EFFECTS OF NIFEDIPINE ON DIASTOLIC ABNORMALITIES IN LOW-FLOW AND PACING-INDUCED ISCHEMIA IN ISOLATED RAT HEARTS

SHIN-ICHI MOMOMURA, M.D., TAKASHI SERIZAWA, M.D., HIROSHI IKENOUCHI, M.D., TSUNEKI SUGIMOTO, M.D., AND MASAHICO IIZUKA, M.D.

An animal experimental model which simulates human effort angina, especially in terms of diastolic abnormalities, was developed using isovolumically beating perfused rat hearts. Using this model, we studied the effects of nifedipine, a Ca²⁺ channel blocker, on diastolic properties during pacing-induced ischemia. When the preload of the left ventricle was set at a low level, low-flow ischemia (coronary perfusion pressure of 40 mmHg plus tachycardia (480 beats/min for 4 min) did not induce an increase in left ventricular end-diastolic pressure (LVEDP). However, with a high preload, low-flow ischemia plus pacing tachycardia induced an increase in LVEDP of 8.4 ± 5.4 mmHg (p < 0.01) and a prolongation of the time constant of ventricular pressure decline (6.8 ± 4.6 msec, p < 0.05) immediately after pacing tachycardia. Pretreatment with nifedipine (3 × 10⁻⁴M) prevented the rise in LVEDP induced by pacing tachycardia. Thus, in isolated perfused hearts, diastolic abnormalities similar to those seen in angina pectoris were obtained by low-flow ischemia plus pacing tachycardia. The response to nifedipine suggested that an alteration of Ca²⁺ movement may play an important role in the increase in left ventricular stiffness under these conditions.

An increase in left ventricular diastolic pressure relative to volume during spontaneous attacks of the angina pectoris and angina attacks provoked by exercise or pacing tachycardia has been reported by many investigators in patients with coronary artery disease. This decreased left ventricular diastolic distensibility usually accompanied by a decrease in the rate of left ventricular relaxation. Similar changes in diastolic properties have been observed in open-chest dogs with severe coronary stenoses and superimposed pacing tachycardia. Several possible mechanisms producing such changes have been proposed and one of the most likely of these is an alteration in intrinsic myocardial properties due to ischemia-induced calcium overload. However, investigations using open-chest dogs could not completely exclude other mechanisms, such as extrinsic diastolic compression by the right ventricle, or dyssynchrony between the ischemic and normal myocardium. In fact, it is well known that these factors may alter left ventricular diastolic properties. Therefore, to accurately assess the role of intrinsic factors in the alteration of left ventricular diastolic properties in ischemia, the development of an experimental model of angina in the isolated

Key words:
Diastratic stiffness
Relaxation
Perfused rat heart
Ca²⁺ antagonist

(Received August 00, 1990; accepted October 11, 1990)
The Second Department of Internal Medicine, Faculty of Medicine, University of Tokyo, Tokyo, Japan
This study was supported in part by a grant from the Japanese Ministry of Health and Welfare (461-1).
Mailing address: Shin-ichi Momomura, M.D., The Second Department of Internal Medicine, Faculty of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113, Japan

Japanese Circulation Journal Vol. 55, June 1991 623
(body weight 270–350g) were used in this study.

The isolated isovolumically beating heart preparation is illustrated in Fig. 1. After decapitation, the thorax was opened and the heart was excised quickly. The aorta was cannulated and the heart was perfused in a retrograde fashion with oxygenated modified Krebs-Henseleit buffer (gassed with 95% O₂+5% CO₂) at 37°C using a constant-flow pump (Masterflex, Cole-Parmer). The modified Krebs-Henseleit buffer used in the present study had the following composition: 118 mM NaCl, 4.7 mM KCl, 2.0 mM CaCl₂, 1.2 mM KH₂PO₄, 1.2 mM MgSO₄, 25 mM NaHCO₃, 0.4 mM Na₂-EDTA, 55 mM glucose, and 1.0 mM lactate. The oxygen tension of the oxygenated buffer was approximately 530–570 mmHg, and the pH was 7.40–7.46 at the level of the aortic cannula.

After the initiation of perfusion, a small plastic cannula was placed in the left ventricle via a stab incision at the apex to drain the Thebesian flow. Then another thin cannula was inserted into the right ventricle via the pulmonary artery. This cannulation was aimed at decompressing the right heart by completely collecting coronary sinus flow and was also performed to obtain samples for lactate measurements. A latex balloon with a long axis slightly longer than that of the left ventricle was then inserted into the left ventricle via the left atrial appendage, and the base of the left atrium was ligated to prevent the balloon from prolapsing. The heart was paced at a rate of 210 beats/min with an electric stimulator (Nihon Kohden) via a pair of hook-shaped electrodes attached to the right ventricular outflow tract. At this heart rate, diastole was long enough for left ventricular pressure to decay fully and a flat diastolic portion of the left ventricular pressure curve was obtained at each beat.

**METHODS**

*Isolated isovolumically beating heart preparation*

Ten- to 12-week-old male Wistar rats

heart is necessary. It should be noted that a beneficial effect of Ca²⁺ antagonists on diastolic abnormalities induced by pacing tachycardia¹²,¹³ has been reported.

The present study was not intended to resolve whether or not right ventricular diastolic compression or dysynchrony between normal and ischemic myocardium contribute to altered left ventricular diastolic distensibility during ischemia. Instead, focusing on the role of the alteration of intrinsic factors in the impaired left ventricular diastolic properties, we first developed a model of pacing-induced ischemia in which changes in diastolic properties mimicked those seen in angina pectoris. In addition, we examined the effects of nifedipine, a Ca²⁺ antagonist, on the diastolic abnormalities in this model.
fidelity pressure transducer. Initially, the left ventricular end-diastolic pressure was set to approximately 10 mmHg by injecting bubble-free water into the balloon.

Coronary perfusion pressure was monitored by another pressure transducer, and set to 100 mmHg by adjusting the flow rate of the perfusion pump: The coronary flow was fixed during the control perfusion period.

Left ventricular isovolumic pressure, coronary perfusion pressure, and pacing signals were recorded on magnetic tape using an 8-channel data recorder (Sony Magnescale Inc.), as well as on a thermal array recorder (Nihon Kohden). Left ventricular pressure data stored on magnetic tape were digitized every two seconds using a 12-bit A/D converter. From the digitized data, the following indices were determined using a personal technical computer (Packet IIE, Anritsu Co.): left ventricular peak systolic pressure (LVSP), left ventricular end-diastolic pressure (LVEDP), left ventricular developed pressure (LVEDP=LVSP−LVEDP), left ventricular peak positive dP/dt (+dP/dt), and left ventricular peak negative dP/dt (−dP/dt). In this model, since the left ventricle was contracting isovolumically, left ventricular end-diastolic pressure was a direct index of left ventricular stiffness. As an index of the rate of left ventricular relaxation, the time constant (τBP) of left ventricular pressure decay was calculated from the digitized pressure data. Calculation of the time constant was based on the assumption that the asymptote of left ventricular pressure decay is not always zero but may vary.

As mentioned previously, in the isovolumic preparation the diastolic portion of the left ventricular pressure curve was usually flat. However, during pacing tachycardia this flat diastolic portion disappeared due to incomplete relaxation relative to the shortened diastole, and the end-diastolic pressure apparently increased. During such tachycardia, the succeeding systolic pressure development commences even before peak negative dP/dt has been achieved and the determination of peak positive dP/dt, peak negative dP/dt and the time constant may be invalid. Therefore, only left ventricular developed pressure and left ventricular end-diastolic pressure were calculated as indices of left ventricular function during pacing tachycardia.

**Lactate extraction rate**

Effluent obtained from the pulmonary arterial cannula was collected and analyzed to determine its lactate concentration, which was measured by the lactate oxidase method using a photospectrometer (Hitachi U-3200). Lactate data were expressed as the lactate extraction rate: $LER (\mu M/min/g) = \frac{CF}{[\text{Lac}]_{a} - [\text{Lac}]_{v}}$, where $CF$ is the perfusion flow per gram of heart tissue, $[\text{Lac}]_{a}$ is the lactate concentration in the oxygenated buffer, and $[\text{Lac}]_{v}$ is the lactate concentration in the effluent from the coronary sinus.

**Experimental protocol**

1) Pacing tachycardia with normal coronary flow (n=11): After several readjustments of left ventricular balloon volume and coronary flow, hemodynamic stabilization was obtained. Pacing tachycardia at a rate of 480 beats/min was then performed for 4 min. Left ventricular and coronary perfusion pressures were measured until 10 min after the cessation of pacing tachycardia.

2) Pacing-induced ischemia with a low preload: In 8 other hearts, coronary perfusion pressure was decreased to 45 mmHg by reducing the flow rate. As coronary flow was reduced, left ventricular end-diastolic pressure was also decreased because of loss of the turgor effect. Therefore left ventricular end-diastolic pressure was readjusted to 10 mmHg by infusing additional bubble-free water into the balloon. After low-flow ischemia was initiated, left ventricular pressure was observed for 15 min, and pacing tachycardia (480 beats/min) was then superimposed for 4 min. The heart rate was then returned to 210 beats/min and left ventricular pressure and coronary perfusion pressure were followed for 10 more minutes during the post-pacing tachycardia period.

3) Pacing-induced ischemia with a high preload: In 12 other hearts, coronary perfusion pressure was lowered to 45 mmHg by decreasing perfusion flow, while left ventricular end-diastolic pressure was simultaneously raised to 30 mmHg by inflating the balloon with bubble-free water to increase ventricular preload. After low-flow
TABLE I  CHANGES IN PARAMETERS OF LEFT VENTRICULAR FUNCTION DURING PACING TACHYCARDIA WITHOUT ISCHEMIA

<table>
<thead>
<tr>
<th></th>
<th>LVDP (mmHg)</th>
<th>+dP/dt (mmHg/sec)</th>
<th>LVEDP (mmHg)</th>
<th>-dP/dt (mmHg/sec)</th>
<th>TB (msec)</th>
<th>Pb (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before PT</td>
<td>143.1±22.7</td>
<td>3248±632</td>
<td>10.0±1.1</td>
<td>2490±404</td>
<td>27.5±3.9</td>
<td>0.5±3.2</td>
</tr>
<tr>
<td>PT (2 min)</td>
<td>58.9±13.7**</td>
<td>—</td>
<td>34.6±12.3**</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>PT (4 min)</td>
<td>62.9±15.2**</td>
<td>—</td>
<td>32.2±9.1**</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>After PT (0 min)</td>
<td>85.2±27.4**</td>
<td>1965±632**</td>
<td>10.4±1.8</td>
<td>1256±502**</td>
<td>30.2±5.6</td>
<td>0.1±3.1</td>
</tr>
<tr>
<td>After PT (30 sec)</td>
<td>120.7±26.9</td>
<td>2633±677</td>
<td>9.8±1.6</td>
<td>1766±501</td>
<td>30.4±6.0</td>
<td>-2.0±3.1</td>
</tr>
<tr>
<td>After PT (1 min)</td>
<td>128.1±25.4</td>
<td>2720±697</td>
<td>10.3±1.6</td>
<td>1834±516</td>
<td>30.0±5.2</td>
<td>-1.9±2.3</td>
</tr>
<tr>
<td>After PT (10 min)</td>
<td>141.9±22.9</td>
<td>3269±662</td>
<td>9.1±2.2</td>
<td>2204±432</td>
<td>25.6±3.1</td>
<td>-1.6±5.1</td>
</tr>
</tbody>
</table>

PT = pacing tachycardia, LVDP = left ventricular developed pressure, LVEDP = left ventricular end-diastolic pressure, + dP/dt = peak positive dP/dt, - dP/dt = peak negative dP/dt, TB = time constant of relaxation, Pb = asymptote of left ventricular pressure decay. *p<0.05, **p<0.01 vs. before PT.

TABLE II  CHANGES IN PARAMETERS OF LEFT VENTRICULAR FUNCTION DURING ISCHEMIA PLUS PACING TACHYCARDIA WITH A LOW PRELOAD (n=8).

<table>
<thead>
<tr>
<th></th>
<th>LVDP (mmHg)</th>
<th>+dP/dt (mmHg/sec)</th>
<th>LVEDP (mmHg)</th>
<th>-dP/dt (mmHg/sec)</th>
<th>TB (msec)</th>
<th>Pb (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ischemia (0 min)</td>
<td>86.1±9.8</td>
<td>1973±275</td>
<td>10.5±0.9</td>
<td>1229±231</td>
<td>29.9±4.7</td>
<td>0.7±3.2</td>
</tr>
<tr>
<td>Before PT</td>
<td>86.2±10.7</td>
<td>1989±283</td>
<td>10.9±2.3</td>
<td>1246±249</td>
<td>28.6±5.5</td>
<td>1.1±3.8</td>
</tr>
<tr>
<td>PT (2 min)</td>
<td>34.9±12.1**</td>
<td>—</td>
<td>19.8±3.8**</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>PT (4 min)</td>
<td>35.0±11.7**</td>
<td>—</td>
<td>20.6±4.9**</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>After PT (0 min)</td>
<td>45.2±14.6**</td>
<td>1138±258**</td>
<td>11.7±3.6</td>
<td>600±210**</td>
<td>37.1±8.9</td>
<td>3.5±5.4</td>
</tr>
<tr>
<td>After PT (30 sec)</td>
<td>74.0±10.0*</td>
<td>1611±239*</td>
<td>11.3±2.8</td>
<td>935±211*</td>
<td>37.9±9.2*</td>
<td>1.6±5.2</td>
</tr>
<tr>
<td>After PT (1 min)</td>
<td>79.7±10.1</td>
<td>1721±233</td>
<td>11.0±2.6</td>
<td>1035±220*</td>
<td>32.7±5.5</td>
<td>2.2±4.1</td>
</tr>
<tr>
<td>After PT (10 min)</td>
<td>84.5±10.1</td>
<td>2004±300</td>
<td>10.6±2.6</td>
<td>1253±237</td>
<td>27.7±4.4</td>
<td>2.1±3.4</td>
</tr>
</tbody>
</table>

See Table I for abbreviations. *p<0.05, **p<0.01 vs. before PT.

ischemia was initiated, left ventricular pressure was observed for 15 min, and pacing tachycardia (480 beats/min) was then superimposed for 4 min. Left ventricular pressure and coronary perfusion pressure were observed for 10 more min during the post-tachycardia period.

4) Nifedipine pretreatment: In 7 hearts, oxygenated buffer containing 3×10^{-8}M of nifedipine was perfused after the perfusion of normal oxygenated buffer. Ischemia was then induced by decreasing the coronary perfusion pressure to 45 mmHg, and left ventricular end-diastolic pressure was elevated to 30 mmHg. Pacing tachycardia was superimposed and changes in left ventricular performance in the post-tachycardia period were observed as described above.

After each experiment, the whole heart and the left ventricle were weighed, and myocardial flow per gram of wet heart tissue was calculated.

**Statistical analysis**

Indices of left ventricular function and coronary perfusion between the hearts with and without nifedipine treatment were first examined by analysis of variance for differences of the mean. When differences of the mean were significant, a modified t-test was used to compare each index between groups. Hemodynamic parameters in the same group at different time periods were also compared by analysis of variance fol-

NII-Electronic Library Service
### TABLE III

<table>
<thead>
<tr>
<th></th>
<th>LVDP (mmHg)</th>
<th>(\frac{dP}{dt}) (mmHg/sec)</th>
<th>LVEDP (mmHg)</th>
<th>(\frac{-dP}{dt}) (mmHg/sec)</th>
<th>(T_{pr}) (msec)</th>
<th>Pb (mmHg/sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Without nifedipine, increased preload (n=12)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ischemia (0 min)</td>
<td>67.8±17.1</td>
<td>1430±379</td>
<td>30.2±3.0</td>
<td>896±253</td>
<td>34.0±7.4</td>
<td>23.7±5.3</td>
</tr>
<tr>
<td>Before PT</td>
<td>68.3±17.5</td>
<td>1455±402</td>
<td>30.2±3.1</td>
<td>906±265</td>
<td>33.4±6.8</td>
<td>22.4±4.2</td>
</tr>
<tr>
<td>PT (2 min)</td>
<td>25.2±10.2**</td>
<td>—</td>
<td>40.7±8.2**</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>PT (4 min)</td>
<td>23.0±10.2**</td>
<td>—</td>
<td>46.7±6.4**</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>After PT (0 min)</td>
<td>41.5±15.6**</td>
<td>881±294**</td>
<td>38.4±5.5**</td>
<td>540±253**</td>
<td>40.2±9.0*</td>
<td>33.5±7.0**</td>
</tr>
<tr>
<td>After PT (30 sec)</td>
<td>52.2±17.0*</td>
<td>1073±329*</td>
<td>38.2±4.2**</td>
<td>667±252*</td>
<td>41.0±9.1*</td>
<td>30.8±5.8**</td>
</tr>
<tr>
<td>After PT (1 min)</td>
<td>58.0±17.9</td>
<td>1183±350</td>
<td>37.5±4.2**</td>
<td>736±271</td>
<td>40.3±11.0</td>
<td>29.8±5.0**</td>
</tr>
<tr>
<td>After PT (10 min)</td>
<td>66.4±16.0</td>
<td>1432±359</td>
<td>34.3±3.1**</td>
<td>898±243</td>
<td>33.4±7.3</td>
<td>26.9±3.9*</td>
</tr>
<tr>
<td><strong>With nifedipine (3×10^{-4}M), increased preload (n=7)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ischemia (0 min)</td>
<td>66.1±16.9</td>
<td>1493±384</td>
<td>30.2±3.1</td>
<td>865±233</td>
<td>33.6±6.2</td>
<td>22.5±4.5</td>
</tr>
<tr>
<td>Before PT</td>
<td>66.2±16.9</td>
<td>1496±380</td>
<td>30.2±3.1</td>
<td>818±277</td>
<td>32.6±4.8</td>
<td>22.7±4.6</td>
</tr>
<tr>
<td>PT (2 min)</td>
<td>25.3±24.9**</td>
<td>—</td>
<td>37.2±4.0*</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>PT (4 min)</td>
<td>23.0±8.9**</td>
<td>—</td>
<td>38.8±3.8*a</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>After PT (0 min)</td>
<td>44.3±12.0*</td>
<td>1051±248*</td>
<td>32.3±2.9a</td>
<td>588±136</td>
<td>37.7±4.2</td>
<td>24.7±6.5a</td>
</tr>
<tr>
<td>After PT (30 sec)</td>
<td>58.5±21.2</td>
<td>1299±410</td>
<td>32.2±2.9a</td>
<td>739±226</td>
<td>38.1±8.2</td>
<td>23.5±6.0a</td>
</tr>
<tr>
<td>After PT (1 min)</td>
<td>61.1±20.1</td>
<td>1349±416</td>
<td>32.1±2.7a</td>
<td>773±237</td>
<td>35.1±8.0</td>
<td>23.9±5.4</td>
</tr>
<tr>
<td>After PT (10 min)</td>
<td>66.5±18.1</td>
<td>1512±446</td>
<td>32.0±1.8</td>
<td>897±261</td>
<td>32.8±6.2</td>
<td>24.1±5.0</td>
</tr>
</tbody>
</table>

See Table I for abbreviations. *p<0.05, **p<0.01 vs. before PT.

\(a=p<0.05\) vs. without nifedipine.

...followed by a modified t-test.

**RESULTS**

**Pacing tachycardia without ischemia** (Table I)

Myocardial flow per gram of heart tissue was 15.3±2.1 ml/g min when the coronary perfusion pressure was set at 100 mmHg. With pacing tachycardia superimposed on normal coronary perfusion, left ventricular developed pressure decreased from 143.1 ±22.7 mmHg before tachycardia to 62.9 ±15.2 mmHg at the end of 4 min of pacing tachycardia (p<0.01), and was 85.2±27.4 mmHg immediately after the cessation of the tachycardia (p<0.01). Left ventricular developed pressure recovered completely to 141.9±22.9 mmHg by 10 min after the cessation of pacing tachycardia. Similarly, peak positive \(\frac{dP}{dt}\) decreased from 3248±632 mmHg/sec before pacing to 1965±632 mmHg/sec immediately after pacing (p<0.01), and then returned to the baseline level by 10 min after the cessation of pacing. Regarding the indices of the diastolic properties, after the cessation of pacing, left ventricular end-diastolic pressure immediately returned to the pre-pacing value (10.0±1.1 mmHg before pacing vs. 10.4±1.8 mmHg immediately after pacing). Peak negative \(\frac{dP}{dt}\) was transiently decreased following pacing tachycardia and recovered to the pre-pacing level after 10 min. Time constant of relaxation and the asymptote of LV relaxation were unchanged.

**Effects of low-flow ischemia and superimposed pacing tachycardia with a low preload** (Table II)

When the coronary perfusion pressure was lowered to 45 mmHg and left ventricular end-diastolic pressure was set to 10 mmHg, the coronary perfusion flow was 7.8±0.9 ml/g min. Upon initiation of low-flow ischemia, left ventricular developed pressure was
86.1 ± 9.8 mmHg and time constant was 29.9 ± 4.7 msec. During the 10 min of ischemia, the left ventricular pressure and coronary perfusion pressure were stable, and no deterioration of left ventricular performance occurred.

With pacing tachycardia, the left ventricular systolic pressure was decreased and the minimum pressure elevated. In the immediate post-pacing period, a rise in the left ventricular end-diastolic pressure compared to the pre-pacing period did not occur. Transient mild prolongation of the time constant was observed 30 sec after pacing tachycardia.

Changes in left ventricular performance in pacing-induced ischemia with an elevated preload (Upper panel of Table III)

Coronary perfusion flow per gram of heart tissue was 6.4 ± 1.4 ml/g min when the coronary perfusion pressure was lowered to 45 mmHg and the preload was increased (left ventricular end-diastolic pressure = 30 mmHg). During pacing tachycardia, the left ventricular end-diastolic pressure elevated and left ventricular developed pressure declined as observed in ischemia with a low preload. After pacing tachycardia, the left ventricular peak systolic pressure and the peak positive dP/dt were decreased transiently but returned to pre-pacing values within 10 min. In contrast to ischemia with a low preload, left ventricular end-diastolic pressure gradually increased during pacing and remained elevated after pacing was terminated. It was 30.2 ± 3.0 mmHg before pacing tachycardia and 38.4 ± 5.5 mmHg immediately after pacing (p < 0.01 vs. before

Japanese Circulation Journal  Vol.55, June 1991
pacing). It returned to $34.3 \pm 3.1$ mmHg 10 min after pacing tachycardia. Peak negative dP/dt was decreased transiently after pacing, but recovered completely by 10 min afterwards. Time constant was prolonged from $33.4 \pm 6.8$ to $41.0 \pm 9.1$ msec at 30 sec after pacing ($p<0.05$), and this prolongation of the time constant was also transient.

**Effects of nifedipine on normal perfusion and pacing-induced ischemia** (Table IV and the lower panel of Table III)

The effects of nifedipine on hemodynamics during normal coronary perfusion are shown in Table IV. At $3 \times 10^{-8}$M of nifedipine, left ventricular developed pressure decreased ($p<0.05$), peak positive dP/dt was unchanged, and left ventricular end-diastolic pressure and time constant were unchanged, while peak negative dP/dt was slightly decreased.

With low-flow ischemia (coronary perfusion pressure: 45 mmHg) and high preload (initial left ventricular end-diastolic pressure of 30 mmHg), the coronary flow per gram of heart tissue was 5.9 ml/g min. This did not differ from that seen in low flow ischemia without nifedipine. As in ischemia without nifedipine, left ventricular performance was stable during ischemia until pacing tachycardia was superimposed. Changes in left ventricular developed pressure during pacing were similar to those seen during pacing-induced ischemia without nifedipine. The gradual increase in the left ventricular end-diastolic pressure observed during pacing tachycardia in the untreated high-preload hearts was not seen under the presence of
nifedipine. Decreases in left ventricular developed pressure, peak positive dP/dt, and peak negative dP/dt after pacing were also similar to those observed in the case of ischemia without nifedipine. However, in contrast to without nifedipine, left ventricular end-diastolic pressure returned nearly to the pre-pacing level immediately after pacing. Also the rise in left ventricular end-diastolic pressure from before pacing to immediately after pacing was not significant. Similarly, the increase in the asymptote of exponential fit of the left ventricular pressure decline was not significant. Finally, the time constant of left ventricular pressure decline showed a tendency to be prolonged only with pacing tachycardia. Typical recordings of left ventricular pressure during pacing-induced ischemia with and without nifedipine treatment are shown in Fig. 2.

To summarize the effects of nifedipine on pacing-induced ischemia, changes in the indices of left ventricular performance from before pacing to 30 sec after pacing with increased preload were compared between the nifedipine-treated and untreated hearts (Table V). Nifedipine did not alter the changes in systolic function or the rate of relaxation when compared with the untreated hearts, and its effect on left ventricular performace during pacing-induced ischemia seemed to be limited to the indices of left ventricular stiffness, i.e., left ventricular end-diastolic pressure and asymptote.

**Comparison between index of left ventricular stiffness and the rate of relaxation**

Changes in left ventricular end-diastolic pressure (ΔEDP) and time constant (ΔT) are shown in Fig. 3. During pacing-induced ischemia without nifedipine, the increase in left ventricular end-diastolic pressure after pacing tachycardia was accompanied by a comparable prolongation of the time constant. This means that the increase in left ventricular stiffness and the slowing of the rate of relaxation were in accordance. However, during pacing-induced ischemia with nifedipine, the prolongation of time constant was more prominent than the increase in left ventricular end-diastolic pressure. Thus, the ΔEDP–ΔT relation was different between the two groups.

**Changes in metabolic parameters during low coronary perfusion and superimposed tachycardia with or without nifedipine**

With ischemia and an elevated preload, the lactate extraction rate was $-90.6\pm36.4\,\mu\text{M/min}$. After 4 min of tachycardia, the lactate extraction rate tended to decrease further to $-128.4\pm39.2\,\mu\text{M/min}$. In the presence of nifedipine, the lactate extraction rate during ischemia was $-104.3\pm42.9\,\mu\text{M/min}$, and pacing tachycardia did not alter the lactate extraction rate ($-104.3\pm51.8\,\mu\text{M/min}$).

**DISCUSSION**

**Experimental models of angina pectoris**

One of the objectives of this study was to create a model in which the hemodynamic changes mimicked those seen in effort angina pectoris. In open-chest dogs, attempts to develop such a model have been reasonably successful. Serizawa et al produced severe stenoses in the left anterior descending and circumflex coronary arteries, and then superimposed pacing tachycardia? After

*Japanese Circulation Journal Vol.35, June 1991*
pacing, they observed an upward shift in the left ventricular diastolic pressure-volume relationship, which was identical to that observed clinically during attacks of angina pectoris. In their canine model, the effects of extrinsic factors were minimized because the pericardium was widely opened. The mechanism underlying this upward shift thus seemed likely to be the sustained interaction between contractile proteins (i.e., actin and myosin) in diastole. However, since ischemia of the myocardium was limited to the regions perfused by the stenosed arteries, the effects of dys synchrony between ischemic and normal myocardium on left ventricular diastolic distensibility could not be completely excluded in their model.

In the isolated perfused heart, global and homogeneous ischemia can be easily achieved by decreasing the perfusion flow. Recently, Isoyama et al demonstrated a transient increase in left ventricular chamber distensibility during demand ischemia created by decreased perfusion flow and pacing tachycardia in blood-perfused, isolated, isovolumically beating rat hearts. However, attempts to induce diastolic abnormalities similar to those seen in angina pectoris using buffer-perfused hearts have not been very successful so far. The reason for this failure to induce decreased diastolic distensibility in buffer-perfused isolated hearts is not clear. An insufficient increase in energy demand during pacing tachycardia in the buffer-perfused model may be one of the important causes. Serizawa et al, were able to obtain decreased diastolic distensibility in a buffer-perfused model only when the systolic pressure was maintained during pacing tachycardia. In the present model, elevation of the left ventricular preload allowed left ventricular systolic pressure to be maintained at a relatively high level during pacing tachycardia. Increased left ventricular size should augment systolic wall stress according to Laplace's law, so the increase in energy demand during pacing tachycardia would be greater in the high-preload hearts. Furthermore, in hearts with a high preload, more severe subendocardial ischemia should have been induced than in those with a low preload. Another advantage of elevating the preload is that the change in diastolic distensibility is exaggerated, because the left ventricular diastolic pressure-volume relationship is operating on the steeper portion of its curve.

The mechanism of this decrease in distensibility is still unclear, although residual interaction between action and myosin due to calcium overload is an attractive and likely possibility. As mentioned above, myocardial energy consumption increases with tachycardia, but energy supply may be limited in hearts with coronary stenoses. It is thought that under such circumstances, the sarcoplasmic reticulum cannot obtain sufficient energy to take up cytosolic calcium, so that a residual interaction between actin and myosin and a consequent decrease in myocardial diastolic distensibility may ensue. Increased slow-cannel calcium influx during tachycardia may potentiate this process.

Recent studies of intracellular Ca2+ levels in the isolated perfused hearts using Ca2+ indicators have demonstrated that the diastolic Ca2+ level increases during brief global ischemia. These findings may support the concept that the intracellular diastolic Ca2+ level is increased in our model. Application of these techniques and ATP measurement may be able to elucidate the mechanism of the alteration of diastolic properties during pacing-induced ischemia.

Effects of nifedipine on left ventricular function in the normoxic heart

In the present model, nifedipine depressed systolic function in hearts with normal coronary perfusion, although the time constant of left ventricular relaxation was unchanged. This effect on the rate of relaxation was inconsistent with the results of Walsh et al. They studied the effects of calcium channel blocking agents in conscious dogs, and found both negative inotropic and lusitropic effects. They attributed the decreased rate of relaxation to a decrease in systolic shortening. It appears that the effect of nifedipine on relaxation may vary according to the experimental model and methods for calculation of relaxation indices as well as due to the concentration or the route of administration of the drug.

Effects of Ca2+ blocking agents on diastolic abnormalities in diseased hearts

We showed that nifedipine inhibited the
increase in left ventricular stiffness during ischemia plus pacing tachycardia. The effects of Ca\textsuperscript{2+} blockers on diastolic abnormalities in coronary heart disease and experimentally induced ischemia have been observed by a few investigators. Lorell et al demonstrated that in patients with coronary artery disease the upward shift of the left ventricular diastolic pressure-volume relation induced by pacing tachycardia was attenuated by nifedipine.\textsuperscript{12} Nakamara et al also reported a preventive effect of nifedipine on diastolic abnormalities in the post-pacing period in patients with ischemic heart disease.\textsuperscript{13} Similar effects of calcium antagonists on diastolic properties have been reported in other types of heart disease, including hypertrophic cardiomyopathy.\textsuperscript{22} In an experimental setting, Henry et al have demonstrated that nifedipine pretreatment prevented ischemic contracture in the isolated rabbit hearts.\textsuperscript{23} Nayler et al showed that verapamil, another calcium antagonist, attenuated the increase in resting tension in isolated guinea pig hearts.\textsuperscript{24} A direct effect of calcium antagonists on the relaxation process is unlikely, but they may improve diastolic abnormalities by increasing regional blood flow to the ischemic myocardium in patients with coronary heart disease. However, in the present model, coronary flow was constant and myocardial perfusion should have been uniformly depressed during ischemia. Thus the suppression of the inward calcium current by nifedipine may be the most important mechanism of its beneficial effect on left ventricular diastolic properties. Decreased Ca\textsuperscript{2+} influx may attenuate the calcium overload in ischemia, while negative inotropicism due to decreased Ca\textsuperscript{2+} influx may preserve myocardial high-energy phosphates and enable the sarcoplasmic reticulum to utilize more ATP to accumulate calcium. Kohmoto et al demonstrated verapamil decreased resting calcium level and delayed the onset of contracture and depletion of ATP in cultured chick embryo ventricular myocytes.\textsuperscript{25} It is possible that nifedipine attenuates ventricular diastolic abnormalities through similar effects on intracellular Ca\textsuperscript{2+} and high energy phosphates.

Interestingly, the present study showed that nifedipine did not alter the showing in the rate of relaxation very much, despite its beneficial effect on diastolic stiffness. This discrepancy between in the rate of relaxation and stiffness may indicate that the determinants of these two properties are not always identical. Dissociation of left ventricular stiffness and the rate of relaxation has been reported by Momomura et al during hypoxia with isoproterenol in isovolumically beating perfused rat heart.\textsuperscript{14} Nayler and Williams demonstrated that depletion of myocardial ATP occurred prior to the rise in resting tension during hypoxia in guinea pig hearts.\textsuperscript{26} Therefore, diastolic stiffness may be determined by the amount of ATP available relative to the amount of calcium which the sarcoplasmic reticulum has to accumulate, while the rate of relaxation may depend on factors other than the amount of ATP available, such as phosphorylation of phospholamban or ATPase activity in the sarcoplasmic reticulum.

In summary, under increased preload, ischemia plus pacing tachycardia induced diastolic abnormalities, including decreased distensibility and a slowed rate of relaxation. These changes in diastolic properties mimicked the diastolic abnormalities which occur during angina pectoris. Pretreatment with nifedipine prevented the decrease in left ventricular diastolic distensibility induced by ischemia plus pacing tachycardia. This beneficial effect of nifedipine was probably due to its suppression of the calcium influx and energy consumption. In contrast, the slowed rate of relaxation was less improved by this agent.

REFERENCES

4. MANN T, BRODIE BR, GROSSMAN W, McLaurin LP: Effect of angina on the left ven-

Japanese Circulation Journal Vol. 55, June 1991


