's one-factor theory of excitation (18) based on a first-order linear differential equation which, in this case, must be interpreted in a modified fashion:

$$\frac{dc(t)}{dt} = A\psi - Bc(t),$$

where $c(t)$ stands for the temporal concentration of acetylcholine, $A$ is an activating constant, $B$ is an inactivating constant, and $\psi$ is the release of acetylcholine in the extracellular cleft fluid ($\psi = \psi_0 \exp[-kt]$). Solution of Eq. 17 leads immediately to an expression like that in Eq. 16. Presented in this way, this model also describes a two-compartment system.

The shape of the peak envelope of the myogram, either after single-pulse stimulation (Fig. 2) or during repetitive vagal excitation (Figs. 3 and 4),
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strongly suggests dilution of the transmitter substance in the extracellular environment facing the atrial membrane. Furthermore, these experiments tend to support the assumption of a linear relationship between concentration of acetylcholine and reduction in force of contraction. This dilution phenomenon is due to simple passive diffusion and to the inactivation process of acetylcholineesterase. Brown and Eccles (12) obtained inhibitory curves for heart rate in cats after single-shock stimulation of the vagus that were similar to the type of inotropic response described in this paper. However, the build-up time, $t_{ap}$, was considerably shorter, about 0.5 seconds.

The study made by Glaze and Dong (19) on heart rate responses to vagal stimulation concluded that acetylcholine concentration at the sinoatrial node did not directly control the decay of cardiac frequency inhibition. This conclusion is in opposition to the assumption underlying Eq. 1 used in this study.

Two equal compartments in series seem to account reasonably well for the negative inotropic effect of vagal stimulation on atrial muscle. The first compartment can be ascribed to the intracellular fluid held in the vagal nerve endings, and the second compartment corresponds to the extracellular fluid in the cleft (Fig. 1). Another possibility is to consider the cleft as the first compartment and the atrial intracellular fluid as the second. However, we do not favor this alternative, because in all probability no acetylcholine actually enters into the muscle intracellular fluid.

The two-equal-compartment model leads to a critically damped second-order system (17). In fact, 42 of the 67 fits (or 63%) performed with the 48 single shock responses fell within this category, suggesting that the vagal nerve ending-atrial membrane system behaves as if it were a second-order system with a damping coefficient equal to unity. The ratio of acetylcholine to acetylcholineesterase is probably a factor which determines the degree of damping. Some of the curves showed a persistency of the inotropic effect (Fig. 5) that might be interpreted as a slight overdamping, but other curves showed the opposite effect (Fig. 6), i.e., the equivalent of underdamping. It is suggested that experiments might be performed with drugs that either increase or decrease the amounts of available acetylcholine or acetylcholineesterase so that the overdamped or underdamped conditions can be revealed. No clear interpretation of the second-order system parameters can be made, because, although diffusion might be equated to friction or resistance and the amount of acetylcholine might be equated to mass or inductance, the system does not provide an equivalent for compliance or capacitance. Nonetheless, we may expect that in chemical systems in which two or more substances are involved the concentrations may show temporal periodicity (17).

The average time constant can be regarded as a parameter measuring the dynamics of the system, which include processes of passive diffusion and inactivation by acetylcholineesterase. Its reciprocal is the rate constant that describes the disappearance of substance from each compartment. However, the values obtained by the single-shock method were different from the values calculated using the repetitive-stimulation curves (the ratio of the two average time constants, 4.7/0.84, is 5.6). A possible explanation could be the accumulation of acetylcholine due to repetitive releases, which would cause a quicker establishment of the negative inotropic effect.

At the same time, more acetylcholine would call for either larger amounts of acetylcholineesterase or for greater activity of the enzyme. If the relationship is not linear, the damping condition would also be modified. Termination of repetitive stimulation was always followed by significant overshoots (positive inotropic effect, Fig. 4). These results would generally agree with findings described by Endoh et al. (6) in canine papillary muscle: "a negative inotropic response occurred immediately after administration of 0.01 to 10 $\mu$g of ACh and was followed by a positive response 1 to 2.5 minutes later." However, some kind of accommodation phenomenon to acetylcholine excess during repetitive stimulation might explain the poststimulation positive inotropic effect without postulating larger amounts (or activity) of acetylcholineesterase.

Repetitive stimulation might also involve a decrease in the liberation of acetylcholine due to exhaustion of the vesicles. If this were the case, then the theory of constant release proposed in this paper would not be applicable.

In the case of single-pulse stimulation, the results indicate that in 68% of the fitted curves (48 curves) the appearance time is between 0.22 and 1.34 seconds (mean 0.78 seconds). These values are similar to those obtained for repetitive stimulation, with 68% of the curves (11 curves) within the range of 0.35 to 1.16 seconds (mean 0.75 seconds). This time is an estimate of how long it takes for the first molecules of mediator (the fastest molecules) to
reach and act on transducer T2 of the qualitative model in Figure 1. It might be questioned why the average time for the appearance of the inotropic effects for single and multiple stimuli should be the same. One simple answer is that the first stimulus, whether alone or the first of a series, is the determining factor in the latency. It is entirely possible that very rapidly repetitive stimuli might produce a shortening of the latency, especially when its relatively long value (~0.8 seconds) is considered. In these experiments, however, repetitive stimuli were only of the order of 10 pulses/sec or less to prevent both chronotropic and dromotropic effects.

References

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