Effects of Sympathetic and Parasympathetic Stimulation on the Induction of Atrial Flutter in Dogs with Aseptic Pericarditis

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SUMMARY

To study the effects of sympathetic stimulation (SS) and vagal stimulation (VS) on the induction of atrial flutter (AF) and its cycle length, aseptic pericarditis was produced surgically in 17 adult mongrel dogs. Programmed atrial stimulation was used to induce AF and to determine the effective refractory periods (ERP) and maximum conduction delay (maxCD) of the atrium. These tests were performed before and during stimulation of the right cervical vagus nerve and the right stellate ganglion. Results showed: (1) AF could be induced in animals with short ERP and large maxCD before SS; (2) ERP was significantly shortened, maxCD was significantly increased by VS; (3) During SS, ERP was slightly shortened, but there were no changes in maxCD and the induction rate of AF; (4) The cycle length of AF was shortened by SS, however, the cycle length of AF was shortened more notably by VS than by SS. From these findings, a shortening of the ERP and an increase in maxCD appear to be related to an increased AF induction rate by VS.

Key Words:
Atrial flutter Aseptic pericarditis Sympathetic stimulation Vagal stimulation Induction of atrial flutter Cycle length of atrial flutter

Induction of atrial fibrillation (AF) has been reported to be promoted by sympathetic stimulation (SS)\(^1,^2\) and vagal stimulation (VS)\(^3,^4\) but there have been few reports on the effects of SS and VS on the induction of atrial flutter (AF) or the cycle length of induced AF. Sharma et al\(^3\) reported that AF was induced by electrical stimulation of the atrium during intravenous administration of adrenaline. Their study primarily concerned the effects of catecholamines on acetylcholine-induced AF and they did not evaluate the
effects of SS on electrically-induced AF. Scherf et al\textsuperscript{3}) reported the induction of AF and AF after application of acetylcholine on the atrial epicardium in the dog. However, we are unaware of any systematic studies of the effects of VS on induction of AF. Rosenblueth et al\textsuperscript{9}) reported that the cycle length of AF is slightly shortened by submaximum VS and intravenous adrenaline injection. However, they did not evaluate either the magnitude of this shortening of the cycle length or its relationship to changes in the atrial refractory period. This study examined the effects of SS and VS on induction of AF and its cycle length in a dog model with aseptic pericarditis, in which stable and sustained AF can be really induced by electrical stimulation.

**METHODS**

**Preparation of aseptic pericarditis model**

Seventeen adult mongrel dogs weighing 9–12 kg were used. The dogs were anesthetized by intravenous injection of pentobarbital sodium (30 mg/kg), intubated, and an aseptic thoracotomy was performed at the right fourth intercostal space under artificial ventilation with a Harvard respirator. The heart was exposed by pericardial incision. For the subsequent recording of atrial electrograms, bipolar electrodes (electrode diameter 1 mm, inter-electrode distance 2 mm) were sutured to the epicardial surface of the high lateral right atrium, sulcus terminalis, low lateral right atrium, Bachmann's bundle, and low posterior left atrium (Fig. 1). Another bipolar electrode (diameter 1 mm, inter-electrode distance 2 mm) was sutured to a site near the recordings on the high lateral right atrium for electrical stimulation (Fig. 1). A single sheet of sterilized gauze was then sutured to the right atrial surface, powdered talc was applied to the area, and the pericardium was closed.

![Fig. 1. Positions of bipolar electrodes on the atrium.](image)

BB = Bachmann's bundle; HLRA = high lateral right atrium; LA = left atrium; LLRA = low lateral right atrium; LPLA = low posterior left atrium; RA = right atrium; ST = sulcus terminalis.
The wires from the electrodes were passed through the right fourth intercostal space out of the thorax and led subcutaneously to the back, where it was exposed and fixed on the body surface. After closing the thoracotomy, penicillin G (100,000 units) was administered intramuscularly to prevent infections, and the animals were allowed to recover.

**Installation of electrodes for SS and VS and stimulation conditions**

(a) Dissection and separation of the right stellate ganglion and attachment of stimulation electrodes

The animals were anesthetized by intravenous injection of pentobarbital sodium (30 mg/kg) 3 days after surgical induction of experimental aseptic pericarditis. After endotracheal intubation, a thoracotomy was performed by median sternal incision under artificial ventilation. After removing connective tissues around the right stellate ganglion, all nerve branches except the cardiac branch (ansae subclaviae) arising from the right sympathetic ganglion were separated to block stimulation from the center. Next, a bipolar electrode was inserted into the right stellate ganglion, which was then covered with petrolatum. The left stellate ganglion was exposed and removed.

(b) Installation of electrode on the right vagus nerve

The vagal trunks were exposed bilaterally in the neck and were cut proximally. A bipolar electrode was inserted into the distal stump of the right vagal trunk.

**Experimental protocol**

The experiment was carried out according to the protocol shown in

![Experimental protocol diagram](image)

Fig. 2. Experimental protocol.

AF = atrial flutter; PAS = programmed atrial stimulation; SS = sympathetic stimulation; VS = vagal stimulation.
Fig. 2. First, the effective refractory period (ERP) of the atrium and the maximum conduction delay (maxCD) of the atrium were determined during sinus rhythm before autonomic stimulation (control) (Fig. 2-a). AF was induced by stimulation of the high lateral right atrium. ERP, maxCD and induction of AF were also studied during SS and VS. The cycle length of induced AF was examined before autonomic stimulation, during SS, and during VS (Fig. 2-b).

**Stimulation methods**

(a) Condition of SS and VS

The bipolar electrode in the right stellate ganglion was connected to an electrical stimulator (E2991, Device Co). The bipolar electrode in the right vagal trunk was connected to another stimulator (SEC2102, Nihon Kohden Co). The right stellate ganglion was stimulated with rectangular electric waves with a frequency of 4 Hz and a duration of 2 msec. The intensity of stimulation was adjusted to the level at which the heart rate was increased by 30–50% (1–5 volts). The right cervical vagal trunk was stimulated with rectangular electric waves with a frequency of 20 Hz and duration of 2 msec. The intensity of stimulation was adjusted to the level at which the heart rate was reduced by 30–50% (1–4 volts).

(b) Methods and conditions of atrial stimulation

ERP and maxCD were determined by programmed atrial stimulation. A single stimulus was delivered through the atrial electrode. AF was induced by continuous atrial stimulation. A programmable stimulator (BC-02A, Fukuda Denshi) was used to deliver rectangular electric waves of 2 msec duration at an intensity twice the diastolic threshold.

**Recording methods**

All atrial recording electrodes were connected to an amplifier (UA-210, Fukuda Denshi) and a recorder (Mingograf 800, Siemens). For recording the body surface electrocardiogram, needle electrodes were inserted into the 4 limbs of the animals and connected to the same recording apparatus. Epicardial electrograms from the 5 atrial sites were recorded at a time constant of 0.003 sec simultaneously with body surface electrocardiograms (leads II, III and aVf). The recording paper speed was 100 mm/sec.

**Items examined and methods of measurement**

(a) Atrial ERP

Progressively premature atrial stimuli (S2) were introduced after 8 basal atrial stimulation pulses at a cycle length of 300 msec (S1). The coupling
interval (S1–S2) was shortened by 5 msec at each stimulation, and the longest coupling interval (S1–S2) at which S2 did not evoke an atrial response was regarded as the atrial ERP.

(b) Atrial maxCD

Figure 3 shows a schematic atrial electrogram recorded from the low lateral right atrium during programmed atrial stimulation of the high lateral right atrium. As indicated in the figure, the difference between S2A2 and S1A1 of the 8th basal stimulation pulse was regarded as the atrial conduction delay, and its maximum value at various coupling periods was expressed as maxCD. The maxCD was determined only during basal stimulation at a cycle length of 300 msec.

(c) Induction of AF

1) Method of induction

AF was induced by brief electrical stimulation of the high right atrium. The duration of stimulation was 1 sec, and the frequency of stimulation was 20, 25 or 33 Hz. The stimulation was repeated 10 times at 7-sec intervals for each frequency.

2) Definition of induced AF and af

Atrial flutter (AF) was defined as an electrically induced atrial arrhythmia lasting more than 30 sec; it was characterized by atrial electrograms with a regular, short cycle length and a saw tooth appearance of surface ECG isoelectric lines. Atrial fibrillation (af) was defined as an electrically induced atrial arrhythmia lasting at least 10 sec, characterized by atrial electrograms with extremely irregular cycle length and amplitude, and a surface ECG with both an irregular cycle length and an amplitude that lacked isoelectric lines.

3) Induction rate of AF

The induction rate was expressed as the percentage of animals that dis-
played at least one period of AF during programmed atrial stimulation.

4) Measurement of cycle length of induced AF

To increase the precision of measurement of the cycle length, the sum of the intervals of 10 consecutive AF waves was divided by 10.

**Statistical analysis**

All results are expressed as the means±standard deviations. Statistical analysis was performed by analysis of variance, Bonferroni t-test and Chi-square test, as appropriate. Correlation coefficients were obtained by single regression analysis. The differences in the values were considered to be significant when the p value was less than 0.05.

**Animal care**

Experiments were conducted according to the Nagasaki University Guidelines for the Care and Use of Laboratory Animals.

**RESULTS**

**Induction of AF**

Figure 4 shows an example of atrial flutter (AF), which was induced immediately after brief electrical stimulation in the control-state. Electrocardiographic leads II, III and aVF were recorded simultaneously with atrial electrograms at sulcus terminalis, low lateral right atrium, Bachmann’s bundle and low posterior left atrium. Atrial electrograms showed a regular, short cycle length and surface ECGs showed atrial waves without isoelectric lines.

![Fig. 4. Induction of atrial flutter by electrical stimulation. ST=sulcus terminalis; LLRA=low lateral right atrium; BB=Bachmann's bundle; LPLA=low posterior left atrium; AF=atrial flutter.](image-url)
Induction rate of AF

Before autonomic stimulation, the AF induction rate was 17.7% (3/17); 2 animals displayed both AF and atrial fibrillation (af) and one animal displayed only AF (Fig. 5). During VS, the AF induction rate was 35.3% (6/17), which did not differ from the control rate of 3/17. The af induction rate was 29.4% (5/17) before stimulation, slightly reduced to 23.6% (4/17) during SS, but increased significantly (p<0.05) to 64.7% (11/17) during VS. During SS, the AF induction rate was the same as control.

ERP of the atrium

As shown in Table I, the ERP of the high lateral right atrium was reduced during both SS and VS (p<0.01). The ERP during VS was also lower than during SS (p<0.01). Figure 6 compares the effects of SS and VS on atrial ERP as a function of induction of AF and af. The control ERP was longer in animals that did not display AF or af (negative group; 167±18 msec) than in animals displaying AF (AF (+) group; 130±18 msec) and animals displaying af (af (+) group, 133±16 msec) (p<0.01). During SS, the ERP of the atrium shortened significantly to 148±15 msec in the negative group, 122±10 msec in the AF (+) group and 123±13 msec in the af (+) group.
Table I. The Atrial ERP and maxCD in the Atrium during SS and VS

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maxCD = effective refractory period; maxCD = maximum conduction delay; C = control; SS = sympathetic stimulation; VS = vagal stimulation. * p < 0.01 vs. control, ** p < 0.01 vs. SS.

group (p < 0.01). During VS, it further shortened to 143 ± 12 msec in the negative group, 108 ± 15 msec in the AF (+) group and 111 ± 15 msec in the af (+) group (p < 0.01). The ERP did not differ significantly between the AF (+) and af (+) groups before stimulation (control), during SS, or during VS, but it was significantly shorter in these groups than in the negative group (p < 0.01).

**maxCD**

As shown in Table I, there was no significant change in maxCD during SS. However, maxCD was notably increased during VS, though statistically not significant. Figure 7 compares maxCD in the AF (+), af (+) and negative groups during SS and VS. Before autonomic stimulation (control), maxCD was 57 ± 2 msec in the AF (+) group, 48 ± 13 msec in the af (+) group and 28 ± 14 msec in the negative group. During SS, maxCD was 45 ± 4 msec in the AF (+) group, 39 ± 7 msec in the af (+) group and 33 ± 17 msec in the negative group. During VS, maxCD was 57 ± 15 msec in the AF (+) group, 55 ± 16 msec in the af (+) group and 33 ± 16 msec in the negative
Fig. 6. Comparison of the atrial ERP in the AF (+), af (+) and negative groups during SS and VS.

The ERP was not significantly different between the AF (+) and af (+) groups in the control period, during SS, and during VS, but it was significantly shorter in these groups in the negative group.

○ = AF (+) only; ▲ = af (+) only; ◆ = both AF (+) and af (+); × = AF (-) and af (-); SS = sympathetic stimulation; VS = vagal stimulation; ERP = effective refractory period.

Fig. 7. Comparison of the maxCD in the AF (+), af (+) and negative groups before and during SS and VS.

The maxCD was not significantly different between the AF (+) and af (+) groups in control, during SS, or during VS. However, it was significantly greater in these groups than in the negative group (p<0.01).

Relationship of ERP of the atrium and maxCD with induction of AF and af

Figure 8 shows the relationship between the ERP and maxCD during control, SS and VS periods. The maxCD was greater in animals displaying
Fig. 8. Relationship of the atrial ERP and maxCD before and during SS and VS.

\( \bigcirc = \text{AF (+) only}; \; \blacktriangle = \text{af (+) only}; \; \Box = \text{both AF (+) and af (+)}; \; \times = \text{AF (--) and af (--)}; \; \text{SS = sympathetic stimulation; VS = vagal stimulation; maxCD = maximum conduction delay; ERP = effective refractory period.} \)

Fig. 9. Effects of sympathetic and vagal stimulation on the cycle length of induced AF.

\( \text{SS = sympathetic stimulation; VS = vagal stimulation; ST = sulcus terminalis; LLRA = low lateral right atrium; BB = Bachmann's bundle; LPLA = low posterior left atrium; F-F indicates cycle length of induced AF. See text for details.} \)

AF and/or af (AF and/or af (+) group) than in animals that did not display both AF and af (negative group) during control (49±12 vs. 28±14 msec), SS (40±7 vs. 33±17 msec) and VS (55±16 vs. 33±16 msec), though statistically not significant. On the other hand, the ERP was significantly shorter in the AF and/or af (+) group than in the negative group during control (134±15 vs. 167±18 msec, \( p<0.01 \)), SS (122±11 vs. 148±15 msec, \( p<0.01 \)) and VS (111±15 vs. 143±12 msec, \( p<0.01 \)). Thus, both AF and
Table II. The Cycle Length of AF during SS and VS

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mean±SD | 153±16 | 143±13* | 128±12*,**

AF=atrial flutter; C=control; SS=sympathetic stimulation; VS=vagal stimulation. * p<0.05 vs. control, ** p<0.05 vs. SS.

Fig. 10. Relationship between the cycle length of AF and the ERP before and during SS and VS.

○=control; ●=sympathetic stimulation; Δ=vagal stimulation; AF=atrial flutter; ERP=effective refractory period.
Changes in the cycle length of AF during SS and VS

Changes in the cycle length of AF were studied in 6 dogs. Figure 9 shows typical records of the sympathetic and vagal stimulation periods during induced AF. The cycle length of induced AF was 162 msec before autonomic stimulation (control). It reduced slightly to 142 msec during SS and reduced considerably to 128 msec during VS. As shown in Table II, the cycle length of induced AF was 153±16 msec before autonomic stimulation (control). It reduced significantly to 143±13 msec during SS (p<0.05) and reduced more notably to 128±12 msec during VS (p<0.05). Figure 10 shows the relationship between the cycle length of AF and the ERP during these stimulus conditions. When the values before stimulation, during SS and during VS were evaluated as a whole, a significant positive correlation was observed between the cycle length of AF and the ERP (p<0.05).

Discussion

It is recognized that VS and SS can induce AF. Reports on the effects of SS and VS on induction of AF, though, are rare, and these effects have not been evaluated quantitatively. Sharma et al1) reported that continuous atrial stimulation could induce AF during intravenous adrenaline installation in 4 of 14 normal dogs. However, they did not compare the results before and after adrenaline administration. Moreover, their study does not exclude the possibility that intravenous adrenaline infusion and stimulation of the stellate ganglion may have different effects on induction of AF.

The effects of acetylcholine administration and VS on induction of AF have been reported by a number of investigators.3)-7) However, to our knowledge, there are no quantitative reports on changes in the induction rate of AF by VS. In this study, the effects of SS and VS on induction of AF were examined in a model with aseptic pericarditis described by Waldo et al.8) Since stable and sustained AF can be readily induced in this model by electrical atrial stimulation under appropriate conditions, it is suitable for quantitative evaluation of AF induction. Moreover, unlike other models such as local atrial injection of aconitine11) or crushing of the inter-venae-caval bridge (Rosenblueth model),9) the induction of AF with atrial injury due to aseptic pericarditis is similar to clinically observed AF in pericarditis or after cardiac operations. Therefore, our model is considered to be advantageous for evaluation of the effects of autonomic activity on AF.
**Effects of autonomic activities on induction of AF**

The AF induction rate increased during VS in the aseptic pericarditis model. This observation was similar to the earlier reports that induction of af was promoted by VS. Scherf et al reported that AF and af were induced by application of acetylcholine-saturated filter paper on the dog sinus node. Our results also suggest that VS facilitates induction of AF.

There was no effect of SS on induction of either AF or af in the aseptic pericarditis model. Hashimoto et al contended that adrenergic mechanisms play an important role in the development of af, because acetylcholine-induced af is suppressed by reserpine. Although the reason for the discrepancy between our results and these reports is unknown, its possible causes include: (a) Sharma et al used adrenaline, a sympathomimetic agent, while we directly stimulated the sympathetic nerve (stellate ganglion); (b) Hashimoto et al and Sharma et al used acetylcholine and evaluated its interactions with sympathetic drugs, but we examined the effects of direct sympathetic efferent stimulation; and (c) experimental models without atrial injury were used in their studies.

**Electrophysiologic conditions for AF induction**

Both AF and af tended to be induced more frequently in animals with shorter ERP and greater maxCD (Fig. 8). Buxton et al reported that the maxCD was greatest in patients with paroxysmal AF or paroxysmal af. Cosio et al also observed a shorter ERP and a greater maxCD in the AF (+) group than in the AF (−) group, and suggested that these changes contribute to the establishment of intra-atrial reentry. In our study, the induction rates of AF or af were not affected by SS, probably because the shortening of the ERP was mild and the maxCD did not increase. On the other hand, VS probably increased the induction rates of AF and af by markedly shortening the ERP and increasing the maxCD.

**Effects of autonomic activities on the cycle length of AF**

In this study, the AF cycle length was significantly shortened during both SS and VS, but the degree of this shortening was greater during VS. Rosenblueth et al reported a slight shortening of AF cycle length during either intravenous injection of adrenaline or submaximum VS in an AF model prepared by crushing of the inter-venae-caval tissue (Rosenblueth model); however, they did not detail the extent of this shortening quantitatively. In our study, the AF cycle length was shortened by even moderate SS or VS that increased or decreased the rate of sinus rhythm by 30–50%. The shortening was only 10 msec during SS but 25 msec during VS. Although we
cannot explain why the AF cycle length was shortened by both SS and VS, the sympathetic nerve\(^\text{14,15}\) and the vagus nerve\(^\text{16-18}\) may each influence intra-atrial conduction time, thus shortening the cycle length by introducing reentry with an excitable gap. Furthermore, if the AF in this aseptic pericarditis model is assumed to be caused by reentry via a leading circle, the reentry cycle is determined by the refractory periods.\(^\text{10}\) Therefore, the AF cycle length may have been shortened as a consequence of shortening of the refractory period by SS and VS.

**Limitation of the study**

We studied the effects of autonomic activity on AF induction and cycle length during separate periods of SS and VS. However, our results may include the effects of interactions of the two nervous systems in nerve terminals\(^\text{19-21}\) as well as the effects of either system alone. In addition, since the ERP was measured at the high lateral right atrium, and the maxCD was determined as a delay of conduction between the high lateral right atrium and low lateral right atrium, they may not necessarily reflect electrophysiologic characteristics of all sites involved in the reentry responsible for AF.

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