Nitrendipine Binding in Congestive Heart Failure Due to Myocardial Infarction

Ian M.C. Dixon, Sheu-Lun Lee, and Naranjan S. Dhalla

Depressed cardiac pump function is the hallmark of congestive heart failure, and it is suspected that decreased influx of Ca\(^{2+}\) into the cardiac cell is responsible for depressed contractile function. Since Ca\(^{2+}\) channels in the sarcolemmal membrane are considered to be an important route for the entry of Ca\(^{2+}\), we examined the status of Ca\(^{2+}\) receptors/channels in failing rat hearts after myocardial infarction of the left ventricular free wall. For this purpose, the left coronary artery was ligated and hearts were examined 4, 8, and 16 weeks later; sham-operated animals served as controls. Hemodynamic assessment revealed decreased total mechanical energy (left ventricular systolic pressure×heart rate), increased left ventricular diastolic pressure, and decreased positive and negative dP/dt in experimental animals at 4, 8, and 16 weeks. Although accumulation of ascites in the abdominal cavity was evident at 4 weeks, other clinical signs of congestive heart failure in experimental rats were evident from the presence of lung congestion and cardiac dilatation at 8 and 16 weeks after induction of myocardial infarction. The density of Ca\(^{2+}\) receptors/channels in crude membranes, as assessed by \(^{[3]}\)H nitrendipine binding assay, was found to be decreased in the uninfarcted experimental left ventricle at 8 and 16 weeks; however, no change in the affinity of nitrendipine was evident. A similar depression in the specific binding of another dihydropyridine compound, \(^{[3]}\)H PN200-110, was also evident in failing hearts. Brain and skeletal muscle crude membrane preparations, unlike those of the right ventricle and liver, revealed a decrease in Ca\(^{2+}\) receptors/channels density in experimental animals at 16 weeks. Reduction in the Ca\(^{2+}\) channel number was also seen in heart homogenate as well as purified sarcolemmal preparations from failing animals at 16 weeks. These data support the view that mechanisms governing the entry of Ca\(^{2+}\) in the myocardium may be depressed in moderate to severe stages of congestive heart failure, and such changes may be a consequence of events occurring during the development of congestive heart failure. (Circulation Research 1990;66:782–788)

Disturbances in Ca\(^{2+}\) metabolism are suspected to be involved in a wide variety of experimental models of heart disease.\(^1\)–\(^3\) Ca\(^{2+}\) entry to the interior of the myocardium is believed to occur mainly via voltage- or receptor-operated calcium channels located in the sarcolemmal membrane.\(^4\) and it has been shown that the functional status of these Ca\(^{2+}\) channels in the cell can be monitored by determining the specific binding of Ca\(^{2+}\) antagonists.\(^5\) Reports of increased density of Ca\(^{2+}\) channels/receptors in crude membrane preparations from hearts of genetically cardiomyopathic hamsters\(^5\)–\(^6\) raise the possibility that intracellular Ca\(^{2+}\) overload via augmented sarcolemmal Ca\(^{2+}\) influx may be the molecular basis of characteristic pathological alterations seen in these hearts. However, no change in Ca\(^{2+}\) channel density was seen in older cardiomyopathic hamsters with extensive cardiac hypertrophy and congestive heart failure.\(^7\) Furthermore, previous studies have shown that \(^{[3]}\)H nitrendipine and \(^{[3]}\)H verapamil binding sites are reduced when hearts are subjected to global ischemia or hypoxia-reoxygenation injury associated with intracellular Ca\(^{2+}\) overload.\(^8\)\(^9\) Thus, it appears that the status of Ca\(^{2+}\) channels in the myocardium may depend on the type of heart disease. To determine the status of these channels during the development of congestive heart failure, we have undertaken a study of the density and affinity of Ca\(^{2+}\) channels in cardiac sarcolemmal membranes of rats with congestive heart failure as a result of infarction of the left ventricular free wall. Our results indicate that the density of cardiac Ca\(^{2+}\) channels/receptors in exper-

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ponential animals is decreased without changes in the \(^{3}H\)nitrendipine binding affinity.

**Materials and Methods**

**Experimental Model**

Myocardial infarction was produced in male Sprague-Dawley rats (200–250 g) by occlusion of the left coronary artery as described by Johns and Olson\(^{10}\) and as modified by Selye et al.\(^{11}\) After the animals were anesthetized with ether, the skin was incised along the left sternal border, the incised heart was exteriorized through the intercostal space. The left coronary artery was ligated approximately 2 mm from its origin with a suture of 6-0 silk, and the heart was exteriorized in the chest. Throughout the course of the operation, rats were maintained on a positive-pressure ventilation system delivering a mixture of 95% \(O_{2}\)-5% \(CO_{2}\) mixed with ether. Closure of the wound was accomplished by a purse-string suture. The mortality of all animals operated on in this fashion was approximately 35% within 48 hours. Sham-operated animals were treated similarly except that the suture around the coronary artery was not tied. Animals were allowed to recover, received food and water ad libitum, and were maintained for a period of 4, 8, and 16 weeks before hemodynamic and biochemical assessment.

**Hemodynamic Studies**

After 4, 8, or 16 weeks of operation, the animals were anesthetized with an injection of sodium pentobarbital (50 mg/kg i.p.). To maintain adequate ventilation, the trachea was intubated, the right carotid artery was exposed, and a microtip pressure transducer (model SPR-249, Millar Instruments, Houston, Texas) was introduced through a proximal arteriotomy.\(^{12,13}\) The catheter was advanced carefully through the lumen of the carotid artery until the tip of the transducer entered the left ventricle. The catheter was secured with a silk ligature around the artery, and readings were taken from a dynograph recorder (model R511A, Beckman, Fullerton, California). Left ventricular pressures, heart rate, rate of contraction (+dP/dt), rate of relaxation (−dP/dt), and arterial systemic and diastolic pressures were recorded.

**Crude Membrane Preparation**

Rats were killed by decapitation and their hearts were removed. The atria, connective tissue, and right ventricle were trimmed away, and the remaining left ventricle was processed for the preparation of membranes according to the method described by Wagner et al.\(^{5}\) In all experimental animals, the uninfarcted ventricular tissue was used after removing the scar. In some experiments, the gastrocnemius muscle, the brain cortex, liver, and right ventricle were also processed for the preparation of crude membranes. Briefly, the tissue was washed, minced, and then homogenized in 50 mM Tris-HCl, pH 7.4 (15 ml/g tissue) with a PT-20 polytron (Brinkman Instruments, Westbury, New York; two times, 20 seconds each, setting 5). The resulting homogenate was centrifuged at 1,000g for 10 minutes, and the pellet was discarded. The supernatant was centrifuged at 48,000g for 25 minutes. The resulting pellet was resuspended and centrifuged twice in the same buffer at the same speed; the final pellet was resuspended in 50 mM Tris-HCl, pH 7.4. This preparation has been commonly used by various investigators for studying receptor mechanisms in the cell.

**Preparation of Cardiac Sarcolemmal Membranes**

Purified light sarcolemmal membrane fraction was isolated from left ventricular tissue according to the method of Pitts,\(^{14}\) and the heavy sarcolemmal membranes were isolated by the hypotonic shock/lithium bromide method.\(^{15}\) All isolation steps were carried out at 0–4°C, and membrane fractions were frozen immediately after isolation in liquid \(N_{2}\) and stored up to 3 weeks at −80°C before binding studies. Such freezing of membranes had no effect on the binding values. Protein concentration of all membranes was determined by the method of Lowry et al.\(^{16}\)

\[^{3}H\]Nitrendipine Binding Assay

Binding of \[^{3}H\]nitrendipine to membrane fractions was monitored according to a method reported earlier.\(^{5,8}\) Membrane preparations (0.08–0.1 g protein/tube) were incubated with 0.035–5 nM \[^{3}H\]nitrendipine, unless otherwise indicated in the text, in the absence or presence of 2.5 \(\mu\)M unlabeled nifedipine, a concentration sufficient to inhibit more than 95% of the specific \[^{3}H\]nitrendipine binding. Assays were terminated after 1 hour at room temperature (22–23°C) by filtration (GF/C filters, Whatman, Clifton, New Jersey). Filters were washed twice with 5 ml cold Tris-HCl buffer. The radioactivity of the filters was counted in a scintillation counter (model LS7500, Beckman Instruments, Fullerton, California) at an efficiency of 39–41%. The nonspecific \[^{3}H\]nitrendipine binding (in the presence of nifedipine) was subtracted from the total binding (in the absence of nifedipine) to obtain the specific binding of \[^{3}H\]nitrendipine. Binding of another dihydropyridine compound, \[^{3}H\]PN200-110, to crude membrane fraction was also carried out to determine whether the observed changes with nitrendipine were due to any artifact of the radioligand employed here. Bindings of these radioligands were also measured by using heart homogenates. Assay standards of \[^{3}H\]nitrendipine and \[^{3}H\]PN200-110 were prepared by dilution with 0.25% ethanol. Control experiments indicated that 0.25% ethanol did not significantly alter specific binding of either dihydropyridine. \[^{3}H\]Nitrendipine and \[^{3}H\]PN200-110 were obtained from New England Nuclear Medicine, Boston, Massachusetts. \[^{3}H\]Nitrendipine was 5-methyl-\[^{3}H\]nitrendipine, with a specific activity of 80.9 Ci/mmol, whereas \[^{3}H\]PN200-110 was obtained from American Radiolabeled Chemicals, St. Louis, Missouri, with a specific activity of 110 Ci/mmol.
Table 1. General Characteristics of Experimental Rats 4, 8, and 16 Weeks After Induction of Myocardial Infarction

<table>
<thead>
<tr>
<th>Parameters</th>
<th>4-Week Sham</th>
<th>4-Week Experimental</th>
<th>8-Week Sham</th>
<th>8-Week Experimental</th>
<th>16-Week Sham</th>
<th>16-Week Experimental</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left ventricle weight (g)</td>
<td>0.825±0.091</td>
<td>0.782±0.102</td>
<td>0.91±0.09</td>
<td>1.25±0.16</td>
<td>1.03±0.10</td>
<td>1.45±0.13</td>
</tr>
<tr>
<td>Right ventricle weight (g)</td>
<td>0.230±0.025</td>
<td>0.307±0.039</td>
<td>0.239±0.023</td>
<td>0.384±0.042</td>
<td>0.258±0.034</td>
<td>0.409±0.045</td>
</tr>
<tr>
<td>Left ventricle wt/body wt (10⁻³)</td>
<td>1.86±0.21</td>
<td>1.95±0.25</td>
<td>1.92±0.17</td>
<td>2.71±0.28</td>
<td>1.84±0.24</td>
<td>2.79±0.29</td>
</tr>
<tr>
<td>Scar weight (g)</td>
<td>ND</td>
<td>0.357±0.061</td>
<td>ND</td>
<td>0.369±0.086</td>
<td>ND</td>
<td>0.396±0.044</td>
</tr>
<tr>
<td>Ascites (ml)</td>
<td>ND</td>
<td>3.2*±1.1</td>
<td>ND</td>
<td>4.3*±1.8</td>
<td>0.5±0.8</td>
<td>11.3*±2.1</td>
</tr>
<tr>
<td>Lung wet weight (g)</td>
<td>1.67±0.16</td>
<td>1.72±0.21</td>
<td>1.81±0.14</td>
<td>2.57*±0.16</td>
<td>1.94±0.12</td>
<td>3.15*±0.17</td>
</tr>
<tr>
<td>Lung dry weight (g)</td>
<td>0.47±0.03</td>
<td>0.45±0.07</td>
<td>0.50±0.08</td>
<td>0.53±0.08</td>
<td>0.51±0.03</td>
<td>0.58±0.04</td>
</tr>
<tr>
<td>Lung wet wt/dry wt</td>
<td>3.55±0.36</td>
<td>3.82±0.46</td>
<td>3.62±0.28</td>
<td>4.85*±0.30</td>
<td>3.80±0.23</td>
<td>5.43*±0.24</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SEM of eight experiments. Left ventricular weight indicated for experimental animals does not include scar tissue. ND, not detectable.
*p<0.05.

(+) -5-methyl-[3H]PN200-110, with a specific activity of 70.2 Ci/mmol.

Statistical Analysis

Results are presented as mean±SEM. The statistical differences between mean values for the two groups were evaluated by Student’s t test. A value of p<0.05 was considered a significant difference between the two groups. Estimates of equilibrium binding parameters (dissociation constant [Kₐ] and maximal density [B_max]) were obtained from Scatchard plot analysis.

Results

A study of left ventricle, right ventricle, scar weight, left ventricle/body weight ratio, appearance of abdominal ascites, and lung weight revealed significant differences between experimental (16 weeks after coronary occlusion) and sham-operated animals (Table 1). Specifically, evidence of cardiac hypertrophy in experimental animals was noted by the increased mass of the remaining viable left ventricle as well as right ventricular myocardium. Furthermore, left ventricle/body weight ratio was increased and the accumulation of ascites in the abdominal cavity was evident in the experimental animals. Congestion of lungs in experimental animals was noted by increased wet lung weight and wet/dry lung weight ratio. Although no difference in liver weight or wet/dry liver weight ratio between sham-operated and experimental animals was seen, the livers of experimental animals had rounded edges and yellowish coloration. Signs of clinical congestive heart failure were also evident in experimental rats 8 weeks after myocardial infarction, but the hearts of these animals were hypertrophied to a lesser extent than were those of the 16-week experimental group. No difference in the scar weights of the left ventricular free wall was seen among the 4-week, 8-week, or 16-week experimental groups. At a period of 4 weeks after surgery, the experimental animals were not significantly different from the sham-operated animals in any of the parameters indicated above except in the accumulation of abdominal ascites (Table 1).

Hemodynamic Parameters

Assessment of hemodynamic performance of the 16-week experimental group revealed decreases in mean arterial pressure, left ventricular systolic pressure, left ventricular end-diastolic pressure, +dP/dt,

Table 2. Hemodynamic Characteristics of Experimental Rats 4, 8, and 16 Weeks After Myocardial Infarction

<table>
<thead>
<tr>
<th>Group</th>
<th>MAP (mmHg)</th>
<th>LVSP (mmHg)</th>
<th>LVEDP (mmHg)</th>
<th>+dP/dt</th>
<th>−dP/dt</th>
<th>HR</th>
<th>Total mechanical energy</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-wk</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>120±2</td>
<td>144±4</td>
<td>3.68±0.8</td>
<td>5,568±107</td>
<td>4,781±249</td>
<td>420±7</td>
<td>60,615±1,781</td>
</tr>
<tr>
<td>Experimental</td>
<td>109±12</td>
<td>122±10</td>
<td>11.3*±1.2</td>
<td>4,500±477</td>
<td>3,318*±404</td>
<td>385±28</td>
<td>47,380*±4,854</td>
</tr>
<tr>
<td>8-wk</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>117±8</td>
<td>135±7</td>
<td>3.49±0.73</td>
<td>5,413±332</td>
<td>5,312±180</td>
<td>391±15</td>
<td>54,180±2,574</td>
</tr>
<tr>
<td>Experimental</td>
<td>90*±6</td>
<td>119±9</td>
<td>12.6*±1.4</td>
<td>4,457*±138</td>
<td>3,985*±210</td>
<td>357±9</td>
<td>45,036*±2,310</td>
</tr>
<tr>
<td>16-wk</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>115±6</td>
<td>132±7</td>
<td>3.44±0.92</td>
<td>5,762±528</td>
<td>5,400±672</td>
<td>380±16</td>
<td>50,705±4,483</td>
</tr>
<tr>
<td>Experimental</td>
<td>65*±4</td>
<td>90*±6</td>
<td>14.2*±0.8</td>
<td>3,769*±213</td>
<td>3,043*±303</td>
<td>242*±5</td>
<td>21,987*±1,330</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SEM of eight experiments. All measurements were made on Beckman dynograph using a Millar microcatheter; the catheter was inserted into the left ventricle via cannulation of the right carotid artery. MAP, mean arterial pressure; LVSP, left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure; +dP/dt, rate of contraction; −dP/dt, rate of relaxation; HR, heart rate. Total mechanical energy=HR×LVSP.

*p<0.05.
Nitrendipine Binding in Congestive Heart Failure

Specific binding of [3H]nitrendipine was significantly reduced in the unscarred left ventricular myocardium in experimental animals 16 weeks after infarction of the left ventricular free wall (Figure 1 and Table 3). Figure 1 shows the saturation curves and Scatchard plots of [3H]nitrendipine binding with crude cardiac membranes from control and experimental animals. Although two populations of [3H] nitrendipine binding sites were detected in left ventricular preparations (high and low affinity), no changes in [3H]nitrendipine binding characteristics (K_d and B_max) of the low-affinity population were observed between control and experimental preparations. The data revealed that B_max was decreased in animals suffering from congestive heart failure without any change in K_d (Table 3). It can also be seen from Table 3 that the [3H]nitrendipine binding in failing heart was also decreased when homogenate was employed for the assay. Table 4 illustrates the binding data of left ventricular preparations from control and experimental animals at different times after the induction of myocardial infarction. Both 8- and 16-week experimental groups showed significantly decreased [3H]nitrendipine receptor binding density with no change in K_d when compared with control values. No differences in binding characteristics of control and the 4-week experimental group were observed. The observed changes in [3H]nitrendipine binding characteristics of the failing myocardium were also compared with normalized left ventricular hypertrophy in different experimental groups. It can be seen from Table 4 that a significant increase in cardiac hypertrophy 4 weeks after coronary ligation was not associated with any change in the [3H]nitrendipine binding properties. Although B_max values for [3H]nitrendipine binding were depressed 8 and 16 weeks after coronary ligation, no relation between the extent of hypertrophy and changes in [3H]nitrendipine binding was evident (Table 4). Figure 2 shows that the nonspecific binding of [3H]nitrendipine to membrane preparations of left ventricle from control and 16-week experimental animals was similar when bound [3H]nitrendipine was maximally displaced with high concentrations of unlabeled nifedipine; no further decrease in the total [3H]nitrendipine

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**Figure 1.** Scatchard plot analysis of specific [3H]nitrendipine binding to a crude membrane preparation of left ventricular tissue from control and failing (16-week) rats. Inset: Specific binding of [3H]nitrendipine to crude membrane preparations of left ventricle in control and failing animals at different concentrations of [3H]nitrendipine. Values represent mean ± SEM of eight experiments. Uninfarcted tissue from the left ventricle of failing hearts was used. K_d, dissociation constant; B_max, maximal density. *p < 0.05.

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**Table 3.** [3H]Nitrendipine Binding (Specific) With Heart Homogenate and Crude Membranes From Control and 16-Week Failing Animals

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Failing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yield of crude membranes (mg/g)</td>
<td>10.6±0.6</td>
<td>10.9±0.8</td>
</tr>
<tr>
<td>Heart homogenate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>K_d (µM)</td>
<td>0.43±0.06</td>
<td>0.49±0.04</td>
</tr>
<tr>
<td>B_max (fmol/mg)</td>
<td>12.4±1.02</td>
<td>6.8±0.42*</td>
</tr>
<tr>
<td>Crude membranes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>K_d (nM)</td>
<td>0.41±0.06</td>
<td>0.40±0.09</td>
</tr>
<tr>
<td>B_max (fmol/mg)</td>
<td>183±12</td>
<td>103±9*</td>
</tr>
</tbody>
</table>

The data expressed are mean±SEM of six experiments. Uninfarcted tissue from the left ventricle of failing rat hearts was used. K_d dissociation constant; B_max, maximal density. *p < 0.05.
binding was seen when 5–10 μM unlabeled nifedipine was used in the incubation medium.

To determine whether changes in [3H]nitrendipine binding with failing hearts were of a specific nature, we prepared the medial gastrocnemius (skeletal) muscle, brain cortex, cardiac right ventricle, and liver membranes from controls and 16-week experimental groups. In brain and skeletal muscle preparations, the density of [3H]nitrendipine receptor sites was significantly decreased in the experimental animals when compared with control, with no change in \( K_d \) (Table 5). Right ventricle and liver preparations of control and experimental groups were not changed with respect to receptor \( B_{\text{max}} \) or \( K_d \) (Table 5).

[3H]Nitrendipine binding characteristics of two types of purified sarcolemmal membranes were also compared to rule out the possibility of any artifacts (Table 6). No difference in protein yield between experimental and control groups was seen in each of these membrane preparations. Similarly, the \( K_d \) was unchanged in both types of preparations from the experimental and control left ventricles, whereas the [3H]nitrendipine receptor density was significantly decreased in these membranes from the 16-week experimental group. To test whether the observed changes in failing hearts were limited to the [3H]nitrendipine binding, another radioligand ([3H]PN200-110) was used to study the binding characteristics. Scatchard plot analysis of [3H]PN200-110 binding to homogenate and crude membrane fraction derived from the 16-week sham-operated and experimental groups is given in Table 7. A reduction in the receptor density without any changes in \( K_d \) was evident in failing heart homogenate as well as crude membrane preparations.

### Discussion

Congestive heart failure secondary to myocardial infarction of the left ventricular free wall has been reported to occur in rats after surgical ligation of the left coronary artery. Animals with large healed infarcts were reported to show characteristic rightward movement on the pressure-volume relation (ventricular dilatation), elevated left and right filling pressures, and signs of pulmonary edema. In this study we have examined changes in cardiac function during the course of 16 weeks from the induction of myocardial infarction to investigate the possibility of graded heart failure. We are able to confirm the presence of an early stage of failure and moderate failure in the 4- and 8-week experimental groups, respectively; severe congestive heart failure was present in the 16-week experimental group. This classification of varying degrees of heart failure subsequent to myocardial infarction in rats was based on our observations regarding general characteristics of the experimental animals and hemodynamic data. However, it is understood that such a categorization is arbitrary and is meant to examine the relation between different degrees of heart failure and biochemical alterations. Although we have demonstrated that a progressive loss of cardiac function in congestive heart failure was paralleled by a significant decrease in Ca2+ channel density at 8 and 16 weeks after induction of myocardial infarction, it should be noted that alterations in heart function seen at 4 weeks were not accompanied by any
changes in the characteristics of Ca\(^{2+}\) channels. Thus, it is apparent that the observed changes in Ca\(^{2+}\) channels in moderate to severe stages of congestive heart failure are a consequence of events that occur during the progression of disease. Nevertheless, such changes in the Ca\(^{2+}\) channel activities in failing heart seem specific in nature because the ???-adrenergic receptor density as measured by [\(^3\)H]dihydropyridine was decreased at 4–16 weeks, whereas the ???-adrenergic receptor density as measured by [\(^3\)H]prazosin was increased at 8–16 weeks after coronary ligation in rats (I.M.C. Dixon and N.S. Dhall, unpublished observations).

Depression in Ca\(^{2+}\) channel density as monitored by [\(^3\)H]nitrendipine binding in failing hearts was not only seen in crude membrane fraction, which is commonly used for the detection of these receptor sites, but it was also evident in heart homogenate as well as purified sarcolemmal preparations obtained by two different methods. Furthermore, such changes were also seen when another radioligand, [\(^3\)H]PN200-110, was employed. It should be pointed out that the observed decrease in Ca\(^{2+}\) channel density in failing heart was not due to any alterations in the nonspecific binding of the radioligands because values for the nonspecific binding of [\(^3\)H]Ca\(^{2+}\) antagonists in control and failing heart preparations were not different from each other. Because hypertrophy of the right ventricle in experimental animals did not exhibit any changes in Ca\(^{2+}\) channel density, myocardial hypertrophy of the left ventricles cannot be considered to account for the observed changes in [\(^3\)H]nitrendipine binding. The decrease in Ca\(^{2+}\) channel density in the failing left ventricle was not specific because a marked depression in [\(^3\)H]nitrendipine binding with brain and skeletal muscle from the experimental animals was also seen. Since [\(^3\)H]nitrendipine binding densities in right ventricular and liver preparations from animals with congestive heart failure were not decreased, it is unlikely that the observed decrease in Ca\(^{2+}\) channels is due to a generalized down-regulation of these sites caused by some circulating hormones and other factors in congestive heart failure. Thus, the exact mechanisms for the observed decrease in Ca\(^{2+}\) channel density are far from clear; however, it can be argued that such changes are due to functional ischemia commonly seen in congestive heart failure.\(^{21}\) This view is supported by the fact that both myocardial ischemia and hypoxia-reoxygenation were reported to result in a decrease of Ca\(^{2+}\) channel density.\(^{3,9}\)

Since Ca\(^{2+}\) channels in the cell membrane are considered to play a critical role in the proper functioning of the excitation-contraction coupling in the myocardium,\(^{2,22}\) a loss of functional Ca\(^{2+}\) channels at moderate and severe stages of heart failure can cause a derangement of cardiac function. On the other hand, various vasodilator substances such as nitroglycerin, hydralazine, and captopril, which may not have any direct effect on the myocardium, have been shown to exert a beneficial effect in congestive heart failure.\(^{20,23-28}\) This is considered to be due to a reduction in the afterload imposed on the heart. Furthermore, the use of Ca\(^{2+}\) antagonists in congestive heart failure is controversial\(^{23-28}\) despite their marked vasodilator effect, which results in lowering the afterload on the failing hearts. Such a controversy may be due to the varying degrees of the negative inotropic effects of Ca\(^{2+}\) antagonists in view of the differences in the Ca\(^{2+}\) channel densities observed at different stages of heart failure. It should be pointed out that a decrease in the sensitivity of failing heart to extracellular Ca\(^{2+}\) has been demonstrated in this experimental model\(^{17}\) and this can be seen to be due to the observed decrease in Ca\(^{2+}\) channel density.

### Table 5. Binding (Specific) Characteristics of [\(^3\)H]Nitrendipine to Crude Membrane Fractions of Various Tissues in Rats 16 Weeks After Myocardial Infarction

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Control</th>
<th>Experimental</th>
<th>Control</th>
<th>Experimental</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right ventricle</td>
<td>0.40±0.06</td>
<td>0.44±0.07</td>
<td>92±12</td>
<td>85±8</td>
</tr>
<tr>
<td>Brain (cortex)</td>
<td>0.27±0.06</td>
<td>0.23±0.09</td>
<td>117±12</td>
<td>77±9</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>5.9±0.6</td>
<td>6.5±0.9</td>
<td>1,361±216</td>
<td>750±186</td>
</tr>
<tr>
<td>Liver</td>
<td>8.3±0.6</td>
<td>9.7±1.8</td>
<td>69.2±8.1</td>
<td>79.8±7.6</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SEM of eight experiments. \(K_d\), dissociation constant; \(B_{\text{max}}\), maximal density. \(^*p<0.05\).

### Table 6. Comparison of [\(^3\)H]Nitrendipine Binding (Specific) to Purified Sarcolemmal Preparations Isolated by Two Different Methods

<table>
<thead>
<tr>
<th>Sarcolemma</th>
<th>Sucrose gradient</th>
<th>Hypotonic shock</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yield (mg/g)</td>
<td>1.3±0.4</td>
<td>3.7±0.7</td>
</tr>
<tr>
<td>(K_d) (nM)</td>
<td>0.45±0.08</td>
<td>0.39±0.07</td>
</tr>
<tr>
<td>(B_{\text{max}}) (fmol/mg)</td>
<td>162±14</td>
<td>148±13</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SEM of eight experiments. The light sarcolemmal preparation was obtained by the sucrose density gradient method, whereas the heavy sarcolemmal preparation was obtained by the hypotonic shock-lithium bromide treatment method. Uninfarcted tissue from the left ventricle of failing rat hearts 16 weeks after induction of myocardial infarction was used. \(K_d\), dissociation constant; \(B_{\text{max}}\), maximal density. \(^*p<0.05\).
However, changes in the Ca\textsuperscript{2+} channel density may be only one of several mechanisms responsible for disturbance of Ca\textsuperscript{2+} movements in the failing heart,\textsuperscript{1,2} and thus it is not our intention to rule out other molecular abnormalities to explain cardiac dysfunction in congestive heart failure. Nonetheless, a depression in Ca\textsuperscript{2+} channel density would result in a decrease in Ca\textsuperscript{2+} influx in myocytes to moderate to severe degrees of failing hearts, and under these conditions, therapeutic applications promoting the entry of Ca\textsuperscript{2+} through mechanisms other than Ca\textsuperscript{2+} channels would be beneficial in congestive heart failure.

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I M Dixon, S L Lee and N S Dhalla

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