Inotropic, chronotropic, and dromotropic effects mediated via parasympathetic ganglia in the dog heart

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Inotropic, chronotropic, and dromotropic effects mediated via parasympathetic ganglia in the dog heart. Am J Physiol Heart Circ Physiol 279: H1201–H1207, 2000.—Some parasympathetic ganglionic cells are located in the epicardial fat pad between the medial superior vena cava and the aortic root (SVC-Ao fat pad) of the dog. We investigated whether the ganglionic cells in the SVC-Ao fat pad control the right atrial contractile force, sinus cycle length (SCL), and atrioventricular (AV) conduction in the autonomic or decentralized heart of the anesthetized dog. Stimulation of both sides of the cervical vagal complexes (CVS) decreased right atrial contractile force, increased SCL, and prolonged AV interval. Stimulation of the rate-related parasympathetic nerves to the sinoatrial (SA) node (SAPS) increased SCL and decreased atrial contractile force. Stimulation of the AV conduction-related parasympathetic nerves to the AV node prolonged AV interval. Trimethaphan, a ganglionic nicotinic receptor blocker, injected into the SVC-Ao fat pad attenuated the negative inotropic, chronotropic, and dromotropic responses to CVS by 33–37%. On the other hand, lidocaine, a sodium channel blocker, injected into the SVC-Ao fat pad almost totally inhibited the inotropic and chronotropic responses to CVS and partly inhibited the dromotropic one. Lidocaine or trimethaphan injected into the SAPS locus abolished the inotropic responses to SAPS, but it partly attenuated those to CVS, although these treatments abolished the chronotropic responses to SAPS or CVS. These results suggest that parasympathetic ganglionic cells in the SVC-Ao fat pad, differing from those in SA and AV fat pads, nonselectively control the atrial contractile force, SCL, and AV conduction partially in the dog heart.

Parasympathetic ganglionic cells; atrial contractility; sinus rate; atrioventricular conduction; trimethaphan

Efferent vagal nerve fibers control the heart via ganglionic cells in the walls of the heart. Almost all parasympathetic ganglionic cells for sinus nodal pacemaker activity exist in the fatty tissue overlying the right pulmonary veins (SA fat pad), and those for atrioventricular (AV) conduction exist in the fatty tissue at the junction of the inferior vena cava and left atrium (AV fat pad) (1, 7–9, 19, 20). The parasympathetic neural elements controlling sinus rate also exist in the fatty tissue overlying the right pulmonary veins of the human hearts (3). However, clustered parasympathetic ganglionic cells controlling atrial contractile force have not been identified yet in the mammalian heart, although stimulation of the parasympathetic neural elements at the SA fat pad increases spontaneous sinus cycle length (SCL) and partly decreases the atrial contractile force in the dog heart (9). Recently, Chiou et al. (4) have reported that there are ganglionic cells in the fat pad located between the medial superior vena cava and aortic root (SVC-Ao fat pad) and that radiofrequency catheter ablation to the SVC-Ao fat pad led to complete vagal denervation or attenuation of vagally induced effective refractory period (ERP) shortening of the right and left atria of the dog. However, many autonomic nerve fibers pass through and around the SVC-Ao fat pad in the dog (17, 21, 22). In the present study, therefore, we examined whether the parasympathetic ganglionic cells in the SVC-Ao fat pad selectively and totally control the right atrial contractile force and electrical responses to the activation of the vagus nerves in the anesthetized dog. To accomplish this purpose, we studied the effects of the topical injection of a ganglionic nicotinic receptor blocker, trimethaphan, and a sodium channel blocker, lidocaine, into the SVC-Ao fat pad on the first derivative of the right atrial pressure (RA dP/dt), SCL, and AV conduction time (AVCT) in response to parasympathetic nerve stimulation. We electrically stimulated both sides of the right and left vagus nerves, sinus rate-related parasympathetic neural elements in the SA fat pad, or AV conduction-related parasympathetic neural elements in the AV fat pad separately (8, 9).

METHODS

Preparation. Our animal experiments were approved by the Shinshu University School of Medicine Animal Studies Committee. Thirty-one mongrel dogs, weighing 10–23 kg, were anesthetized with pentobarbital sodium (30 mg/kg iv), and supplemental doses were given to maintain stable anesthesia. A tracheal cannula was inserted, and intermittent positive-pressure ventilation was started. The chest was opened transversely at the fourth intercostal space. To block

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neural conduction, both cervical vagus nerves were ligated tightly and crushed at the neck, and both stellate ganglia were crushed with a tight ligature at their junctions with the ansa subclaviae. These maneuvers remove almost all tonic neural activity to the heart (10).

To record the electrical activity of the right atrium and ventricle, two bipolar electrodes were placed on the base of the epicardial surface of the right atrial appendage and on the epicardial surface of the right ventricle, respectively. The spontaneous SCL and AVCT were measured and displayed on a thermowriting rectigraph (model WT 685T; Nihon Kohden, Tokyo, Japan). The right atrial pressure was measured by a catheter-tip pressure transducer (model TCP2, Nihon Kohden) that was inserted into the middle of the right atrial cavity via the right jugular vein. Right atrial pressure and RA dP/dt were recorded on the rectigraph. Systemic arterial pressure was also measured via the right femoral artery.

Two bipolar silver electrodes, at a 2-mm interelectrode distance, were used to stimulate the intracardiac parasympathetic neural elements. One was placed on the fatty tissue overlaying the right atrial side of the juncture of the right pulmonary veins (8, 19); we refer to this electrical stimulation of the intracardiac parasympathetic neural elements to the SA nodal region as SAPS. Another was placed on the fatty tissue at the juncture of the inferior vena cava and left atrium; we refer to this electrical stimulation of the intracardiac neural elements to the AV nodal region as AVPS. Both electrodes were connected an electrical stimulator (model SEN 7103, Nihon Kohden). Stimulation was subthreshold for activation of pacemaker cells and cardiac muscle cells when a quite narrow stimulation pulse duration (0.01–0.06 ms) for parasympathetic nerve stimulation was used (8). To stimulate extracardiac parasympathetic efferent nerves to the heart, two fine copper needle electrodes were inserted into each cervical vagus nerve at the neck; we refer to such electrical stimulation as the cervical vagal complex (CVS). Before the experiment, we arbitrarily determined the pulse duration and a frequency of the stimulation (SAPS and AVPS, 0.01–0.06 ms and 10–30 Hz; CVS, 0.01–0.04 ms and 5–20 Hz) to increase the SCL by 300 ms and prolong the AVCT by 30 ms. The voltage amplitude of the stimulation was 10 V.

Protocols. We conducted two series of experiments. In the first series, to determine the role of the parasympathetic ganglionic cells in the SVC-Ao fat pad on the cardiac responses, we investigated the effects of topical injection of trimethaphan (n = 8), a nicotinic ganglionic receptor antagonist, or lidocaine (n = 6), a sodium channel blocker, into the SVC-Ao fat pad on the inotropic, chronotropic, and dromotropic responses to CVS in the anesthetized dogs. Trimethaphan was injected at a dose of 0.3 mg in a volume of 0.2 ml saline, and lidocaine was injected at a dose of 3.0 mg in a volume of 0.2 ml saline. Used doses of trimethaphan or lidocaine did not significantly influence the SCL, the AVCT, and atrial contractility. Direct cardiac effects of a blocker were determined 3 min after the drug administration, and then the drug effects on the cardiac responses to CVS were determined at the end of a 30-s stimulation.

In the second series, to determine the different roles between the parasympathetic ganglionic cells in the SVC-Ao fat pad and those in the SAPS locus, we studied the effects of topical injection of trimethaphan at a dose of 0.3 mg (n = 8) or lidocaine at a dose of 3.0 mg (n = 5) in a volume of 0.2 ml saline into the SAPS locus on the inotropic, chronotropic, and dromotropic responses to CVS, SAPS, or AVPS. Additionally, we also studied the effects of topical injection of trimethaphan into the SVC-Ao fat pad followed by injection of trimethaphan into the SAPS locus on the inotropic and chronotropic responses to CVS or SAPS in four anesthetized animals. Each stimulation was separated by intervals of 1 min or more to allow a sufficient recovery time.

Drugs. Drugs used in the experiments were trimethaphan camsylate (Nippon Rosch, Tokyo, Japan) and lidocaine hydrochloride (Fujisawa, Osaka, Japan).

Statistical analysis. All data are means ± SE. ANOVA with the Bonferroni test was used for the statistical analysis of multiple comparisons of the data. Student’s t-test for paired data was used for comparison between the two groups. P values less than 0.05 were considered statistically significant.

RESULTS

Effects of trimethaphan or lidocaine injected into the SVC-Ao fat pad. Before treatment with trimethaphan or lidocaine into the SVC-Ao fat pad, we determined the atrial contractile force, the spontaneous SCL, and AVCT in responses to stimulation of both sides of CVS, stimulation of the rate-related parasympathetic neural elements to the SA nodal region at the SA fat pad (SAPS), or stimulation of the AV conduction-related parasympathetic neural elements to the AV nodal region at the AV fat pad (AVPS) as shown in Fig. 1. CVS decreased right atrial pressure and RA dP/dt, increased SCL, and prolonged AVCT (Fig. 1A). We used the RA dP/dt as an indicator of the atrial contractile force. On the other hand, SAPS increased the SCL with a decrease in atrial pressure responses but did not prolong the AVCT (Fig. 1B), and AVPS prolonged the AVCT without changes in SCL and atrial pressure responses (Fig. 1C). Summarized data are shown in Table 1.

To determine how the parasympathetic ganglionic cells in the SVC-Ao fat pad control the cardiac responses, we then studied the effects of trimethaphan injected into the SVC-Ao fat pad on the changes in right atrial contractile force, SCL, and AV conduction in response to CVS. Three minutes after topical injection of trimethaphan into the SVC-Ao fat pad, basal heart rate and arterial blood pressure of the anesthetized dog did not change significantly from the predrug control levels.

Topical injection of trimethaphan at a dose of 0.3 mg in a volume of 0.2 ml saline into the SVC-Ao fat pad similarly attenuated decreases in RA dP/dt, increases in SCL, and the prolongation of AVCT in response to CVS by 37.4 ± 4.7%, 34.3 ± 5.4%, and 33.1 ± 6.5% from the respective control level (100%) in eight experiments (Fig. 2). The 0.2 ml of saline injected into the SVC-Ao fat pad did not affect the cardiac responses and arterial blood pressure.

To inhibit the action of the nerve fibers passing through the SVC-Ao fat pad as well as the ganglionic nicotinic receptor mediated action, we studied the effects of lidocaine injected into the SVC-Ao fat pad on the cardiac responses to CVS. Topical injection of lidocaine at a dose of 3.0 mg depressed decreases in RA dP/dt, increases in SCL, and the prolongation of AVCT in response to CVS by 83.1 ± 2.4%, 89.0 ± 2.2%, and 53.2 ± 13.1% from the respective control level (100%)
in six experiments (Fig. 3). Three minutes after lidocaine treatment, basal heart rate and arterial blood pressure did not change significantly from the predrug control levels.

Effects of trimethaphan or lidocaine injected into the SAPS locus. To investigate the relationship between the functional role of the parasympathetic ganglionic cells in the SVC-Ao fat pad and those of the SAPS locus, we studied the effects of trimethaphan injected into the SAPS locus on the changes in right atrial contractile force, SCL, and AVCT in response to CVS, SAPS, or AVPS. Topical injection of trimethaphan into the SAPS locus suppressed the negative chronotropic and inotropic responses to SAPS by 98.0 ± 1.0% and 95.8 ± 2.3% from the respective control level (100%), respectively, and it also suppressed the negative chronotropic response to CVS by 86.0 ± 3.5% (Fig. 4). However, trimethaphan injected into the SAPS locus attenuated the negative inotropic response to CVS partially by 42.4 ± 3.5%. Dromotropic responses to

Table 1. Inotropic, chronotropic, and dromotropic responses to stimulation of both sides of the cervical vagus nerves, stimulation of SAPS, and stimulation of AVPS in anesthetized dog hearts

<table>
<thead>
<tr>
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<th>n</th>
<th>RAP, mmHg</th>
<th>RA dP/dt, mmHg/s</th>
<th>SCL, ms</th>
<th>AVCT, ms</th>
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<tr>
<td>CVS</td>
<td></td>
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<tr>
<td>Control</td>
<td>12</td>
<td>5.3 ± 0.2</td>
<td>40.7 ± 2.1</td>
<td>451 ± 13</td>
<td>133 ± 6</td>
</tr>
<tr>
<td>Stimulation</td>
<td>12</td>
<td>2.8 ± 0.3**</td>
<td>12.4 ± 1.7***</td>
<td>778 ± 41***</td>
<td>176 ± 8***</td>
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<tr>
<td>SAPS</td>
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<tr>
<td>Control</td>
<td>12</td>
<td>5.3 ± 0.2</td>
<td>40.6 ± 1.7</td>
<td>451 ± 14</td>
<td>133 ± 6</td>
</tr>
<tr>
<td>Stimulation</td>
<td>12</td>
<td>4.0 ± 0.3*</td>
<td>24.6 ± 1.8**</td>
<td>782 ± 50***</td>
<td>123 ± 6</td>
</tr>
<tr>
<td>AVPS</td>
<td></td>
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</tr>
<tr>
<td>Control</td>
<td>11</td>
<td>5.1 ± 0.2</td>
<td>38.2 ± 1.3</td>
<td>454 ± 13</td>
<td>133 ± 6</td>
</tr>
<tr>
<td>Stimulation</td>
<td>11</td>
<td>5.1 ± 0.2</td>
<td>39.6 ± 1.7</td>
<td>459 ± 12</td>
<td>165 ± 8**</td>
</tr>
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</table>

Data are shown as means ± SE; n, number of hearts. RAP, right atrial a wave pressure; RA dP/dt, first derivative of the RAP; SCL, sinus cycle length; AVCT, atrioventricular conduction time; CVS, cervical vagus complex; SAPS, parasympathetic nerves to the sinoatrial node; AVPS, parasympathetic nerves to the atrioventricular node. *P < 0.05, **P < 0.01, and ***P < 0.001 vs. control.
CVS and AVPS were not affected by trimethaphan injected into the SAPS locus.

We also studied the effects of lidocaine injected into the SAPS locus on the cardiac responses to CVS, SAPS, or AVPS (Fig. 5). Topical injection of lidocaine at a dose of 3.0 mg in a volume of 0.2 ml saline into the SAPS locus abolished the negative chronotropic responses to SAPS and CVS and the negative inotropic response to SAPS. Lidocaine attenuated the negative inotropic response to CVS partly by 56.0 ± 6.7% from the respective control level (100%). These effects of lidocaine on the cardiac responses to CVS and SAPS were similar to those effects of trimethaphan injected into the SAPS locus (Figs. 4 and 5). Lidocaine did not affect the dromotropic responses to each parasympathetic stimulation.

Additionally, we investigated the effects of trimethaphan on the cardiac responses to CVS or SAPS when trimethaphan was injected into the SVC-Ao fat pad followed by injection into the SAPS locus in four anesthetized dogs (Fig. 6). Topical injection of trimethaphan into the SVC-Ao fat pad attenuated the negative inotropic (Fig. 6A) and chronotropic (Fig. 6B) responses to CVS by 29.9 ± 6.4% and 35.6 ± 9.3% from the control level (100%), respectively. The injection of trimethaphan into the SAPS locus following the trimethaphan injection into the SVC-Ao fat pad then further attenuated the negative inotropic response to CVS by 49.9 ± 2.4% from the predrug control level and suppressed the residual negative chronotropic response to CVS by 91.8 ± 2.0%. The negative inotropic (Fig. 6C) and chronotropic (Fig. 6D) responses to SAPS were slightly but not significantly attenuated by the injection of trimethaphan into the SVC-Ao fat pad and suppressed by the following injection of trimethaphan into the SAPS locus. The inhibitions by trimethaphan injected into the SVC-Ao fat pad and SAPS locus of the negative cardiac responses to CVS or SAPS were not additive to the inhibition by trimethaphan treatment alone.

**DISCUSSION**

Parasympathetic ganglionic cells in the SAPS locus and AVPS locus selectively control the atrial pacemaker activity and AV conduction, respectively, in the dog heart (5, 7). Since the SAPS caused a negative chronotropic and inotropic effect and was blocked by treatment with hexamethonium (7) or with trimethaphan in this study, ganglionic cells might not be activated directly by the stimulus but rather the preganglionic fibers were. Miyazaki et al. (13) demonstrated that 1) vagal neuronal transmission in the heart was readily inhibited by either hexamethonium, a ganglionic blocker, or tetrodotoxin, an axonal blocker, and 2) sympathetic neurotransmission was blocked by tetrodotoxin but not by hexamethonium. In the present study, we showed that trimethaphan, a ganglionic blocker, readily blocked SAPS-induced negative chronotropic and inotropic effects, confirming the results demonstrated by Miyazaki et al. (13). To inhibit the shortening of the atrial refractory period by parasympathetic activation, Chiu et al. (4) applied epica-
dial radiofrequency catheter ablation to the SVC-Ao fat pad in the dog heart. From their results, they thought that the parasympathetic neural elements in the SVC-Ao fat pad were the head station of vagal fibers to both atria and to the sinus and AV nodes in the dog. They investigated changes in atrial refractory period in the fat pads, but they did not study other cardiac responses, although there are parasympathetic ganglionic cells in the SVC-Ao fat pad of the dog. In 1992, Mick et al. (12) reported that another fat pad controlling the sinus function exists at the posterior atrial site (PAFP, posterior atrial fat pad) neighbor to the SVC-Ao fat pad in the dog heart. However, the PAFP is not identical with SVC-Ao fat pad that Chiou et al. (4) reported, in anatomical location, because the SVC-Ao fat pad is located between the medial SVC and aortic root, superior to the right pulmonary artery, and the PAFP is located the posterior atrial site. In the present study we demonstrated first that parasympathetic ganglionic cells in the SVC-Ao fat pad control the right atrial contractile force, sinus node activity, and AV conduction partially and nonselectively in the dog heart. These results suggest that the parasympathetic ganglionic cells in the SVC-Ao fat pad are functionally different from those in the SAPS locus and AVPS locus.

The parasympathetic ganglionic cells in the SAPS locus and AVPS locus selectively control the chronotropic and dromotropic activity, respectively. On the other hand, those in the SVC-Ao fat pad affect to some degree inotropic, chronotropic, and dromotropic activity, respectively.

Control of the sinus node pacemaker activity. Effect of the application of lidocaine or trimethaphan into the SAPS locus or the SVC-Ao fat pad (Figs. 2–5) suggests that 1) almost all parasympathetic nerve fibers that induce the negative chronotropic effect pass through the SVC-Ao fat pad, 2) they change the ganglionic neurotransmission at the parasympathetic ganglionic cells in the dog heart, and 3) some of the cervical nerve fibers change the synapses in the SVC-Ao fat pad and/or the SAPS locus in the dog heart.

Control of atrial contractile force. We studied the a wave pressure and its first derivative of the right atrium (RA) of the dog heart in response to stimulation of both sides of the carotid sinus (CVS) and the rate-related SAPS in anesthetized dogs. Changes of basal state: decreases in RA dP/dt by CVS and SAPS from 39.4 ± 3.0 to 11.6 ± 2.8 mmHg (69.6%) and from 37.4 ± 3.2 to 17.4 ± 3.5 mmHg (54.3%), respectively; increases in SCL by CVS and SAPS from 494 ± 32 to 822 ± 79 ms (69.6%) and from 490 ± 29 to 840 ± 104 ms (69.0%), respectively; and prolongations of AVCT by CVS and AVPS from 130 ± 7 to 185 ± 9 ms (44.0%) and from 130 ± 9 to 166 ± 8 ms (29.4%), respectively. Open and solid columns present responses to each stimulation before and after lidocaine treatment, respectively. *P < 0.001 vs. control.
atrium as an indicator of the right atrial myocardial contractility in the dog heart. The first derivative of the a wave pressure reflects the sum of the contraction of the right atrial muscles forming the right intra-atrial cavity, although the a wave pressure is influenced by timing of the closure of the tricuspid valves and other factors, e.g., the venous return as preload, and the right ventricular pressure as afterload (16).

In the present study, we confirm that parasympathetic ganglionic cells in the SAPS locus from both sides of CVS control the right atrial myocardial contractility partially in the dog heart (9). Furthermore, lidocaine injected into the SVC-Ao fat pad suppressed the negative inotropic response to CVS (Figs. 3, and trimethaphan injected into the SVC-Ao fat pad alone and into the SVC-Ao fat pad and the SAPS locus also attenuated the inotropic response to CVS in part (Figs. 2 and 6). These results, therefore, suggest that one-half of the parasympathetic ganglionic cells controlling the right atrial contractility exist in the SAPS locus and SVC-Ao fat pad, and there may be no selective cluster of the intracardiac parasympathetic ganglia for the control of atrial contractile force in the dog heart. There are many clusters of the ganglionic cells in the dog heart (2, 24), but AVPS or stimulation of the clusters of the ganglionic cells at the AV fat pad did not affect the right atrial contractility in the anesthetized dog (14). Cardiac responses to vagus stimulation are also regulated by intracardiac and extracardiac neural regulation in the dog heart (11). Therefore, to control the atrial contractile force at the parasympathetic ganglia, we need further studies to define the residual parasympathetic ganglionic cells in the heart or extra-cardiac sites.

In the present study, we have focused on the selective control of the parasympathetic ganglionic cells in the SVC-Ao fat pad on the atrial contractile force. Thus we did not investigate precisely the relationship between the parasympathetic ganglionic cells in the SVC-Ao fat pad and those in the AVPS locus. However, from present results and previous reports (7, 18), we can speculate that control of the dromotropic response to parasympathetic nerve activations similarly involves both the SVC-Ao fat pad and the SAPS locus.

There are three functional parasympathetic ganglionic groups in the dog heart: parasympathetic ganglionic cells in the SA fat pad for the pacemaker activity and those in the AV fat pad for the AV conduction (1, 8, 19), and parasympathetic ganglionic cells in the SVC-Ao fat pad for the pacemaker activity, atrial contractility, and AV conduction shown in the present study. The parasympathetic ganglia in the SA fat pad work as a controller of the atrial rate and those in the AV fat pad work as a controller of the AV conduction. We have investigated whether those parasympathetic ganglia control respective cardiac functions at the presynaptic site or at the heart (6—9, 14, 23). Some of the parasympathetic ganglionic cells in the SA fat pad regulate the atrial contractile force and refractory period only in part (9, 23). On the other hand, Chiou et al. (4) thought that the parasympathetic neural elements including parasympathetic ganglia in the SVC-Ao fat pad were the head station of the vagal fibers to both atria and to the SA and AV nodes in the dog. However, in the present study, we presented that the parasympathetic ganglionic cells in the SVC-Ao fat pad might not be the head station of vagal fibers to the right atrium and to the sinus and AV nodes in the dog. They might work as an overall modulator of the atrial pacemaker activity, atrial contractility, and AV conduction in the dog heart. This modulator may balance pacemaker activity and AV conductivity to complete heartbeat as previously suggested (15).

REFERENCES


