Role of the Vagus Nerve in the Antidysrhythmic Effect of Dexmedetomidine on Halothane/Epinephrine Dysrhythmias in Dogs

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Background: Dexmedetomidine, an α₂-adrenergic agonist, can prevent the genesis of halothane/epinephrine dysrhythmias through the central nervous system. Because stimulation of α₂ adrenoceptors in the central nervous system enhances vagal neural activity and vagal stimulation is known to inhibit digitalis-induced dysrhythmias, dexmedetomidine may exert the antidysrhythmic property through vagal stimulation. To address this hypothesis, the effect of dexmedetomidine in vagotomized dogs was examined and compared with that in intact dogs. In addition, the effect of vagotomy on the antidysrhythmic action of doxazosin, an α₁ antagonist, was studied.

Methods: Adult mongrel dogs were anesthetized with halothane (1.3%) and monitored continuously for systemic arterial pressure and premature ventricular contractions. Animals were divided into two groups receiving bilateral vagotomy or sham operation. The dysrythmia threshold was expressed by the dysrhythmogenic dose of epinephrine, defined as the smallest dose producing four or more premature ventricular contractions within a 15-s period, and plasma concentration of epinephrine at the time when the dysrhythmogenic dose was reached. The threshold was determined in the presence of dexmedetomidine (a selective α₂ agonist that crosses the blood-brain barrier) and doxazosin (a selective α₁ antagonist that does not penetrate the blood-brain barrier) in the two groups. In addition, the effect of dexmedetomidine in the presence of atropine methylbromide instead of vagotomy was examined.

Results: Vagotomy did not affect the basal vulnerability to halothane/epinephrine dysrhythmias significantly. Although dexmedetomidine dose-dependently prevented the genesis of the dysrythmias in intact dogs, the beneficial effect of dexmedetomidine was abolished in both the vagotomized and the atropine-treated dogs. On the other hand, vagotomy did not change the antidysrhythmic property of doxazosin.

Conclusions: The vagus nerve plays an important role in the prevention of halothane/epinephrine dysrhythmias by dexmedetomidine in dogs. However, resting vagal tone neither modulates the onset of halothane/epinephrine dysrythmias nor affects the antidysrhythmic action of doxazosin. (Key words: Anesthetics, volatile; halothane. Autonomic nervous system: vagus nerve. Heart: dysrhythmia. Pharmacology: dexmedetomidine; doxazosin. Receptors: α₂-adrenergic; α₁-adrenergic.)

THE myocardial sensitization of halothane to dysrhythmogenic action of epinephrine is a well known phenomenon.1,2 Although the role of adrenergic receptors in the cardiovascular system in the genesis of the halothane/epinephrine dysrhythmias has been well explored,3-6 the detailed mechanism involved in producing the dysrhythmia is obscure. Several reports indicate that α₂ adrenoceptors in the central nervous system modulate digitalis-induced cardiac dysrhythmias.9-11 We previously reported that dexmedetomidine, a selective α₂ agonist, prevented halothane/epinephrine dysrhythmias through a central nervous system pathway.12 However, the detailed mechanism whereby the central nervous system alters the onset of dysrhythmias has not been elucidated. Considering that an α₂ agonist increases vagal outflow13 and increased
vagal tone appears to be protective against several types of dysrhythmias.\textsuperscript{14-16} Eisenach\textsuperscript{17} pointed out a possible contribution of vagal activation in the antidyssrhythmic action of dexmedetomidine. To test this possibility, we compared the antidyssrhythmic action of dexmedetomidine in vagotomized \textit{versus} intact animals. In addition, we examined whether bilateral vagotomy affects the antidyssrhythmic property of doxazosin, an $\alpha_1$ antagonist,\textsuperscript{18} which acts exclusively through a peripheral mechanism.\textsuperscript{5}

Materials and Methods

The studies were conducted under guidelines approved by the Animal Care Committee of Osaka University Faculty of Medicine.

Fifty-nine adult mongrel dogs of either sex and weighing 7.5–12 kg were used in this study. Anesthesia was induced with halothane alone and maintained at an end-tidal concentration of 1.3%, which was monitored continuously by an anesthetic gas analyzer (Datex model AA 102-30-00, Helsinki, Finland). A different dog was used for each experiment; thus, only one dyssrhythmic dose was determined in any individual dog. The trachea of each animal was intubated with auffed tracheal tube, and the lungs were mechanically ventilated (Aika R60, Tokyo, Japan). The end-tidal carbon dioxide concentration was continuously monitored with an expired gas monitor (Minato 1121 A, Osaka, Japan) and maintained at a level of 35–40 mmHg. A heating lamp and circulating water blanket were used to maintain the esophageal temperature at 37.0–38.5°C. A femoral artery catheter was inserted for both pressure monitoring and blood gas and serum electrolyte sampling. Lead II of the electrocardiogram was monitored continuously. The arterial pressure and electrocardiogram were recorded for review with a thermal array recorder (Nihon Kohden WS-641G, Tokyo, Japan). A femoral vein was cannulated for administration of drugs and lactated Ringer’s solution, which was infused at a rate of 10 ml·kg$^{-1}$·h$^{-1}$. Serum K$^+$ was maintained between 3.5 and 4.5 mEq/l by infusing K$^+$ at a rate of 1–10 mEq/h. Arterial pH, oxygen tension, and serum Na$^+$ were maintained within the ranges of 7.35–7.45, 85–100 mmHg, and 135–150 mEq/l, respectively.

The dysrhythmia threshold was achieved when four or more premature ventricular contractions occurred within 15 s. The dysrhythmic dose of epinephrine was defined as the smallest dose producing dysrhythmias. According to our previous method,\textsuperscript{19} the dysrhythmic dose of epinephrine was determined with standardized logarithmically spaced infusions of epinephrine lasting 5 min with 10–30 min recovery periods between infusions. The infusion was started at 0.67 μg·kg$^{-1}$·min$^{-1}$, and the dose was increased by $e^{0.4}$ until dysrhythmias occurred. If dysrhythmias occurred at one of these doses, a smaller dose, divided by $e^{0.2}$, was tested. At the time when the criterion for dysrhythmic dose had been satisfied, an arterial blood sample was collected to measure the plasma concentration of epinephrine, as described previously, using a diphenylethylenediamine condensation method (model HLC-8030 catecholamine analyzer, Tosoh, Tokyo, Japan).\textsuperscript{20} It has a limit of sensitivity of 10 pg/ml for epinephrine and norepinephrine and an inter-and intra-assay variation of less than 3%.

The animals were randomly assigned to two groups: the intact group and the vagotomized group. In the vagotomized dogs, bilateral vagotomy was performed by sectioning both vagus nerves at level of C4, and more than 30 min later when hemodynamic variables became stable, the experiments were started. All dogs in the intact group received a sham operation instead of vagotomy. At first, the dysrhythmic dose of epinephrine was determined in the presence of dexmedetomidine, a highly selective $\alpha_2$ agonist,\textsuperscript{21,22} at 0 (control), 0.2, and 0.5 μg·kg$^{-1}$·min$^{-1}$ intravenously, in intact dogs. The same protocol was repeated in separate vagotomized dogs to examine the role of the vagus nerve in alternation of the halothane/epinephrine dysrhythmias by dexmedetomidine. In the separate six intact dogs, because the vagus nerve is composed of afferent and efferent fibers, we examined the effect of the largest dose of dexmedetomidine (0.5 μg·kg$^{-1}$·min$^{-1}$ intravenous) in the presence of atropine methyl nitrate (3.0 mg·kg$^{-1}$ intravenous) in a dose blocking afferent vagal outflow to the heart.\textsuperscript{23} Secondly, we examined the effect of vagotomy on the antidyssrhythmic effect of doxazosin, a selective $\alpha_1$ antagonist that does not cross the blood-brain barrier.\textsuperscript{18} In this experiment, an initial dose of 200 μg·kg$^{-1}$ of doxazosin was administered intravenously, followed by 0.5 μg·kg$^{-1}$·min$^{-1}$ to maintain steady plasma concentration of 40 ng·ml$^{-1}$ according to a previous pharmacokinetic data.\textsuperscript{24,25} Plasma concentration of doxazosin was measured by a similar method described by Kaye \textit{et al.}\textsuperscript{21} In brief, 1 ml of plasma sample was alkaline.
with 0.5 M Na₂CO₃ and extracted with diethyl ether. The ether layer was recovered and evaporated to dryness with nitrogen, followed by reconstitution with mobile phase. The sample was injected into the high performance liquid chromatography system combined with an ultraviolet detector (model UV-8010, Tosoh). The limit of this assay was 1 ng·ml⁻¹. We examined the effect of doxazosin in intact and vagotomized dogs with the same experimental protocol.

Data were expressed as mean ± SEM. The results of multiple groups were analyzed by one-way analysis of variance, and comparisons between groups were assessed by Scheffé’s test. Comparison between two groups was assessed by Student’s t test for unpaired data. P < 0.05 was considered statistically significant.

Results

The effect of dexmedetomidine on the dysrhythmia thresholds in intact and vagotomized dogs are shown in figures 1 and 2, respectively. Although the dysrhythmogenic dose of epinephrine in vagotomized dogs was significantly less than that in intact dogs at the control state (dexmedetomidine = 0; P = 0.032), the plasma concentration of epinephrine in the vagotomized group was not significantly less (P = 0.41). Dexmedetomidine treatment significantly increased both the dysrhythmogenic dose and the plasma concentration of epinephrine in a dose-dependent manner in intact dogs (fig 1). However, this antidysrhythmic action of dexmedetomidine was abolished in vagotomized dogs (fig 2). Also, the beneficial action of dexmedetomidine was not observed in the presence of atropine methyl nitrate (fig 3). The systolic arterial pressure was significantly increased, heart rate significantly decreased, and the diastolic arterial pressure unchanged at the time when the dysrhythmias were observed after the dexmedetomidine treatment in intact dogs (table 1), whereas hemodynamic parameters were unaffected by dexmedetomidine at the time of dysrhythmias in vagotomized dogs (table 2). In contrast to dexmedetomidine, doxazosin inhibited the halothane/epinephrine dysrhythmias in both intact and vagotomized animals (fig 4). In this experiment, the plasma concentrations of doxazosin in the intact and vagotomized groups were 45.0
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![Graph showing the effect of atropine methyl nitrate on dysrhythmogenic doses (DD) and plasma concentrations (PC) of epinephrine during halothane anesthesia.](http://anesthesiology.pubs.asahq.org/pdfaccess.ashx?url=/data/journals/jasa/931827/)

Fig. 3. The effect of atropine methyl nitrate (0 and 3.0 mg·kg⁻¹ intravenous) in the presence of dexmedetomidine (0.5 mg·kg⁻¹·min⁻¹ intravenous) on dysrhythmogenic doses (DD) and plasma concentrations (PC) of epinephrine during halothane anesthesia (mean ± SEM; number of observations is shown in parentheses). *P < 0.05, compared with the 0 dose.

± 2.6 and 46.4 ± 2.1 ng·ml⁻¹, respectively. Doxazosin significantly decreased systolic and diastolic arterial pressure in the both groups, but it did not affect heart rate (table 3).

Discussion

The principal finding in the current study in intact dogs was that the antidiarrhythmic action of dexmedetomidine, a selective α₂ agonist, was abolished by bilateral vagotomy. On the other hand, the antidiarrhythmic action of doxazosin, a selective α₁ antagonist that does not cross the blood-brain barrier, was not affected by vagotomy. When we compared the dysrhythmogenic dose and plasma concentration of epinephrine in intact dogs with those in vagotomized dogs in the absence of any pretreatment (dexmedetomidine = 0), vagotomy decreased the dysrhythmogenic dose, whereas the plasma concentration was not reduced (figs. 1 and 2 and Results). Because we investigated seven and eight animals in the intact and the vagotomized groups, respectively, one may deduce that the number of animals was so small as to cause a type II error, failing to detect a difference. However, a P value about plasma concentration between the two groups was relatively large (P = 0.41, Results). Thus, we thought that the possibility of the type II error was small, although the possibility was not eliminated. The reason for the dissociation between the dysrhythmogenic dose and plasma concentration of epinephrine is obscure. One possible explanation of this phenomenon is that vagotomy might affect metabolism of catecholamines. One previous report documented that the plasma concentration is a more reliable indicator than the dysrhythmogenic dose of epinephrine to evaluate the dysrhythmia threshold.¹² Thus, we may consider that resting vagal tone does not appear to affect the vulnerability to epinephrine-induced dysrhythmias during halothane anesthesia.

Halothane has been known to sensitize the heart to dysrhythmogenic effect of epinephrine, and various studies have attempted to determine the precise mechanism for this type of dysrhythmia.¹³ Most of these reports focused on interaction of halothane and epinephrine in myocardium and changes in hemodynamic variables such as arterial blood pressure and heart rate. Previously, several studies⁹–¹¹,¹⁴,¹⁷ demonstrated the important role of the central nervous system in the modification of the genesis of digoxin-induced dys-

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<th>Table 1. Hemodynamic Data during Dysrhythmias in the Presence of Dexmedetomidine during Halothane Anesthesia in Intact Dogs</th>
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<tr>
<td><strong>Dose of Dexmedetomidine (µg·kg⁻¹·min⁻¹)</strong></td>
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<td>--------------------------------</td>
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<tr>
<td>0</td>
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<tr>
<td>0.2</td>
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<td>0.5</td>
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SAP = systolic arterial pressure; DAP = diastolic arterial pressure; HR = heart rate.

Data are mean ± SEM.

*P < 0.05 versus dexmedetomidine 0.
rhythmias, and other reports\textsuperscript{28,29} suggested that a similar central contribution exists in the halothane/epinephrine dysrhythmias. In our previous report,\textsuperscript{12} we demonstrated that dexmedetomidine prevented the genesis of halothane/epinephrine dysrhythmias through a central nervous system action. Because an $\alpha_2$ agonist enhances the baroreflex response to increase in blood pressure,\textsuperscript{10} and inhibits neural firing rate from the locus ceruleus,\textsuperscript{31} suggesting an increase in vagal tone and a decrease in sympathetic outflow, we considered the significant role of the vagus in the antidysrhythmic property of dexmedetomidine. In the current study, although dexmedetomidine significantly prevented the epinephrine-induced dysrhythmias, the antidysrhythmic action of dexmedetomidine was nullified in vagotomized dogs, indicating that the vagal activity plays a critical role in the antidysrhythmic action of the $\alpha_2$ agonist. Because the vagus nerve includes afferent and efferent fibers, we determined the protective effect of dexmedetomidine in the presence of pharmacologic blockade of the efferent pathway. The prevention of the antidysrhythmic action of dexmedetom- idine by atropine (fig. 3) suggests that the efferent activity in the heart is the critical component.

It is well known that adrenergic $\alpha_2$ binding sites are widely distributed in the brain and regulate various physiologic processes, including autonomic control.\textsuperscript{32} Concerning vagal activity, the dorsal motor nucleus of vagus is an important region, where an efferent para-sympathetic nerve originates, and the activity of this region is directly regulated by nucleus tractus solitarius, where an afferent vagal sensory input terminates.\textsuperscript{33} These two nuclei are rich in $\alpha_2$ binding sites.\textsuperscript{34} In addition, these two sites are innervated and functionally modulated by higher brain regions, including locus ceruleus, nucleus amygdala, hypothalamus, and hippocampus. These areas also express the high concentrations of $\alpha_2$ binding sites.\textsuperscript{32} Because dexmedetomi-
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Fig. 4. Dysrhythmogenic doses (DD) and plasma concentrations (PC) of epinephrine in the presence of doxazosin during halothane anesthesia in intact (A) and vagotomized (B) dogs, respectively. The animals in each group received a sham operation or a bilateral vagotomy, respectively (mean ± SEM; number of observations is shown in parentheses). *P < 0.05, compared with the control value.

Table 3. Hemodynamic Data during Dysrhythmias in the Presence of Doxazosin during Halothane Anesthesia in Intact and Vagotomized Dogs

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>SAP (mmHg)</th>
<th>DAP (mmHg)</th>
<th>HR (beats/min)</th>
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</thead>
<tbody>
<tr>
<td><strong>Intact dogs</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Control</td>
<td>7</td>
<td>233 ± 6</td>
<td>127 ± 7</td>
<td>129 ± 9</td>
</tr>
<tr>
<td>Doxazosin</td>
<td>7</td>
<td>175 ± 12</td>
<td>90 ± 3*</td>
<td>138 ± 10</td>
</tr>
<tr>
<td><strong>Vagotomized dogs</strong></td>
<td>8</td>
<td>198 ± 10</td>
<td>113 ± 5</td>
<td>203 ± 23</td>
</tr>
<tr>
<td>Control</td>
<td>7</td>
<td>156 ± 9*</td>
<td>91 ± 4*</td>
<td>225 ± 16</td>
</tr>
<tr>
<td>Doxazosin</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SAP = systolic arterial pressure; DAP = diastolic arterial pressure; HR = heart rate.

Data are mean ± SEM.

* P < 0.05 versus control.

anism of the beneficial property of doxazosin is still controversial.4–6,41

Arterial blood pressure has been suggested to be an important factor in the genesis of halothane/epinephrine dysrhythmias.1,2 Vasoconstriction by activation of peripheral α₂ adrenoceptor in arterial vessels facilitates elevation of arterial blood pressure,42 and this effect would become more prominent in vagotomized dogs in whom central hemodynamic effect of dexmedetomidine attenuates. Although this action may potentiate the halothane/epinephrine dysrhythmias, there were no significant differences in hemodynamic variables at the dysrhythmias in the vagotomized group. Thus, this hemodynamic effect would not affect the current results significantly. In comparison, central withdrawal of sympathetic tone by dexmedetomidine might counteract the vasoconstriction followed by peripheral stimulation of α₂ adrenoceptor in intact dogs. However, arterial pressure at the dysrhythmias increased significantly with the increased dysrhythmogenic dose of epinephrine, suggesting that the hemodynamic changes were dependent largely on the dose of epinephrine (table 1). In our doxazosin experiment, although doxazosin required more epinephrine dose to achieve the dysrhythmia threshold, arterial blood pressures were lower in the presence of doxazosin. These results may support the earlier data, which suggest that the myocardial α₁ adrenoceptor may play an important role in the genesis of halothane/epinephrine dysrhythmias.4–6

We conclude that the vagus nerve plays a critical role in the antiadysrhythmic effect of dexmedetomidine on halothane/epinephrine dysrhythmias in dogs, although
resting vagal tone neither exerts a significant protection against the dysrhythmias nor affects the antidyssrhythmic effect of doxazosin.

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