Vagal Stimulation Prior to Atrial Rapid Pacing Protects the Atrium From Electrical Remodeling in Anesthetized Dogs

Manabu Takei, MD; Masato Tsuboi, MD; Tatsuya Usui, MD; Takeshi Hanaoka, MD; Fumio Kurogouchi, MD; Masakazu Aruga, MD; Yuuichi Katagiri, MD; Mafumi Owa, MD*; Keishi Kubo, MD*; Kendo Kiyosawa, MD

Atrial electrical remodeling is thought to be the cause of the maintenance of atrial fibrillation (AF). Although the initiation and maintenance of AF is partially associated with autonomic nervous tone, vagally mediated AF does not tend to become permanent. Therefore, the effects of preceding vagal stimulation (VS) on the atrial effective refractory period (ERP) under electrical remodeling conditions were investigated in anesthetized dogs. Atrial ERPs were measured at 5 sites before and after a 7-h period of atrial rapid pacing in the control group. In the VS group, the vagus nerve was stimulated for 20 min before a period of atrial rapid pacing. Atrial rapid pacing shortened the ERP at each site in the control group (electrical remodeling). On the other hand, atrial rapid pacing after VS did not shorten the ERP at any site in the VS group. Tetrodotoxin, which was administered into the fatty tissue overlying the right atrial side of the right pulmonary vein junctions, blocked the protective effect of VS against the shortening of the ERP induced by atrial rapid pacing. In contrast, atropine did not interfere with such protective effects. These results suggest that VS prior to atrial rapid pacing protects the atrium from atrial electrical remodeling. (Jpn Circ J 2001; 65: 1077–1081)

Key Words: Atrium; Autonomic nervous system; Electrophysiology; Fibrillation; Vagus nerve

Methods

The animal experiments were approved by the Shinshu University School of Medicine Animal Experimentation Committee, and the animals were obtained through the Animal Laboratory for Research at Shinshu University School of Medicine.

Twenty-three mongrel dogs of either sex weighing 10–20 kg were anesthetized with sodium pentobarbital (30 mg/kg, iv), and supplemental doses were given to maintain stable anesthesia. A tracheal cannula was inserted, and intermittent positive-pressure ventilation was initiated. 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, in order to remove virtually all tonic neural activity to the heart. A fluid-filled catheter was placed in the right femoral artery and connected to a transducer for the monitoring of arterial blood pressure. The right femoral vein was cannulated with an 8 Fr sheath for the infusion of physiological saline at 100–200 ml/h to replace spontaneous fluid losses. An operating table lamp was used to maintain the epicardial temperature, and the thoracotomy was covered with a vinyl sheet.

Four quadripolar electrodes (diameter, 1.5 mm; interelectrode distance, 5 mm) were placed on the epicardial surface of the right atrial appendage (RAA epi), high right atrium (HRA), low right atrium (LRA) and left atrial free wall (LAF) in order to determine the epicardial ERPs. We studied the effects of vagal stimulation (VS) on the changes in atrial ERPs induced by atrial rapid pacing and investigated the mechanism of these effects.

Preparations

The animal experiments were approved by the Shinshu University School of Medicine Animal Experimentation Committee, and the animals were obtained through the Animal Laboratory for Research at Shinshu University School of Medicine.

Twenty-three mongrel dogs of either sex weighing 10–20 kg were anesthetized with sodium pentobarbital (30 mg/kg, iv), and supplemental doses were given to maintain stable anesthesia. A tracheal cannula was inserted, and intermittent positive-pressure ventilation was initiated. 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, in order to remove virtually all tonic neural activity to the heart.

A fluid-filled catheter was placed in the right femoral artery and connected to a transducer for the monitoring of arterial blood pressure. The right femoral vein was cannulated with an 8 Fr sheath for the infusion of physiological saline at 100–200 ml/h to replace spontaneous fluid losses. An operating table lamp was used to maintain the epicardial temperature, and the thoracotomy was covered with a vinyl sheet.

Four quadripolar electrodes (diameter, 1.5 mm; interelectrode distance, 5 mm) were placed on the epicardial surface of the right atrial appendage (RAA epi), high right atrium (HRA), low right atrium (LRA) and left atrial free wall (LAF) in order to determine the epicardial ERPs. We
inserted a steerable 6Fr Franz MAP/Pacing combination catheter (model 1675, EP Technologies, Sunnyvale, CA, USA) through the right femoral vein and positioned it at the endocardial RAA (RAA endo) near the quadripolar electrode at the RAA epi. This electrode was used to stimulate the atrium with rapid pacing and to determine the atrial ERP.

To stimulate the vagal nerves to the heart, bipolar wire electrodes were hooked into the cardiac end of each cervical vagus nerve and connected to a stimulator (BC-02, Fukuda Denshi, Tokyo, Japan). We used a steady stimulation of 5–10 V, 0.5 ms pulse duration, and at 5 Hz. We selected an intensity of vagal stimulation that avoided second- or third-degree atrioventricular block and permitted atrial pacing to be conducted to the ventricle during vagal stimulation. This chosen vagal stimulation intensity generally decreased the heart rate (HR) by ~30% from its control level and shortened the atrial ERP, as reported previously.10

Measurement of ERP
ERP was determined at each test site by an extrastimulus technique that involved the use of an additional electrical stimulator (SEC-3102, Nihon Kohden, Tokyo, Japan). The bipolar electrode at each test site was driven with a 2-ms rectangular stimulus at 2-fold the diastolic voltage threshold for activation. A train of 8 stimuli (S1) at a constant cycle length of 400 ms was followed by a premature stimulus (S2). The atrial response to S2 was recorded as the atrial deflection, which was obtained from the second bipolar electrode in the quadripolar electrode. Atrial ERP was defined as the longest S1–S2 interval that failed to result in atrial depolarization. The S1–S2 interval was shortened in steps of 10 ms until S2 failed to produce a propagated response. The S1–S2 interval was then increased by 10 ms and shortened in 1 ms increments until S2 failed to capture.

Experimental Protocols
In the first series of experiments (Control group), we measured atrial ERPs at 5 sites (RAA endo, RAA epi, HRA, LRA, LAF) in 6 dogs before and after a 7-h period of atrial rapid pacing, which was performed at a rate of 800 beats/min and a current at twice the threshold level and a 2-ms pulse duration.

We used 8 dogs in the second series of experiments (VS group). After determining the atrial ERPs at the same 5 sites, we performed VS for 20 min and atrial ERPs were measured 3 min after VS began. After that, we performed atrial rapid pacing at 800 beats/min for 7 h and the atrial ERPs at the 5 sites were again determined.

We used 4 dogs in the third series of experiments (atropinized VS group, AVS). At the start of the experiment, the dogs were given atropine sulfate (0.04 mg/kg, iv) and the same dose was administered each hour thereafter to block the responses mediated by the muscarinic receptors. We then carried out the same protocol as that used for the second series.

We used 5 dogs in the fourth series of experiments (tetrodotoxin-treated VS group, TVS). Electrical stimulation of the fatty tissue overlaying the right atrial side of the junctions of the right pulmonary veins elicited a marked slowing of the sinus rate, together with nonuniform shortening of the atrial ERP, thus identifying the parasympathetic neuronal pathway to the sinoatrial node and right atrial musculature in this fatty tissue.10 At the start of the experiment, tetrodotoxin (3 μg/0.01 ml) was applied topically to the fatty tissue in order to block parasympathetic axonal conduction and then we followed the same protocol as that used in the second series of the present experiments.

The order of the choice of sites for ERP measurements was randomized in all experimental protocols.

Statistical Analysis
All composite data are expressed as means±standard error (SE). Data from the 5 sites were analyzed by means of a one-way analysis of variance (ANOVA), and Bonferroni’s test was used for the comparison of mean values. The comparisons of the control value and each stimulation value were analyzed by one-way repeated-measures ANOVA followed by contrasts for the mean values comparison. A value of p<0.05 was considered significant.

Results
Effects of Atrial Rapid Pacing on Cardiac Parameters
The control HR and systolic blood pressure (SBP) in the 6 neurally decentralized, anesthetized open-chest dogs were 119±11 beats/min and 120±14 mmHg, respectively. Although the 7-h atrial rapid pacing (800 beats/min) decreased the HR by 22±13 beats/min from the basal level, it did not significantly change the SBP (120±14 mmHg vs
**Vagal Protection From Atrial Remodeling**

**Atrial Effective Refractory Periods (ERPs) Induced by Atrial Rapid Pacing**

The control ERPs of RAA endo, RAA epi, HRA, LRA, and LAF were 168±6, 173±8, 170±4, 169±8, and 157±6 ms, respectively (no significant difference among sites). The atrial rapid pacing period of 7 h significantly (p<0.05) abbreviated the atrial ERPs at RAA endo, RAA epi, HRA, LRA, and LAF. Although the ERP at LAF increased after atrial rapid pacing tended to be slightly shortened, the decrease was not significant (p=0.056). The shortening of the ERPs at RAA endo, RAA epi, HRA, LRA, and LAF induced by atrial rapid pacing was 14±4, 13±3, 12±3, 16±4, and 6±2 ms, respectively. The dispersion of repolarization after atrial rapid pacing (38±3 ms) was increased (p<0.05) compared with the control value (28±4 ms).

**Effects of VS Prior to Atrial Rapid Pacing on Changes in Cardiac Parameters Induced by Atrial Rapid Pacing**

The control HR and SBP in the 8 PVS dogs were 125±5 beats/min and 119±4 mmHg, respectively. Vagal stimulation decreased the HR and SBP by 38±4 beats/min and 17±5 mmHg, respectively, from the basal level. Control ERPs at RAA endo, RAA epi, HRA, LRA, and LAF were 161±6, 163±7, 172±6, 161±8, and 155±9 ms, respectively. During VS before atrial rapid pacing, the ERP at each locus was nearly abolished by atropine, the protective effect of VS against the shortening of the ERP induced by atrial rapid pacing was maintained (Table 1, Fig. 2).

**Effects of Atropine and Tetrodotoxin on the Protective Effects of Vagal Stimulation Against the Shortening of ERP Induced by Atrial Rapid Pacing**

The control HR and SBP in the APVS group were 135±14 beats/min and 135±8 mmHg, respectively, and after atropine treatment, they were 136±12 beats/min and 140±8 mmHg, respectively. Vagal stimulation did not affect the HR (137±8 beats/min) or the SBP (139±6 mmHg). Although the effect of VS on the ERP at each site was nearly abolished by atropine, the protective effect of VS against the shortening of the ERP induced by atrial rapid pacing was maintained (Table 1, Fig. 2).

**Discussion**

The major finding of this study was that VS prior to atrial rapid pacing protected the atrium from atrial electrical remodeling.

Over the past few years, some studies have demonstrated a progressive shortening of atrial ERP in response to brief or long-term episodes of atrial rapid pacing, and as in those studies, atrial rapid pacing observed in the present study reduced the duration of the atrial ERP and increased the ERP dispersion in the control group. This shortening of the atrial ERP has been referred to as ‘atrial electrical remodeling’ and has been assumed to be the cause of the induction and maintenance of AF. The shortening of the atrial ERP induced by atrial rapid pacing has been shown to be blocked by verapamil. In patients with chronic AF, a
peak incidence of AF occurs during the first 5 days after cardioversion, and recurrence of AF can be reduced by intracellular calcium-lowering medication. Therefore, atrial electrical remodeling was mediated by rate-induced intracellular calcium overload. Yue et al showed that atrial rapid pacing reduced transient outward currents (Ito) and L-type Ca²⁺ currents (Ica), and that the reduced Ica appeared to decrease accommodation to changes in rate.

It is well known that vagal tone is one of the most important factors in the induction of APD and this induction of AF by VS might be caused by nonuniform shortening of the atrial ERP. It may also increase ERP dispersion. Cardiac vagal tone declines with age and the smaller the tachycardiae induced response to isometric exercise in older humans is associated with an inability to decrease cardiac vagal tone below an already reduced baseline. Clinically, patients with vagally mediated AF are younger in age, always have idiopathic AF, have occurrences at night, and do not have a tendency toward permanent AF. In the present study, although VS prior to atrial rapid pacing nonuniformly shortened atrial ERPs, the abbreviation of the atrial ERPs produced by atrial rapid pacing did not occur after VS (Table 1). This protective effect of VS on the shortening of the ERP induced by atrial rapid pacing may explain why patients with vagally mediated AF do not tend to develop permanent AF.

Although the effects of VS on HR, SBP, and ERP at each site were nearly abolished by atropine, protective effects were maintained by VS, thus decreasing the risk of shortening the duration of the ERP, which is otherwise induced by atrial rapid pacing (Table 1). Our results are in good agreement with those of Wijffels et al who demonstrated that the electrical remodeling by AF was not influenced by atropine. They also demonstrated that electrical remodeling by AF was not mediated by changes in ischemia, stretch or atrial natriuretic factor. Tetrodotoxin, when applied to the fatty tissue overlying the right atrial side of the right pulmonary vein junctions, blocked this protective effect of VS (Fig 2). Tetrodotoxin is a selective axonal conduction blocker and the fatty tissue overlying the right atrial side of the right pulmonary vein junctions contains the parasympathetic neuronal pathway to the right atrial musculature. Therefore, we concluded that this protective effect was not mediated by muscarinic receptors, even though it was still the result of VS. We do not know the mechanism of the protective effect exerted by VS against the shortening of ERP induced by atrial rapid pacing. Vasoactive intestinal peptide (VIP) is thought to be co-stored and co-released with acetylcholine and its release induced by electrical stimulation was blocked by tetrodotoxin. Halimi et al demonstrated that VIP lengthened atrial APD. One possible mediator of the protective effects of VS on atrial remodeling may be neuropeptides such as VIP. The effect of VIP injection on HR lasted for the periods of up to 100 min in an in vivo preparation but to our knowledge, there were no reports that the effects of VIP induced by VS lasted 7h. Therefore, further studies are needed to elucidate the mechanisms involved.

Study Limitations

The first limitation is that the atrial ERP was measured at only 5 sites, and therefore the responses of other areas of the atrium to atrial rapid pacing and VS are unknown. A second limitation is that the duration of atrial rapid pacing in the present study was only 7h; thus, we still do not have information concerning the duration of the protective effects of VS on the shortening of atrial ERP induced by atrial rapid pacing. Fifteen minutes are required to measure 5 ERP sites; hence we were unable to measure atrial ERPs during atrial rapid pacing. Therefore, we still do not know what initiates this protective effect. A third limitation is that the length and intensity of VS that produce this protective effect are unknown. This issue is important because the vagal tone 5 min prior to the onset of AF is higher than that 20 min prior to the onset of AF. A fourth limitation is that we did not investigate the reversion of the physiological rate adaptation of the atrial ERP. Furthermore, pentobarbital is known to prolong atrial ERP compared with the unanesthetized state and it affects sympathetic and parasympathetic nervous tone. It is, therefore, possible that we overestimated the effects of VS and underestimated the effects atrial rapid pacing on atrial ERP.

Conclusion

Our results suggest that in dog hearts VS prior to atrial rapid pacing protects the atrium from atrial electrical remodeling, and also suggest that one possible mediator of the protective effects of VS on atrial remodeling may be neuropeptides.

References


Japanese Circulation Journal Vol.65, December 2001
before onset of paroxysmal atrial fibrillation by power spectral analysis of heart rate. *J Am Coll Cardiol* 1994; 457A


