Temporal Changes in the Sympathetic-Parasympathetic Interactions that Occur in the Perfused Canine Atrium

Yasuyuki Furukawa and Matthew N. Levy

From the Division of Investigative Medicine, The Mt. Sinai Medical Center and Case Western Reserve University, Cleveland, Ohio

SUMMARY. In the isolated, blood-perfused, canine right atrium, stimulation of the intramural autonomic nerves evoked negative chronotropic and inotropic responses. The responses were not maintained at a constant level during tonic neural stimulation, but they tended to drift back toward their control levels. These time-dependent changes in the cardiac responses were more pronounced the higher the frequency of stimulation. After the β-adrenergic receptors were blocked in half of the preparations, the negative cardiac responses to autonomic neural stimulation were more pronounced, but the time dependency of those responses was less. After the muscarinic receptors were blocked in the other half of the preparations, only positive responses to neural stimulation were observed. These responses faded significantly at a high stimulation frequency (30 Hz), but not at a lower frequency (5 Hz). The cardiac responses to combined autonomic neural stimulation were substantially more negative than the algebraic sum of the individual responses to sympathetic and parasympathetic stimulation. The extent of this interaction was most pronounced near the beginning of stimulation, but it became less pronounced as the stimulation progressed. Hence, the cardiac sympathetic-parasympathetic interactions change appreciably with time during a continuous train of autonomic neural stimulation. (Circ Res 55:835-841, 1984)

THE sympathetic and parasympathetic divisions of the autonomic nervous system exert antagonistic effects on the heart. Numerous studies have shown that these opposing influences are not algebraically additive, but complicated interactions exist (Levy, 1971; Levy and Martin, 1979). In previous studies, the effects of autonomic nerve stimulation on the cardiac responses have usually been examined over only a limited range of stimulation frequencies, and usually only the steady state responses have been analyzed.

The inotropic and chronotropic responses to tonic sympathetic stimulation increase to a maximum value, and tend to remain at that value when the stimulation frequency is relatively low (Levy and Blattberg, 1976a). At higher stimulation frequencies, the responses tend to fade after the maximum value has been reached. The cardiac responses to tonic vagal stimulation tend to fade below the maximum value even when the stimulation frequencies are low, but the extent of fade does not vary appreciably with frequency within the low frequency range (Martin et al., 1982; Martin, 1983). The fade may be more pronounced at high frequencies, however (Jalife et al., 1980).

It has not yet been determined whether such temporal changes in the cardiac effects of autonomic nervous stimulation influence the extent of the interaction between the two components of this system. The present study of isolated, blood-perfused, canine right atrium preparations was designed to determine the cardiac responses to separate and combined sympathetic and parasympathetic stimulation in order to analyze the temporal changes in the interactions between these two autonomic components.

Methods

Isolated atria were obtained from 14 donor dogs, and each preparation was perfused with arterial blood from a second, support dog. The details of this preparation have been described previously (Chiba et al., 1975a, 1975b).

The support dogs, which weighed from 19 to 30 kg, were anesthetized with sodium pentobarbital (30 mg/kg, iv). Sodium heparin (500 USP units/kg) was administered intravenously to each dog at the beginning of the perfusion, and 200 USP units/kg were given each hour thereafter. The donor animals weighed 16-28 kg, and they also were anesthetized with sodium pentobarbital. Heparin (200 USP units/kg, iv) was given, and the right atria were excised and immersed in ice-cold physiological saline. The wet weights of the isolated right atrial preparations varied from 9.5 to 15.5 g. In each preparation, the sinus node artery was cannulated via the right coronary artery, and it was perfused with blood conducted from the femoral artery of the support dog with the aid of a roller pump. A pneumatic resistance was placed in parallel with the perfusion system so that the perfusion pressure could be maintained constant at 100 mm Hg. The blood flow rate to the isolated atrium was 3-7 ml/min. The venous effluent from the preparation was led to a collecting funnel,
from which it was returned continuously to the support dog via an external jugular vein.

The ventricular margin of the atrium was attached to a rigid stainless steel bar, and the preparation was placed in a glass container. The superior part of the atrium was connected to a force transducer (Grass FT03C) by silk thread. The arterial muscle was usually stretched to a resting tension of 2 g. The isometric tension and the maximum rate of tension development were recorded on a direct-writing Brush oscillograph (Mark 260).

Two pairs of silver electrodes were brought into contact with the epicardial surface of the isolated atrium. The diameter of each electrode was 0.3 mm, and the distance between the electrodes in each pair was 1.5 mm. The first pair of electrodes, placed on the caval margin of the atrium, was used to stimulate the intramural nerves. The second pair of electrodes, placed on the atrial free wall, was used to record the electrogram. The atrial rate was derived from the atrial electrogram.

Experimental Protocols

The experiments were subdivided into two groups, each involving seven perfused atria. In group 1, the intramural autonomic nerves were stimulated before and after the administration of propranolol. In group 2, the nerves were stimulated before and after the administration of atropine. In all experiments, the intramural sympathetic and parasympathetic nerves were stimulated electrically (Grass S4) at frequencies of 5 and 30 Hz. To stimulate the intramural nerves, we arbitrarily set the duration of the electrical pulses at 1 msec, and adjusted the voltage so that it was just below the threshold for the automatic and myocardial fibers, but above the threshold for the intramural nerve fibers (Furukawa et al., 1980). The mean voltage was 5.3 V. Each train of stimulation was continued for 2 minutes, so that the responses would approach a steady state level. Sufficient recovery time (more than 4 minutes) was permitted after cessation of each stimulation train to allow control levels to be restored.

In group 1, the cardiac responses to autonomic nerve stimulation at the two frequencies were first determined before autonomic blockade (period 1). Propranolol hydrochloride (1 mg/kg) was then given intravenously to the support dog. Propranolol did not reduce significantly the basal levels of heart rate or atrial contractile force. However, it did completely block the positive cardiac responses of the isolated atrium to sympathetic nerve stimulation; this is documented below. At least 10 minutes were allowed to elapse after the injection of propranolol before the nerves were again stimulated (period 2). The order of applying the two stimulation frequencies was randomized in each observation period of each experiment. The chronotropic and inotropic responses of the isolated atrium to sympathetic nerve stimulation in each group were analyzed by means of a four-way, mixed model analysis of variance (Sokal and Rohlf, 1969). The drug treatment, stimulation frequency, and elapsed time after the beginning of stimulation were considered to be fixed factors. The individual preparations were considered to comprise a random factor. Scheffé's test was used for comparisons of mean values (Scheffé, 1953).

Results

Representative Experiments

Figure 1 displays the chronotropic and inotropic responses of two representative atrial preparations.

The nature of the responses to stimulation, and the alteration of those responses by atropine and propranolol, constituted the evidence that the intramural nerves were being stimulated effectively. The absence of prominent rhythm irregularities during stimulation prior to the administration of atropine or propranolol and the absence of any detectable effect of stimulation after both of these drugs had been given demonstrated that the stimuli were subthreshold for the automatic and myocardial fibers. Furthermore, atropine was given after completion of two group 1 type experiments, and propranolol was given after completion of two group 2 type experiments. Thus, at the end of these four experiments, both atropine and propranolol had been given. Stimulation of the intramural nerves then had no detectable affect. These results demonstrate the efficacy of the blocking drugs, and support the contention that the stimuli were below threshold for the automatic and myocardial fibers.

The cardiac responses to intramural sympathetic and parasympathetic nerve stimulation in each group were analyzed by means of a four-way, mixed model analysis of variance (Sokal and Rohlf, 1969). The drug treatment, stimulation frequency, and elapsed time after the beginning of stimulation were considered to be fixed factors. The individual preparations were considered to comprise a random factor. Scheffé's test was used for comparisons of mean values (Scheffé, 1953).

Results

Representative Experiments

Figure 1 displays the chronotropic and inotropic responses of two representative atrial preparations.

![Figure 1: Changes in Cardiac Cycle Length and Right Atrial Contractile Force](image-url)
to intramural autonomic nerve stimulation at a frequency of 5 Hz. In the absence of propranolol or atropine (left panels), the cycle length responses rapidly increased to a maximum value, and then they "faded" back during stimulation toward the prestimulation control levels. The atrial contractile force responses diminished rapidly to a minimum value, and then faded back toward (A) or beyond (B) the control values during stimulation. After cessation of stimulation, there was a transient overshoot of the chronotropic and inotropic responses.

After propranolol administration (Fig. 1A, right panel), similar negative chronotropic and inotropic effects were induced by nerve stimulation, but the fade was less pronounced after propranolol than before. In the other preparation (Fig. 1B), the chronotropic and inotropic responses to autonomic nerve stimulation became positive after atropine (right panel), and these responses faded only slightly during the train of nerve stimulation.

Composite Data

**Group 1: Effects of Propranolol**

The negative chronotropic and inotropic responses to autonomic neural stimulation were significantly more pronounced after, than before, propranolol (Fig. 2). Both responses faded ($P < 0.001$) back toward their prestimulation levels during the 120-second train of stimulation. The extents of the fade were significantly less after propranolol treatment than before; i.e., the interactions between propranolol treatment and time during stimulation were highly significant ($P < 0.001$). This fading of the chronotropic response was more pronounced at the higher than at the lower stimulation frequency ($P < 0.001$). The effects of stimulation frequency on the inotropic response were less pronounced after propranolol than before ($P = 0.05$). However, the effects of stimulation frequency on the chronotropic response were not altered significantly by propranolol.

**Group 2: Effects of Atropine**

Before atropine, autonomic nerve stimulation evoked only negative chronotropic and inotropic responses (Fig. 3). Hence, before atropine, the inhibitory parasympathetic effects on heart rate and atrial contractility must have been preponderant over the facilitatory sympathetic effects. After atropine, however, autonomic nerve stimulation evoked only positive chronotropic and inotropic responses.

The negative chronotropic and inotropic re-
Responses obtained before atropine were significantly greater at the higher than at the lower stimulation frequency ($P < 0.05$). Similarly, the positive chronotropic and inotropic responses that were elicited after atropine tended to be greater at the higher than at the lower stimulation frequency, but the difference for the inotropic response was not significant. The chronotropic and inotropic responses all faded significantly with time. The tendency to fade was significantly greater at the higher than at the lower stimulation frequency (Fig. 3). Also, the tendencies for the chronotropic and inotropic responses to fade were significantly less after than before atropine.

Discussion

Simultaneous stimulation of the intramural sympathetic and parasympathetic nerve fibers evoked negative chronotropic and inotropic responses of the isolated right atrium during the initial control period before autonomic blocking drugs had been given. Hence, the inhibitory effects of the parasympathetic stimulation were preponderant over the facilitatory effects of the concomitant sympathetic stimulation. These cardiac responses were not maintained at constant levels during the continuous trains of neural stimulation, but they tended to drift back toward their control levels (Figs. 1–3). These time-dependent changes (fading) of the responses were more pronounced the higher the frequency of stimulation.

Individual Responses to Sympathetic and Parasympathetic Stimulation

After treatment with propranolol (Fig. 2), autonomic neural stimulation evoked greater reductions in heart rate and atrial contractile force than it did before propranolol. Also, the time-dependent changes in the cardiac responses were more pronounced when the stimulation frequency was increased. However, the time dependency was less pronounced after propranolol than before propranolol (Figs. 1 and 2). The negative cardiac responses to combined sympathetic and parasympathetic nerve stimulation were converted to positive responses by treatment with atropine (Figs. 1 and 3). These positive responses to neural stimulation tended to fade when the stimulation frequency was 30 Hz, but this tendency was much less prominent when the stimulation frequency was only 5 Hz.

At the dosage levels used in these experiments, atropine and propranolol completely blocked the responses of the isolated atria to stimulation of the intramural parasympathetic and sympathetic nerve fibers, respectively. Furthermore, the electrical stimuli had no detectable effects on the isolated atria once both blocking drugs had been given. Hence, the responses evoked prior to the administration of both antagonists must have been ascribable entirely to the stimulation of intramural autonomic nerve fibers.

The time-dependent changes in the cardiac responses may be ascribable to events occurring at prejunctional or postjunctional sites in the autonomic neuroeffector junctions in the heart. With respect to prejunctional mechanisms, a progressive reduction of neurotransmitter release during a continuous train of autonomic neural stimulation has been observed in a variety of tissue preparations. Time-dependent reductions in norepinephrine overflow have been detected in the heart during sympathetic nerve stimulation (Siegel et al., 1961; Huković and Muscholl, 1962; Yamaguchi et al., 1973; Levy and Blattberg, 1976a), particularly at high stimulation frequencies. Similar time-dependent changes in acetylcholine overflow from the isolated chicken heart during continuous trains of vagal stimulation have also been described (Klibinger and Löllheloh, 1976). The overflow of acetylcholine was maintained almost constant for 5 minutes in such preparations when the stimulation frequency was 3 Hz, but the overflow progressively diminished when the frequency was 20 Hz.

With regard to postjunctional mechanisms, studies conducted with exogenous agonists have shown that fading of the cardiac responses may also be ascribable to "postjunctional" phenomena, such as desensitization of receptors and of certain post-receptor mechanisms (Galper et al., 1977; Gertjegerdes et al., 1979; Jalife et al., 1980; Chang et al., 1982; Hasuo et al., 1982). The relative importance of these various mechanisms remains to be established, however.

Sympathetic-Parasympathetic Interactions

If the sympathetic-parasympathetic interactions are assumed to be negligible, the cardiac responses ($R_{sp}$) to simultaneous sympathetic and parasympathetic nerve stimulation would equal the algebraic sum of the individual responses ($R_s$ and $R_p$) to separate sympathetic and parasympathetic nerve stimulation; that is

$$R_{sp} = R_s + R_p.$$

In the present study, the cardiac responses to combined autonomic neural stimulation were determined before and after treatment with autonomic blocking drugs in two groups of animals. The same experimental conditions prevailed in both groups during the initial observation period before drug treatment. Therefore, it is reasonable to expect that the cardiac responses to combined neural stimulation were similar in the two groups. Table 1 confirms that the responses during the first observation period were not significantly different in the two groups.

In the above equation, therefore, we have allowed $R_{sp}$ to represent the mean response of all the animals in both groups to combined autonomic neural stimulation at a given frequency of stimulation and at a given time after the beginning of stimulation. The responses to such combined stimulation were always...
TABLE 1

Analysis of Variance of the Temporal Changes in the Chronotropic and Inotropic Responses to
Autonomic Neural Stimulation at Two Frequencies

<table>
<thead>
<tr>
<th></th>
<th>Chronotropism</th>
<th>Inotropism</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DF</td>
<td>MS</td>
</tr>
<tr>
<td>Group (G)</td>
<td>1, 12</td>
<td>285</td>
</tr>
<tr>
<td>Stim. freq. (S)</td>
<td>1, 12</td>
<td>19,451</td>
</tr>
<tr>
<td>Time (T)</td>
<td>4, 12</td>
<td>5,558</td>
</tr>
<tr>
<td>G × S</td>
<td>1, 12</td>
<td>696</td>
</tr>
<tr>
<td>G × T</td>
<td>4, 12</td>
<td>47</td>
</tr>
<tr>
<td>S × T</td>
<td>4, 12</td>
<td>2,646</td>
</tr>
<tr>
<td>G × S × T</td>
<td>4, 12</td>
<td>46</td>
</tr>
</tbody>
</table>

* In 14 isolated, perfused atrial preparations during the initial observation period before an autonomic
   blocking drug had been administered. DF = degree of freedom; MS = mean square; P = probability;
   NS = not significant.

recorded during the first observation period, prior
to drug administration. R5 represents the mean re-
response of the animals in group 2 to sympathetic
stimulation alone (i.e., after atropine), at the given
frequency of stimulation and at the given time after
stimulation. Similarly, Rp represents the mean re-
response of the animals in group 1 to parasympathetic
stimulation alone (i.e., after propranolol), and at the
given frequency of stimulation and at the given time
after stimulation. Implicit in this formulation are the
assumptions that atropine does not directly alter the
responses to sympathetic stimulation and that pro-
pranolol does not directly alter the responses to
parasympathetic stimulation.

If the responses (Rsp) to combined stimulation
equaled the algebraic sum of the individual re-
ponses to sympathetic (Rs) and parasympathetic
(Rp) stimulation, the difference between Rsp and the
sum of Rs + Rp [i.e., the value of Rsp – (Rs + Rp)]
would equal zero. By definition, a significant differ-
ence between Rsp and (Rs + Rp) would denote a
substantial sympathetic-parasympathetic interac-
tion. A difference greater than zero (i.e., a positive
response) would indicate that during combined
autonomic neural stimulation, the sympathetic ef-
effects predominated over the parasympathetic ef-
effects. Conversely, a difference less than zero (i.e., a
negative response) would signify that during com-
bined autonomic stimulation, the parasympathetic
effects predominated over the sympathetic effects.

We calculated the differences between Rs + Rp and
(Rsp + Rp) at various times after the beginning of stimu-
lation, and plotted the data in Figure 4. The graphs
show that, for the chronotropic and inotropic re-
sponses, the differences between Rs + Rp and (Rsp + Rp)
were all negative. Hence, at both stimulation fre-
quencies and at all times during stimulation, the
parasympathetic influences predominated over the
sympathetic influences. This finding of vagal pre-
dominance is consistent with previous investiga-
tions. In the dog heart in situ, the effects of parasym-
pathetic stimulation predominated over those of
sympathetic stimulation in the control of sinoatrial
(SA) node pacemaker activity (Samaan, 1935; Levy

![Figure 4](http://circres.ahajournals.org/)

**FIGURE 4.** The percentage differences between the cardiac responses
to simultaneous stimulation of the intramural sympathetic and para-
sympathetic nerve fibers (Rs + Rp) and the algebraical sum of the cardiac
responses [(Rs + Rp)] to separate parasympathetic and sympathetic
stimulation, respectively. The combined responses were determined
before any autonomic blocking drugs were given. The separate re-
sponses were obtained after the administration of the propranolol and
atropine, respectively.
and Zieske, 1969; Warner and Russell, 1969) and atrial contractile force (Stuesser et al., 1979). In isolated rat atria also, the effects of acetylcholine were predominant over those of norepinephrine on the SA node pacemaker activity (Grodner et al., 1970).

The differences between $R_n$ and $(R_n + R_p)$ were more pronounced at the higher than at the lower frequency of stimulation (Fig. 4). At comparable times and stimulation frequencies, the differences were greater for the inotropic responses than for the chronotropic responses. The differences between $R_n$ and $(R_n + R_p)$ became less pronounced with time after the beginning of stimulation. By linear regression analysis, the slopes of all of the curves in Figure 4 were substantially greater than zero, which indicates that these temporal changes were statistically significant ($P \leq 0.01$).

The accentuated antagonism between the sympathetic and parasympathetic effects on the heart may be mediated at prejunctional as well as at postjunctional levels (Levy, 1971; Levy and Martin, 1979; Vanhoutte and Levy, 1980). At the prejunctional level, the acetylcholine released from vagal endings interacts with muscarinic receptors on nearby postganglionic sympathetic nerve terminals and inhibits the release of norepinephrine (Löffelholz and Muscholl, 1969, 1970; Levy and Blattberg, 1976b; Lavallée et al., 1978). At the postjunctional level, acetylcholine acts to attenuate the rise in the intracellular concentration of cAMP that is induced by sympathomimetic agents (Bailey et al., 1979; Lavallée et al., 1978). The cholinergic antagonism may also be mediated in part through a rise in the intracellular levels of cGMP (Watanabe et al., 1981; Watanabe, 1984).

The accentuated antagonism was most pronounced early in the stimulation period, and then it gradually became less pronounced as the train of neural stimulation was continued (Fig. 4). These temporal changes probably depend in part on the progressive changes in the rate of neurotransmitter liberation in the tissue level of acetylcholine would tend to attenuate the sympathetic-parasympathetic interaction at prejunctiornal and postjunctional levels. The precise mechanisms responsible for the temporal changes in the autonomic interactions in the heart remain to be delineated, however.

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