Experimental Studies

Vagal Modulation of Ventricular Tachyarrhythmias
Induced by Left Ansae Subclaviae
Stimulation in Rabbits

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SUMMARY

Previous evidence has shown that vagal nerve activity modulates ventricular arrhythmias in patients and in animal models. However, the effects of direct vagus nerve stimulation on ventricular tachyarrhythmias brought by direct sympathetic nerve stimulation have not been elucidated. In the present study, the effects of electrical stimulation of the left cervical vagus nerve on ventricular tachycardia (VT) which was induced by electrical stimulation of the left ansae subclaviae (LAS) in rabbits were assessed. Monophasic action potentials (MAPs) of the left ventricular endocardium were recorded simultaneously with surface ECG. In 27 rabbits tested, the stimulation of LAS induced VT in 19 rabbits. Polymorphic VT (PVT) was induced in 14 rabbits (PVT group), whereas monomorphic VT (MVT) was induced in 5 rabbits (MVT group). Vagus nerve stimulation during the sympathetically-induced PVT led to a restoration of sinus rhythm in 10 of 14 rabbits, while that during the sympathetically-induced MVT did not restore sinus rhythm in any of the 5 rabbits. Vagus nerve stimulation during the stimulation of LAS, which was of subthreshold intensity for the induction in VT, did not result in an induction of PVT in any of the 14 rabbits in the PVT group, while it induced slow MVT in 3 of 5 rabbits in the MVT group. These results indicate that vagus nerve stimulation preferentially exerts protective antiarrhythmic effects against PVT induced by sympathetic neural stimulation in this model, while it occasionally induces slow MVT under conditions in which sympathetic nerve activity is potentially elevated. (Jpn Heart J 1998; 39: 503-511)

Key words: Monophasic action potentials, Ventricular tachycardia, Left ansae subclaviae, Vagus nerve

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CREASED sympathetic neural activity facilitates the prevalence of ventricular tachyarrhythmias in patients and in animal models.\(^1\)\(^-\)\(^4\) Conversely, vagus nerve stimulation suppresses ventricular tachyarrhythmias in patients and in animal models.\(^5\)\(^-\)\(^7\) However, information is limited about the direct effects of vagus nerve stimulation on ventricular tachyarrhythmias brought about by direct sympathetic nerve stimulation.\(^8\) Recent experimental and clinical studies indicate that increased vagal activity does not always exert protective antiarrhythmic effects. It has been demonstrated that vagal stimulation failed to terminate ventricular tachycardia (VT) or prevent ventricular fibrillation (VF) experimentally induced by catecholamine.\(^9\)\(^,\)\(^10\) In a recent clinical study, the sudden increase in vagal activity was reported to play an important role in triggering VF in patients with idiopathic VF.\(^11\)

It is therefore of both academic and clinical interest to investigate how direct vagus nerve stimulation modulates the ventricular arrhythmogenesis that is induced by direct stimulation of sympathetic nerves. We examined this by assessing the effects of left cervical vagus nerve stimulation on VT induced by the direct electrical stimulation of left ansae subclaviae (LAS) in rabbits \textit{in vivo}.

**METHODS**

**Preparations:** Male Japanese White rabbits weighing 2.7–3.3 kg were anesthetized with intravenous secobarbital (20 mg/kg), and anesthesia was maintained by supplemental doses as required. Animals were ventilated with room air via a tracheal cannula connected to an artificial ventilator (SN-480–5, Shinano, Tokyo, Japan). The tidal volume and respiratory rate were adjusted to maintain blood gases and pH within the physiological range. Body temperature was monitored rectally, and maintained at 38–39°C by a heating pad. Saline-filled polyethylene catheters (0.8 mm in diameter) were inserted into the femoral artery to monitor blood pressure. After a median sternotomy, the heart was exposed and suspended in a pericardial cradle. Monophasic action potentials (MAPs) of the left ventricular endocardium were recorded using the contact electrode technique, along with the standard limb ECG (leads II) and femoral arterial blood pressure.\(^7\) Recordings were monitored using a heat pen recorder (CP-642G, Nihon Kohden, Tokyo) and stored in a PCM data recorder (RD-111T, TEAC, Tokyo) for later analysis. The left stellate ganglion was isolated from the surrounding tissue and its LAS was carefully isolated and doubly ligated. The left cervical vagus nerve in the neck was also isolated and doubly ligated. Bipolar stimulating electrodes were placed around the LAS and the left vagus nerve to deliver a 2 msec square-wave pulse of 1 to 10 mA and 50 Hz from an electronic stimulator (SEN-7203, Nihon Kohden). The intensity of vagus nerve stimulation was set at the minimum level as determined by the response of the LAS stimulator (P1) to the vagus nerve stimulation. All experiments were conducted in rabbits anesthetized with secobarbital.
was set at a level that prolonged the sinus cycle length about 3 times control and then maintained unaltered throughout the experimental period. The threshold of LAS stimulation required for induction of VT was determined by gradually increasing the intensity of the pulse, and 1.2 times threshold was used for induction of arrhythmias.

All procedures met the guidelines of the Physiological Society of Oita Medical University, Japan, for the care and use of laboratory animals.

**Experimental protocol:** After control recordings were made for 20 minutes, stimulation of LAS was carried out in 27 rabbits. The VT was induced in 19 of 27 rabbits. Therefore, the following protocols were applied to these 19 rabbits. First, during the occurrence of VT that was induced by LAS stimulation, vagus nerve stimulation was added to LAS stimulation, to examine whether or not such stimulation suppresses sympathetically-induced VT. Second, during stimulation of the LAS with intensity set at maximal subthreshold level for an induction of VT, vagus nerve stimulation was added to LAS stimulation, to examine whether the vagus nerve stimulation can induce VT under these conditions. In another 4 rabbits in which rapid polymorphic VT was induced by the LAS stimulation, the LAS stimulation was carried out before and after the intravenous injection of propranolol (0.2 mg/kg, diluted in normal saline) to exclude the possibility that electrical stimulation of the LAS directly affects myocardium, thereby causing VT.

**Definitions:** The following definitions were used for ventricular arrhythmias recorded by ECG: 1) VT, three or more consecutive premature ventricular beats; 2) polymorphic VT (PVT), irregular VT with varying QRS morphologies; and 3) monomorphic VT (MVT), regular VT with a single morphology.

**Statistical analysis:** Data are expressed as mean ± SEM, unless otherwise specified. Efficacy of vagus nerve stimulation on suppression or induction of VT was analyzed by Fisher’s exact test. Changes in numeric data in the same rabbits by vagus nerve stimulation were analyzed using Student’s paired t test. A p value < 0.05 was considered statistically significant.

**Results**

**Characteristics of VT induced by the stimulation of LAS:** In 27 rabbits tested, the LAS stimulation induced PVT in 14 rabbits (PVT group) and MVT in 5 rabbits (MVT group). In 3 of 5 rabbits in the MVT group, increasing the intensity of LAS stimulation transformed the induced MVT into PVT. A typical PVT episode induced by stimulation of the LAS is shown in Figure 1. In this case, PVT with an irregular MAP recording developed approximately 3 sec after the start of LAS stimulation. The development of PVT produced a marked
Figure 1. Polymorphic ventricular tachycardia (PVT) induced by stimulation of the left ansae subclaviae (LAS). Surface ECG (II), monophasic action potential of the left ventricle (LV-MAP), and arterial blood pressure (BP) are shown. In this tracing, PVT with a cycle length of approximately 75 msec with an irregular appearance of the MAP recording is induced 3 sec after the start of stimulation of the LAS. The first premature beat of the PVT is associated with a premature electrical activity arising during phase 4 in the MAP recording. The development of PVT results in the marked decrease in arterial blood pressure. The discontinuing stimulation of LAS leads to the spontaneous termination of PVT to restore a regular sinus rhythm.

Figure 2. Monomorphic ventricular tachycardia (MVT) induced by stimulation of the left ansae subclaviae (LAS) and subsequent vagally-induced slow MVT during subthreshold intensity of the LAS stimulation. Surface ECG (II) and monophasic action potential of the left ventricle (LV-MAP) are shown. In the left side of this tracing, MVT with a cycle length of 102 msec is induced immediately after the initiation of the LAS stimulation alone. The first premature beat of the MVT is associated with a premature electrical activity arising during phase 4 in the MAP recording. The cessation of LAS stimulation promptly restored a sinus rhythm. The vagus nerve stimulation, added to the preceding subthreshold intensity of the LAS stimulation, induced the slow MVT with a cycle length of 125 msec.

decrease in arterial blood pressure. The cessation of LAS stimulation left PVT lasting for approximately 4 sec, which terminated spontaneously to restore a sinus rhythm. Thus, the induced PVT persisted during the LAS stimulation, and discontinuing LAS stimulation terminated the PVT within 10 sec in all 14 rab-
Table I. Suppressive Effect of Vagus Nerve Stimulation on Polymorphic (PVT) and Monomorphic Ventricular Tachycardia (MVT) Induced by the Stimulation of the Left Ansae Subclaviae

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<thead>
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<th></th>
<th>Suppression</th>
<th>Non-suppression</th>
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<tbody>
<tr>
<td>PVT group (n = 14)</td>
<td>10/14</td>
<td>4/14</td>
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<tr>
<td>MVF group (n = 5)</td>
<td>0/5</td>
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Table II. Induction of Polymorphic (PVT) and Monomorphic Ventricular Tachycardia (MVT) by Concurrent Vagus Nerve Stimulation during Subthreshold Stimulation of the Left Ansae Subclaviae

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<th>Induction</th>
<th>Non-induction</th>
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<tbody>
<tr>
<td>PVT group (n = 14)</td>
<td>0/14</td>
<td>14/14</td>
</tr>
<tr>
<td>MVF group (n = 5)</td>
<td>3/5</td>
<td>2/5</td>
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Figure 3. Suppression of the sympathetically-induced polymorphic ventricular tachycardia (PVT) by vagus nerve stimulation. Surface ECG (II) and monophasic action potential of the left ventricle (LV-MAP) are shown. In this tracing, PVT with a cycle length of approximately 80 msec with an irregular appearance of the MAP recording persisted during the LAS stimulation. The additive concurrent application of vagus nerve stimulation terminated the PVT to restore a sinus rhythm.

bits. The mean cycle length of PVT in these 14 rabbits was 77 ± 6 msec. In the MAP recordings, the first ventricular beat of PVT arose during phase 4 in 11 rabbits and during phase 3 in the remaining 3 rabbits. The MVT induced by the LAS stimulation was characterized by a MAP recording with relatively uniform shaped action potentials without electric diastole (left side of Figure 2). In the case shown in Figure 2, MVT developed immediately after the initiation of LAS stimulation. The induced MVT persisted during LAS stimulation, and discontinuing LAS stimulation led it to spontaneous termination. The mean cycle length of MVT in these 5 rabbits was 108 ± 7 msec. In the MAP recordings, the first ventricular beat of the MVT arose during phase 4 in all 5 rabbits.

Effects of vagus nerve stimulation on VT induced by the stimulation of LAS:

In the PVT group, additional application of vagus nerve stimulation during the
sympathetically-induced PVT terminated PVT to restore a sinus rhythm in 10 of 14 rabbits tested. In the remaining 4 rabbits, vagus nerve stimulation failed to terminate PVT (Table I). A typical termination of PVT by vagus nerve stimulation is shown in Figure 3. In this case, PVT developed and persisted during the LAS stimulation. The concurrent application of vagus nerve stimulation terminated the PVT at approximately 1.5 sec after the start of vagal stimulation. The termination of PVT was consistently preceded by a slowing of the VT rate. In the MVT group, additional application of vagus nerve stimulation during the sympathetically-induced MVT led to a termination of VT in none of 5 rabbits tested (Table I). The difference in suppressive efficacy of vagus nerve stimulation on PVT from MVT was statistically significant (p < 0.05).

Effects of vagus nerve stimulation during the subthreshold intensity of LAS stimulation: Subsequently, the effects of vagus nerve stimulation during the subthreshold intensity of LAS stimulation for an induction of VT were assessed. Vagus nerve stimulation following the subthreshold stimulation of LAS did not induce VT in any of the 14 rabbits in the PVT group (Table II). However, the same intervention produced MVT in 3 of the 5 rabbits in the MVT group (right side of Figure 2) (Table II). In the case shown in Figure 2, concurrent application of vagus nerve stimulation produced MVT with a cycle length of 125 msec, slower than that induced by LAS stimulation alone (102 msec, left side of Figure 2). The cycle length of vagally-induced MVT observed in the 3 rabbits was significantly longer than that of the original MVT which was induced by LAS stimulation alone (128 ± 11 vs 106 ± 9 msec; n = 3, p < 0.01). Following the development of vagally-induced MVT, we confirmed that neither LAS stimulation of subthreshold intensity nor vagus nerve stimulation lasting for 20 sec per se caused VT.

Effects of propranolol on the PVT induced by the LAS stimulation: In another 4 rabbits in which PVT was induced by the LAS stimulation, the LAS stimulation was carried out before and after the intravenous injection of propranolol (0.2 mg/kg). In all 4 rabbits, VT was not inducible after the administration of propranolol.

Discussion

Main findings: In the present study, PVT or MVT was induced by direct electrical stimulation of the LAS in 19 of 27 rabbits tested. PVT was predominantly induced rather than MVT (14 vs 5 rabbits). While concurrent left cervical vagus nerve stimulation suppressed PVT in 10 of 14 rabbits of the PVT group, it terminated the MVT in none of 5 rabbits of the MVT group. Vagus nerve stimulation during subthreshold intensity of LAS stimulation induced VT in none
of 14 rabbits in the PVT group. However, it induced slow MVT in 3 of 5 rabbits in the MVT group.

Possible mechanisms for PVT induced by the stimulation of LAS: In the present study, while the PVT was in association with an irregular appearance of MAP recordings, the MVT was with a uniform shaped MAP recording. The responses of PVT to vagus nervous stimulation were clearly different from those of MVT. These findings indicate a distinct underlying electrophysiological mechanism between PVT and MVT.

The electrophysiological mechanisms for the PVT induced by stimulation of the LAS in the present study remain unclear and speculative. It has been demonstrated that catecholamines shorten the refractory period of Purkinje fibers and ventricular muscle tissue and enhance diastolic depolarization in Purkinje fibers, which may induce phase 4-dependent depression of conduction. Therefore, the heterogenous sympathetic innervation of the ventricle and subsequent heterogenous changes in the electrophysiologic properties, produced by stimulation of the LAS, could be attributed to the generation of the reentrant VT. At triggering mechanism may also be responsible for the generation of PVT observed because delayed (DADs) and early afterdepolarizations (EADs) were induced and enhanced in vitro by catecholamines and in vivo by the stimulation of cardiac sympathetic nerves.

Vagally-induced MVT during the subthreshold stimulation of LAS: The vagally-induced MVT was analogous in morphology, of ECG and MAP recordings, to the original sympathetically-induced MVT with a slower tachycardia rate, indicating essentially an identical underlying mechanism. It is tempting to speculate that the intense sympathetic nerve stimulation augmented the ventricular automaticity which exceeds the sinus rhythm, resulting in an induction of MVT, and that the potentially enhanced ventricular automaticity produced by the subthreshold intensity of LAS stimulation was unmasked by concurrent application of vagus nerve stimulation, leading to manifestation of the slow MVT.

Suppressive effects of vagal stimulation on sympathetically-induced PVT: Acetylcholine released by vagus nerve stimulation can slow ventricular automaticity, depress Purkinje fiber automaticity and increase the refractory period of ventricular muscle. In addition, acetylcholine inhibits the release of norepinephrine from sympathetic nerves at prejunctional and postjunctional levels. Thus, vagus nerve stimulation and subsequently released acetylcholine, directly and indirectly, offset the actions of catecholamines released by the LAS stimulation. These effects of vagus nerve stimulation may have combined to suppress PVT which was induced by the stimulation of LAS.

Limitations: The present study has several reservations. First, we used secobarbital, a known as an adrenergic stimulating agent for anesthesia which
might have influenced the results. Second, the stimulus frequency of 50 Hz with an intensity up to 10 mA used for LAS stimulation was not physiologic. Finally, the sympathetically-induced PVT observed was indistinguishable from VF on the ECG and MAP recordings. This is due to an experimental limitation of using rabbits, i.e. the size of the myocardial mass is too small to demonstrate VF.

Conclusions: In the present study, we demonstrated that direct stimulation of the LAS produced PVT and MVT, most likely due to different mechanisms. Although direct vagus nerve stimulation preferentially antagonized the sympathetically-induced PVT, it occasionally induced MVT under conditions in which the sympathetic tone was potentially elevated.

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REFERENCES

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