Interactive Effects of Verapamil and Sympathetic Nerve Stimulation on the Sinus and AV Nodes in the Canine Hearts

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SUMMARY

The interactive effects of verapamil and sympathetic nerve stimulation on the sinus and atrioventricular (AV) nodes were studied in 33 bilaterally stellactomized and vagotomized dogs. Sinus cycle length (SCL) and AV nodal conduction time (AH) at a constant drive cycle length (600 msec) were recorded. Sympathetic nerve stimulation (SNS) was performed for 40 sec by electrical stimulation of the right stellate ganglion. Several doses of verapamil (0.5–2.0 µg/ml, 1.0 ml) were injected directly into the sinus and AV node arteries at 20 sec of SNS. In addition, SNS was repeated at two different intensities after verapamil administration. Verapamil significantly increased the SCL and the AH in a dose dependent fashion, which was reduced by SNS. The reductions of SCL and AH by the SNS were attenuated in the presence of verapamil when the stimulation intensity was weak, but not when the stimulation intensity was strong. It is concluded that verapamil and sympathetic nerve activity interact antagonistically on the sinus node automaticity and AV nodal conduction.

Key Words:
Verapamil     Sympathetic nerve stimulation     Sinus node     AV node

VERAPAMIL blocks Ca++ entry into the cell and inhibits the slow inward current (Isi), resulting in inhibition of the sinus node automaticity and depression of atrioventricular (AV) node conductivity. Conversely, an increase in catecholamines increases the Isi, causing increases in the sinus node automaticity and AV node conductivity. Therefore, the action of verapamil theoretically cancels the activation of beta-adrenoreceptors.1) Although a few studies2),3) have suggested an interaction between catecholamines and...
verapamil in the sinus and AV nodes, there are no systematic and conclusive data. Thus, the present study was designed to determine the influence of enhanced sympathetic nerve activity on the direct effects of verapamil on the sinus and AV nodes in dogs. The effects of verapamil administration on sinus cycle length (SCL) and AH interval (AH) were evaluated during two different intensities of stellate ganglion stimulation. These experiments supported the hypothesis that verapamil and sympathetic nerve activity have opposite effects on sinus node automaticity and AV node conduction.

**METHODS**

Thirty-three mongrel dogs (20–40 kg) of both sexes were anesthetized with sodium pentobarbital (25 mg/kg, iv), intubated with a cuffed endotracheal tube and artificially respirated with room air using a Harvard respirator. The chest cavity was opened by a median sternotomy. Both cervical common carotid sheaths were dissected and both vagosympathetic trunks were sectioned.

**Sympathetic nerve stimulation (SNS)**

After removing connective tissue 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 central nervous system. Next, a platinum bipolar electrode (0.08 mm in diameter, 5 mm of inter-electrode-tip distance) was inserted into the right stellate ganglion, covered with petrolatum jelly, and connected to an electrical stimulator (Fukuda Denshi, Cardiac Stimulator BC-02A). The left stellate ganglion was exposed and removed.

The right stellate ganglion was stimulated with rectangular electric waves with a frequency of 50 Hz and a duration of 2 msec. For Protocol SN (see below), the intensity of stimulation (voltage) was adjusted to the two levels at which the sinus cycle length (SCL) in the control was approximately shortened by 25% (weak stimulation, Sw) and 50% (strong stimulation, Ss). For Protocol AVN (see below), the intensity of stimulation was adjusted to levels at which the AH interval (AH) in controls was approximately shortened by 20 msec (weak stimulation, Sw) and 40 msec (strong stimulation, Ss).

The reproducibility of the effects of sympathetic nerve stimulation (SNS) is an important premise in order to evaluate the interaction between verapamil and SNS. Our preliminary studies confirmed that these methods of SNS produced stable effects for at least 3 hours.
Sinus and AV nodal arterial cannulation

After the animals were prepared for SNS, they were placed on their left side. A right thoracotomy was performed at the level of the fourth or fifth intercostal space. The pericardium was incised along the right phrenic nerve. The heart was suspended in a pericardial cradle to enable visualization of the sinus nodal artery or the posterior descending artery and the crux of the heart.

For Protocol SN, the sinus nodal artery was exposed at its origin from the right coronary artery. Tapered polyethylene tubing (Intramedic, No. 7410) was inserted directly into the sinus nodal artery and was positioned with a depth of 10–15 mm within the artery to pass through the large arterial branches.

For Protocol AVN, the AV nodal artery was isolated at the corner where the left circumflex turned into the posterior descending artery. It was cannulated within the "AV nodal branch", which supplies the blood to the AV node, with tapered polyethylene tubing (Intramedic, No. 7410). The cannula was flushed periodically with heparinized saline solution (5 units/ml) to prevent clotting. Acetylcholine chloride (0.1 μg/ml, 1.0 ml) followed by normal physiologic saline (1.0 ml) were administered over 3–4 sec into the cannula to confirm that the cannulated blood vessel was the sinus nodal artery or AV nodal artery. Induction of sinus arrest or complete heart block immediately following acetylcholine administration was considered as a confirmation of successful cannulation. When the SCL or AH showed 5% or more changes within 5 min of ligation of either nodal artery, the sinus or AV node was considered to have become ischemic and such cases were excluded from the analysis.

Recordings

Bipolar electrodes (two 0.08 mm diameter platinum wires) were positioned in the right atrial appendage and in the endocardium of the right atrial free wall using 22 gauge hypodermic needles. The inter-tip distance for each bipolar electrode was approximately 5 mm.

A quadripolar electrode catheter (USCI #6) was introduced through the right femoral vein and positioned across the tricuspid valve for recording of His bundle electrogram. Systemic arterial blood pressure was recorded continuously through the right femoral artery with a pressure transducer (Statham P32Db). The left femoral vein was cannulated for administration of normal physiologic saline and drugs. Intracardiac electrograms, the standard lead ECG (II, III and aVF) and blood pressure were continuously monitored on an oscilloscope (Fukuda Denshi, Monitorscope CS-800) and recorded with a multichannel recorder (Siemens, Mingograf 800) at paper speeds of 100 mm/
Experimental protocol
The experiment was carried out according to the following protocol:
(1) Protocol SN
   A. Protocol SN-1 (changes in SCL after verapamil administration during strong SNS)
      SNS(Ss) was performed for 40 sec. Twenty sec after the initiation of the SNS(Ss), 1.0 ml of physiologic saline as a control was administered rapidly (in less than 1 sec) into the cannula in the sinus nodal artery; 1.0 ml of physiologic saline was administered immediately to flush the cannula. Changes in the SCL were observed during the 40 sec SNS(Ss). After the SCL recovered to the pre-stimulation level, 1.0 ml of verapamil (at concentrations of 1.0 and 2.0 µg/ml) was administered in this order at 15-min intervals. The cannula was flushed and data were recorded as in the control condition.
   B. Protocol SN-2 (changes in SCL during SNS in the presence of verapamil)
      For collection of control data, 1.0 ml of physiologic saline was administered rapidly into the cannula in the sinus nodal artery; the cannula was flushed immediately with 1.0 ml of physiologic saline. After 4 min, SNS(Sw) was performed for 40 sec. Changes in the SCL during each SNS were evaluated. Seven min later, 1.0 ml of verapamil solution (1.0 µg/ml) was administered rapidly into the cannula in the sinus nodal artery and the cannula flushed with saline. After 4 min, SNS(Sw) was performed, and after 2 min, SNS(Ss) was performed, in a manner similar to the control condition. Changes in the SCL during each SNS were evaluated. After 7 min, a similar evaluation was performed with 2.0 µg/ml of verapamil solution.
(2) Protocol AVN
   For the evaluation of AV nodal conduction, a bolus injection of pentobarbital sodium (2.0 ml, 0.1%) was given into the cannulated sinus nodal artery to depress the sinus nodal activity. The atrium was paced continuously at a fixed cycle length (500 msec), at an intensity of twice diastolic threshold. Pacing was performed with rectangular current pulses (2 msec duration) using a programmed stimulator.
   A. Protocol AVN-1 (changes in AH after verapamil administration during SNS)
      This protocol was the same as Protocol SN-1, except 3 different concentrations of verapamil (0.5, 1.0 and 2.0 µg/ml) were administered through the cannula in the AV nodal artery. Changes in AH during a 40-sec SNS period
were observed.

B. Protocol AVN-2 (changes in AH during SNS in the presence of verapamil)

This protocol was the same as Protocol SN-2, except 3 different concentrations of verapamil were administered into the AV nodal artery. Changes in AH during a 40-sec SNS period were evaluated.

**Evaluation**

In the experiments using Protocol SN-1 and AVN-1, interanimal differences precluded a direct comparison of the degree of the SCL or AH prolongation by verapamil. Therefore, \( \Delta \% \text{SCL} \) or \( \Delta \% \text{AH} \) was obtained for comparison by normalizing the changes (\( \Delta \text{SCL}, \Delta \text{AH} \)) in the SCL or AH after verapamil administration to the absolute SCL or AH before verapamil administration. The same approach was used in Protocols SN-2 and AVN-2. The changes in the SCL or AH during SNS were calculated separately for Sw (\( \Delta \text{W}\%\text{SCL} \) or \( \Delta \text{W}\%\text{AH} \)) and Ss (\( \Delta \text{S}\%\text{SCL} \) or \( \Delta \text{S}\%\text{AH} \)), and normalized for the SCL or AH before SNS (\( \Delta \text{W}\%\text{SCL} \) or \( \Delta \text{W}\%\text{AH} \) and \( \Delta \text{S}\%\text{SCL} \) or \( \Delta \text{S}\%\text{AH} \), respectively).

**Statistical analysis**

All results are expressed as the mean±standard deviation. Statistical analysis was performed by analysis of variance, two sided unpaired t-test, as appropriate. Values were considered significant at the level of \( p<0.05 \).

**Animal care**

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

**RESULTS**

1. **Protocol SN-1: Changes in SCL after verapamil administration during SNS (n=8)**

   The results from Protocol SN-1 are shown in Table I. The SNS markedly shortened the SCL. Although the SCL during the SNS was unaffected by administration of physiologic saline (control), it was prolonged significantly in a dose-dependent manner by administration of verapamil. Thus, verapamil dose-dependently inhibits the SNS-induced shortening of the SCL.

2. **Protocol SN-2: Changes in SCL during SNS in the presence of verapamil (n=8)**

   The results from Protocol SN-2 are presented in Table II. The SNS
Table I. Effect of Intra-SN Arterial Verapamil on SCL During Sympathetic Nerve Stimulation

<table>
<thead>
<tr>
<th>SCL (msec)</th>
<th>Control (n=8)</th>
<th>Verapamil (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before SNS (1)</td>
<td>628.1±68.1</td>
<td>648.1±72.9*</td>
</tr>
<tr>
<td>During SNS</td>
<td>451.3±65.5</td>
<td>548.3±90.9*</td>
</tr>
<tr>
<td>After V (2)</td>
<td>466.3±45.7</td>
<td>568.8±91.4*</td>
</tr>
<tr>
<td>ġSCL (msec)</td>
<td>15.0±50.4</td>
<td>87.5±37.3*</td>
</tr>
<tr>
<td>%SCL (%)</td>
<td>4.4±11.9</td>
<td>19.1±10.0*</td>
</tr>
</tbody>
</table>

SNS = sympathetic nerve stimulation; SCL = sinus cycle length; V = verapamil; ġSCL = (3)−(2); %SCL = [ġSCL/(2)]×100.

| P<0.05 vs. Control, ** p<0.05 vs. Verapamil 1.0. |

Table II. Effect of Sympathetic Nerve Stimulation on SCL in the Absence and Presence of Verapamil

<table>
<thead>
<tr>
<th>SCL (msec)</th>
<th>Control (n=8)</th>
<th>Verapamil (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before SNS (B)</td>
<td>608.4±103.0</td>
<td>670.9±81.9*</td>
</tr>
<tr>
<td>Sw</td>
<td>472.5±60.9</td>
<td>543.1±79.1*</td>
</tr>
<tr>
<td>Ss</td>
<td>412.5±67.6*</td>
<td>480.6±48.7*</td>
</tr>
<tr>
<td>ġWSCL (msec)</td>
<td>136.9±78.2</td>
<td>126.9±58.2*</td>
</tr>
<tr>
<td>ġSSCL (msec)</td>
<td>195.0±62.6</td>
<td>191.3±84.8</td>
</tr>
<tr>
<td>ġW%SCL (%)</td>
<td>21.4±8.8</td>
<td>18.8±8.8</td>
</tr>
<tr>
<td>ġS%SCL (%)</td>
<td>31.9±6.5†</td>
<td>27.7±10.2*†</td>
</tr>
</tbody>
</table>

SNS = sympathetic nerve stimulation; Sw = weak SNS; Ss = strong SNS; ġWSCL (ġW) = B−Sw; ġSSCL (ġS) = B−Ss; ġW%SCL = (ġW/B)×100; ġS%SCL = (ġS/B)×100.

* P<0.05 vs. Control, ** p<0.05 vs. Verapamil 1.0, † p<0.01 vs. Sw, ‡ p<0.01 vs. ġW%SCL.

alone (control) produced a significant shortening of the SCL for both Sw and Ss. Verapamil treatment prolonged the SCL in a dose-dependent manner for both Sw and Ss. The SCL during Ss was significantly shorter than that during Sw for the control and two doses of verapamil. In addition, ġS%SCL was also significantly greater than ġW%SCL for both the control and two doses of verapamil. Thus, the SNS-induced shortening of the SCL is inhibited dose-dependently by prior administration of verapamil. This inhibition is more prominent during Sw than during Ss.

(3) Protocol AVN-1: Changes in AH after verapamil administration during SNS (n=25)

The results using Protocol AVN-1 in all animals are shown in Table
Table III. Effect of Intra-AVN Arterial Verapamil on AH During Sympathetic Nerve Stimulation

<table>
<thead>
<tr>
<th>AH (msec)</th>
<th>Control (n=25)</th>
<th>Verapamil (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before SNS (1)</td>
<td>162.9± 22.0</td>
<td>165.7± 23.9</td>
</tr>
<tr>
<td>During SNS</td>
<td>127.1± 17.3</td>
<td>125.0± 18.3</td>
</tr>
<tr>
<td>After V (3)</td>
<td>128.6± 18.0</td>
<td>130.7± 19.9</td>
</tr>
<tr>
<td>Before V (2)</td>
<td>128.6± 18.0</td>
<td>130.7± 19.9</td>
</tr>
</tbody>
</table>

\( \frac{\Delta AH}{msec} \)

| \( \Delta AH \) (msec) | 1.4± 2.4 | 5.7± 7.3* |
| \( \Delta % AH \) (%) | 0.8± 1.4 | 3.3± 4.0* |

SNS=sympathetic nerve stimulation; V=verapamil; AH=AH interval; \( \Delta AH=(3)-(2) \); \( \Delta % AH = (\Delta AH/2)\times 100 \).

* \( p < 0.01 \) vs. Control, ** \( p < 0.01 \) vs. Verapamil 0.5, *** \( p < 0.01 \) vs. Verapamil 1.0.

Table IV. Effect of Sympathetic Nerve Stimulation on AH in the Absence and Presence of Verapamil

<table>
<thead>
<tr>
<th>AH (msec)</th>
<th>Control (n=25)</th>
<th>Verapamil (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before SNS (B)</td>
<td>164.3± 22.3</td>
<td>165.7± 189.3±</td>
</tr>
<tr>
<td>Sw</td>
<td>145.7± 20.1*</td>
<td>148.6± 21.7</td>
</tr>
<tr>
<td>Ss</td>
<td>122.9± 15.5*##</td>
<td>125.7± 17.2*##</td>
</tr>
</tbody>
</table>

\( \frac{\Delta WAH}{msec} \)

| \( \Delta WAH \) (msec) | 18.6± 7.5 | 17.1± 8.1 |
| \( \Delta SAH \) (msec) | 41.4± 15.7 | 40.0± 16.6 |
| \( \Delta W% AH \) (%) | 11.2± 4.0 | 10.4± 4.6 |
| \( \Delta S% AH \) (%) | 24.7± 7.6† | 23.7± 8.2† |

SNS=sympathetic nerve stimulation; Sw=weak SNS; Ss=strong SNS; \( \Delta WAH = (B-Sw) \); \( \Delta SAH = (B-Ss) \); \( \Delta W% AH = (\Delta WAH/B)\times 100 \); \( \Delta S% AH = (S/B)\times 100 \).

* \( p < 0.01 \) vs. Control, ** \( p < 0.01 \) vs. Verapamil 0.5, *** \( p < 0.01 \) vs. Verapamil 1.0, # \( p < 0.05 \) vs. Before SNS, ## \( p < 0.05 \) vs. Sw, † \( p < 0.05 \) vs. \( \Delta W% AH \).

III. The SNS markedly shortened AH. This shortened AH was not prolonged by administration of physiologic saline (control) but was significantly prolonged dose-dependently by administration of verapamil. The \( \Delta % AH \) was significantly increased in a dose-dependent manner by administration of
verapamil. These results suggest that verapamil inhibits dose-dependently the shortening of the AH by the SNS.

(4) Protocol AVN-2: Changes in AH during SNS in the presence of verapamil (n=25)

The results using Protocol AVN-2 in all animals are shown in Table IV. The SNS alone (control) produced a significant shortening of the AH for both Sw and Ss. Prior administration of verapamil prolonged the AH in a dose-dependent manner for both Sw and Ss. The AH during Ss was significantly shorter than that during Sw for control and three doses of verapamil. \( \Delta S\% AH \) was also significantly greater than \( \Delta W\% AH \) for control and three doses of verapamil. Thus, the SNS-induced shortening of the AH is inhibited dose-dependently by prior administration of verapamil. This inhibition is more prominent during Sw than during Ss.

Discussion

In the present study, verapamil administration into sinus and AV nodal arteries resulted in prolongation of the SCL or AH. This prolongation was reduced by SNS. The degree of prolongation of the SCL or AH by verapamil was greater at higher verapamil concentrations, but smaller at higher intensities of SNS. These results suggest that verapamil and SNS act antagonistically on sinus node automaticity and AV nodal conductivity, and this antagonistic action is affected by the concentration of verapamil and the intensity of SNS.

Interaction between verapamil and SNS

Zipes et al. observed that the SCL and AH were prolonged by direct verapamil administration into both nodal arteries of dogs. Since this effect was blocked by administration of isoproterenol (2.0 ml, 0.1 or 2.0 \( \mu g/ml \)) into both nodal arteries, they suggested that isoproterenol antagonized the inhibitory effects of verapamil on both nodes. Subsequently, Lupi et al. reported that administration of verapamil (0.1–2.0 \( \mu g/ml \)) into the sinus nodal artery did not affect either the norepinephrine- or SNS-induced shortening of the SCL and AH in 4 dogs, but that a higher concentration of verapamil (10 \( \mu g/ml \)) reduced the effects of norepinephrine. The discrepancies between our results and Lupi et al. may be due to differences in the method of SNS and the stimulation intensity. For example, our preparations were surgically denervated, while autonomic innervation was intact in their study. This suggests that even the control state was under the influence of the autonomic
nervous system in their experiments. This supposition is supported by their observation that the heart rate in the control dogs was 140–160/min, which was much higher than the heart rate after autonomic blockade in our study. Furthermore, since they performed intense SNS to increase the heart rate from 150/min to 200/min, only verapamil at a high concentration could antagonize the SNS.

**Mechanism of interaction between verapamil and SNS**

The sites and mechanisms of interactions between verapamil and SNS remain unclear. Lindemann et al speculated that catecholamines bind with beta-receptors, resulting in increased intracellular cyclic AMP and an increase in the number of calcium channels; while verapamil, on the other hand, acts directly on the calcium channel, independently of changes in cyclic AMP. According to this hypothesis, catecholamines and verapamil act at different sites to regulate the interspike interval. Although verapamil has also been reported to inhibit Ca++ dependent norepinephrine release from sympathetic nerve terminals, other studies have suggested that verapamil does not affect norepinephrine release. Thus, a presynaptic locus of action seems unlikely in our study.

**Characteristics and limits of this study**

Verapamil was administered to both nodal arteries both to avoid the effects of nerve reflexes following intravenous verapamil administration and to evaluate the direct effects of verapamil on both nodes. Verapamil at concentrations of 0.5–2.0 µg/ml produced adequate effects at both nodes. For detailed evaluation of the dose-dependency of the action of verapamil on the SCL and AH, SNS was performed at two intensities. The sympathetic and vagal nerves were cut bilaterally to eliminate confounding effects of autonomic discharges of central origin.

The sinus and AV nodes in dogs are generally supplied by 2 or more branches of the coronary arteries, but sometimes by 1 coronary branch. Since a 2–3 min ischemic period after ligation of both nodal arteries causes prolongation of the SCL and AH, dogs were excluded from the experiment when the SCL changed by 5% or more within 5 min of ligation.

**Clinical implication**

Breithardt et al reported that the SCL was shortened by intravenous verapamil administration to normal subjects but prolonged by intravenous verapamil administration after blocking the autonomic nerve with propranolol and atropine. Verapamil administration reduces the blood pressure by
vasodilatation, triggering a vasoreflex. This reflex enhances sympathetic nerve activity, reducing the inhibitory effects of verapamil on the sinus node and shortening the SCL. On the other hand, blockade of the vasoreflex with a drug may prolong the SCL because the inhibitory effects of verapamil are not reduced. These findings suggest that the effects of verapamil are modified by enhanced autonomic nerve activity. In pathologic conditions such as sick sinus syndrome, verapamil administration may induce marked bradycardia. Severe unexpected side effects of verapamil have also been reported at routine doses in patients already receiving beta-blockers. On the other hand, a larger amount of verapamil has been reported to be needed to reduce heart rate in patients with atrial fibrillation complicated by heart failure than in those without heart failure. This finding may be associated with increased blood norepinephrine due to enhanced sympathetic nerve activity during heart failure. Thus, the interaction between the verapamil action and sympathetic nerve activity is a potentially important consideration in therapeutic intervention.

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References


