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

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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)
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 to intramural autonomic nerve stimulation were measured at 15, 30, 60, 90, and 120 seconds after the beginning of each train of stimulation.

In group 2, the cardiac responses to stimulation of the intramural autonomic nerves were determined before and after the administration of atropine sulfate (300 μg/kg) to the support dog. This dose of atropine blocked the negative cardiac responses of the isolated atrium to parasympathetic nerve stimulation, as documented below. The atropine did not significantly affect the basal levels of heart rate or atrial contractile force. At least 10 minutes were allowed to elapse after the injection of atropine before the nerves were again stimulated.

Results

Representative Experiments

Figure 1 displays the chronotropic and inotropic responses of two representative atrial preparations.
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 prejunctival or postjunctival sites in the autonomic neuroeffector junctions in the heart. With respect to prejunctival 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; Hubovic 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 (Kilbinger and Löffelholz, 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 postjunctival mechanisms, studies conducted with exogenous agonists have shown that fading of the cardiac responses may also be ascribable to 'postjunctival' phenomena, such as desensitization of receptors and of certain post-receptor mechanisms (Galper et al., 1977; Gertijegerdes 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_s 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></td>
<td></td>
</tr>
<tr>
<td>Stim. freq. (S)</td>
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<td>285</td>
</tr>
<tr>
<td>Time (T)</td>
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<td>19,451</td>
</tr>
<tr>
<td>G x S</td>
<td>4,12</td>
<td>5,558</td>
</tr>
<tr>
<td>G x T</td>
<td>1,12</td>
<td>696</td>
</tr>
<tr>
<td>S x T</td>
<td>4,48</td>
<td>47</td>
</tr>
<tr>
<td>G x S x T</td>
<td>4,48</td>
<td>2,646</td>
</tr>
<tr>
<td></td>
<td>4,48</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. R₄ represents the mean 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, R₅ represents the mean response of the animals in group 1 to parasympathetic stimulation alone (i.e., after propranolol), 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 propranolol does not directly alter the responses to parasympathetic stimulation.

If the responses (Rₛₚ) to combined stimulation equaled the algebraic sum of the individual responses 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 difference between Rₛₚ and (Rₛ + Rₚ) would denote a substantial sympathetic-parasympathetic interaction. A difference greater than zero (i.e., a positive response) would indicate that during combined autonomic neural stimulation, the sympathetic effects predominated over the parasympathetic effects. Conversely, a difference less than zero (i.e., a negative response) would signify that during combined autonomic stimulation, the parasympathetic effects predominated over the sympathetic effects.

We calculated the differences between Rₛₚ and (Rₛ + Rₚ) at various times after the beginning of stimulation, and plotted the data in Figure 4. The graphs show that, for the chronotropic and inotropic responses, the differences between Rₛₚ and (Rₛ + Rₚ) were all negative. Hence, at both stimulation frequencies and at all times during stimulation, the parasympathetic influences predominated over the sympathetic influences. This finding of vagal predominance is consistent with previous investigations. In the dog heart in situ, the effects of parasympathetic stimulation predominated over those of sympathetic stimulation in the control of sinoatrial (SA) node pacemaker activity (Samaan, 1935; Levy...
and Zieske, 1969; Warner and Russell, 1969) and atrial contractile force (Stuesse 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 \( R_{ch} \) and \((R_{ch} + R_{sy})\) 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 \( R_{ch} \) and \((R_{ch} + R_{sy})\) 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 prejunctural as well as at postjunctural levels (Levy, 1971; Levy and Martin, 1979; Vanhoutte and Levy, 1980). At the prejunctural 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 postjunctural level, acetylcholine acts to attenuate the rise in the intracellular concentration of cAMP that is induced by sympathomimetic agents (Bailey et al., 1979; Watanabe et al., 1981; Watanabe, 1984). The cholinergic antagonism may also be mediared 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 release from the autonomic nerve endings (Siegel et al., 1961; Huković and Muscholl, 1962; Yamaguchi et al., 1973; Kilbinger and Löffelholz, 1976; Levy and Blattberg, 1976a), as described above. A progressive decline in the release of acetylcholine during continuous parasympathetic stimulation, for example, would result in decreased concentrations of this transmitter in the cardiac tissues. This diminution in the tissue level of acetylcholine would tend to attenuate the sympathetic-parasympathetic interaction at prejunctural and postjunctural 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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INDEX TERMS: Atropine • Autonomic nervous system • Chronotropic response • Inotropic response • Propranolol
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