

Parasympathetic inhibition of sympathetic effects on atrioventricular conduction in anesthetized dogs

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Hoyano, Yuji, Yasuyuki Furukawa, Miho Kasama, and Shigetoshi Chiba. Parasympathetic inhibition of sympathetic effects on atrioventricular conduction in anesthetized dogs. *Am. J. Physiol.* 273 (Heart Circ. Physiol. 42): H1800–H1806, 1997.—To investigate the selective parasympathetic control of atrioventricular (AV) conduction during sympathetic activation, we studied the effects of cervical vagus nerve stimulation on the positive dromotropic responses to sympathetic interventions before and after surgical dissection of dual fatty tissues at the junction of the inferior vena cava and inferior left atrium and at the right atrial side of the atrial junctions of the right pulmonary veins in open-chest anesthetized dogs. In atrial-paced hearts, vagus stimulation at low frequencies prolonged atrio-His (A-H) interval and at high frequencies induced second- and third-degree AV blocks. Vagus stimulation additively prolonged A-H interval shortened by stimulation of the ansae subclaviae or isoproterenol infusion. After dissection of dual fatty tissues, vagus stimulation prolonged A-H interval by only 7%. However, during sympathetic stimulation but not during isoproterenol infusion, vagus stimulation prolonged the shortened A-H interval. Atropine abolished the responses to vagus stimulation. These results suggest that even during sympathetic activation, regional vagus inputs selectively control A-H interval, and even after denervation of the regional parasympathetic nerves, presynaptic parasympathetic inhibition of the positive cardiac responses to sympathetic activation works in the heart in situ.

atrioventricular node; autonomic nervous system; sinus node; sympathetic-parasympathetic interaction; intracardiac parasympathetic nerve

CARDIAC FUNCTION is regulated by tonic activity of the sympathetic and parasympathetic nervous systems, i.e., activation of the sympathetic nerves normally facilitates cardiac function, whereas activation of the parasympathetic nerves inhibits it. Parasympathetic nerve stimulation decreases heart rate more in the presence of tonic sympathetic nerve stimulation than in its absence (15, 19, 25). On the other hand, the sympathetic-parasympathetic interactions in atrioventricular (AV) conductivity observed in previous studies were not consistent. In some studies in anesthetized dogs, the increase in AV conduction time in response to vagus stimulation was not influenced by sympathetic nerve stimulation (15, 24). In other studies, however, vagus nerve stimulation during sympathetic stimulation produced either a greater increase in AV conduction time in anesthetized dogs (20) or a smaller increase in AV conduction time in anesthetized puppies (23). Thus the sympathetic-parasympathetic interactions in the AV conductivity are different from those of the sinoatrial (SA) nodal pacemaker activity.

The parasympathetic neural elements included in cardiac intrinsic ganglionated plexus in the fatty tissue

overlying the right atrial side of the atrial junction of the right pulmonary veins mediate nearly all parasympathetic control of sinus rate (1, 8, 16). Additionally, it is well recognized that parasympathetic neural elements included in cardiac intrinsic ganglionated plexus in the fatty tissue at the junction of the inferior vena cava and inferior left atrium control AV conduction (1, 8, 16). We refer to the former and latter parasympathetic neural elements as the sinus rate-related parasympathetic nerves (SRRPN) and the AV conduction-related parasympathetic nerves (AVCRPN), respectively. Therefore, we refer to surgical dissection of the right pulmonary vein fatty tissue including SRRPN as SRRPN denervation and dissection of the inferior vena cava-inferior left atrial fatty tissue including AVCRPN as AVCRPN denervation, although these fatty tissues contain multiple neuronal types in addition to the parasympathetic postganglionic nerves (10, 26). We recently observed that after SRRPN denervation, cervical vagus stimulation did not decrease sinus rate itself but decreased the sinus rate increased by infusion of isoproterenol in anesthetized dogs and suggested that a small number of vagus nerves to the SA nodal area different from the SRRPN decrease the sinus rate increased by adrenergic interventions in the dog heart in situ (4). In the present study, therefore, we investigated whether cervical nerve stimulation after AVCRPN denervation also prolonged the AV conduction time shortened by sympathetic interventions as does cervical vagus stimulation on sinus rate in anesthetized dogs.

MATERIALS AND METHODS

Preparation. Eleven mongrel dogs (10–19 kg body wt) were anesthetized with pentobarbital sodium (30 mg/kg iv); supplemental doses of pentobarbital sodium were given as necessary to maintain stable anesthesia. A tracheal cannula was inserted, and intermittent positive-pressure ventilation (tidal volume 20 ml/kg; frequency 14 strokes/min) 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 almost all tonic neural activity to the heart (14).

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 electrically. Atrial rate was derived from the atrial electrogram with a tachometer (model AT-600G, Nihon Kohden, Tokyo, Japan). A bipolar electrode catheter was inserted via the right femoral artery and positioned in the noncoronary cusp of the aortic valve to record His bundle electrical activity. The His bundle electrogram was filtered with a band pass of 30–300 Hz (model AP621G, Nihon Kohden). The electrograms were recorded on the oscillograph (model RTA1200, Nihon Kohden) at paper speeds of 100 mm/s. In these electrograms, the interval between the atrial deflection and the His bundle deflection

was determined as the atrio-His (A-H) interval. Atrial rate and femoral arterial blood pressure were also recorded on the oscillograph.

To stimulate cervical vagus nerves, two copper wire electrodes were placed in each cardiac side of the cervical vagus complexes at the crushed region and connected in parallel to an electrical stimulator (model SEN7103, Nihon Kohden). The cervical vagus nerve fibers were stimulated with a pulse amplitude of 10 V with ≤ 0.03 -ms pulse duration and frequencies of 2, 5, 10, and 30 Hz or 1-ms duration and 30 Hz for 30 s. To stimulate bilateral efferent sympathetic nerve fibers, two bipolar hook electrodes were placed on both sides of the ansa subclavia and connected to an electrical stimulator (model SEN7103, Nihon Kohden).

We removed the fatty tissue, including the intracardiac parasympathetic neural elements, at the junction of the inferior vena cava and inferior left atrium by careful trimming of the fatty tissue using a thermoknife and 10% phenol (4). We refer to this procedure as AVCPRN denervation. Cervical vagus stimulation with 1 ms, 10 V, and 30 Hz caused complete AV block. Thus we thought that the AVCPRN were denervated almost totally when cervical vagus stimulation with 1 ms, 10 V, and 30 Hz hardly increased A-H interval (increases of <20 ms). We also removed the fatty tissue, including the intracardiac parasympathetic neural elements to the SA nodal area overlying the right atrial side of the atrial junctions of the right pulmonary veins, because some of these neural elements affect the negative dromotropic response to cervical vagus stimulation (7, 8). We refer to this procedure as SRRPN denervation. Thus we studied the dromotropic or chronotropic effects of cervical vagus nerve stimulation before and after denervation of both AVCPRN and SRRPN.

Protocols. We investigated the effects of cervical vagus nerve stimulation, before and after AVCPRN and SRRPN denervation, on the decrease in A-H interval in response to sympathetic interventions in the atrial-paced heart of the open-chest anesthetized dog and the effects of cervical vagus stimulation on the increase in sinus rate in response to sympathetic interventions in the unpaced heart of the open-chest dog.

First, we studied the effects of cervical vagus stimulation on the decrease in A-H interval in response to sympathetic nerve stimulation ($n = 6$) or isoproterenol infusion ($n = 6$) before and after AVCPRN and SRRPN denervation in the atrial-paced heart of open-chest anesthetized dogs. To determine the A-H interval, the heart rate was held by electrical atrial pacing. The right atrium was paced with 4 V and 1-ms pulse duration at a rate of ~ 150 beats/min that was 40 beats/min above intrinsic sinus rate but maintained 1:1 AV transmission without Wenckebach-type AV block. Before denervation, we determined the effects of cervical vagus stimulation on A-H interval in the absence or presence of sympathetic nerve stimulation or isoproterenol infusion in the atrial-paced heart as the vagus stimulation level was changed by increasing the stimulation frequencies from 2 to 5, 10, and 30 Hz with 10 V and ≤ 0.03 -ms pulse duration and by prolongation of the stimulation pulse duration from ≤ 0.03 to 1 ms with 10 V and 30 Hz (Fig. 1). The stimulation with 1 ms and 10 V at 30 Hz caused complete AV block. To confirm whether the sympathetic interventions could cause similar cardiac effects before and after denervation, we determined the increase in sinus rate in response to sympathetic nerve stimulation in unpaced hearts. We stimulated the cardiac sympathetic nerves with 10–12 V, 1 ms, and 2 Hz for 6 min, and this stimulation increased the sinus rate ~ 70 beats/min. We also determined the infusion rate of isoproterenol (0.2–0.4

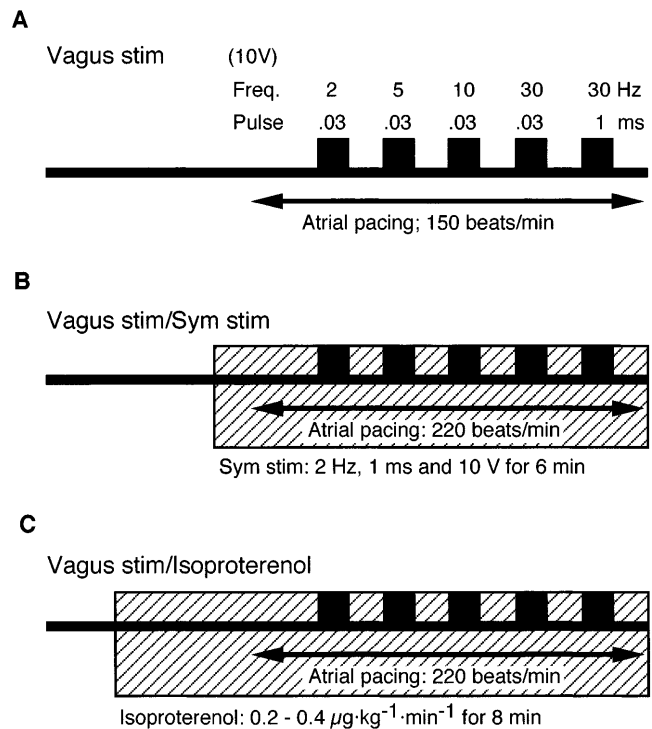


Fig. 1. Experimental protocol. We first determined changes in atrio-His (A-H) interval in response to stimulation of cervical vagus nerves (Vagus Stim; A) with ≤ 0.03 -ms pulse duration and 10-V amplitude at a frequency of 2, 5, 10, or 30 Hz and with 1-ms duration and 10-V amplitude at a frequency of 30 Hz for 30 s with a 30-s interval under electrical atrial pacing. We then tested effects of sympathetic interventions (hatched areas), i.e., sympathetic stimulation (Sym Stim; B) and isoproterenol infusion (C) on negative dromotropic responses to cervical vagus stimulation under atrial pacing. We repeated experiment after both sinus rate- and atrioventricular conduction-related parasympathetic nerve (SRRPN and AVCPRN, respectively) denervation. Freq, frequency.

$\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) that similarly increased sinus rate 70 beats/min. While the right atrium was paced at a rate of 220 beats/min, we determined the effects of cervical vagus stimulation on A-H interval. After each sympathetic intervention, at least 15 min were needed for recovery. Twenty minutes after AVCPRN and SRRPN denervation, we had confirmed the minimum response to cervical vagus stimulation with 1 ms, 10 V, and 30 Hz, and we then studied the effects of cervical vagus stimulation on the positive dromotropic response to sympathetic interventions. Nerve stimulation and isoproterenol infusion with the same conditions were repeated before and after denervation.

In six animals, we tested the effects of atropine at a intravenous dose of 0.2 mg/kg on the negative dromotropic responses to cervical vagus stimulation during sympathetic intervention after AVCPRN and SRRPN were denervated. Two minutes after atropine treatment, the effects of cervical vagus stimulation on the responses to sympathetic intervention were determined again.

In the second series of experiments, we investigated the effects of cervical vagus stimulation on increases in sinus rate in response to sympathetic nerve stimulation or isoproterenol infusion before and after SRRPN and AVCPRN denervation in open-chest anesthetized dog hearts. In these experiments, we also removed dual fatty tissues at the right atrial side of the atrial junctions of the right pulmonary veins and at the junction of the inferior vena cava and inferior left atrium.

First, we determined the changes in sinus rate in response to cervical vagus stimulation as the stimulation level was changed by increasing the stimulation frequencies from 2 to 30 Hz with 10 V and ≤ 0.03 -ms pulse duration and by prolongation of the stimulation pulse duration from ≤ 0.03 to 1 ms with 10 V and 30 Hz. We then studied the effects of cervical vagus stimulation on the positive chronotropic response to stimulation of the ansae subclaviae or isoproterenol infusion. We determined the level of sympathetic nerve stimulation or of infusion rate of isoproterenol that is required to increase sinus rate by ~ 70 beats/min (to 200 beats/min or greater) before the start of the experiment. The sympathetic nerves were stimulated with 2 Hz, 1-ms pulse duration, and 10–12 V for 5 min. Isoproterenol was infused at a rate of 0.2 – $0.4 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ for 7 min. After each sympathetic intervention, at least 15 min of recovery time were needed. When we clearly observed an increase in sinus rate after cessation of cervical vagus stimulation, we stopped further experiments. Twenty minutes after SRRPN and AVCPRN denervation, we had confirmed the minimum response to cervical vagus stimulation with 1 ms, 10 V, and 30 Hz, and we then studied the effects of cervical vagus stimulation on the positive chronotropic responses to sympathetic stimulation and isoproterenol infusion in SRRPN- and AVCPRN-denervated hearts. Nerve stimulation and isoproterenol infusion with the same conditions were repeated before and after SRRPN and AVCPRN denervation.

Statistical analysis. All data are expressed as means \pm SE, and the averages are for each animal. We analyzed the absolute changes in A-H interval and rate in response to interventions except where mentioned. Analysis of variance with Bonferroni's test was used for the statistical analysis of multiple comparisons of data. P values < 0.05 were considered statistically significant.

RESULTS

Cervical vagus stimulation before and after denervation of AVCPRN and SRRPN. Before removal of the fatty tissue at the junction of the inferior vena cava and inferior left atrium including the intracardiac parasympathetic neural elements to the AV nodal region, i.e., AVCPRN, and of the fatty tissue overlying the right atrial side of the atrial junction of the right pulmonary veins including the intracardiac parasympathetic neural elements to the SA nodal region, i.e., SRRPN, cervical vagus nerve stimulation prolonged A-H interval in the anesthetized dog hearts paced electrically (147 ± 9.5 beats/min) (Fig. 2). Vagus stimulation at frequencies of 2 and 5 Hz with 0.01- to 0.03-ms pulse duration and 10-V amplitude prolonged A-H interval, and at 10 and 30 Hz it caused second- and third-degree AV blocks (Fig. 2). When the level of stimulation was increased to 1-ms duration at 30 Hz, vagus stimulation caused a complete AV block. After careful denervation of the AVCPRN and SRRPN, vagus stimulation prolonged A-H interval by only 8 ± 4.5 ms at 30 Hz with 0.01- to 0.03-ms pulse duration. Even when the level of the vagus stimulation was increased by the prolongation of the stimulation pulse duration to 1 ms at 30 Hz, vagus stimulation did not prolong A-H interval (Fig. 2).

Before AVCPRN and SRRPN denervation, cardiac sympathetic nerve stimulation and isoproterenol infusion similarly increased sinus rate and decreased A-H interval in six unpaced hearts of anesthetized dogs

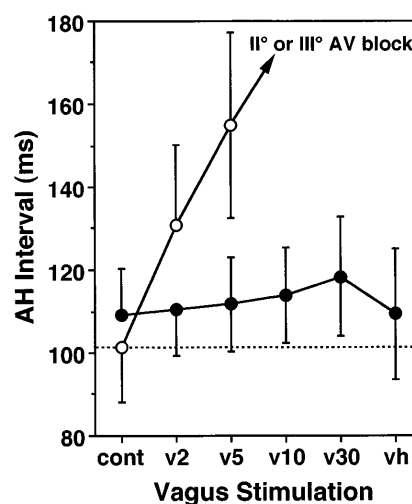


Fig. 2. Changes in A-H interval in response to cervical vagus nerve stimulation before (○) and after (●) denervation of both SRRPN and AVCPRN in 6 open-chest anesthetized dog hearts under atrial pacing. Vertical bars, SE. Dashed line, basal A-H interval (mean 101 ms). Cervical vagus nerves were stimulated at frequencies of 2 (v2), 5 (v5), 10 (v10), and 30 Hz (v30) with ≤ 0.03 -ms pulse duration and 30 Hz with 1-ms pulse duration (vh) for 30 s. Pulse amplitude of stimulation was 10 V. Cont, control.

(Table 1). After denervation, sympathetic stimulation and isoproterenol infusion increased sinus rate similarly to stimulation before denervation. However, the decrease in A-H interval induced by sympathetic stimulation was less than that by isoproterenol infusion in denervated hearts and less than the decreases in A-H interval induced by sympathetic interventions in intracardiac parasympathetic nerve-intact hearts.

Before and after AVCPRN and SRRPN denervation, resting A-H intervals were not significantly different in six atrial-paced hearts of autonomically decentralized, open-chest anesthetized dogs (Table 1). The A-H intervals during sympathetic interventions in atrial-paced hearts were not significantly different before and after denervation (Table 2).

Parasympathetic inhibition of positive dromotropic responses to sympathetic interventions before and after denervation of AVCPRN and SRRPN. Stimulation of the right and left ansae subclaviae decreased A-H interval before and after AVCPRN and SRRPN denervation. However, the positive dromotropic response to sympathetic nerve stimulation after denervation was smaller ($P < 0.05$) than that before denervation (Table 1).

During sympathetic nerve stimulation or isoproterenol infusion, cervical vagus stimulation at 2 and 5 Hz with ≤ 0.03 -ms pulse duration and 10-V amplitude prolonged A-H interval, and at 10 and 30 Hz it caused second- or third-degree AV block when AVCPRN and SRRPN were intact (Figs. 3 and 4). The increases in A-H interval in response to vagus stimulation during sympathetic interventions were not significantly different from those in response to vagus stimulation alone in intracardiac parasympathetic nerve-intact hearts of open-chest anesthetized dogs (Fig. 4).

After AVCPRN and SRRPN denervation, during sympathetic nerve stimulation, cervical vagus stimulation

Table 1. Cardiac values before and during sympathetic nerve stimulation or isoproterenol infusion in AVCRPN- and SRRPN-intact and -denervated, unpaced dog hearts

Experimental Group	<i>n</i>	Before			During Sympathetic Intervention		
		HR, min ⁻¹	AHI, ms	HVI, ms	HR, min ⁻¹	AHI, ms	HVI, ms
AVCRPN and SRRPN intact							
Control	6	107 ± 6.5	80 ± 9.2	36 ± 4.8			
Sympathetic	6	111 ± 8.6	79 ± 9.9	37 ± 3.5	185 ± 6.8	56 ± 6.6	35 ± 4.3
Isoproterenol	6	105 ± 7.0	80 ± 9.3	37 ± 4.6	182 ± 7.2	58 ± 6.8	36 ± 4.5
AVCRPN and SRRPN denervation							
Control	6	98 ± 4.8	77 ± 8.3	43 ± 6.1			
Sympathetic	6	93 ± 5.7	77 ± 8.4	42 ± 5.2	164 ± 8.1	68 ± 9.8	41 ± 5.2
Isoproterenol	6	94 ± 5.7	79 ± 8.5	41 ± 5.7	170 ± 5.9	55 ± 7.6	42 ± 6.4

Values are means ± SE; n, no. of experiments. HR, heart rate; AHI, atrio-His interval; HVI, His-ventricle interval; AVCRPN, atrioventricular conduction-related parasympathetic nerves; SRRPN, sinus rate-related parasympathetic nerves. Cardiac sympathetic nerves were stimulated with 2 Hz, 1-ms pulse duration, and 10–12 V for 6 min, and isoproterenol was infused at a rate of 0.2–0.4 µg·kg⁻¹·min⁻¹ for 8 min. Nerve stimulation and isoproterenol were given with same parameters and rate, respectively, before and after AVCRPN and SRRPN denervation.

prolonged A-H interval slightly but significantly ($P < 0.001$); vagus stimulation at 30 Hz significantly ($P < 0.05$) prolonged A-H interval during sympathetic stimulation (Figs. 3 and 4). On the other hand, however, during isoproterenol infusion vagus stimulation did not prolong A-H interval significantly (Fig. 4). The negative dromotropic response to vagus stimulation during sympathetic stimulation was abolished by atropine at an intravenous dose of 0.2 mg/kg in the atrial-paced heart (Fig. 5).

Parasympathetic inhibition of positive chronotropic responses to sympathetic interventions in unpaced heart before and after SRRPN and AVCRPN denervation. Before and after SRRPN and AVCRPN denervation, sympathetic nerve stimulation and isoproterenol infusion increased sinus rate similarly in five unpaced hearts of open-chest anesthetized dogs (Table 3). Cervical vagus nerve stimulation decreased sinus rate itself and sinus rate during sympathetic interventions in a

stimulation frequency-dependent manner (data not shown). The decreases in sinus rate induced by vagus stimulation were similar in the presence of sympathetic nerve stimulation or isoproterenol infusion.

After SRRPN and AVCRPN denervation, vagus stimulation hardly decreased sinus rate itself but attenuated the positive chronotropic response to sympathetic stimulation and to isoproterenol infusion as we previously reported (4). The inhibition by vagus stimulation of the positive chronotropic response to sympathetic stimulation was greater ($P < 0.01$) than that of the positive chronotropic response to isoproterenol infusion.

DISCUSSION

In the dog heart, stimulation of the intracardiac parasympathetic neural elements at the fatty tissue at the junction of the inferior vena cava and inferior left atrium selectively prolongs AV conduction time (6, 8), and denervation of the regional parasympathetic nerves (AVCRPN) eliminates the prolongation of AV conduc-

Table 2. AHI and HVI before and during sympathetic nerve stimulation or isoproterenol infusion in AVCRPN- and SRRPN-intact and -denervated, electrically paced dog hearts

Experimental Group	n	APR, min ⁻¹	AHI, ms	HVI, ms
AVCRPN and SRRPN intact				
Control	6	147 ± 9.5	101 ± 12.9	36 ± 4.7
Sympathetic	6	220	72 ± 5.9	36 ± 4.6
Isoproterenol	6	220	71 ± 4.8	37 ± 4.5
AVCRPN and SRRPN denervation				
Control	6	137 ± 8.0	109 ± 11.1	42 ± 5.8
Sympathetic	6	220	89 ± 11.4	42 ± 6.0
Isoproterenol	6	220	79 ± 6.5	41 ± 6.5

AHI and HVI values are means ± SE; n, no. of experiments. APR, atrial rate paced electrically. Cardiac sympathetic nerves were stimulated with 2 Hz, 1-ms pulse duration and 10–12 V for 6 min, and isoproterenol was infused at a rate of 0.2–0.4 µg·kg⁻¹·min⁻¹ for 8 min. Nerve stimulation and isoproterenol were given with same parameters and rate, respectively, before and after AVCRPN and SRRPN denervation. In control experiments, atrial pacing rates had to be lowered in 3 of 6 animals after denervation because same pacing rates as those in AVCRPN and SRRPN intact animals had caused Wenckebach-type AV block.

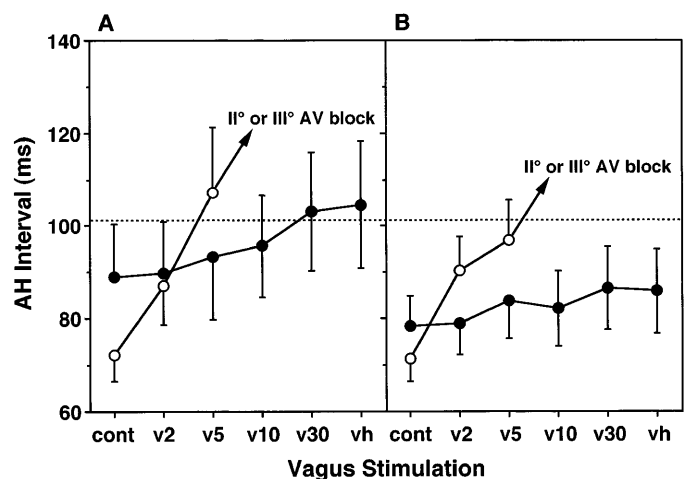


Fig. 3. Effects of cervical vagus stimulation at 5 different levels on positive dromotropic responses to sympathetic nerve stimulation (A) and isoproterenol infusion (B) before (○) and after (●) denervation of SRRPN and AVCRPN in 6 open-chest anesthetized dog hearts under atrial pacing. Vertical bars, SE. Dashed line, basal A-H interval (mean 101 ms). Pulse amplitude of stimulation was 10 V.

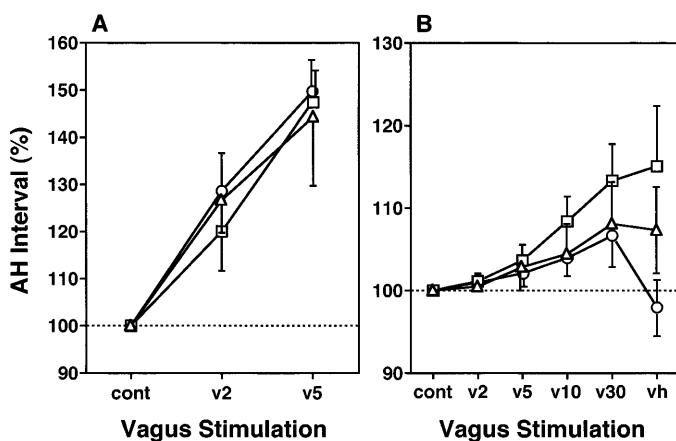


Fig. 4. Effects of cervical vagus stimulation at 5 different levels on basal A-H interval (\circ), positive dromotropic responses to sympathetic nerve stimulation (\square), and isoproterenol infusion (\triangle) before (A) and after (B) denervation of SRRPN and AVCPRN in 6 open-chest anesthetized dog hearts under atrial pacing. Values are percentage of A-H interval just before cervical vagus stimulation. Vertical bars, SE. Pulse amplitude of stimulation was 10 V.

tion time induced by cervical vagus stimulation (1, 16). Stimulation of the intracardiac parasympathetic neural elements at the fatty tissue overlying the atrial junctions of the right pulmonary veins selectively decreases sinus rate (6, 8), and denervation of the regional parasympathetic nerves (SRRPN) eliminates the decrease in sinus rate in response to cervical vagus stimulation (1, 16). Thus activation of AVCPRN and SRRPN prolongs AV conduction time and decreases sinus rate, respectively.

In the present study, we demonstrated that after denervation of AVCPRN and SRRPN, cervical vagus stimulation hardly prolonged A-H interval itself or the A-H interval decreased by isoproterenol infusion in the atrial-paced heart. However, vagus stimulation at a high stimulation frequency, but not at low frequencies, prolonged the A-H interval slightly but significantly when sympathetic stimulation shortened A-H interval in the AVCPRN- and SRRPN-denervated hearts. These

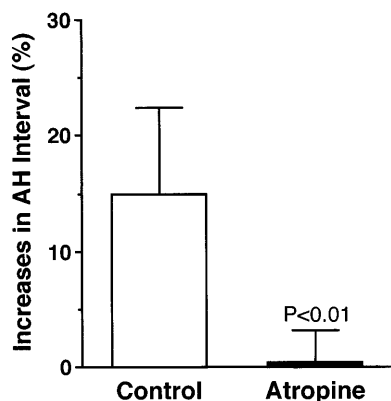


Fig. 5. Effects of atropine (0.2 mg/kg iv) on negative dromotropic responses to cervical vagus stimulation during sympathetic nerve stimulation in 6 anesthetized dog hearts after both SRRPN and AVCPRN were removed. Values are percent changes from level just before cervical vagus stimulation. Vertical bars, SE. Cervical nerves were stimulated with 30 Hz, 1 ms, and 10 V.

Table 3. Sinus rate before and during sympathetic nerve stimulation and isoproterenol infusion in five SRRPN- and AVCPRN-intact and -denervated dog hearts

Experimental Group	Intact	After Denervation
Control	125 \pm 6.5	130 \pm 7.4
Sympathetic stimulation	214 \pm 11.9	195 \pm 11.2
Isoproterenol infusion	212 \pm 13.3	202 \pm 11.6

Values are means \pm SE in beats/min for 5 experiments in each group. Cardiac sympathetic nerves were stimulated with 2 Hz, 1-ms pulse duration, and 10–12 V for 5 min, and isoproterenol was infused at a rate of 0.2–0.4 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ for 7 min. Nerve stimulation and isoproterenol were given with same parameters and rate, respectively, before and after SRRPN and AVCPRN denervation.

results suggest that the negative dromotropic response to cervical vagus stimulation is mediated almost totally by AVCPRN at rest and during sympathetic activity in the heart in situ and that, in addition to the postsynaptic parasympathetic inhibition of the sympathetic effects, presynaptic parasympathetic inhibition works functionally although the parasympathetic inhibition threshold for postsynaptic inhibition is much lower than that for presynaptic inhibition.

AVCPRN denervation. To elucidate the role of the AVCPRN on AV conduction when sympathetic nerves are activated, we investigated the effects of cervical vagus stimulation on A-H interval during sympathetic interventions, i.e., sympathetic nerve stimulation and isoproterenol infusion, before and after AVCPRN and SRRPN denervation in the dog heart. To complete the denervation of AVCPRN, we removed the fatty tissue at the junction of the inferior vena cava and lower left atrium by using a thermoknife with phenol. We considered that denervation had been completed when cervical vagus nerve stimulation did not increase A-H interval ≥ 20 ms, although the same vagus stimulation caused complete AV block before denervation. To elucidate the effects of the denervation procedures on the responses to sympathetic interventions, we compared the changes in sinus rate and in A-H interval in response to sympathetic interventions before and after denervation in spontaneously beating hearts. The decrease in A-H interval in response to sympathetic stimulation after AVCPRN and SRRPN denervation was less than that before the denervation, although the decrease in A-H interval induced by isoproterenol and the increase in sinus rate induced by sympathetic interventions were not different before and after AVCPRN and SRRPN denervation (Table 1). These results indicate that the AVCPRN denervation procedure removes not only AVCPRN but also some of the sympathetic nerves to the AV nodal region in the dog heart. The ventrolateral cardiac nerve is distributed to the inferior atrial, AV junctional, and ventricular tissues, and its stimulation causes shortening of AV conduction time and an induction of AV junctional rhythm (9, 17, 18). Stimulation of AVCPRN decreases AV conduction time and induces an AV junctional rhythm in atropine-treated anesthetized dogs (5, 21). Thus our denervation procedure allows us to analyze

the data of the sympathetic-parasympathetic interactions on AV conduction before and after AVCPRN and SRRPN denervation qualitatively but not quantitatively.

Sympathetic-parasympathetic interactions on AV conduction and sinus rate. In intracardiac parasympathetic nerve-intact hearts, vagus nerve stimulation decreases sinus rate more in the presence than in the absence of tonic sympathetic interventions in anesthetized dogs as shown previously (4, 15, 19, 25), whereas the increase in AV conduction time in response to vagus nerve stimulation was not significantly influenced by sympathetic nerve stimulation (Figs. 3 and 4) as previously reported (15, 24). On the other hand, after AVCPRN and SRRPN denervation, vagus stimulation did not prolong A-H interval during isoproterenol infusion, whereas it decreased sinus rate during isoproterenol infusion. These results suggest that, although a very small number of intracardiac parasympathetic neural elements might exist after AVCPRN and SRRPN denervation, the denervation of AVCPRN and SRRPN abolished the dromotropic effects of vagus stimulation in the dog heart and that the AVCPRN almost totally mediate the negative dromotropic response to cervical vagus stimulation in the presence as well as the absence of adrenergic stimulation. However, stimulation of a small number of parasympathetic neural elements probably decreased sinus rate increased by isoproterenol because of the parasympathetic inhibition of the sympathomimetic effects on sinus rate at the postsynaptic site (4), suggesting that the parasympathetic neural regulation of sinus rate and AV conduction is different and that complex interactions exist in the heart.

After AVCPRN and SRRPN denervation, during sympathetic stimulation, cervical vagus stimulation at a high frequency (30 Hz) increased A-H interval slightly but significantly, but at low frequencies it did not (Fig. 4). These results together with the lack of prolongation of the A-H interval in response to vagus stimulation during isoproterenol infusion suggest that presynaptic parasympathetic inhibition works functionally, although the parasympathetic inhibition threshold for postsynaptic inhibition is lower than that for presynaptic inhibition in the heart in situ. Parasympathetic ganglionic cells as well as postsynaptic parasympathetic nerve fibers exist in the fatty tissues at the junction of the inferior vena cava and lower left atrium and at the right atrial junctions of the right pulmonary veins (3, 6, 16). Because we might have removed those neural elements, including parasympathetic ganglionic cells, the prolongation of A-H interval by vagus stimulation during sympathetic stimulation observed in the present study would be induced at the site proximal to these parasympathetic ganglia. It is well known that sympathetic-parasympathetic interactions on the heart are evoked at both the presynaptic and postsynaptic sites (12, 13). Sympathetic-parasympathetic interaction at the presynaptic site involves interactions between the terminal postganglionic vagal and sympathetic fibers, which often lie in close apposition to each

other in the heart (2, 11, 13). Therefore, it is likely that intraneuronal mechanisms for sympathetic-parasympathetic interactions may partially be an extracardiac neural interaction in addition to an intracardiac neural interaction in the dog heart. However, it has been reported that there are several intrinsic cardiac ganglionated plexuses other than those we have removed, including not only cholinergic neurons but also catecholamine-sensitive and/or -producing neurons and others (10, 26). Thus we cannot rule out the possibility of the participation of other innervation, from nerves that do not pass through the intrinsic cardiac ganglia we have removed, in presynaptic inhibition of the positive dromotropic response to sympathetic nerve activation.

We confirmed that in the SRRPN- and AVCPRN-denervated heart of the anesthetized dog, cervical vagus stimulation hardly decreased sinus rate, but it depressed the sinus rate increased by isoproterenol as well as by sympathetic stimulation as previously reported (4). SRRPN stimulation during sympathetic nerve stimulation decreases sinus rate more than SRRPN stimulation alone in the anesthetized dog heart (22). On the other hand, vagus stimulation after AVCPRN and SRRPN denervation did not affect the A-H interval during isoproterenol infusion significantly (Figs. 3 and 4). Thus the postsynaptic inhibition by vagus stimulation of sinus rate would be more complex than that of AV conduction in the dog heart in situ.

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Received 16 September 1996; accepted in final form 12 June 1997.

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