Cardiac excitation-contraction coupling in the portal hypertensive rat

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Zavecz, James H., Orlando Bueno, Ronald E. Maloney, James M. O'Donnell, Sandra C. Roerig, and Harold D. Battarbee. Cardiac excitation-contraction coupling in the portal hypertensive rat. Am J Physiol Gastrointest Liver Physiol 279: G28–G39, 2000.—Basal contractility and responses to β-adrenoceptor activation are compromised in hearts from rats with chronic portal vein stenosis. Here we report the effect of partial ligation of the portal vein on myocardial G protein expression, β-adrenoceptor-G protein coupling, and excitation-contraction coupling (ECC). Contractility (dT/dt) was reduced 30–50% in right and left ventricles, but the rate of relaxation (−dT/dt) was unaffected. Isoproterenol-induced positive inotropism was diminished, but there was no difference in ED50. The concentration-dependent enol-induced positive inotropism was diminished, but there was no difference in ED50. The concentration-dependent increase in −dT/dt was unaffected. Gqβ and Gqα expression, cholera toxin- and pertussis toxin-induced ADP-ribosylation, and formation of the agonist-receptor-Gs complex were unaffected by portal vein stenosis. Of the components of ECC examined, the caffeine-sensitive sarcoplasmic reticulum Ca2+ uptake and release processes were unchanged: the apparent density of L-type Ca2+ channels decreased 60% with no change in affinity; the dihydropyridine Ca2+ channel agonist BAY K 8644 produced relative changes in dT/dt that were similar in both groups, suggesting normal function in the remaining Ca2+ channels; and Na+/Ca2+ exchange was reduced 50% in the portal vein stenosis group. These data suggest that the effect of portal vein stenosis on myocardial contractility is the result of alterations to ECC.

marked hemodynamic changes occur in humans and experimental animals with cirrhotic liver disease. A hyperdynamic circulation develops that is characterized by increased cardiac output, increased splanchnic blood flow, low total systemic vascular resistance, mild tachycardia, low or normal blood pressure, an increased blood volume, portal hypertension, and reduced responsiveness to vasoconstrictors. These changes can occur whether the disorder is the result of cirrhosis (27, 32, 49), prehepatic portal obstruction (9, 19, 27, 35, 59), or portocaval shunting (18, 33, 58).

Ventricular dilatation, especially of the right heart, and hypertrophy, especially in early cirrhosis, occur in human hearts (43). In animal studies (20), the heart weight in CCl4-induced cirrhotic rats is greater than that in controls, presumably because of chronic ventricular overload. There are no cardiac histological abnormalities in these animals (20). Similarly, Ma and colleagues (45) reported no light microscopic changes in the hearts of bile duct ligation-induced cirrhotic rats. However, these observations do not duplicate what is seen in human cirrhotic patients, most likely because of the long duration of cirrhosis in patients compared with the relatively brief time the experimental animals are cirrhotic. In the portal vein stenosis model, no change in either right or left heart size occurs during the brief interval of 10–12 days postsurgery (Battarbee and Zavecz, unpublished observations), suggesting that either hypertrophy does not occur or impairment is of insufficient duration to result in measurable hypertrophy.

In addition to altered basal hemodynamics, liver disease also disrupts dynamic function. Tilt tests, lower body negative pressure, and other hypotensigenic techniques indicate that both cardiac and peripheral resistance reflex responses are impaired in hepatic disease patients (11, 12, 41, 42) and in experimental portal hypertension (6). Furthermore, cirrhotic patients (1, 8, 25, 34, 38, 46, 54), experimental models of cirrhosis (13, 36), prehepatic portal hypertensive models (6, 7), and cholestatic animals (13–15) exhibit reduced cardiac responses to exogenous and endogenous catecholamines. Studies (26, 43) both in human nonalcoholic cirrhosis and in animal models of nonalcoholic cirrhosis have demonstrated impairment of cardiac contractility in response to various stressors. This development of high-output heart failure with systemic vasodilation has been termed “cirrhotic cardiomyopathy.” Although the ventricular dysfunction coexisting with a high cardiac output secondary to reduced peripheral vascular resistance is common in cirrhotic patients (38, 43),...
the symptoms are usually latent, only appearing under conditions that stress the myocardium, including liver transplantation, surgical portosystemic shunting, transjugular intrahepatic portosystemic shunt, mental stress, physical exercise, and psychological stimulation (26, 43). Interestingly, portocaval shunting, not hepatocellular disease, is the common factor in the hepatic models used to study the cardiovascular effects of liver disease. Thus it appears that the hyperdynamic circulation results from the shunting of visceral venous blood into the systemic circulation (for an in-depth discussion of cirrhotic cardiomyopathy, see Ref. 43).

In our previous studies (7, 65) with the portal vein-stenosed rat, we dissociated hepatocellular dysfunction from the effect of portal hypertension using the chronic portal vein-stenosed rat, a model of chronic liver disease in which a hyperdynamic circulation is present without the hepatocellular damage of cirrhosis. We have observed depressed contractile function in isolated right and left ventricular tissue from chronic portal vein-stenosed rats as well as decreased isoproterenol-induced positive inotropism. β-Adrenoceptors have been shown not to be downregulated in this model, but a greater fraction of cardiac β-adrenoceptors must be occupied to produce equivalent absolute increases in $\frac{dT}{dt}$, the maximum rate of tension development (65), suggesting that the cause of the decreased response to isoproterenol is postreceptor. On the other hand, it is conceivable that the altered responsiveness to β-adrenergic receptor activation results from a mechanism not directly involving β-adrenergic signal transduction. In the present study, we have extended our research to include the effects of portal hypertension with portosystemic shunting on cardiac $G_{\alpha}$ and $G_{\text{i}}$ expression, β-adrenoceptor-$G_{\alpha}$ coupling, and excitation-contraction coupling (ECC).

METHODS

Contractile experiments. Ten to twelve days after portal vein stenosis or sham operation, each animal was decapitated and the heart was immediately excised and transferred to a preparative tissue bath containing Krebs-Henseleit solution (KH) equilibrated with 95% $O_2$-5% $CO_2$. The buffer contained (in mM) 118 NaCl, 5.8 KCl, 27.2 NaHCO$_3$, 1.0 NaH$_2$PO$_4$, 1.2 MgSO$_4$, 2.5 CaCl$_2$, and 11.1 glucose. The temperature of the buffer was maintained at 37°C and the pH at 7.4. A small strip of right ventricle was removed, and a left ventricular papillary muscle was dissected free. The remainder of the heart was immediately frozen in liquid nitrogen and stored at $-70°C$ for use in the binding experiments (see below). One end of each muscle was attached to a rigid support and then placed in an organ bath, where the other end of the muscle was attached to a Grass FT03C force-displacement transducer via a length of surgical silk. Muscles were field stimulated at 2 Hz, with a voltage 50% greater than threshold and a pulse duration of 4 ms. Resting length was set to the peak of each muscle’s length-tension curve. Isometric tension was recorded by means of the force-displacement transducer connected to a Grass model 7D recorder. $\frac{dT}{dt}$ and the maximum rate of relaxation ($-\frac{dT}{dt}$) were obtained by differentiating the output of the channel measuring isometric force. At the end of each experiment, a graticule was used to measure each muscle. Tissues were dried and their weights recorded. These measurements were used to normalize the data for differences in tissue weight and dimensions.

In the experiments in which the effect of portal vein stenosis on the response of the myocardium to β-adrenoceptor activation with isoproterenol was studied, tissues were equilibrated in KH, and when contractions stabilized, isoproterenol was added to the tissue bath. On attainment of a stable positive inotropic effect, isoproterenol was washed out of the tissue. When contractions returned to the control level, the next concentration of isoproterenol was added. Exposure to the different concentrations of isoproterenol was randomized.

In experiments examining the effect of portal vein stenosis on the relationship between contractile strength and the extracellular $Ca^{2+}$ concentration, the tissues were equilibrated with KH containing 2.5 mM $Ca^{2+}$. After force stabilized, the bathing solution was quickly changed to KH with 0.3125 mM $Ca^{2+}$. When force stabilized, which required 2–3 min, an appropriate amount of $Ca^{2+}$ was added to produce the next $Ca^{2+}$ concentration. Concentrations of $Ca^{2+} > 7.5$ mM were not investigated because of the potential for $Ca^{2+}$ precipitation with the buffer system and because the maximal response had already been achieved. The brief period of exposure to low $Ca^{2+}$ did not affect subsequent muscle performance, as indicated by the similarity of force measured at the initial 2.5 mM $Ca^{2+}$ concentration and that measured at 2.5 mM $Ca^{2+}$ during the concentration-response experiment (sham: 1.079 ± 1.17 and 1.147 ± 0.94 mN/cm$^2$, respectively; stenosed: 634 ± 130 and 660 ± 34 mN/cm$^2$, respectively, in papillary muscle).

Experiments with the dihydropyridine L-type $Ca^{2+}$ channel activator BAY K 8644 were performed to assess further $Ca^{2+}$ channel functionality. After contractions stabilized, BAY K 8644 ($10^{-8}$–3 × 10$^{-6}$ M) was added cumulatively. Succeeding concentrations were added when the response to the previous concentration stabilized.

Experiments in permeabilized muscle fibers. To determine the effect of portal vein stenosis on the $Ca^{2+}$ sensitivity of the myofilaments and $Ca^{2+}$ uptake and release from the sarcoplasmic reticulum (SR), left ventricular papillary muscles were chemically skinned with either saponin or Triton X-100 to permeabilize both the sarcolemma and the SR membrane, respectively. In this way, the “intracellular” cytosolic concentration of $Ca^{2+}$ can be controlled and manipulated. The solutions used in these experiments were prepared according to a computer program that takes into account the binding constants of all of the constituents (51). Papillary muscles were removed from left ventricles, and 100- to 150-μm-diameter fiber bundles, 1–2 mm in length, were dissected free in relaxing solution containing 5 mM MgATP, 1 mM Mg$^{2+}$, 5 mM EGTA, 20 mM imidazole, 15 mM creatine phosphate, and potassium methanesulfonate to yield an ionic strength of 200 mM with pCa > 8.5 adjusted to pH 7.0. One end of each bundle was tied with a human hair to a force transducer (Kent Scientific model TRN001) and the other to a support that positioned the fibers horizontally in a 3-ml tissue bath. The fibers were stretched to a point at which passive tension was just measurable (−0.05 mN). The output of the transducer was digitized and stored on disk for subsequent analysis. All experiments were performed at room temperature. To assess the effect of portal vein stenosis on the sensitivity of the myofilaments to $Ca^{2+}$, the fiber bundles were permeabilized by superfusion (2 ml/min) with oxygenated relaxing solution containing 0.5% Triton X-100 for 1 h. This treatment permeabilizes both the sarcoplasmic reticular and sarcosomal membranes (24, 66). Fibers were superfused with relaxing solution for an additional 60 min before exposure to $Ca^{2+}$.
In the experiments that examined the effect of portal vein stenosis on Ca\(^{2+}\) uptake and release from the SR, fibers were chemically permabilized by superfusion with 50 µg/ml saponin for 30 min to permeabilize the sarcolemma only (24, 66). Saponin was washed out of the tissues, which were then superfused for an additional 30 min in relaxing solution before the start of the experiment.

Myofilament Ca\(^{2+}\) sensitivity was measured by superfusing with appropriate incubation vessels. [3H]isradipine bound was determined by the addition of 1 µM nitrendipine to appropriate incubation vessels. [3H]isradipine bound was determined by filtration (65). Each assay was performed in duplicate. Receptor density and the apparent equilibrium dissociation constant \(K_d\) for [3H]isradipine were determined using nonlinear regression (22).

Binding assays were performed at 37°C for 30 min in a total volume of 200 µl containing 50 mM Tris-HCl (pH 7.4), 100 µg/m antigen, 1 mM CaCl\(_2\), and 30–600 pM [3H]isradipine (85.8 Ci/mmol; DuPont NEN, Boston, MA). Nonspecific binding, which amounted to 10–20% of total binding, was determined by the addition of 1 µM nitrendipine to appropriate incubation vessels. [3H]isradipine bound was determined by filtration (65). Each assay was performed in duplicate. Receptor density and the apparent equilibrium dissociation constant \(K_d\) for [3H]isradipine were determined using nonlinear regression (22).

β-Adrenoceptor-G protein coupling. When \(G\) binds to the agonist-β-adrenoreceptor complex, forming an agonist-β-adrenoceptor-G protein complex, the agonist is bound with higher affinity than when it is bound to the receptor only. The fraction of β-adrenoceptors in this “high-affinity” state is indicative of the coupling of the agonist-receptor complex with \(G\). The fraction of β-adrenoceptors in the high-affinity state and the apparent dissociation constant of an agonist for the high-affinity state were determined as described previously (52, 53).

Competition binding experiments were performed using cardiac membranes prepared as previously described (65). Left ventricles were minced finely and homogenized with a Polytron PT10 homogenizer (twice at half-speed for 5 s) in 35 ml of ice-cold buffer containing 5 mM Tris-HCl (pH 7.4), 1 mM MgCl\(_2\), 0.25 M sucrose, 1 mM EDTA, and 1 mM diethiothreitol (DTT). The homogenate was filtered through three layers of cheesecloth and centrifuged at 12,000 g for 10 min. The supernatant was centrifuged at 45,500 g for 25 min, resuspended, and centrifuged twice. The final pellet was suspended in 2 ml of buffer (50 mM Tris-HCl, pH 7.4, 10 mM MgCl\(_2\), 1 mM EDTA, and 1 mM DTT). A 100-µl aliquot was removed for protein determination, and the remainder was stored in liquid nitrogen until used. Approximately 90% of all preparations were used within 48 h, and the remainder were used within 1 wk of freezing. Binding assays were performed at 37°C for 30 min in a total volume of 0.5 ml containing 50
mM Tris·HCl (pH 7.4), 5 mM MgCl$_2$, 1 mM ascorbic acid, 50 µg of protein, 25–50 µM [125I]iodopindolol (46 Ci/mmol; Amersham Life Sciences, Arlington Heights, IL), and 15 different concentrations of isoproterenol (10$^{-11}$–10$^{-4}$M) with or without the addition of 2 mM guanlylimidodiphosphate. Nonspecific binding, which amounted to 10–15% of total binding, was determined by the addition of 100 µM (≠)isoproterenol to appropriate incubation vessels. Binding of [125I]iodopindolol to β-adrenoceptors was assessed by filtration (52, 65). Assays were performed in duplicate. The curves obtained by plotting [125I]iodopindolol bound vs. the log of the isoproterenol concentration were analyzed by nonlinear regression (22), and IC$_{50}$ values for isoproterenol and the percentage bound to high- and low-affinity states were determined. K$_H$ and K$_L$, the apparent K$_D$ for isoproterenol binding to the high- and low-affinity states, respectively, were calculated by the method of Cheng and Prusoff (21).

G protein separation and immunoblotting. G protein expression was assessed using the Western blot technique. Cardiac membranes containing 50 or 100 µg of protein were mixed with an equal volume of a buffer containing 0.125 M Tris·HCl, pH 6.8, 4% SDS, 20% glycerol, and 10% 2-mercaptoethanol (treatment buffer), heated at 95°C for 2 min, and subjected to electrophoresis. Proteins were transferred onto Immobilon-P membranes in TBS (20 mM Tris, pH 7.6, and 137 mM NaCl) containing 0.1% SDS-PAGE, 9% acrylamide in 6 M urea (55). After electrophoresis, membranes were incubated overnight with TBS (20 mM Tris, pH 8.0, 0.25% Lubrol, 20 mM thymidine, 1 mM ATP, 5 µM GTP, 20 mM arginine, 50 mM NaCl, 4 mM MgCl$_2$, 100 mM DTT, 1 µg of PTX, and 3–5 µCl [α-32P]NAD in a final volume of 0.1 ml. The mixtures were incubated for 2 h at 30°C, and then 1 ml of ice-cold HEPES (20 mM, pH 7.7) was added. The activated CTX (5 µg) and 3–5 µCl [α-32P]NAD were added to make a final volume of 0.1 ml. The mixture was incubated for 2 h at 30°C, and then 1 ml of ice-cold HEPES (20 mM, pH 7.7) was added and the mixture was centrifuged for 10 min in a Microfuge at 4°C. The pellet was resuspended in 0.5 ml of the same buffer and centrifuged again for 10 min.

The protein pellets were resuspended in treatment buffer and separated by 6 M urea SDS-PAGE as described above for immunoblot analysis. Gs and Gi were analyzed on at least two different gels.

Table 1 summarizes the effect of 10–12 days of portal vein stenosis on basal developed tension, DT/dt, and −dT/dt in left ventricular papillary muscles and right ventricular strips. Developed tension and DT/dt were decreased 30–46% and 28–50%, respectively. Portal vein stenosis had no significant effect on the rate of relaxation.

### RESULTS

Basal contractile function. Table 1 summarizes the effect of 10–12 days of portal vein stenosis on basal developed tension, DT/dt, and −dT/dt in left ventricular papillary muscles and right ventricular strips. Developed tension and DT/dt were decreased 30–46% and 28–50%, respectively. Portal vein stenosis had no significant effect on the rate of relaxation.

β-Adrenoceptors. Although the absolute increase in contractility induced by isoproterenol was markedly decreased by portal vein stenosis (Fig. 1A), there was no difference in EC$_{50}$ (sham, 28.8 ± 2.8 nM; stenosed, 25.0 ± 2.7 nM). Figure 1B demonstrates that the relative change in DT/dt was not different between the sham-operated and portal vein-stenosed groups. Similar results were observed in both left and right ventricular tissue (right ventricle not shown). The effect of

| Table 1. Effect of portal vein stenosis on DT, DT/dt, and −dT/dt |
|-----------------------|---------------------|---------------------|
|                       | Right Ventricle       | Left Ventricle       |
|                       | DT, mM/cm$^2$ | DT/dt, N·s$^{-1}$·cm$^{-2}$ | −dT/dt, N·s$^{-1}$·cm$^{-2}$ | DT, mM/cm$^2$ | DT/dt, N·s$^{-1}$·cm$^{-2}$ | −dT/dt, N·s$^{-1}$·cm$^{-2}$ |
| Sham                  | 637 ± 79           | 17.5 ± 1.9           | 21.9 ± 3.9           | 1065 ± 116    | 32.9 ± 3.8           | 19.5 ± 4.0           |
| Portal vein stenosis  | 444 ± 50           | 12.6 ± 1.0           | 20.3 ± 4.1           | 570 ± 55      | 16.3 ± 1.9           | 23.6 ± 6.1           |
| n                     | 21/20              | 21/20                | 9/10                 | 21/10         | 21/10                 | 10/10                 |
| P                     | <0.05              | <0.05                | NS                   | <0.01         | <0.01                 | NS                    |

Values are means ± SE; n = no. of sham-operated/no. of portal vein-stenosed rats. DT, basal cardiac developed tension; DT/dt, maximal rate of tension development; −dT/dt, maximal rate of relaxation; NS, not significant.
decrease in G protein expression or a defect in operated rats (65). Therefore, we examined whether an increase in myocardial contractility as in sham-vein-stenosed rats is required to produce the same increase in receptor occupancy in hearts from portal isoproterenol is unaffected (65), a nearly threefold after 2 wk of portal vein stenosis, and their affinity for not shown.

A similar result was observed in the left ventricle (data shown). The effect of portal vein stenosis on the enhancement of $-dT/dt$ by isoproterenol is shown in Fig. 2. Portal vein stenosis was without effect in either absolute (Fig. 2A) or relative terms (Fig. 2B), and the EC50 was not different (sham, 38.5 ± 13.8 nM; stenosed, 41.4 ± 8.4 nM). A similar result was observed in the left ventricle (data not shown).

Although β-adrenoceptors are not downregulated after 2 wk of portal vein stenosis, and their affinity for isoproterenol is unaffected (65), a nearly threefold increase in receptor occupancy in hearts from portal vein-stenosed rats is required to produce the same increase in myocardial contractility as in sham-operated rats (65). Therefore, we examined whether a decrease in G protein expression or a defect in β-adrenoceptor-G protein coupling is responsible for the diminished response to isoproterenol. Figures 3 and 4 illustrate the effect of portal vein stenosis on $G_{i,1}$ and $G_{i,2}$ expression in the left ventricle. Cardiac membranes from both sham-operated and portal vein-stenosed animals were incubated with specific antisera directed against selective peptide sequences in the α-subunits of different G protein subtypes. With the antiserum RM/1, which is specific for the α-subunit of stimulatory G proteins ($G_{s,i}$), 3–4 immunopositive bands with a molecular mass of 42–44 kDa were found to be present in cardiac membranes from sham-operated and portal vein-stenosed rats. Representative immunoblots are shown in Fig. 3C. The relative density of the bands from ventricles taken from eight sham-operated and eight portal vein-stenosed rats, all run on the same gel, was determined, and the results are shown in Fig. 4. No difference in $G_{s,i}$ expression was detectable between the sham-operated and portal vein-stenosed groups. Expression of the inhibitory G protein subtypes $G_{i,1}$ and $G_{i,2}$ was detected using antisera AS/7 ($G_{i,1}$ and $G_{i,2}$) and $G_{i,2}$. EC/2 was used to detect $G_{i,1}$. Results of these studies are shown in Fig. 3A. Spinal cord membranes, which contain $G_{i,1}$ (64), showed two immunopositive bands with this antisera, but no bands were observed in the cardiac membranes. The autoradiographic intensity of the lower bands ($G_{i,2}$) seen in Fig. 3B is shown in Fig. 4 for all eight sham-operated and all eight portal vein-stenosed rats, demonstrating that there is no difference in expression of $G_{i,2}$ in the sham-operated and the portal vein-stenosed groups. No immunopositive bands for $G_{i,3}$ or $G_{i,2}$ were detected in the rat heart (data not shown).

The extent of ADP ribosylation of $G_{i}$ (with PTX) and $G_s$ (with CTX) was examined to determine whether portal vein stenosis altered G protein function. No differences in ADP ribosylation were observed between the sham-operated and portal vein-stenosed groups (data not shown).

The effect of portal vein stenosis on the coupling of the β-adrenoceptor agonist-receptor complex to $G_s$ was determined by quantifying the percentage of β-adrenoceptors in the high-affinity state of the receptor. The results are summarized in Table 2. Although $K_d$ was lower in the portal vein stenosis group, suggesting a difference in the stability of the agonist-receptor-G protein complex, the difference between the sham-operated and portal vein-stenosed groups was not significant (P > 0.05). Furthermore, portal vein stenosis-induced portal hypertension and portosystemic shunting did not affect the fraction of receptors in the high-affinity state.

Extracellular Ca$^{2+}$. The effect of portal vein stenosis on the relationship between extracellular Ca$^{2+}$ and developed tension was studied in left ventricular papillary muscle and right ventricular strips. Developed tension was significantly reduced before any manipulation of the extracellular Ca$^{2+}$ concentration in portal
vein-stenosed rats (Table 1) and, as Fig. 5A demonstrates for papillary muscle, it remained consistently less over the complete range of Ca\(^{2+}\) concentrations examined. Maximal force development was observed at the same extracellular Ca\(^{2+}\) concentration in the sham-operated and portal vein-stenosed groups. Using force developed in 2.5 mM Ca\(^{2+}\) before the experimental protocol was performed to normalize the data in Fig. 5A, the two groups could be compared despite the lower control tension in the portal vein stenosis group. This comparison is shown in Fig. 5B and demonstrates that the relationship between the extracellular Ca\(^{2+}\) concentration and the relative magnitude of force was similar in the sham-operated and portal vein-stenosed groups when the data were normalized to account for the lower control tension in the stenosed group. Similar results were obtained in right ventricle (data not shown). Furthermore, the time course of the contractile response to stepped changes in the extracellular Ca\(^{2+}\) concentration was the same in the sham-operated and portal vein-stenosed groups (data not shown). There was no difference in the small rise in diastolic tension between the sham-operated and portal vein-stenosed groups at 5 and 7.5 mM Ca\(^{2+}\).

**Fig. 3.** G protein \(\alpha\)-subunits in membranes from mouse spinal cord (SC) and rat ventricles [sham and portal vein-stenosis (PVS)]. Representative immunoblots of \(G_{a1c}\) (A), \(G_{a5}\), and \(G_{a12}\) (B), and \(G_{d1}\), expression in ventricles from sham-operated and portal vein-stenosed rats. Spinal cord expression was included as an internal control because spinal cord expresses \(G_{a1}, G_{a5}\), and \(G_{d1}\). A: antisera selective for \(G_{a1c}\); B: antisera AS/7 selective for \(G_{a1}\) and \(G_{a5}\); C: antisera RM/1 selective for \(G_{d1}\).
promoting Ca\(^{2+}\) release through the SR Ca\(^{2+}\) release channels was used to assess the SR Ca\(^{2+}\) content. Ca\(^{2+}\) uptake and release were not different in the sham-operated and portal vein-stenosed groups as evidenced by the nearly identical responses to caffeine (Fig. 7B).

Loading and subsequent release by caffeine was performed three times in each muscle with identical results, indicating that Ca\(^{2+}\) uptake by the SR under these conditions was consistent and reproducible (not shown). Furthermore, there was no difference between the sham-operated and portal vein-stenosed groups in the rate of tension developed in response to caffeine.

SR Ca\(^{2+}\) content and Na\(^+/Ca^{2+}\) exchange in intact muscle fibers. Figure 8 illustrates the effect of portal vein stenosis on caffeine-induced Ca\(^{2+}\) release in nonpermeabilized muscle. Figure 8A demonstrates that the stimulation of Na\(^+/Ca^{2+}\) exchange and the resultant increase in Ca\(^{2+}\) efflux with Ca\(^{2+}\)-free solution with normal Na\(^+\) reduces the SR Ca\(^{2+}\) content. Using this protocol, the effect of portal vein stenosis on SR Ca\(^{2+}\) content and Na\(^+/Ca^{2+}\) exchange was investigated. Figure 8B presents a comparison between the sham-operated and portal vein-stenosed groups in normal KH buffer solution (2.5 mM Ca\(^{2+}\)) and in Ca\(^{2+}\)-free solution with normal Na\(^+\), which reduces SR reuptake.

Table 3. \[^{3}H\]isradipine binding to L-type Ca\(^{2+}\) channels in cardiac membranes from left ventricles of sham-operated and portal vein-stenosed rats

<table>
<thead>
<tr>
<th></th>
<th>K(_{D}), mM</th>
<th>B(_{\text{max}}), fmol/mg</th>
<th>n(_{H})</th>
</tr>
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<tbody>
<tr>
<td>Sham</td>
<td>0.138 ± 0.016</td>
<td>122.9 ± 8.0</td>
<td>1.15 ± 0.11</td>
</tr>
<tr>
<td>Portal vein stenosis</td>
<td>0.148 ± 0.021</td>
<td>45.4 ± 10.9*</td>
<td>1.05 ± 0.03</td>
</tr>
</tbody>
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Values are means ± SE from 4 membrane preparations from sham-operated rats and 10 membrane preparations from portal vein-stenosed rats. B\(_{\text{max}}\), receptor density; n\(_{H}\), Hill coefficient. *P < 0.001.

Fig. 5. A: effect of extracellular Ca\(^{2+}\) concentration on developed tension in left ventricular papillary muscles from sham-operated and portal vein-stenosed rats. Values are means ± SE of 7–8 muscles. Each papillary muscle was equilibrated in normal KH containing 2.5 mM Ca\(^{2+}\) (control data point) before switching to the lowest concentration of Ca\(^{2+}\) (0.3125 mM). Subsequent addition of Ca\(^{2+}\) to attain the next higher Ca\(^{2+}\) concentration was made after force had reached a plateau. B: relative changes in papillary muscle force generation with changes in extracellular Ca\(^{2+}\) concentration. Values represent mean ± SE percentage of the control value in 2.5 mM Ca\(^{2+}\). Similar results were observed in right ventricle (data not shown). *P < 0.05, **P < 0.01 significantly different from sham.

Fig. 6. Effect of portal vein stenosis on pCa-force relationship in skinned left ventricular papillary muscle fiber bundles. Values represent means ± SE %maximum response obtained in tissues from sham-operated rats; n = 5. Data from each group were fit to Hill equation and gave a half-maximal pCa of 5.57 and 3.55 for fibers from sham-operated and portal vein-stenosed rats, respectively. Maximal response was 520 ± 100 and 520 ± 180 µN in sham and stenosed rats, respectively. Hill coefficients for sham and stenosed groups were 2.2 ± 0.4 and 2.7 ± 0.4, respectively.

Fig. 7. A: caffeine-induced Ca\(^{2+}\) release from sarcoplasmic reticulum (SR) in permeabilized papillary muscle from a sham-operated rat. After permeabilization and release of SR Ca\(^{2+}\) content with caffeine, muscles were exposed to the following solutions: a, relaxing solution for 2 min; b, Ca\(^{2+}\) loading (pCa 6.0) for 3 min; c, low EGTA for 1 min; d, 50 mM caffeine; e, low EGTA; f, caffeine; and g, relaxing solution. Rapid upward deflection seen in tracing at arrows is an artifact from replacement of bathing solution. B: compilation of the effect of portal vein stenosis on caffeine-induced Ca\(^{2+}\) release determined as shown in A. Values are means ± SE of response to 50 mM caffeine; n = 5–8.
of Ca\(^{2+}\) leaked during rest by stimulating Na\(^{+}\)/Ca\(^{2+}\) exchange. There was a decrease in the response to caffeine in both left ventricular papillary muscles (not shown) and right ventricle (Fig. 8B), although the difference was significant only in the right ventricle. As expected, stimulation of Na\(^{+}\)/Ca\(^{2+}\) exchange for 5 min with Ca\(^{2+}\)-free solution with normal Na\(^{+}\) significantly decreased the response to caffeine in the sham-operated and portal vein-stenosed groups. However, the magnitude of the decrease was greater in the sham-operated group in absolute as well as relative terms (sham: -379.1 mN/cm\(^2\), -62%; stenosed: -155.3 mN/cm\(^2\), -39%).

Effect of BAY K 8644. BAY K 8644, a dihydropyridine that prolongs the average open time of L-type Ca\(^{2+}\) channels, increased the development of force as a function of concentration between 10 nM and 1 \(\mu\)M. As demonstrated by Fig. 9, BAY K 8644 produced comparable increments in dT/dt at each concentration in tissues from sham-operated and portal vein-stenosed animals.

DISCUSSION

Studies (7, 10, 13, 16, 29, 37, 40) in both human alcoholic and nonalcoholic cirrhosis and in nonalcoholic animal models of cirrhosis have shown that liver disease is associated with impaired basal cardiac contractile function and diminished responses to \(\beta\)-adrenergic stimulation. In cirrhotic humans, this altered cardiac function has been termed cirrhotic cardiomyopathy (43). In earlier studies (7, 65), our laboratory has used the chronic portal vein-stenosed rat, a hepatic model with extensive portosystemic shunting without hepatocellular disease, to dissociate the cardiac effects of portosystemic shunting and hepatocellular disease. The site of cardiac impairment responsible for the decrease in basal contractile function is largely unexplored in this model. In the present study, we have extended our work to include the effect of chronic portal vein stenosis on 1) sites in myocardial ECC coupling that could reduce basal contractility as well as the contractile response to \(\beta\)-adrenoceptor activation and 2) postreceptor sites that could alter responses to \(\beta\)-adrenoceptor activation.

In the current study, basal myocardial contractility was reduced 30–50% in both the right and left ventricle of the chronic portal vein-stenosed rat (Table 1), and \(\beta\)-adrenoceptor responsiveness was also diminished (Fig. 1), in agreement with previous reports from our laboratory (7, 65). However, the positive lusitropic action of isoproterenol was unaltered by portal vein stenosis (Fig. 2).

ECC. The effect of portal vein stenosis on ECC was tested initially by estimating sarcolemmal dihydropyridine receptor density as an indication of L-type Ca\(^{2+}\) channels.
channel density. The decrease in dihydropyridine binding sites observed in hearts from portal vein-stenosed animals (Table 3) suggests that the density of L-type Ca\(^{2+}\) channels was reduced. If the remaining channels still gate the inward movement of Ca\(^{2+}\) normally, relative changes in cardiac force in sham-operated and portal vein-stenosed rats would be expected to be similar, and the maximum force developed in both groups should be achieved at the same extracellular Ca\(^{2+}\) concentration. Muscles from the stenosed group, however, would not be expected to generate the same absolute force as the sham group. A between-group difference in the relative change in force development would suggest the possibility of additional effects of portal vein stenosis. This expectation is, of course, founded on the proviso that the sensitivity of the myofilaments to Ca\(^{2+}\) and SR Ca\(^{2+}\) uptake and release are unaffected by portal vein stenosis.

In actively contracting muscle, an increase or decrease in extracellular Ca\(^{2+}\) concentration results in a parallel increase or decrease in Ca\(^{2+}\) entry during the action potential because of the change in its electro motive force (57). This increase or decrease in Ca\(^{2+}\) influx is accompanied by enhancement or attenuation of the intracellular Ca\(^{2+}\) transient (2) and increased or decreased force development (31). In the present study, this relationship between extracellular Ca\(^{2+}\) concentration and force development held for myocardium from both groups. However, at all extracellular Ca\(^{2+}\) concentrations, the force developed by the stenosed group was significantly lower than in the sham-operated group (Fig. 5A). Although this observation could be the result of dysfunction in any of several steps in ECC, it is consistent with decreased Ca\(^{2+}\) influx during muscle contraction.

Additional indirect support for the hypothesis that the cardiac effect of portal vein stenosis results from decreased density of L-type Ca\(^{2+}\) channels was obtained by determining the effects of stenosis on the sensitivity of the myofilaments to Ca\(^{2+}\), SR Ca\(^{2+}\) uptake and release, and the positive inotropic effect of the dihydropyridine agonist BAY K 8644. Although a decrease in myofilament Ca\(^{2+}\) sensitivity and/or SR Ca\(^{2+}\) transport would not necessarily negate the hypothesis, the absence of an effect of portal vein stenosis on these aspects of ECC would support the hypothesis. As Fig. 6 illustrates, no difference in myofilament Ca\(^{2+}\) sensitivity was observed (half-maximal pCa was 5.57 and 5.55 in sham-operated and portal vein-stenosed rats, respectively), and maximum tension development occurred at the same Ca\(^{2+}\) concentration in the two groups. With respect to SR Ca\(^{2+}\) uptake and release, a lack of effect of portal vein stenosis on the SR is suggested by the absence of an effect on caffeine-induced Ca\(^{2+}\) release in permeabilized fibers. In permeabilized fibers, where the Ca\(^{2+}\) concentration can be rigidly controlled and uptake into the SR limited to a fixed time interval, there was no difference in the contraction caused by caffeine between the sham-operated and stenosed groups (Fig. 7). Two conclusions about the effect of portal vein stenosis on SR Ca\(^{2+}\) handling are suggested by the absence of a difference between the two experimental groups: 1) there is no direct effect of portal vein stenosis on SR uptake and release; and 2) there is no change in the number of release channels, because the magnitude of a caffeine contraction is dependent not only on the amount of Ca\(^{2+}\) in the SR but also on the number of release channels available to interact with caffeine.

The experiments performed with the L-type Ca\(^{2+}\) channel agonist BAY K 8644 are also consistent with a decrease in Ca\(^{2+}\) channel density contributing to the reduced contractile function in portal vein-stenosed rats. BAY K 8644 increased the transsarcolemmal Ca\(^{2+}\) influx by lengthening the mean open time of L-type Ca\(^{2+}\) channels. Because the SR Ca\(^{2+}\) and myofilament sensitivity to Ca\(^{2+}\) were unaffected by portal vein stenosis, if the remaining L-type Ca\(^{2+}\) channels function normally, BAY K 8644 should increase force by the same relative increments in tissues from both sham-operated and stenosed animals because the only difference between the two groups is the number of Ca\(^{2+}\) channels. As can be seen in Fig. 9, BAY K 8644 produced similar relative increments in myocardial force in sham-operated and portal vein-stenosed rats. It should be noted that although these experiments on ECC suggest that Ca\(^{2+}\) entry into the myocytes from portal vein-stenosed rats is decreased, actual measurement of the current carried by the L-type Ca\(^{2+}\) channel is necessary to confirm unequivocally the role of the L-type Ca\(^{2+}\) channel in the myocardial action of portal vein stenosis.

Aside from the decrease in dihydropyridine binding sites, the only other difference between the portal vein-stenosed and sham-operated groups was a decrease in the response to caffeine in nonpermeabilized muscles in normal KH buffer (Fig. 8B). Although these data do not permit a conclusion to be made concerning the cause of the decrease, possible explanations include a reduced SR Ca\(^{2+}\) content, a change in Na\(^{+}/\)Ca\(^{2+}\) exchange, and greater leakage of Ca\(^{2+}\) from the SR during rest (diastole). As mentioned above, the caffeine-induced SR Ca\(^{2+}\)-release data shown for skinned fibers (Fig. 7B) indicate that SR Ca\(^{2+}\) uptake and release in the portal vein-stenosed group is unaffected when loading conditions are controlled. These data are inconsistent with a direct effect of portal vein stenosis on SR Ca\(^{2+}\) content.

Because no effect of portal vein stenosis on SR Ca\(^{2+}\) content was observed in permeabilized muscle, the decreased response to caffeine in intact fibers would appear to be caused by a mechanism other than SR Ca\(^{2+}\) uptake and release. However, in functioning, nonpermeabilized muscle, a change in the normal relationship between SR uptake of cytosolic Ca\(^{2+}\) and transport of Ca\(^{2+}\) out of the cell by Na\(^{+}/\)Ca\(^{2+}\) exchange during rest (diastole) could affect the SR Ca\(^{2+}\) content. In the rat heart, nearly all of the Ca\(^{2+}\) released from the SR is sequestered into the SR by SR Ca\(^{2+}\)-ATPase (5). Na\(^{+}/\)Ca\(^{2+}\) exchange plays only a minor role in the removal of Ca\(^{2+}\) from the cytoplasm relative to the SR (5). A substantive increase or decrease in Na\(^{+}/\)Ca\(^{2+}\) exchange activity in portal vein-stenosed rats, however, might have an inverse effect on the SR Ca\(^{2+}\) content.
and the amount of Ca\(^{2+}\) available to induce subsequent contractions. To test this hypothesis, nonpermeabilized fibers were exposed to a Ca\(^{2+}\)-free solution with normal Na\(^{+}\), which stimulates Na\(^{+}/Ca\(^{2+}\) exchange, and under these conditions at rest, a decrease in SR Ca\(^{2+}\) content sensitive to caffeine is observed in the rat heart (Ref. 3; compare the response to caffeine with and without Ca\(^{2+}\) in the sham and stenosed groups in Fig. 8B). Therefore, Na\(^{+}/Ca\(^{2+}\) exchange can be assessed using caffeine to determine the relative amount of Ca\(^{2+}\) remaining in the SR after stimulation of Na\(^{+}/Ca\(^{2+}\) exchange. If portal vein stenosis was associated with either increased or decreased Na\(^{+}/Ca\(^{2+}\) exchange, a change in the response to caffeine could be expected. In our study, the decrease in the response to caffeine after Ca\(^{2+}\)-free solution with normal Na\(^{+}\) was considerably greater in sham-operated rats in absolute as well as relative terms (sham: \(-379.1 \text{ mN/cm}^2\), \(-62\%\); stenosed: \(-155.3 \text{ mN/cm}^2\), \(-39\%\)).

The greater decrease in the sham-operated rats (Fig. 8B) suggests that Na\(^{+}/Ca\(^{2+}\) exchange activity was greater in the sham-operated group than in the portal vein-stenosed group. Because the response to caffeine in the presence of extracellular Ca\(^{2+}\) was greater in sham-operated rats than in portal vein-stenosed rats, a difference in absolute terms might be expected, but not in relative terms if Na\(^{+}/Ca\(^{2+}\) exchange was not affected. One could speculate that an apparent decrease in Na\(^{+}/Ca\(^{2+}\) exchange would produce a greater response to caffeine in Ca\(^{2+}\)-free solution with normal Na\(^{+}\) in the portal vein-stenosed group because the exchanger is less effective, and therefore, a greater amount of Ca\(^{2+}\) can be taken up by the SR. However, the effect of caffeine in intact fibers suggests that there is less, not more, Ca\(^{2+}\) in the SR of the portal vein-stenosed group. A possible explanation for the reduction in Na\(^{+}/Ca\(^{2+}\) exchange may lie in the relationship between Ca\(^{2+}\) entry through L-type Ca\(^{2+}\) channels and extrusion by Na\(^{+}/Ca\(^{2+}\) exchange. It has been suggested that, at equilibrium, the amount of Ca\(^{2+}\) that is removed by Na\(^{+}/Ca\(^{2+}\) exchange varies directly with the amount of Ca\(^{2+}\) that enters via Ca\(^{2+}\) channel current (I_{Ca}) (50, 62) and that the amount of Ca\(^{2+}\) entering via I_{Ca} is balanced by an equal amount of Ca\(^{2+}\) leaving the cell at equilibrium. In that case, a chronically reduced Ca\(^{2+}\) entry associated with downregulation of L-type Ca\(^{2+}\) channels could result in decreased Na\(^{+}/Ca\(^{2+}\) exchange. Although this is circumstantial evidence, it does fit the data. A decrease in Na\(^{+}/Ca\(^{2+}\) exchange expression would lend credence to this hypothesis.

Alternatively, the reduced response of intact fibers to caffeine in normal KH buffer solution in the portal vein-stenosed group could be the result of a greater leakage of Ca\(^{2+}\) from the SR during rest. In the rat, however, this is complicated by the fact that nearly all Ca\(^{2+}\) leaked from the SR is taken back up into the SR and a decline in the response to caffeine is not observed (5). However, a decrease in the response to caffeine can be observed if Na\(^{+}/Ca\(^{2+}\) exchange is stimulated during the period of rest (5). The data from permeabilized tissues suggest that the SR from the stenosed group takes up the same amount of Ca\(^{2+}\) as the sham group, at least under controlled loading conditions (Fig. 7B). A larger Ca\(^{2+}\) leak after portal vein stenosis would be expected to produce greater Ca\(^{2+}\) efflux when Na\(^{+}/Ca\(^{2+}\) exchange is stimulated by Ca\(^{2+}\)-free solution with normal Na\(^{+}\), and therefore, a reduced response to caffeine. However, our data showed smaller absolute and relative decrements in the response to caffeine in the portal vein-stenosed group than in the sham-operated group after a 5-min period in Ca\(^{2+}\)-free solution with normal Na\(^{+}\) (sham: \(-379.1 \text{ mN/cm}^2\), \(-62\%\); stenosed: \(-155.3 \text{ mN/cm}^2\), \(-39\%\)) (Fig. 8B). This smaller decrement suggests that Na\(^{+}/Ca\(^{2+}\) exchange was less in the portal vein-stenosed group and is not indicative of increased leakage of Ca\(^{2+}\) from the SR during rest.

One question that can be asked is, How do the experiments performed in permeabilized and nonpermeabilized quiescent fibers apply to the observations of depressed contractile function in paced right ventricles and left ventricular papillary muscles (Table 1 and Fig. 1)? The experiments in permeabilized tissues had as their goal the determination of whether the sensitivity of the myofilaments to Ca\(^{2+}\) and SR Ca\(^{2+}\) uptake and release were directly impaired by portal vein stenosis. The results from these experiments suggest that these processes were not directly affected by portal vein stenosis. If these processes are altered in intact contracting muscle, the effect would have to be an indirect one that is removed by permeabilizing the sarcolemma. The data from caffeine-induced contraction in quiescent, intact muscle are probably more indicative of the situation in contracting muscle. It has been shown that the effect of a high concentration of caffeine in intact muscle is similar to the effect of rapid administration of caffeine during a contraction cycle in rat ventricular myocytes, i.e., there is an increase in the Ca\(^{2+}\) transient, a decrease in SR Ca\(^{2+}\) content, and increased extrusion of Ca\(^{2+}\) via Na\(^{+}/Ca\(^{2+}\) exchange in response to the increase in Ca\(^{2+}\) release (50, 62).

\(\beta\)-Adrenoceptor responsiveness. Although we had previously demonstrated that hearts from stenosed rats require a threefold greater \(\beta\)-adrenoceptor occupancy to produce the same absolute increase in force in response to isoproterenol (65), no alteration in myocardial \(\beta\)-adrenoceptor density and affinity occurred in hearts from portal vein-stenosed rats (44, 65). In the present study, we investigated whether a change in G protein expression or coupling of \(\beta\)-adrenoceptors to Gs could explain the effect of portal vein stenosis on \(\beta\)-adrenoceptor activation. As demonstrated by Figs. 3 and 4, portal vein stenosis did not alter G protein expression.

Failure of the coupling of the \(\beta\)-adrenoceptor with Gs could cause the effect of portal vein stenosis on \(\beta\)-adrenoceptor-mediated responses in the heart. It is well known that the \(\beta\)-adrenoceptor has two affinity states for a bound agonist (28) and that only \(\beta\)-adrenoceptors coupled to Gs are in the high-affinity state. Fewer \(\beta\)-adrenoceptors in the high-affinity state would be expected to decrease the efficacy of GTP because there is less agonist-receptor-G protein complex with which GTP can interact. \(\beta\)-Adrenoceptor-Gs coupling has been shown to be significantly decreased in some models of cardiac hypertrophy and failure, even though
β-adrenoceptor density, determined by antagonist binding, was not reduced (63). This suggests that for a given number of β-adrenoceptors to which an agonist has bound, there will be a reduced positive inotropic effect (63). Because we (65) had previously found that hearts from portal vein-stenosed rats required a greater β-adrenoceptor fractional receptor occupancy to produce the same positive inotropic response to isoproterenol as sham-operated rats, the effect of portal vein stenosis on the high- and low-affinity state of the β-adrenoceptor was investigated. The unchanged fraction of receptors in the high-affinity state (Table 2) clearly shows that portal vein stenosis was without effect on β-adrenoceptor-G protein coupling. Therefore, β-adrenoceptor signaling does not appear to be influenced by portal vein stenosis. Indeed, one could argue that the lack of a relative change in the response to β-adrenoceptor activation in the presence of a decrease in basal cardiac force generation is indicative of no effect on β-adrenoceptor responsiveness at all. If this is so, then another process must be responsible. Although the process of ECC has not been studied in animals subjected to portal vein stenosis until now, there is evidence from several models of hypertrophy and heart failure in different species, as well as in human congestive heart failure, demonstrating, even in mild pathological states where basal L-type Ca²⁺ channel peak current density or dihydropyridine binding was unaltered, reduced β-adrenoceptor responsiveness that coincides with a decrease in the β-adrenoceptor-mediated enhancement of L-type Ca²⁺ channel current density (30, 47, 48, 56).

In summary, myocardial contractility was depressed by the induction of portal hypertension by chronic ligation of the prehepatic portal vein. Various aspects of β-adrenoceptor signaling and ECC were examined in rat right ventricular strips and left ventricular papillary muscles. In permeabilized muscle, myofilament Ca²⁺ sensitivity or SR Ca²⁺ uptake and release did not differ between the sham and the stenosed groups. The number of dihydropyridine binding sites was reduced, and in intact muscles, Na⁺/Ca²⁺ exchange and the SR Ca²⁺ content were reduced in the portal vein-stenosed group. No effect of portal vein stenosis on β-adrenoceptor-G protein coupling and G protein expression or function was observed. The data suggest that in the rat heart, portal vein stenosis-induced myocardial dysfunction is associated with alterations in ECC but not β-adrenoceptor signaling.

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