Verapamil Preserves Myocardial Contractility in the Hereditary Cardiomyopathy of the Syrian Hamster


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SUMMARY. We attempted to alter the inherited myocardial damage and loss of contractility of the cardiomyopathic Syrian hamster (strain U-MX7-1) by giving cardiac drugs that altered intracellular calcium and myocardial workload. Thirty-seven 21-day-old cardiomyopathic and thirty-seven 21-day-old normal hamsters were divided into five groups each: verapamil-, propranolol-, digoxin-, hydralazine-, and saline-injected. On their 90th day of life, the hamsters were killed. Of the five cardiomyopathic groups, only verapamil reduced myocardial damage. When both “control” and cardiomyopathic hamsters were treated with saline, digoxin, or propranolol, the cardiomyopathic hamsters had significantly less contractile force, maximal rate of force development, and maximum velocity of unloaded shortening. When both groups were treated with verapamil or hydralazine, there were no significant group differences in the indices of contractility. However, when saline-treated cardiomyopathic hamsters were compared with drug-treated cardiomyopathic hamsters, only verapamil preserved myocardial contractility. There was also a weak correlation between the $V_{\text{max}}$ and the actin-activated ATPase activity of the cardiomyopathic hamsters ($r = 0.63, P < 0.001$). We conclude that verapamil helped protect the myocardium of genetically cardiomyopathic hamsters against structural damage, and helped preserve myocardial contractility (Circ Res 50:405-412, 1982)

As cardiac muscle fails, its contractility decreases (Spann et al., 1967). The reason for this loss of contractility is not clear, although several associated biochemical abnormalities have been identified. Of these, three may be important. First, sarcoplasmic reticular function is abnormal, leading to a slower calcium uptake and release (Harigaya and Schwartz, 1969), and to an abnormal intracellular calcium distribution (Ito and Chidsey, 1971). As a result, less calcium is available for myofibrillar activation, relaxation is impaired (Dhalla, 1975), and mitochondrial function is impaired (Peng et al., 1977). Second, because mitochondrial function is impaired and because of an increased energy demand on the remaining cells, the concentration of high energy phosphates falls (Pool et al., 1967). Although the availability of ATP is thought to be adequate for mechanical function, a regional lack of energy for cellular reconstruction cannot be ruled out (Katz, 1975). Finally, associated with the decrease in contractility there is a decrease in myofibrillar (Chandler et al., 1967) and actin-activated myosin ATPase activity (Wikman-Coffelt et al., 1979).

It has been hypothesized that one factor that contributes to the pathogenesis of some forms of heart failure is calcium overload. The cardiomyopathic hamster has a defect in calcium handling that leads to intracellular calcium overload (Lossnitzer, 1975; Ma and Bailey, 1979). The necrotic lesions peak at about the same time as the calcium overload and disappear by the 100th day of the hamster's life (Lossnitzer, 1975). As many as 25% of the cardiac cells die, leaving the remaining 75% to do the work (Jasmin and Bajusz, 1974). The remaining myocardium hypertrophies and eventually fails (Forman et al., 1972). The reflex increase in sympathetic tone and myocardial norepinephrine production may only serve to worsen both the calcium overload and necrosis (Lossnitzer, 1975). The failing myocardium of the cardiomyopathic hamster resembles that of the failing human myocardium in its biochemical and mechanical properties (Gertz et al., 1970; Forman et al., 1972; Lossnitzer, 1975; Pang and Weglicki, 1980).

In this study, by giving four different cardiac drugs, we attempted to alter the degree of structural damage and the decrease in contractility which typifies the failing heart of the cardiomyopathic Syrian hamster. In line with the calcium overload hypothesis, verapamil and propranolol were given to decrease intracellular calcium and to decrease oxygen consumption (Shand, 1975; Singh et al., 1978). Hydralazine was given in an attempt to decrease afterload (Ablad, 1963; Chatterjee, et al., 1976), and digoxin was given, presumably to increase intracellular calcium (Smith and Haber, 1973).

Methods

Drug Protocol

Twenty-one-day-old cardiomyopathic Syrian hamsters (n = 37) of strain U-MX7-1 were chosen for the study (Jasmin and Bajusz, 1974). The hamsters were divided into four groups of seven and one group of nine, with one littermate and the same number of females in each group.
Muscle Mechanics

The hamsters were killed by a guillotine, the heart removed, and the left ventricle cut open. The tendinous end of the best papillary muscle was tied with a piece of 7-0 Deknatel suture, excised, and mounted in an isolated muscle bath. The base of the muscle was held in a Lucite clamp and the tendinous end was tied to a lever with an electromagnetic feedback system to allow control of force, length, and velocity (Brutsaert et al., 1973). The muscle was bathed in Krebs-Henseleit solution containing (in mM) (NaCl, 118.5; KCl, 4.74; MgSO₄, 2.43; KH₂PO₄, 1.18; NaHCO₃, 24.9; CaCl₂, 2 H₂O, 2.54, and dextrose, 5.0), which was kept at 37°C and a pH of 7.4, and was gassed with 95% oxygen and 5% CO₂. The maximum velocity of shortening at L_max length. The average cross-sectional area of muscle was calculated by dividing the muscle weight by its length, assuming a general cylindrical shape and a specific gravity of 1.0.

To assess contractility, the maximal force and dF/dt were measured at the peak of the force-frequency curve at three stimuli per minute (Forman et al., 1972). After adjusting the elastic damping of the force-length velocity lever feedback system to compensate for the electromechanical transients, the maximum velocity of unloaded muscle shortening was obtained at six stimuli per minute, by abruptly decreasing the load of the muscle at the time of activation. The lever system is identical to that described in detail by Brutsaert et al. (1973). This measurement was used as a close approximation of V_max and is more reproducible than an "extrapolated V_max" from a series of isotonic afterloaded contractions. The equivalent mass of the lever and coil was 190 mg, the static compliance was 3.5 μm, and the dynamic compliance was 0.5 μm/sec.

Histological Protocol

In an attempt to better quantify the degree of protection afforded to the left ventricular myocardium against spontaneously occurring structural damage, we developed a morphometric counting system, building on the semiquantitative approach used earlier (Bajusz, 1969). The strength of the morphometric approach, i.e., its surprising reproducibility from relatively small numbers of animals, comes from the randomization of sampling and counting (Weibel, 1969).

As each hamster was killed, a sample approximately 0.5 cm × 0.5 cm in size was excised from the entire thickness of the left ventricular myocardium, midway along the ventricular free wall, and fixed in 6% glutaraldehyde buffered to pH 7.2 with 0.1 M sodium cacodylate. It was subsequently dehydrated in ethanol and embedded with random orientation in glycol methacrylate (GMA) plastic. The sample was cut into 4 μm-thick sections on three levels, each separated from the next by at least 100 μm. The sections were staged with lead hematoxylin (Solcia et al., 1969). Each level was analyzed separately and the results pooled for each hamster.

As controls, 37 normal golden hamsters aged 21 days were separated into five groups and received the same drug regimen as the cardiomyopathic Syrian hamsters.

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Biochemical Protocol

Actin-activated myosin ATPase activity was measured because of its known association with V_max, an index of muscle contractility (Barany, 1967). The remaining portion of the hamster hearts were immediately frozen (−80°C) after excision from the hamsters. Myosin was purified from the frozen hamster hearts using a high acceleration-deceleration centrifuge (Wikman-Coiffet and Coiffet, 1981). Briefly, the frozen heart was minced in 10 volumes (wt/vol) of buffer #1, which contained (in mM 1.0 MgCl₂, 0.1 EGTA,
left ventricular thrombi, and one also had left atrial
mean preloads were the same for all 10 groups. Four
weight ratio, or in the myocardial wet-to-dry weight
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normal hamsters by an unpaired t-test.
Neumann-Keuls test. The variables of the cardiomyopathic
tological, and biochemical characteristics were compared
using a Student-Newmann-Keuls test. The mechanical, his-
Statistics
The hamster and myocardial characteristics for all 10
groups were compared by a one-way analysis of variance
using a Student-Newmann-Keuls test. The mechanical, hist-
ological, and biochemical characteristics were compared
separately in cardiomyopathic hamsters and in the normal
hamsters by one-way analysis of variance using the Student-
Newmann-Keuls test. The variables of the cardiomyopathic
hamsters were compared to those of the corresponding
normal hamsters by an unpaired t-test.

Results
Hamster and Myocardial Characteristics
The mean weight of the propranolol-injected car-
diomyopathic hamsters was lower than that of all
other groups (Table 1). There was no difference for
any group in the heart weight, the heart weight:body
weight ratio, or in the myocardial wet-to-dry weight
ratio. The muscle length and cross-section and the
mean preloads were the same for all 10 groups. Four
of the digoxin-injected cardiomyopathic hamsters had
left ventricular thrombi, and one also had left atrial
thrombi and a pericardial effusion. Only one saline-
injected, and none of the other cardiomyopathic ham-
sters had left ventricular thrombi.

Muscle Mechanics
The papillary muscle force and dF/dt were greater
in cardiomyopathic hamsters given verapamil than in
cardiomyopathic hamsters given any other drug (Fig.
1; Table 2). Force, but not dF/dt, was greater after
hydralazine than after digoxin. In the normal ham-
sters, the force and dF/dt of the different groups was
the same. After verapamil and hydralazine, but not
after the other drugs, force and dF/dt were similar to
those in normal hamsters given the same drugs (Fig.
1).
The mean Vmax was greater in cardiomyopathic
hamsters given verapamil than in cardiomyopathic
hamsters given any other drugs except hydralazine
(Fig. 2, Table 2). The Vmax after hydralazine was
greater than after digoxin. In the normal hamsters, the
Vmax of the different groups was the same. After
verapamil and hydralazine, but not after the other
drugs, Vmax was similar to those in hamsters given the
same drugs (Fig. 2).

Histology
Qualitative Features
The histological characteristics of the sponta-
eneously occurring myocardial lesions could be catego-
ized into muscle damage, healing, and calcification
(Fig. 3). Features of damage to myocytes included:
clear areas ("halos") around nuclei, loss of cellular
stainability usually accompanied by localized myoфи-
bril breakdown, hypercontraction and disintegration
of individual myocytes. Features of healing included
infiltration of damaged areas and coating of the fascia
of blood vessels by nonmuscle cells. Calcification
usually occurred superimposed on the zones of heal-
ning calcified lesions, but occasionally smaller calcified
areas were present at foci of myocyte breakdown.
The effect of each drug tested was to accent or
suppress some of these features in a fairly character-
istic fashion. Myocardium from the cardiomyopathic
hamsters given digoxin was the least unique, but was
characterized by a high frequency of nuclear "halos"
and stages of myocyte damage short of breakdown.
Propranolol-treated cardiomyopathic myocardium
characteristically displayed myocyte breakdown in
some areas, but somewhat fewer areas of calcification.
Hydralazine-treated cardiomyopathic myocardium
displayed generalized infiltration by fibroblastic cells.
Verapamil-treated cardiomyopathic myocardium was
unique in displaying large areas of lighter staining
myocytes which appeared otherwise undamaged, and
by an obvious lack of calcification (Fig. 4).

Quantitative Features
Morphometric derivation of the percentage of un-
damaged (microscopically normal) myocardium of
hamsters administered the various drugs is presented
in Table 3. Only verapamil-treated hamsters dis-
played an amount of undamaged myocardium (84.2 ± 16.7%) significantly better than the saline-treated or the other groups, means for which ranged from 66.9 to 71.5%. Thus, within the limits of the prepara-

tion and statistical techniques used, only verapamil clearly provided structural protection.

Biochemistry

The actin-activated myosin ATPase activity of the various groups were not statistically different (Table 2). There was, however, a significant correlation between the actin-activated myosin ATPase activity and the V_{max} of the cardiomyopathic hamsters (r = 0.63, P < 0.001) (Fig. 5). There was no difference between the actin-activated myosin ATPase activities for any of the drug-treated normal hamsters (Table 3), but the actin-activated myosin ATPase activity of each of the normal drug-treated groups was higher than that of their cardiomyopathic counterparts (Fig. 2).

Discussion

This study demonstrates that verapamil helps protect the myocardium of the cardiomyopathic hamsters from damage and preserves the contractility of the remaining overloaded myocardium.

Since only single dose levels of each drug were used, however, any comparison between drugs is difficult, without a knowledge of their comparative dose-response relationships. As detailed in the Methods, however, the dose was selected to be a relatively large one.

In our study, verapamil, a calcium-blocking agent (Singh et al., 1978) decreased myocardial damage and preserved the contractility of the remaining myocardium. Verapamil is thought to prevent damage in the cardiomyopathic hamster by decreasing intracellular calcium and thus preventing the toxic effects of calcium overload (Jasmin and Jajusz, 1974; Lossnitzer, 1975). Verapamil may also prevent focal damage by...
preventing coronary microvascular spasm (Factor et al., 1980). How verapamil preserves contractility is purely speculative and probably multifactorial. Mechanisms which need further investigation include decreasing inotropy and systemic vascular resistance (Singh et al., 1978), thus preserving ATP; and by decreasing calcium, which, besides decreasing the use of ATP, may prevent the calcium-mediated inhibition of mitochondrial ATP production (Peng et al., 1977).

Hydralazine, a potent vasodilator (Ablad, 1963), did not prevent myocardial damage, but may have helped preserve the contractility of the remaining muscle. In hydralazine-treated cardiomyopathic animals, there was no significant reduction in force, df/dt, or V_{max}, as compared to normal animals treated with hydralazine. These three indices of contractility, however, were not statistically better than the saline-treated cardiomyopathic animals. Thus, the effects of hydralazine on preserving contractility were equivocal. The potential mechanism for preserving contractility is uncertain, but might include reduction of microvascular spasm (Factor et al., 1980), or "vasodilator therapy" of the accompanying heart failure (Chatterjee et al., 1976; Cohn, 1978). However, since the in vivo hemodynamic effects of hydralazine could not be measured, its effects on contractility may have been mediated by mechanisms other than afterload reduction.

Propranolol, a ß-blocker (Shand, 1975), did not decrease myocardial damage or preserve contractility. A previous study (Jasmin and Bajusz, 1978) found that propranolol partially protected against damage. Why propranolol did not prevent damage or preserve contractility in our study is not clear, but a number of possibilities exist. We treated our hamsters more than twice as long as Jasmin did but used only half the daily dose. Thus, our lower dose may not have provided sufficient protection. Alternatively, the longer time interval before sacrifice may have been an adverse factor. Digoxin, a positive inotropic agent (Smith and Haber, 1973), did not protect the myocardium from damage or loss of contractility. On gross examination, the cardiomyopathic hamsters treated with digoxin had the most severe failure, with most of them having left ventricular thrombi and one having left atrial thrombi and a pericardial effusion. Although these hamsters had the lowest force, df/dt, V_{max}, and myosin ATPase, these changes were not significantly different from those of the saline-injected cardiomyopathic hamsters, because of the large variability in the severity of disease from litter to litter. Digoxin did not prevent myocardial damage and may have caused a further decrease in contractility by increasing the intracellular calcium and the work of already overloaded cells (Smith and Haber, 1973).
FIGURE 3. Saline-treated myopathic myocardium magnified ×3,150. This badly deteriorated area displays partial myofibril breakdown, total myofibril breakdown and myocytes collapse (hatched area), supercontracted myocytes (arrows), and zones of beginning calcification (arrowhead). At the bottom right, an infiltrate of nonmuscle cells is evident.

FIGURE 4. Verapamil-treated myopathic myocardium magnified ×3,150. This representative area is indistinguishable from nonmyopathic hamster myocardium, except for subtle myofibril damage seen in the hatched zone.
An interrelationship has been established between maximum rate of skeletal muscle shortening and myosin ATPase activity (Barany, 1967), cardiac muscle shortening, and myosin ATPase activity from the same species (Hamrell and Low, 1978; Carey et al., 1978), and the cardiac muscle shortening and actomyosin ATPase activity from the same animal (Alpert et al., 1974). However, this is the first study correlating maximum rate of cardiac muscle shortening with actin-activated myosin ATPase activity from the same animals. However, despite maintaining a normal \( V_{\text{max}} \), the hydralazine and verapamil cardiomyopathic groups had lower actin-activated myosin ATPase activities than did their normal counterparts. This could be the result of a genetically predetermined myosin isozyme (Affara et al., 1980), or the result of in vivo factors not adequately considered in situ. Some of these factors are: magnesium (Best et al., 1977), calcium (Potter and Gergely, 1975), ATP (Cooke and Bialek, 1979), pH (Wikman-Coffelt et al., 1975), temperature (Alpert, 1979), and other factors which regulate myosin in vivo. Also, calmodulin, which influences many cellular reactions via phosphorylation, may not have influenced the biochemical studies, but could have influenced the isolated muscle studies. Finally, the degree of in vivo hydrolysis shown to be present in muscle of dystrophic animals (Stracher et al., 1976), but not present in our purified proteins used for in vitro measurements, may have played an important role in the in vivo activity of myosin, and thus be reflected only in the physiological measurements.

In conclusion, verapamil reduced the degree of myocardial damage and preserved the contractility of the cardiomyopathic Syrian hamster. There was a weak correlation between the maximum velocity of shortening and the actin-activated myosin ATPase activity. Although the verapamil results are compatible with the calcium overload hypothesis of myocardial damage, further studies will be required to firmly establish this hypothesis.

![Figure 5](image-url)

**FIGURE 5.** Correlation between the maximum velocity of shortening \( (V_{\text{max}}) \) of the papillary muscles and the actin-activated myosin ATPase activity of the myocardium in the same cardiomyopathic animals. Each point represents data from a single animal \( (n = 36) \). \( r = 0.63, P < 0.001, L_{\text{max}} = \text{muscle length at the peak of the length-tension curve} \).


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Circ Res. 1982;50:405-412
doi: 10.1161/01.RES.50.3.405

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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