Differential Intracardiac Sympathetic and Parasympathetic Innervation to the SA and AV Nodes in Anesthetized Dog Hearts

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ABSTRACT—Stimulation of discrete intracardiac sympathetic nerves to the SA (SAS stimulation) or AV nodal region (AVS stimulation) increased the heart rate or decreased AV conduction time and caused an AV junctional rhythm, respectively, in anesthetized dogs treated with atropine. Topical application of tetrodotoxin (TTX) at the SAS or AVS stimulation locus totally inhibited the response to each stimulation, whereas each TTX treatment slightly attenuated the chronotropic response to the right ansa stimulation by 23 ± 7.7% and the dromotropic response to the left ansa stimulation by 7 ± 7.5%. TTX abolished AVS stimulation-induced one. Before atropine, topical application of hexamethonium at the locus for stimulation of intracardiac parasympathetic nerves to the SA (SAP stimulation) or AV nodal region (AVP stimulation) abolished almost totally negative chronotropic responses to SAP and cervical vagus stimulation or negative dromotropic responses to AVP and cervical vagus stimulation, respectively. These results demonstrate that activation of a very small population of intracardiac sympathetic nerves to target cells is enough to induce positive chronotropic and dromotropic responses in the heart in situ, and that SA and AV nodal pacemaker activity and AV conductivity are controlled multi-directionally by intracardiac sympathetic nerves in contrast with parasympathetic ones.

Activation of both right and left sympathetic nerves to the heart increases heart rate, shortens atrioventricular (AV) conduction time (AVCT), and increases myocardial contractile force (1, 2). Right sided sympathetic projections exert more pronounced effects on rate (2–4), whereas left sided sympathetic projections predominantly modulate AV conduction (5–8). The more peripherally located the point of electrical activation of cardiac sympathetic nerves, the more distinct the effects on control of regional cardiac function (2, 9). Projections of extracardiac sympathetic efferents to automatic, conductile and contractile tissues of the dog heart are parallel, but distinct (10). Recently, we have found that stimulation of the intracardiac sympathetic nerve fibers to the SA (SAS stimulation) and AV nodal regions (AVS stimulation) results in selective positive chronotropic and dromotropic responses, respectively, in the atropine-treated dog heart (11). There are loci for SAS and AVS stimulation, i.e., the right atrial side of junctions of the right pulmonary veins and

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the junction of the inferior vena cava and left atrium, respectively.

The fatty tissues at the intracardiac sympathetic stimulation sites contain parasympathetic ganglion cells (12–14). When we used a level of stimulation with quite a narrow pulse duration, stimulation at the same sites where the intracardiac sympathetic stimulation had been done induced selective negative chronotropic and dromotropic responses in the dog heart (12, 15, 16). Dissection of each fatty tissue of the stimulation locus totally inhibited the respective vagus input to the SA and AV nodes of the dog heart (17, 18). Dissection of both fatty tissues produced minor effects on chronotropic, dromotropic and inotropic responses to stimulation of stellate ganglia (10).

In the present study, therefore, we investigated whether intracardiac innervation of the sympathetic nerves to the SA and AV nodal regions is similar to that of the parasympathetic ones in the dog heart. To achieve these aims, we studied the effects of a selective axonal conduction blocker, tetrodotoxin (TTX) (19), applied topically into the fatty tissue at the SAS and AVS stimulation loci on the cardiac electrical responses to stimulation of the discrete intracardiac sympathetic nerves or the extracardiac sympathetic nerves in anesthetized, open-chest dog heart after atropine had been given. We also studied the effects of a ganglionic nicotinic blocker, hexamethonium (C6) (20), on the chronotropic and dromotropic responses to stimulation of the discrete intracardiac parasympathetic nerves to the SA and AV nodal regions (15, 16) and to stimulation of the cervical vagus nerves.

MATERIALS AND METHODS

Preparation

We used 12 mongrel dogs that weighed 10 to 20 kg. Each dog was anesthetized with sodium pentobarbital (30 mg/kg, i.v.). A tracheal cannula was inserted and intermittent positive-pressure ventilation was started. The chest was opened transversely at the fifth intercostal space. Each cervical vagus nerve was crushed with a tight ligature, and each stellate ganglion was ligated tightly at its junction with the ansa subclavia. These maneuvers remove virtually all tonic neural activity to the heart (21).

Two bipolar electrodes were placed on the base of the epicardial surface of the right atrial appendage to record the electrical activity and to pace the atrium. A bipolar recording electrode was also placed on the epicardial surface of the right ventricle. The heart rate which was derived from the atrial electrogram and atrioventricular (AV) conduction time (AVCT; AV interval) were measured and displayed on a thermo-writing rectigraph (Nihon Kohden WT685T). An AV junctional rhythm was determined as that which is obtained when the ventricular deflection precedes the atrial deflection. Systemic arterial pressure was also recorded.

Two bipolar silver electrodes, 2-mm inter-electrode distance, were used to stimulate the intracardiac autonomic nerve fibers (11, 15). One bipolar electrode was placed on the fatty tissue overlying the right atrial side of the junctions of the right pulmonary veins or SA fat pad (Fig. 1) and used to stimulate the intracardiac sympathetic and parasympathetic nerve fibers to the SA nodal region; we refer to stimulation of the intracardiac sympathetic

Fig. 1. Dorsal view of the dog heart showing loci (shaded area) for intracardiac autonomic nerve stimulation to the SA nodal region (SA Fat Pad) and to the AV nodal region (AV Fat Pad). AV, atrioventricular; SA, sinoatrial; LV, left ventricle; RA, right atrium; RV, right ventricle; PA, pulmonary artery; PV, pulmonary vein; IVC, inferior vena cava; SVC, superior vena cava.
nerve fibers to the SA nodal region as “SAS stimulation” and stimulation of the intracardiac parasympathetic nerves to the SA nodal region as “SAP stimulation”. The second electrode was placed on the fatty tissue at the junction of the inferior vena cava and left atrium or AV fat pad (Fig. 1). It was used to stimulate the intracardiac sympathetic and parasympathetic nerve fibers to the AV nodal region; we refer to stimulation of the intracardiac sympathetic nerves to the AV nodal region as “AVS stimulation” and to stimulation of the intracardiac parasympathetic nerves to the AV nodal region as “AVP stimulation”.

When we studied the cardiac responses to sympathetic nerve stimulation, a dog was given atropine (0.2 mg/kg, i.v.) before starting the experiment. We used repetitive bursts of stimuli (Nihon Kohden SEN 7103) with a 5-mA pulse amplitude and 1-msec pulse duration (11, 22). The interpulse interval within each burst was 10 msec. A brief burst of stimuli was delivered in each cardiac cycle. Each stimulation burst contained from 1 to 7 pulses. Stimulation was triggered by atrial depolarization. The time from the beginning of the atrial depolarization to the beginning of the stimulus burst was 10 msec. Each 30-sec train of stimuli to the nerves was followed by a sufficient recovery period to allow return to the prestimulation control level. The pulse amplitude and duration were adjusted so that the stimuli were above threshold for neighboring nerve fibers but below threshold for the cardiac cells.

To stimulate extracardiac sympathetic nerves or both sides of ansa subclaviae, two bipolar hook iridium electrodes were placed on the cardiac side of each stellate ganglion and connected to an electrical stimulator (Nihon Kohden SEN 7103). We used repetitive bursts of stimuli. First, we adjusted the level of stimulus to obtain cardiac responses similar to those to SAS or AVS stimulation and if necessary, changed the stimulus voltage, frequency or/and number of pulses per burst to get greater responses to stimulation. We refer to stimulation of the right or left ansa subclavia as “RS stimulation” or “LS stimulation”, respectively.

Before atropine, to study cardiac responses to SAP or AVP stimulation, we stimulated parasympathetic nerves by a steady stimulation with 5-mA pulse amplitude, a very narrow pulse duration or less than 0.05 msec pulse duration and a frequency of 10 to 30 Hz for 30 sec (15, 16). The stimulation intensity was subthreshold for activation of sympathetic nerves, pacemaker cells and cardiac muscle cells.

To stimulate extracardiac parasympathetic nerves to the heart, bipolar wire electrodes were hooked in the cardiac end of each cervical vagus nerve, and the wires were connected with a stimulator. We used a steady stimulation with a narrow pulse duration for stimulation of cervical vagus nerves. The level of stimulation was adjusted to obtain responses similar to those to SAP or AVP stimulation but not to induce sinus arrest or second and third degree AV block. We refer to stimulation of the right and left vagus nerve fibers as “RV stimulation” and “LV stimulation”, respectively.

**Protocols**

We carried out two groups of experiments. In the first group, to study whether activation of a very small population of sympathetic nerve fibers elicits an increase in sinus rate, we investigated chronotropic responses to SAS (n = 10), RS (n = 10), and LS stimulation (n = 10) before and after topical application of TTX (3 μg/0.01 ml) into the fatty tissue at the SAS stimulation locus. We also studied the dromotropist response to AVS (n = 10), LS (n = 10), and RS stimulation (n = 4) before and after topical application of TTX into the fatty tissue at the AVS stimulation locus in order to demonstrate whether activation of a very small population of sympathetic nerves elicits a decrease in AVCT and an induction of AV junctional rhythm. We assessed maximum cardiac responses as data when AVS stimulation caused more than 20-msec decreases in AVCT. The order of stimulation was randomized. AVS stimulation induced a positive
dromotropic effect without changes in heart rate until an AV junctional rhythm preponderated the sinus rhythm. On the other hand, LS and RS stimulation evoked both positive chronotropic and dromotropic responses. Thus, we assessed the dromotropic responses to each stimulation during atrial pacing at a rate of 150 or 180 beats/min in 8 experiments. In another 2 experiments on spontaneously beating hearts, the effects of TTX on the dromotropic responses to AVS and LS stimulation were similar to those in paced hearts. Thus, we combined the data together.

In the second group, before atropine was given to the dog, we assessed the chronotropic response to SAP (n = 6), RV (n = 6), and LV stimulation (n = 2) before and after C₆ (1 mg/0.04 ml or 3 mg/0.12 ml) treated topically into the fatty tissue at the SAP stimulation locus and dromotropic response to AVP (n = 6), LV (n = 5), and RV stimulation (n = 4) before and after C₆ topically applied into the fatty tissue at the AVP stimulation locus. The order of stimulation was randomized. Physiological saline (0.12 ml) injected into the SAP or AVP stimulation locus did not change the response to each parasympathetic stimulation.

Drugs

Drugs used in the experiments were atropine sulfate (Wako Pure Chemicals), hexamethonium bromide (C₆, Yamanouchi), and tetrodotoxin (TTX, Sankyo Central Laboratory).

Statistical analysis

All data were expressed as the mean ± S.E.M. Student’s t-test for paired or unpaired data was used for comparison between the two groups. P values less than 0.05 were considered statistically significant.

RESULTS

Effects of TTX applied to the SAS and AVS stimulation loci

Representative experiments: Stimulation of the intracardiac sympathetic nerves to the SA nodal region (SAS stimulation) consistently increased heart rate with variable changes in AV interval in an anesthetized, open-chest dog (Fig. 2). Stimulation of the right ansa subclavia (RS stimulation) also increased heart rate with decreases in AV interval. RS₁ stimulation induced an increase in heart rate similar to that by SAS stimulation. RS₂ stimulation at a level of stimulation stronger than that of RS₁ stimulation evoked a greater positive chronotropic effect. A level of stimulation was arbitrarily determined by changing stimulation parameters. Three minutes after TTX injected into the fatty tissue at the SAS stimulation locus, SAS, RS₁, and RS₂ stimulations were repeated. As shown in Fig. 2 (right panel), SAS stimulation did not increase

![Fig. 2. Effects of topical application of tetrodotoxin (TTX) on the positive chronotropic response to stimulation of the intracardiac sympathetic nerves to the SA nodal region (SAS Stim) and stimulation of the right ansa subclavia (RS₁ and RS₂ Stim) in an anesthetized dog treated with atropine. TTX (3 μg) was injected into the fatty tissue at the SAS stimulation locus (SAFT). SAS stimulation (horizontal bar) consisted of repetitive brief bursts of stimuli of 1-msec pulse duration, 5-mA pulse amplitude and 5 pulses per burst for 30 sec. RS₁ and RS₂ stimulation consisted of repetitive brief bursts of stimuli of 0.1-msec pulse duration, 3-mA pulse amplitude and 1 pulse per burst, and 1-msec pulse duration, 5-mA pulse amplitude and 5 pulses per burst, respectively, for 30 sec.](image-url)
heart rate at all, but RS₁ and RS₂ stimulations still increased heart rate.

Stimulation of the intracardiac sympathetic nerves to the AV nodal region (AVS stimulation) in the spontaneously beating heart induced a decrease in AV interval followed by an AV junctional rhythm when the level of AVS stimulation was strong (Fig. 3). When the AV junctional rhythm during AVS stimulation was faster than the sinus rate, the junctional rhythm was predominant (upper panel). Stimulation of the left ansa subclavia (LS stimulation) also induced a decrease in the AV interval followed by an AV junctional rhythm. Three minutes after topical application of TTX into the fatty tissue at the AVS stimulation locus, AVS stimulation decreased the AV interval little and did not induce the AV junctional rhythm. However, LS stimulation still similarly induced a decrease in the AV interval followed by the junctional rhythm.

Composite data: The prestimulation control values of the heart rate and AVCT in 10 anesthetized dogs were respectively 105 ± 3.0 beats/min and 134 ± 5.9 msec in spontaneously beating hearts and 164 ± 8.6 msec in paced hearts. These values were not significantly different from those after treatment with TTX or C₆.

Before TTX had been given topically, SAS stimulation increased heart rate by 41 ± 4.7 beats/min from the prestimulation control level in 10 anesthetized dogs. TTX applied to the fatty tissue at the SAS stimulation locus inhibited the positive chronotropic response to SAS stimulation completely (P < 0.001; Fig. 4, left panel). On the other hand, similar increases in heart rate in response to RS stimulation were attenuated by TTX slightly but significantly (P < 0.01) from 56 ± 5.5 (100%) beats/min to 41 ± 4.3 (77 ± 7.7%) beats/min. When we used a high level of stimulation, RS stimulation (e.g., RS₂ stimulation in Fig. 2) evoked almost the maximum increases in heart rate. The positive chronotropic responses to RS stimulation at a high level were reduced slightly (P < 0.05) from 84 ± 3.9 beats/min increases in heart rate to 69 ± 6.0 (83 ± 5.5%) beats/min in 6 experiments. The positive chronotropic responses to LS stimulation at the used levels of stimulation were not attenuated by TTX significantly from 20 ± 4.9 beats/min increases in heart rate to 18 ± 6.4 (80 ± 24.1%) beats/min in 10 anesthetized dogs. LS stimulation induced lower atrial and AV junctional rhythm in 4 of 10 experiments.

AVS stimulation decreased AV conduction time (AVCT) by 46 ± 7.2 msec from the prestimulation control value. After TTX was applied into the fatty tissue at the AVS stimulation site, the positive dromotropic response to AVS stimulation was suppressed completely (Fig. 5), whereas similar decreases in AVCT

Fig. 3. Effects of topical application of tetrodotoxin (TTX) on a decrease in AV interval followed by an AV junctional rhythm induced by stimulation of the intracardiac sympathetic nerves to the AV nodal region (AVS Stim) and stimulation of the left ansa subclavia (LS Stim) in a spontaneously beating dog heart treated with atropine. TTX (3 μg) was injected into the fatty tissue at the AVS stimulation locus (AVFT). AVS and LS stimulation (horizontal bar) consisted of repetitive brief bursts of stimuli of 2-msec pulse duration, 5-mA amplitude and 5 pulses per burst for 30 sec.
in response to LS stimulation were not significantly attenuated by TTX treatment from $51 \pm 5.4$ msec to $49 \pm 7.1$ (93 ± 7.5%) msec in 10 experiments. The positive dromotropic responses to RS stimulation were also not attenuated in 4 animals (Fig. 5, right panel).

AVS stimulation at the used levels induced a subsidiary pacemaker dominance in 5 spontaneously beating dog hearts; an AV junctional rhythm in 4 dogs and a lower atrial rhythm in another. An injection of TTX at the AVS stimulation locus completely inhibited the AV junctional and lower atrial rhythms induced by AVS stimulation, but the subsidiary pacemaker activities induced by LS stimulation in 4 experiments still remained even after TTX treatment as shown in Fig. 3.

Effects of $C_6$ applied to the SAP and AVP stimulation loci

Stimulation of the intracardiac parasymathetic nerve fibers to the SA nodal region (SAP stimulation) and right vagus stimulation (RV stimulation) similarly decreased heart rate and induced sinus arrest when the level of stimulation was high. RV stimulation also increased AVCT with the decrease in heart rate. SAP stimulation and RV stimulation at the used levels of each stimulation decreased the heart rate by 47 ± 8.7 beats/min and 57 ± 8.4 beats/min, respectively. The negative chronotropic responses to SAP and RV stimulation were almost totally ($P < 0.01$) inhibited by topical application of $C_6$ (1 or 3 mg) into the fatty tissue at the SAP stimulation locus (Fig. 6). Effects of $C_6$ on the negative chronotropic responses to left cervical vagus stimulation (LV stimulation) were examined in 2 anesthetized dogs. LV stimulation usually induced second or third degree AV block before reaching a heart rate decrease similar to that induced by SAP stimulation. $C_6$ inhibited the negative chronotropic responses to LV stimulation by 83 and 94% in each case. $C_6$ at the SAP stimulation locus did not significantly affect the negative dromotropic effect of cervical stimulation.

Stimulation of the intracardiac parasym-

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**Fig. 4.** Effects of topical application of tetrodotoxin (TTX) on the increases in heart rate in response to stimulation of the intracardiac sympathetic nerves to the SA nodal region (SAS Stim), the right ansa subclavia (RS Stim), and the left ansa subclavia (LS Stim) in the anesthetized dog heart treated with atropine. TTX (3 µg) was injected into the fatty tissue at the SAS stimulation locus. A fine line shows the effect of TTX on the chronotropic response to each stimulation in each experiment. Open circles and vertical bars show mean values and S.E.M., respectively. Asterisks show levels of statistical significance when each response is compared with its control: ** * P < 0.01 and *** P < 0.001.

**Fig. 5.** Effects of topical application of tetrodotoxin (TTX) on the decreases in AV interval in response stimulation of the intracardiac sympathetic nerves to the AV nodal region (AVS Stim), the left ansa subclavia (LS Stim), and the right ansa subclavia (RS Stim) in the paced heart of the dog treated with atropine. TTX (3 µg) was injected into the fatty tissue at the AVS stimulation locus. Fine lines show the effect of TTX on the dromotropic response to each stimulation in each experiment. Open circles and vertical bars show mean values and S.E.M., respectively. Asterisks show the level of statistical significance when each response is compared with its control: *** P < 0.001.
pathetic nerve fibers to the AV nodal region (AVP stimulation) prolonged AVCT and caused complete AV block when the level of stimulation was high. Cervical vagus stimulation also induced the negative dromotropic and chronotropic effects. The negative dromotropic responses to AVP, LV and RV stimulations were similarly suppressed by C6 injected into the fatty tissue at the AVP stimulation locus (Fig. 7). However, C6 at the AVP stimulation locus did not significantly affect the negative chronotropic effect of cervical vagus stimulation.

**DISCUSSION**

In the present study, we directly demonstrated that activation of a very small population of intracardiac sympathetic nerves is enough to induce increases in sinus rate, shortening of AV conduction time (AVCT), and an induction of AV junctional rhythm in dog hearts in situ, and the intracardiac innervation of the sympathetic nerves differs from that of the parasympathetic nerves.

Right sided cardiac sympathetic projections exert more pronounced effects on heart rate than left sided projections do (2–4), whereas left sided sympathetic inputs predominantly modulate AV conduction (5, 7, 8, 23), but right sided sympathetic inputs also modulate AV conduction consistently (1, 7). We stimulated the intracardiac sympathetic nerves to the AV nodal region at the fatty tissue of the junction of the inferior vena cava and left atrium, and this stimulation activates at least a part of the ventrolateral cardiac nerves from the left ansa subclavia (11). The ventrolateral cardiac nerve is distributed to the inferior atrial, AV junctional, and ventricular tissues because stimulation of the ventrolateral cardiac nerves causes shortening of AVCT and an AV junctional rhythm as well as lower atrial and ventricular rhythms (7, 24–26). The more peripherally located the point of electrical activation of cardiac sympathetic nerves, the more distinct the effects on control of regional cardiac function (2, 9).

Stimulation of the intracardiac sympathetic nerves to the SA nodal region (SAS stimula-
tion) increases heart rate (sinus rate) with variable changes in AVCT which are not observed in the paced heart (11). In the present study, the positive chronotropic response to SAS stimulation was completely inhibited by topical application of TTX into the fatty tissue of the junctions of the right pulmonary veins (Figs. 2 and 4). In contrast, the TTX treatment only slightly attenuated the positive chronotropic response to stimulation of the right ansa subclavia (RS stimulation) by 17 to 23%. A decrease in AVCT and an AV junctional rhythm induced by stimulation of the intracardiac sympathetic nerves to the AV nodal region (AVS stimulation) were abolished by topical application of TTX into the fatty tissue at the AVS stimulation locus (Figs. 3 and 5). On the other hand, the treatment with TTX only slightly attenuated the similar extent of the positive dromotropic response to stimulation of the left ansa subclavia (LS stimulation) by 7 ± 7.5% (not significantly different from the control response). The positive dromotropic response to RS stimulation was also not significantly attenuated by TTX. AV junctional and lower atrial rhythms during LS stimulation still remained even after TTX treatment (Fig. 3). Therefore, these results indicate that activation of a very small population of the cardiac sympathetic nerves can induce profound changes in SA nodal pacemaker activity, AVCT and an AV junctional rhythm in the dog heart in situ. Our results also suggest that activation of a very small population of intracardiac sympathetic nerves evoked under pathophysiological conditions such as ischemia and myocardial infarction may activate an ectopic focus in the heart. If rhythmicity is enhanced in some ectopic site by local activation of sympathetic nerves in ischemia, the ectopic focus may become the dominant pacemaker for the heart (27, 28).

We injected TTX into the fatty tissue of which the diameter is less than 1 cm and the effect of TTX would be limited. TTX treatment completely inhibited the response to SAS or AVS stimulation but slightly attenuated the response to RS or LS stimulation. These results suggest that intracardiac sympathetic nerves innervate the SA and AV nodes multi-directionally in the heart. We obtained the positive chronotropic and dromotropic responses to discrete intracardiac sympathetic nerve stimulation independently (11). On the other hand, an excitation of the extracardiac individual nerve trunks in the cardiac plexus induces discrete responses in localized regions of the heart (4, 29, 30). After sequential dissection around the major cardiac vessels of the dog heart, the resulting deficits in the responses to ansa subclavia stimulation indicate that parallel, but distinct, sympathetic efferent fibers project to the automatic, conductile, and contractile tissues (10). Thus, when we want to prevent all sympathetic inputs to the SA node or AV node of the heart, we need to interrupt the sympathetic input at the points of extracardiac individual trunks surgically, or epicardial superfusion of TTX pharmacologically (31).

On the other hand, C6 treated at the fatty tissue of SAP and AVP stimulation loci totally inhibited the negative chronotropic response to SAP stimulation and the negative dromotropic response to AVP stimulation (Figs. 6 and 7). Furthermore, C6 treated at the SAP stimulation locus also prevented the chronotropic responses to cervical vagus stimulation and C6 treated at the AVP stimulation locus inhibited the dromotropic response to cervical vagus stimulation as previously reported (13). These results suggest that SAP and AVP stimulation excites preganglionic parasympathetic nerves to the SA and AV nodal regions, respectively; and each stimulation may activate almost all of the vagus inputs to the SA and AV nodal regions in the dog heart. It was also reported that resection of the fatty tissues at junctions of the right pulmonary veins and at the junction of the inferior vena cava and left atrium abolished vagus input to the SA and AV nodal regions, respectively, in anesthetized dogs (17, 18) and monkeys (32). Thus, in contrast with intracardiac sympathetic innervation, it may be possible to control the vagus effects on cardiac electrical responses at
the intracardiac parasympathetic ganglia in the fatty tissues.

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