EFFECT OF $\alpha_1$-ADRENOCEPTOR BLOCKADE ON THE LEFT VENTRICULAR FORCE-LENGTH RELATIONSHIP IN DOGS

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We used the left ventricular (LV) end-systolic force-diameter ($F_{es}$-$D_{es}$) relation to evaluate the effect of an alpha$_1$-adrenoceptor antagonist (bunazosin hydrochloride) on the contractility of the beta-blocked left ventricle. Nine adult mongrel dogs were instrumented with ultrasonic crystals to measure LV diameter and a micromanometer to measure LV pressure. Beta-adrenergic and vagal blockade was induced with intravenous propranolol (2 mg/kg) and atropine (0.2 mg/kg), respectively, and preload was decreased by inferior vena caval occlusion. The slope ($E_c$) and extrapolated diameter intercept ($D_0$) of the LV $F_{es}$-$D_{es}$ relation were derived from end-systolic data obtained in the control state (after beta-blockade) and after bunazosin infusion (1 mg/kg). $E_c$ was used as a new index of LV contractility. After bunazosin infusion, the heart rate and $E_c$ were decreased by 7 and 22%, respectively, in comparison with the control state, whereas $D_0$ did not change. These results indicate that alpha$_1$-adrenoceptor blockade significantly reduces myocardial contractility in the beta-blocked canine heart, perhaps by decreasing the intracellular calcium concentration and/or myosin ATPase activity. (Jpn Circ J 1992; 56: 929-935)

$\alpha_1$-ADRENOCEPTOR antagonists are commonly used for the treatment of patients with essential hypertension. Stimulation of the cardiac alpha$_1$-adrenoceptors produces a positive inotropic effect in various animal species$^{1-5}$ and in humans$^{6-8}$ However, it also appears to be involved in the process of myocardial hypertrophy$^9$ and in the genesis of ventricular reperfusion arrhythmias.$^{10}$ The alpha$_1$-adrenoceptor antagonist, bunazosin hydrochloride, has been reported to have a blocking effect on the slow calcium channel in animals.$^{11-13}$ Accordingly, although bunazosin has been used clinically to treat several types of cardiovascular disease, this agent may have the potential to produce a decrease in myocardial contractility. Since contractility is an important factor in determining myocardial oxygen consumption, particularly in the presence of ischemic heart disease, this is a subject of some interest. Furthermore, no convincing evidence has yet been presented that alpha$_1$-adrenoceptor antagonists reduce myocardial contractility in the in situ left ventricle.

Although many contractility indices have been used to assess left ventricular (LV) performance (e.g., $E_{max}$, $dP/dt_{max}$, $mV_{ef}$, or the ejection fraction), to the best of our know-

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METHODS

Animal Preparation

Nine healthy adult mongrel dogs (12±1 kg) were anesthetized with intravenous sodium pentobarbital (26 mg/kg) and instrumented as described in detail elsewhere. In brief, positive pressure ventilation was provided via an endotracheal tube. A thoracotomy was performed in the left fourth intercostal space, the pericardium was opened, and the heart was suspended in a pericardial cradle. A micromanometer-tipped catheter (MPC500, Millar Instruments) was balanced in a constant-temperature water bath (37 °C). It was then inserted through the LV apex and held in place by a purse-string suture. A catheter was inserted into the right femoral vein for the infusion of drugs and fluid, and another catheter was inserted into the right femoral artery for blood gas analysis. An occluder was set around the inferior vena cava (IVC) to produce a decrease in preload. A pair of ultrasonic crystals (diameter 4 mm, frequency 5 MHz) was implanted in the LV endocardium of each dog to provide continuous measurements of the anteroposterior LV diameter [Ultrasonic dimension system (model UDM-5C), MECC, Japan] (Fig. 1). The stability of the LV pressure-diameter loops was tested with an oscilloscope during the steady state and also during preload manipulations. Lead II of the surface electrocardiogram was recorded and the following variables were measured: LV pressure, the time derivative of LV pressure (dP/dt), and the LV anteroposterior diameter. The data for these variables were simultaneously stored in a hard disk memory at 1-msec intervals using a computer system (PC9801VX21, NEC, Japan). The digital data on the hard disk were then evaluated without the use of digital filtering.

Experimental Protocol

The open-chest dogs were mechanically ventilated and arterial blood gases were analyzed (model ABL4, Radiometer, Copenhagen). Arterial PO₂ and PCO₂ were tried to maintain at greater than 100 mmHg and less than 40 mmHg, respectively, irrespective of alterations in the inspired oxygen fraction and/or the ventilation rate.

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Beta-adrenergic and vagal blockade were induced intravenously with propranolol (2 mg/kg) and atropine (0.2 mg/kg) in all dogs. A major reflex change of autonomic tone was defined as an increase or decrease in heart rate of greater than 10 beats/min over the course of caval occlusion. Initial data for the control values were recorded during a steady-state period at least 10 min after the initiation of beta-adrenergic and vagal blockade. During the collection of control data, the IVC was gradually occluded. To eliminate the effects of changes in lung volume due to respiration, data were recorded while the dogs were kept apneic for 20 sec. Caval occlusion resulted in an 18–51 mmHg fall in LV end-systolic pressure. After these control measurements, the dogs received bunazosin hydrochloride intravenously at a dose of 1 mg/kg, and at least 5 min were allowed to elapse before measurements were repeated. Then, IVC occlusion was performed again by the same procedure as before. Occlusion resulted in a 21–33 mmHg fall in LV end-systolic pressure. No antiarrhythmic agent was used during these manipulations.

After the study, arterial blood gas analysis was repeated, and the positions of the ultrasonic crystals were examined at necropsy. In all animals, no arrhythmias occurred during the manipulation of preload, the final arterial blood gas analysis gave a PO₂ of greater than 90 mmHg and a PCO₂ of less than 45 mmHg, the micromanometer drift was less than 1.0 mmHg throughout the course of the study, and the positions of the ultrasonic crystals were appropriate.

Data Analysis and Theoretical Background

Instantaneous LV pressure (P, mmHg) and diameter (D, cm) data recorded from several cardiac cycles during the manipulation of preload were used for the construction of LV force-diameter loops. LV circumferential force (F, g) was calculated from the equation \[ F = P \cdot D / 2a \] (where "a" denotes a conversion factor of 0.735 mmHg·g⁻¹·cm²)⁴⁵

The loops derived for a representative dog are shown in Fig. 2. Endsystole was determined to be at the upper left corner of the LV force-diameter loop, i.e., the point where the ratio of myocardial force to length \[ [F/(L−L_0)] \] was maximal⁶⁻¹⁰. In the present study, the LV endsystolic circumferential force \[ F_{es} = P_{es} \cdot D_{es} / 2a \] (where \( P_{es} \) denotes LV endsystolic pressure (mmHg) and \( D_{es} \) is the LV end-systolic diameter (cm)) was assumed to be linearly proportional to muscle length \( \pi D_{es} \). Thus, \( F_{es} \) was expressed by the following equation⁴⁵:

\[ F_{es} = \pi E_{c} (D_{es} - D_{o}) \]  \hspace{1cm} (1)

where \( E_{c} \) (g/cm) denotes the slope of the LV endsystolic force-length relation (which was used as an index of the inotropic state of the LV myocardium) and \( D_{o} \) (cm) is the basal LV diameter. Instantaneous LV endsystolic force \( F_{es} \) and diameter \( D_{es} \) data were used to obtain the \( E_{c} \) and \( D_{o} \) values. The linear regression equation for the LV \( F_{es} \cdot D_{es} \) relation was obtained as equation (Eq.) 2:

\[ F_{es} = A \cdot (D_{es} - B) \]  \hspace{1cm} (2)

where A is the regression coefficient, and B is the x-axis intercept. Thus, \( E_{c} \) and \( D_{o} \) were derived as follows from Eqs. 1 and 2, respectively:
TABLE 1  EFFECTS OF INFUSION OF BUNAZOSIN (N=9)

<table>
<thead>
<tr>
<th></th>
<th>control (C)</th>
<th>bunazosin (BUN)</th>
<th>% change</th>
<th>p value (C vs. BUN)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate [bpm]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>before</td>
<td>122±24</td>
<td>113±22</td>
<td>−7</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>after</td>
<td>122±24</td>
<td>112±22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LV end-systolic pressure [mmHg]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>before</td>
<td>115±13</td>
<td>99±10</td>
<td>−14</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>after</td>
<td>84±9</td>
<td>73±9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LV end-systolic diameter [cm]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>before</td>
<td>2.00±0.48</td>
<td>2.09±0.49</td>
<td>+5</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>after</td>
<td>1.78±0.45</td>
<td>1.82±0.42</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LV end-systolic force [g]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>before</td>
<td>155±34</td>
<td>139±29</td>
<td>−10</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>after</td>
<td>101±25</td>
<td>89±16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LV F_{es}-D_{es} relation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E_{c} [g/cm]</td>
<td>80±20</td>
<td>62±16</td>
<td>−22</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>D_{o} [cm]</td>
<td>1.36±0.36</td>
<td>1.34±0.36</td>
<td>−1</td>
<td>NS</td>
</tr>
</tbody>
</table>

before: before IVC occlusion. after: after IVC occlusion to the minimum pressure. Symbols: bunazosin = after bunazosin infusion (1 mg/kg). $E_c$: slope of the LV end-systolic force-length relation. $D_o$: extrapolated diameter intercept of the LV end-systolic force-diameter relation. Data were expressed as the mean±SD.

\[ E_c = \frac{A}{\pi} \]  
threeq
\[ D_o = B \]  
fourtheq

The digital data stored on the hard disk were processed using a computer system (PC9801VX21, NEC, Japan) and software developed at our laboratory. We obtained force-diameter loops, determined the end-systolic point in each cardiac cycle, estimated the regression equation for the LV $F_{es}$-$D_{es}$ relation as well as the $E_c$ and $D_o$ values, and drew entire curves for the $F_{es}$-$D_{es}$ relation. Results are expressed as the mean±SD. The statistical significance of differences between the means was assessed using the paired t-test. Linear regression by the least squares method was used to fit data to the LV $F_{es}$-$D_{es}$ relation. Correlation coefficient value ($r$) was obtained.

RESULTS

The findings are summarized in Table I. The change in heart rate was less than 10 beats/min over the course of any IVC occlusion for the manipulation of preload. With IVC occlusion, the LV $P_{es}$ decreased from 115±13 to 84±9 mmHg in the control state and from 99±10 to 73±9 mmHg after bunazosin.

The $F_{es}$, $E_c$, and $D_o$ values were calculated for each dog before and after bunazosin infusion and used for making comparisons between the two states of contractility. Following bunazosin infusion, the baseline heart rate (113±22 vs 122±24 bpm, $p<0.01$), the LV $P_{es}$ value (99±10 vs 115±13 mmHg, $p<0.01$), and the LV $F_{es}$ value (139±29 vs 155±34 g, $p<0.05$) were significantly decreased, but the LV $D_{es}$ value was significantly increased (2.09±0.49 vs 2.00±0.48 cm, $p<0.05$) when compared with the control state.

In all dogs, the LV $F_{es}$-$D_{es}$ relation was near linear before ($r=0.993±0.010$) and after bunazosin infusion ($r=0.995±0.007$). The $E_c$ value was significantly decreased by bunazosin infusion (62±16 vs 80±20 g/cm, $p<0.05$), whereas the $D_o$ value did not change significantly (1.34±0.36 vs 1.36±0.36 cm, NS) (Fig. 3). These results suggested that in the beta-adrenergic receptor blocked canine left ventricle, bunazosin produced a 7% reduction of the chronotropic state and a 22% reduction of the myocardial inotropic state.

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active cross-bridges at that time. 3) The myocardium continues to contract until almost all the free Ca\(^{2+}\) (assumed to be 95% of the initial Ca\(^{2+}\) concentration) has become bound to troponin C at the end of systole. The value of 95% may be taken to denote Ca\(^{2+}\) utilization for cross-bridge activation or the Ca\(^{2+}\) affinity of troponin C. 4) The force at the end of systole remains linearly proportional to the muscle length over the entire tested range of force, according to the proportionality constant E\(_c\).

On the basis of these assumptions, the E\(_c\) value (the slope of the LV F\(_e\)-L\(_e\) relation) theoretically reflects at least four biochemical and mechanical parameters of cross-bridge activation and cycling\(^{21}\) These are: 1) the initial calcium concentration released from the sarcoplasmic reticulum \(\sigma (\text{Ca}^{2+})\), 2) the affinity of troponin C for calcium (\(\alpha\)), 3) the force generated by an active cross-bridge (f), which may depend on myosin ATPase activity, and 4) the cross-sectional area of the myocardium (S). Thus:

\[ E_c = \sigma (\text{Ca}^{2+}) \cdot f \cdot S \cdot a \]  
(See Equation A12 of Ref. 21). The calcium affinity of troponin C (\(\alpha\)) was found to have a value of 0.95 in normal dog hearts\(^{21}\). This suggests that the calcium affinity is not a significant determinant of increases in E\(_c\) in the normal heart, because the maximum value of the calcium affinity is theoretically 1.0.

The regulation of myocardial force by beta-adrenoceptors is believed to be mediated by the intracellular accumulation of cyclic AMP and the subsequent phosphorylation of myocardial functional proteins. On the other hand, the intracellular mechanisms involved in the positive inotropic effects mediated by alpha\(_1\)-adrenoceptors are not yet completely understood. It has been shown that stimulation of myocardial alpha\(_1\)-adrenoceptors increases the intracellular calcium transient, probably by an increase in calcium influx through the myocardial cell membrane\(^{22}\) and has a positive inotropic effect in myocardial preparations.

The present study provided experimental evidence that an alpha\(_1\)-adrenoceptor antagonist, bunazosin hydrochloride, significantly reduced myocardial contractility in the in situ beta-blocked canine LV wall. The occurrence of a decrease in E\(_c\) without any

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**DISCUSSION**

It is necessary first to present briefly the concepts underlying our models. Detailed descriptions of the models have been given in previous reports\(^{14,15,20}\) As a partial LV model, we used a ringlike structure of unit height (1 cm) (Fig 1 of Ref. 14), and each ringlike structure was regarded as approximating a cylinder. The total circumferential force (F) on this cylinder of myocardium during systole was defined as the sum of two forces, which were an active force and a resting force that represented muscular diastolic stiffness. The following assumptions were made\(^{21}\): 1) A cross-bridge is functionally "activated" by the binding of one free Ca\(^{2+}\) ion with the regulatory protein troponin C on the myocardial actin filaments, and rate constant K\(_a\) applies to this binding process. 2) The active myocardial force at a given time is related to the total number of
change in the diameter intercept (D₀) of the LV F maxi-D₀ relation could have resulted from a reduction in the intracellular calcium concentration and/or the myosin ATPase activity induced by the infusion of bunazosin (See Equation D1). This hypothesis derived from Eq. D1 is partly in agreement with the known pharmacological effects of bunazosin on the intracellular mechanisms involved in the positive inotropic response mediated by α₁-adrenoceptors.

We can suggest two possible explanations for the present experimental finding that bunazosin infusion had a negative inotropic effect on the LV myocardium. The infusion of an α₁-adrenoceptor antagonist may decrease α₁-mediated inotropic stimulation, even if the α₁-adrenoceptors are in a hypersensitive state as a result of beta-blockade. The second possibility is that there was an increase in the contribution of α₁-adrenoceptors to the mediation of a positive inotropic effect as a compensation for the reduced beta-adrenoceptor activity in our beta-blocked dogs. Such a situation may develop in some pathological states where the beta-adrenoceptor system is compromised, e.g., hypothyroidism, or after treatment with beta-adrenoceptor antagonists. However, this speculation should be validated by examining the effects of bunazosin on the E₀ value in the presence and absence of autonomic blockade under in vivo conditions.

Accordingly, although the detailed mechanism underlying the positive inotropic effect of the α₁-adrenoceptor remains unclear, it may have an important role in maintaining cardiac contractility, particularly when cardiac beta-adrenergic stimulation is weakened. In addition, this positive inotropic effect of α₁-adrenoceptors may be generated by an increase in the intracellular calcium concentration and/or the myosin ATPase activity in the LV myocardium, because the Ca²⁺ affinity of troponin C is thought to be near maximal.

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