ALTERATIONS IN CALCIUM ANTAGONIST RECEPTORS AND SODIUM-CALCIUM EXCHANGE IN CARDIOMYOPATHIC HAMSTER TISSUES

John A. Wagner, Harlan F. Weisman, Adele M. Snowman, Ian J. Reynolds, Myron L. Weisfeldt, and Solomon H. Snyder

The Syrian cardiomyopathic (CM) hamster (BIO 14.6) develops a progressive cardiomyopathy characterized by cellular necrosis, hypertrophy, and, eventually, cardiac dilatation and congestive heart failure. Several lines of evidence implicate cellular calcium overload as an important etiologic factor. We previously reported an increased number of receptors for calcium antagonist drugs, which block voltage-dependent calcium channels, in heart, skeletal muscle, and brain tissue of these hamsters in the early necrotic stage of the disease. To better characterize the pathophysiological significance of this abnormality we evaluated calcium antagonist receptor binding and Na⁺-Ca²⁺ exchange in CM and control hamsters at different stages of disease as documented by quantitative histopathologic assessment. In CM hamsters as young as 10 days, an age previously thought to be before the onset of disease, we identified cardiac myocyte hypertrophy, a twofold increase in calcium antagonist receptor binding in heart and brain, and a 50% increase in skeletal muscle. Overt histological lesions were present in skeletal muscle at 25 days and in heart between 28-30 days. The size of cardiac lesions increased over time and changed from necrotic foci with cellular infiltration to fibrotic or calcified lesions by 360 days. Myocardial cellular hypertrophy persisted through the late stages of the disease (360 days), but increased calcium antagonist binding was present in heart only to 6 months of age, in skeletal muscle to 90 days, and in brain to 30 days. Na⁺-Ca²⁺ exchange in heart was normal until 15 days and then increased by 400% at 30 days suggesting that this augmentation might be a secondary response to the earlier increase in calcium antagonist receptors. At 360 days cardiac Na⁺-Ca²⁺ exchange was decreased by 50%, likely reflecting progressive cardiac damage. The increase in calcium antagonist receptors in CM animals as young as 10 days supports the hypothesis that abnormalities in voltage-dependent calcium channels play a role in the pathophysiology of CM hamsters. (Circulation Research 1989;65:205-214)
to the beginning of the tail. The hearts were excised, placed in iced 30 mM KCl to achieve diastolic arrest, blotted dry, weighed, and fixed by immersion in a 4% buffered formaldehyde solution for 24 hours. The brains were also removed, weighed after blot drying, and fixed whole by immersion in the formaldehyde solution. Small samples of skeletal muscle were obtained from the tongue, acromiotrapezius, biceps femoris, rectus abdominis, and external oblique and also fixed in formaldehyde.

The fixed hearts were serially sliced into 2-mm rings parallel to the atrioventricular groove from base to apex. Each of the slices was embedded in glycol methacrylate. The plastic-embedded blocks were then cut in 3-μm thick serial sections with a glass knife and Sorvall JB-4 microtome. The serial sections were cut in groups of four with 200 μm between groups. The sections were mounted on glass slides, and the first section from each group was stained with toluidine blue, the second with Masson trichrome, or Von Kossa/Kernechtrot stained. The bones of cells were outlined at a magnification of 7.5-10.5 depending on the size of the cell. The areas encompassed by the epicardial contour, the endocardial contour and lesions, were digitized using a Videoplan image analysis microcomputer (Carl Zeiss, Thornwood, New York). Cross-sectional left ventricular cavity diameter was measured along a line connecting the centers of mass of the right ventricular and left ventricular chambers. The myocardial area of each section was calculated by subtracting the endocardial area from the epicardial area. For each heart, total left ventricular myocardial volume, left ventricular cavity volume, and lesion volume (total and by each subtype) were estimated by serial reconstruction of integrated cross-sectional areas of the histological heart rings. The amount of each type of myocardial injury as well as total amount of tissue injury were expressed as a percentage of total calculated ventricular volume.

To assess the development and extent of myocyte hypertrophy, maximal myocyte diameter was determined from the serial sections. Cross-sectional profiles of cells were outlined at a magnification of ×500 by projecting the cursor of the Videoplan computer through a camera lucida attached to a light microscope. The maximal diameter of the digitized profile was then automatically measured and recorded. To assure that measurements were made near the cell centers, only cell profiles containing nuclei were measured. Between 25 and 50 cells per serial section were measurable with these criteria. Because the 3-μm sections from which the measurements were made were taken at 200-μm intervals—a distance two to three times the length of myocytes—no cell was measured more than once. At least 200 cells from each of the original heart rings were measured, resulting in a minimum of 600 measured cells for the smallest hearts to over 1,000 cells for the largest hearts. Differences in cell diameter between CM and control hamsters at each age were tested for significance by Student's t tests.

Materials and Methods

Histopathologic Studies

Male hamsters (BIO 14.6 and age-matched FIB controls; Bio Breeders) were killed at various ages to document the presence and extent of disease. At sacrifice, the animals received sodium heparin (100 units i.p.) and were anesthetized with intraperitoneal sodium methohexitol (35 mg/kg). They were then weighed, and their body lengths were measured from nose tip to the beginning of the tail. The hearts were excised, placed in iced 30 mM KCl to achieve diastolic arrest, blotted dry, weighed, and fixed by immersion in a 4% buffered formaldehyde solution for 24 hours. The brains were also removed, weighed after blot drying, and fixed whole by immersion in the formaldehyde solution. Small samples of skeletal muscle were obtained from the tongue, acromiotrapezius, biceps femoris, rectus abdominis, and external oblique and also fixed in formaldehyde.

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The image of each histological heart section was projected at a magnification of 7.5-10.5 depending on the heart size and traced onto paper. Intramyocardial lesions were identified and also traced. The lesions were classified into four subtypes: 1) cellular: myocytolysis and contraction band necrosis with cellular infiltration; 2) mixed: cellular infiltration with fibrosis; 3) fibrotic: collagen fibers and fibroblasts without other cell types; and 4) calcified: lesions with calcification with or without cellular infiltration or fibrosis. The presence, type, and extent of cardiac lesions were confirmed by micros-
maximal for 30 seconds). Homogenates were centrifuged at 1,000g for 10 minutes, and the resulting supernatants were filtered through four layers of gauze and centrifuged at 10,000g for 20 minutes. Supernatants were diluted with enough 3 M KCl, 50 mM HEPES, pH 7.4, to bring the final KCl concentration to 1 M, and the suspensions were incubated on ice for 30 minutes followed by centrifugation at 150,000g for 60 minutes. Resulting pellets were resuspended in 50 mM HEPES, pH 7.4, 5 mg protein/ml buffer, and stored at -20°C until use. Skeletal muscle was prepared similarly. Calcium antagonist binding activity remains unaltered in samples frozen up to 6 months.

Tissue Preparation for Na⁺-Ca²⁺ Exchange Assay

Hamsters were killed, and brains and hearts were rapidly removed and placed on ice. All further steps took place at 4°C. Synaptosomes were prepared according to the method of Hajas18 with the following modifications. Brains were homogenized in 260 mM sucrose and 50 mM HEPES, pH 7.4, using a motor-driven, loose-fitting Teflon-glass homogenizer (12 up-and-down strokes). The homogenate was centrifuged at 1,000g for 10 minutes, and the pellet was discarded. The supernatant was diluted with an equal volume of basal buffer (mM: NaCl 160, Tris 20, pH 7.4) and centrifuged at 9,000g for 15 minutes. The resulting pellet was gently resuspended in 3 ml of 260 mM sucrose and 50 mM HEPES, pH 7.4, using the Teflon-glass homogenizer, and the suspension was layered over 20 ml of sucrose, 5 mM MgSO₄, 10 mM imidazole, pH 7.0. This two-step gradient was centrifuged at 15,000g for 15 minutes. The resulting pellet was resuspended in 2 ml basal buffer. The synaptosomes were loaded with Na⁺ by overnight incubation in basal buffer at 4°C.

Hearts were prepared as described.19 Briefly, hearts were rinsed with ice-cold 300 mM sucrose, 5 mM MgSO₄, 10 mM imidazole, pH 7.0, minced with scissors, and combined with 4 volumes of 300 mM sucrose, 5 mM MgSO₄, 10 mM imidazole, pH 7.0. All further steps took place at 4°C. This mixture was subjected to four 5-second bursts with a Brinkmann polytron at setting 8. The mixture was homogenized using a motor-driven Teflon-glass homogenizer (two up-and-down strokes). The sucrose concentration was adjusted to 600 mM with a 1 M sucrose solution and centrifuged at 28,000g for 30 minutes. The supernatant was diluted 1.5-fold with basal buffer and centrifuged at 43,600g for 30 minutes. The resulting pellet was resuspended in basal buffer and incubated overnight at 4°C.

Calcium Antagonist Binding

Di hydropyridine (DHP) binding using [³H]nitrendipine was assayed as described.11 Membranes, diluted with 50 mM HEPES, pH 7.4, to a concentration of 100-150 μg protein/ml assay, were incubated with 0.1-0.3 nM [³H]nitrendipine and varying concentrations of cold nitrendipine. Assays were terminated after 1 hour at room temperature (22-24°C) by filtration over glass fiber filters (Schleicher and Schuell, Keene, New Hampshire) using a Brandel cell harvester (Brandel, Gaithersburg, Maryland). Filters were washed with three 4 ml aliquots of ice-cold 50 mM NaCl. Filters were placed in 4 ml of Formula 963 liquid scintillation cocktail (NEN-DuPont, Boston, Massachusetts), and trapped radioactivity was counted in a Beckman LS8000 scintillation counter (Beckman Instruments, Fullerton, California) at an efficiency of 55%. Specific binding was defined as total binding less binding in the presence of 1 μM nitrendipine. Saturation plots were analyzed with the LIGAND computer program.20 Protein was measured with the BCA assay (Pierce, Rockford, Illinois) with BSA fraction V (Sigma Chemical, St. Louis, Missouri) as protein standard.

⁴Ca²⁺ Flux Measurements

⁴Ca²⁺ uptake through N-type Ca²⁺ channels into hamster brain synaptosomes was measured as described.21 Measurement of Na⁺-Ca²⁺ exchange was essentially as described.19 Briefly, Na⁺-loaded synaptosomes and cardiac membrane vesicles were briefly preincubated at 30°C; 25 μl aliquots were injected into 975 μl of basal buffer or Na⁺-free buffer (160 mM choline chloride recrystallized from ethanol, 20 mM Tris HCl, pH 7.4) and incubated for 1 second at 30°C. Reactions also contained appropriate concentrations of ⁴CaCl₂. Reactions were terminated by the addition of 3 ml ice-cold stop buffer (160 mM choline chloride, 1 mM CaCl₂, 20 mM Tris HCl, pH 7.7, 1.8 mM LaCl₃) mixed with the use of an electronic metronome. Vesicles were collected on glass fiber filters, as above, using a Brandel cell harvester and rinsed twice with 4 ml ice-cold stop buffer without LaCl₃. Retained radioactivity was determined as above. Na⁺-Ca²⁺ exchange was defined as the difference between uptake in Na⁺-free and basal buffers. Data were analyzed by Lineweaver-Burke transformation and simple linear regression.

Materials

[³H]Nitrendipine (specific activity 78 Ci/mmol) and ⁴CaCl₂ were obtained from NEN-DuPont. Nitrendipine was obtained from sources described previously.11 Choline chloride was obtained from Calbiochem, La Jolla, California; choline used for Na⁺-Ca²⁺ exchange incubation buffers was recrystallized from ethanol and stored under high vacuum until use. All other chemicals were reagent grade from commercial sources.

Results

Gross Pathological Findings

Animal weight, body length, heart weight, and brain weight increased progressively with age.
Between 10 and 30 days of age, there were no significant differences in these values between control and CM hamsters at each age (data not shown). At 180 days, control hamsters had significantly greater body weight (141±1 g vs. 114±2 g) and greater body length (18.8±0.8 cm vs. 15.9±0.1 cm). By contrast, heart weight at 180 days of age was greater body length (18.8±0.8 cm vs. 15.9±0.1 cm). This pattern of greater body weight and length, near equal brain weight, but lower heart weight in controls, compared with CM animals was also seen at 240 and 360 days (data not shown). Left ventricular cavity size was not significantly different between the younger control and CM hamsters (e.g., 30 day mean cavity diameter was 1.9±0.1 mm in the controls and 2.1±0.2 mm in the CM hamsters). At 180 days, mean cavity diameter was 2.9±0.1 mm in controls and 3.8±0.2 mm in CM animals (p<0.05). Thereafter, the CM hamsters exhibited progressively greater cavity dilatation.

**Histological Findings**

Cardiac myocyte hypertrophy was apparent in CM hamsters as early as 10 days, the youngest age we examined (Table 1). This is striking because it is generally felt that the animals are free of disease at this early age and by gross measurement there is no significant increase in heart mass.1-3 As in previous studies, we did not identify any other histological abnormalities at this age. CM hamster myocytes were significantly larger at all other ages, but grosser indexes of hypertrophy, such as the ratios of heart weight to body weight and heart weight to brain weight, did not show significant differences between the control and CM hamsters until 180 days of age. Although the ratio of heart weight to body weight is more often used as an index of gross hypertrophy, the ratio of heart weight to brain weight may be preferable, because the CM hamsters have slower increases in their body weight than controls, which alters the usual relation between heart and body weight.

Heart lesions were not usually seen until the animals were 30 days of age, but a few animals showed small myocytolytic necrotic lesions at 28 days (Table 1B). Although some CM animals at 30 days showed myocytolysis without cellular infiltration, these lesions comprised less than 0.1% of the myocardium and thus are not shown separately in the table. The typical lesion at 30 days showed necrosis including the presence of contraction bands and a dense cellular infiltrate composed of cells resembling histiocytes, lymphocytes, and monocytes (Figure 1A, Table 1B). There was evidence of early healing in some lesions with fibroblasts and collagen deposition. A large proportion of the cellular and mixed lesions showed at least some calcification on the Von Kossa stained sections (Figure 1B). Aside from a small dip in the amount of calcium deposition between 180 and 240 days, which likely was due to aberrant sampling, the amount of calcium deposition became more prevalent with time (Table 1B). Widespread fibrosis with no exclusivity cellular lesions was evident at 180 days and more pronounced at 240 and 360 days. These quantitative morphometric findings are in agreement with earlier qualitative descriptions of the histological features of the cardiomyopathy.4-6

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**Table 1. Morphologic Features of Cardiomyopathic Hearts**

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Heart wt/body wt (×10⁻³)</th>
<th>Heart wt/brain wt</th>
<th>Maximum cell diameter (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FIB 14.6</td>
<td>FIB 14.6</td>
<td>FIB 14.6</td>
</tr>
<tr>
<td>10</td>
<td>4.6±1.8</td>
<td>4.6±0.1</td>
<td>0.07±0.03</td>
</tr>
<tr>
<td>15</td>
<td>4.9±0.1</td>
<td>4.9±0.1</td>
<td>0.14±0.02</td>
</tr>
<tr>
<td>30</td>
<td>4.1±0.1</td>
<td>4.1±0.1</td>
<td>0.24±0.02</td>
</tr>
<tr>
<td>180</td>
<td>3.9±0.1</td>
<td>5.4±0.2*</td>
<td>0.52±0.02</td>
</tr>
<tr>
<td>240</td>
<td>3.8±0.2</td>
<td>6.2±0.6*</td>
<td>0.56±0.01</td>
</tr>
<tr>
<td>360</td>
<td>4.1±0.1</td>
<td>7.4±1.5*</td>
<td>0.48±0.02</td>
</tr>
</tbody>
</table>

Results are expressed as mean±SEM. Four to 11 animals for each group were studied except that the volume fraction of lesions was measured in only one 360-day-old CM (BIO 14.6) hamster. Comparisons between cardiomyopathic hearts and control (FIB) hamsters were made with Student's t tests.

¹p<0.05.
²p<0.001.
Wagner et al. Ca²⁺ Antagonist Receptors and Na⁺-Ca²⁺ Exchange in Hamster Cardiomyopathy

Focal myocytolysis was seen in skeletal muscle as early as 25 days (Figure 2). In general, the progression of skeletal muscle lesions paralleled those of the heart but was somewhat accelerated. No brain lesions were apparent.

Ontogeny of Altered Calcium Antagonist Receptor Binding Sites in Cardiomyopathic Hamsters

In our initial studies, we showed increases in numbers of dihydropyridine calcium antagonist receptors as well as phenylalkylamine calcium antagonist receptors at 30, 60, and 90 days in several CM hamster tissues. An important concern is whether alterations in receptor binding sites are secondary to cardiac pathology. Accordingly, in the present study we have examined [³H]nitrendipine binding to dihydropyridine receptors at ages ranging from 10 to 360 days (Table 2; Figures 3 and 4). At 10 days, the earliest time examined, [³H]nitrendipine binding was enhanced about twofold in membranes of heart and brain and almost 50% in skeletal muscle. Statistically significant increases continued to be apparent for [³H]nitrendipine binding to 180 days in heart, 90 days in skeletal muscle, and 30 days in brain. To ascertain whether alterations in [³H] nitrendipine binding derived from abnormalities in the affinity of the ligand for binding sites or in the numbers of binding sites, we conducted saturation analysis monitoring binding of different concentrations of the [³H]nitrendipine. The [³H]nitrendipine increases in binding can be attributed solely to augmented Bₘₐₓ values with no change in Kₘ levels in the three tissues (Figures 3 and 4; data for skeletal muscle not depicted).

Na⁺-Ca²⁺ Exchange in Heart and Brain of Cardiomyopathic Hamsters

Previously we reported augmented ⁴⁰Ca²⁺ accumulation in nerve ending preparations (synaptosomes) of CM hamster brain. Calcium accumulation can be mediated either by voltage-dependent calcium channels or by Na⁺-Ca²⁺ exchange. Recently, we and others have observed...
that, when substantial levels of sodium are included in the incubation buffer, synaptosomal \(^{45}\)Ca\(^{2+}\) accumulation is attributable primarily to Na\(^+\)-Ca\(^{2+}\) exchange rather than to voltage-dependent calcium channels. Since sodium was included in incubation buffers employed for our studies of synaptosomal calcium uptake in CM hamsters, the resultant calcium accumulation presumably involved Na\(^+\)-Ca\(^{2+}\) exchange. To examine this question directly, we compared synaptosomal \(^{45}\)Ca\(^{2+}\) accumulation with sodium in the buffer as in earlier experiments and with choline in the buffer under conditions in which we have shown that calcium uptake involves only N-type voltage-dependent calcium channels. As observed previously, when sodium was included in the incubation buffer, synaptosomes from CM hamsters displayed augmented \(^{45}\)Ca\(^{2+}\) accumulation (control = 1.22±0.23 and CM = 1.78±0.19 nmol/mg; \(p<0.05\)). Nitrendipine (1 µM) did not alter control or CM \(^{45}\)Ca\(^{2+}\) accumulation in the presence of sodium. However, with choline in the buffer, no abnormality in \(^{45}\)Ca\(^{2+}\) uptake was detected (control = 3.60±0.41 and CM = 3.73±0.62 nmol/mg). Therefore, the increase in Ca\(^{2+}\) uptake by synaptosomes is not mediated through n-type Ca\(^{2+}\) channels.

To directly examine Na\(^+\)-Ca\(^{2+}\) exchange, we measured \(^{45}\)Ca\(^{2+}\) uptake into membrane vesicles prepared from brain and heart (Figures 5 and 6). Vesicles were loaded for 16 hours with 160 mM NaCl and \(^{45}\)Ca\(^{2+}\) uptake then monitored in a 1-second incubation in buffers containing either 160 mM sodium or 160 mM choline. In the presence of choline, the large inside-outside gradient for sodium will result in sodium efflux associated with \(^{45}\)Ca\(^{2+}\) influx via the Na\(^+\)-Ca\(^{2+}\) exchange mechanism whereas the Na\(^+\)-Ca\(^{2+}\) exchange mechanism should not be operative with identical concentrations of sodium on the inside and outside of the vesicles. Accordingly, Na\(^+\)-Ca\(^{2+}\) exchange can be monitored as the difference between \(^{45}\)Ca\(^{2+}\) uptake in buffers containing choline in the medium minus uptake in buffers containing sodium in the medium. In these experiments, we evaluated Na\(^+\)-Ca\(^{2+}\) exchange in the presence of different concentrations of calcium to ascertain whether any alterations would be determined by changes in affinity (\(K_m\)) or maximal velocity of uptake (\(V_{max}\)).

Na\(^+\)-Ca\(^{2+}\) exchange was increased in both brain and heart of CM hamsters. However, unlike calcium antagonist receptor binding, which was most elevated in young hamsters, no changes were evident in Na\(^+\)-Ca\(^{2+}\) exchange in 10- and 15-day-old hamsters, while \(V_{max}\) (nmoles per milligram protein ±SEM) was doubled at 30 days in CM brain (control 25±3; CM 53±6) and increased fivefold at 30 days in CM heart (control 22±3; CM 110±15). At 360 days...
TABLE 2. [3H]NITRENIDIPINE BINDING IN THE HEART, BRAIN, AND SKELETAL MUSCLE OF CARDIOMYOPATHIC HAMSTERS

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Bmax (fmol/mg protein)</th>
<th>FIB</th>
<th>CM 14.6</th>
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<tbody>
<tr>
<td>Heart</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>120±35</td>
<td>234±38*</td>
<td></td>
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<tr>
<td>15</td>
<td>140±35</td>
<td>410±120</td>
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<tr>
<td>30</td>
<td>210±22</td>
<td>370±37</td>
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<tr>
<td>180</td>
<td>220±32</td>
<td>320±28*</td>
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<tr>
<td>360</td>
<td>200±25</td>
<td>260±35</td>
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</tr>
<tr>
<td>Brain</td>
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<tr>
<td>10</td>
<td>150±35</td>
<td>310±61*</td>
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<td>15</td>
<td>134±32</td>
<td>250±40*</td>
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<td>110±10</td>
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<td>90</td>
<td>120±22</td>
<td>150±17</td>
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<tr>
<td>180</td>
<td>125±15</td>
<td>126±11</td>
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<tr>
<td>240</td>
<td>138±21</td>
<td>130±29</td>
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<tr>
<td>360</td>
<td>135±27</td>
<td>147±32</td>
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<tr>
<td>Skeletal Muscle</td>
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<tr>
<td>10</td>
<td>1,210±100</td>
<td>1,780±190*</td>
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<td>1,260±80</td>
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<td>1,410±140</td>
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<td></td>
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<tr>
<td>90</td>
<td>1,350±180</td>
<td>1,870±190*</td>
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<tr>
<td>180</td>
<td>1,430±210</td>
<td>1,880±210</td>
<td></td>
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<tr>
<td>240</td>
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<tr>
<td>360</td>
<td>1,490±200</td>
<td>1,620±230</td>
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</table>

Results are expressed as mean±SD for groups of six to 10 animals of each strain for each age. Kd values did not differ significantly between groups and were similar to previously reported values. In brain, Kd values ranged from 0.22 to 0.42 nM. In heart, Kd values varied from 0.15 to 0.33 nM. In skeletal muscle, Kd values ranged from 2.9 to 4.3 nM. There was a small, but not statistically significant, tendency for Kd values to increase in all three tissues in older animals (180-360 days). Comparisons between CM (BIO 14.6) and control (FIB) hamsters were made with Student's t tests.

*p<0.05.

Discussion

In the present study we have confirmed and extended our previous observations that calcium antagonist receptor binding levels are augmented in tissues of CM hamsters. Using the same BIO 14.6 strain of CM hamsters, Finkel et al12-13 and Kobayashi et al14 have confirmed the increase in cardiac [3H]nitrendipine binding. Some investigators have been unable to demonstrate this increase in dihydropyridine binding sites in related cardiomyopathic strains. Howlett and Gordon26 did not find an increase in the number of dihydropyridine binding sites in cardiac muscle homogenates from 50- to 60-day-old CHF 146 CM hamsters compared with matched controls (CHF 148). However, this CM strain is not as well characterized as the BIO 14.6 strain, and it is possible that the progression of disease differs between the two strains. Because we found that the presence and extent of the abnormality in dihydropyridine binding sites changes as the disease progresses, differences in disease progression between the two strains may explain the differences in our results. In another study, no increase in [3H]PN 200-110 binding was observed in crude heart homogenates from 35- to 41-day-old 53.58 CM hamsters.27 However, when Kuo et al15 examined

FIGURE 3. Saturation analysis of [3H]nitrendipine binding in heart of 10- (A), 30- (B), and 360-day-old (C) control (FIB) and cardiomyopathic (CM) (BIO 14.6) hamsters. The concentration of nitrendipine varied between 0.05 and 100 nM. Data shown are from typical experiments performed in duplicate. Experiments were repeated six to 10 times. The total number of binding sites (Bmax) is increased in CM hamsters, with no change in binding affinity.
FIGURE 4. Saturation analysis of \(^{3}H\)nitrendipine binding in brain of 10- (A), 30- (B), and 360-day-old (C) control (F1B) and cardiomyopathic (CM) (BIO 14.6) hamsters using the same techniques as described for heart (Figure 3). The total number of binding sites \((B_{\text{max}})\) is significantly increased in CM hamsters at 10 and 30 days of age but similar to controls at 360 days. As with heart tissue the increase in \(B_{\text{max}}\) is seen with no change in binding affinity.

\[^{3}H\]PN 200-110 binding to a purified cardiac sarcolemmal preparation from 2-month-old hamsters of the same 53.58 strain, they found an augmentation in calcium antagonist receptor number compared with matched controls. In addition, in another study, Kuo et al.\(^{16}\) observed an increase in dihydropyridine calcium antagonist receptors in cardiac sarcolemma from 30-day-old BIO 14.6 CM hamsters using the reversible ligand \(^{3}H\)PN 200-110 as well as photoaffinity techniques with the dihydropyridine \(^{3}H\)azidonopine. The two studies that failed to demonstrate an increase in calcium antagonist receptor sites used crude muscle homogenates rather than purified membrane preparations employed in studies showing an increase in dihydropyridine binding. Other tissue components in the homogenates might have masked increases in dihydropyridine binding.

One striking finding of the present study was that the augmented number of calcium antagonist binding sites was most apparent in younger hamsters and was no longer detectable in older animals with more extensive cardiac hypertrophy and congestive heart failure. The fact that the largest increases occur in the very young hamsters before the development of necrotic lesions or heart failure suggests...
FIGURE 6. Lineweaver-Burke plots of heart Na\(^{+}\)-Ca\(^{2+}\) exchange in 10- (A), 30- (B), and 360-day-old (C) control and cardiomyopathic (CM) hamsters. Typical plots are depicted of experiments repeated on seven or eight occasions.

that the abnormalities are not secondary to cardiac pathology. Moreover, the increased numbers of receptors in brain and skeletal muscle support this conclusion.

We found evidence of myocardial cell hypertrophy in CM animals at the same early age that the increase in calcium antagonist receptors was identified. Other investigators have suggested that myocardial hypertrophy is a secondary phenomenon in the CM hamster, serving as a compensatory response to myocardial tissue injury and resultant loss of ventricular function.\(^{28}\) However, in the present study, myocyte hypertrophy was present at a time before either structural or functional impairment had been documented.\(^{22-24}\) The discrepancy between the quantitative morphometric data indicating an early increase in cardiac myocyte diameter and the grosser estimates of cardiac hypertrophy (heart weight-to-body weight and heart weight-to-brain weight ratios) is probably seen because these latter techniques have less sensitivity in detecting modest degrees of hypertrophy, especially in the extremely small hearts of the younger hamsters. For example, the approximate 10% greater cell diameter seen in the CM animals 30 days old or younger would account for an approximate 20% increase in cell mass, using a cylindrical model for myocytes. This difference was detected by several thousand cell diameter determinations in each group of animals. Assuming \(a < 0.05\) and \(\beta < 0.2\), we would have had to weigh about 100 hearts in each group to be certain that we could detect the same degree of hypertrophy, given the variance present in our heart weight determinations. Nevertheless, we cannot exclude the possibility that the CM hamsters have fewer, but larger, left ventricular myocytes than the control hamsters, accounting for the increased cell mass with little or no change in overall heart mass.

In the present study, we provide evidence that the increased synaptosomal accumulation of calcium in brains of CM hamsters is attributable to augmented Na\(^{+}\)-Ca\(^{2+}\) exchange rather than to an abnormality in voltage-dependent calcium channels in nerve terminals. The calcium antagonist receptor binding sites we have monitored are associated with voltage-dependent calcium channels that mediate calcium influx. At least three subtypes of voltage-dependent, calcium channels have been differentiated,\(^{29,30}\) and the calcium antagonist receptors are only associated with one of these, the L subtype.

N-type calcium channel activity is readily observed in buffers containing choline in place of sodium.\(^{21}\) L-type calcium channel activity is not reliably detected in synaptosomal preparations. We monitored voltage-dependent \(^{4}\)Ca\(^{2+}\) accumulation into brain synaptosomes mediated by the N-type calcium channel and detected no abnormality in CM hamsters. Accordingly, it appears that the genetically determined defect in CM hamsters is restricted to the L-type calcium channel.

The increased Na\(^{+}\)-Ca\(^{2+}\) exchange occurs subsequent to the alteration in calcium antagonist receptors and so may reflect a secondary response to increased calcium influx following the increased numbers of L-type voltage-dependent channels. The notion that the increased Na\(^{+}\)-Ca\(^{2+}\) exchange is a secondary compensatory phenomenon may also explain why more pronounced increases in Na\(^{+}\)-Ca\(^{2+}\) exchange are seen in heart, which displays pathological alterations, than in the brain, where no alterations are apparent. Increases in the Na\(^{+}\)-Ca\(^{2+}\) exchange system, which physiologically extrudes Ca\(^{2+}\) from cells, may occur in response to the intracellular Ca\(^{2+}\) overload characteristic of CM hearts.

In summary, the present study supports conclusions from our earlier work\(^{11}\) suggesting that abnormalities in calcium antagonist receptors associated with voltage-dependent calcium channels play a
major role in the pathophysiology of CM hamsters. The CM hamsters may provide a model for human hypertrophic cardiomyopathy, which shares some pathophysiological features as well as the selective therapeutic response to calcium antagonist drugs. Supporting this possibility, Ferry and Kaumann \(^{31}\) as well as ourselves \(^{32}\) have observed increased calcium antagonist receptor binding in cardiac tissue from these patients comparable in degree to the alterations in CM hamsters.

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