ABNORMAL STRETCHING OF the myocardium is a common feature of cardiac contractile problems. Acute contractile dysfunction resulting from ischemia-reperfusion, for instance, is associated with both paradoxical systolic bulging and diastolic lengthening (“creep”; see Refs. 12, 26, 31). Similarly, chronic heart failure due to valvular regurgitation or cardiomyopathy is frequently associated with excessive cardiac dilatation. Downing et al. (11) have previously demonstrated that acute, global contractile dysfunction can be induced simply by overdistention of the potassium-arrested ventricle. Others have shown that both acute and chronic forms of contractile dysfunction can be reversed by ventricular decompression (i.e., destretching) with a mechanical ventricular assist device (10, 15, 26).

The mechanism by which overstretching may alter contractile function has not been explained. It is known, however, that stretching of the cell membrane induces ion currents in a variety of cell types, including cardiac myocytes, via non-voltage-gated, stretch-activated (SA) ion channels (6–8, 18–20, 22, 24, 30, 33, 34). Changes in intracellular ion concentrations that could potentially result from prolonged overstretching could have profound influences on contractile function. Along these lines, Clemo et al. (6, 7) have recently demonstrated that certain SA channels mediate abnormal currents in cardiomyocytes taken from dogs with pacing-induced dilated cardiomyopathy, although they have not linked SA channels to the contractile defect per se. We hypothesized that overstretching could induce and/or exacerbate cardiac contractile dysfunction via an effect on SA channels. To test this hypothesis, we measured the effects of overstretching on developed tension in isolated, guinea pig papillary muscles. To determine the role of SA channels, we used Gd3+, an SA channel antagonist (6, 7, 19, 22, 30, 33, 34), to modulate any effects of stretch on contractile function. Because Gd3+ may have nonspecific effects on voltage-gated calcium channels (5), we compared its effects with nifedipine, a dihydropyridine (DHP) antagonist of L-type calcium channels.

METHODS

Preparation. The methods used for isolation and superfusion of guinea pig papillary muscles have been described previously (3, 4, 23). Hartley guinea pigs (200–250 g) were anesthetized by intraperitoneal pentobarbital sodium (50 mg/kg) and were given supplemental oxygen to prevent hypoxic preconditioning. The chest was opened, and the heart was rapidly removed to a 5-ml bath, where it was superfused at 15 ml/min with 37°C oxygenated Krebs solution containing (in mM) 129 NaCl, 4.0 KCl, 0.9 NaH₂PO₄, 20.0 NaHCO₃, 2.5 CaCl₂, 0.5 MgSO₄, and 5.5 dextrose. The solution contained 302 mosmol/l and had a pH of 7.4 when bubbled with a 95% O₂:5% CO₂ gas mixture. The right ventricle was opened, and a suitable papillary muscle (diameter <1 mm) was removed with chordae tendineae and a base of surrounding muscle intact. The base was pinned to the floor of the

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bath using 25-μm stainless steel pins. A monofilament suture (10-0 gauge) was tied around the chordae and then looped around a custom-made, miniature, isometric force transducer (model BG-10SP Load Cell; Kulite Semiconductor Products, Leonia, NJ). The muscle was electrically stimulated to contract (0.5 Hz; 2-ms pulses at slightly higher than threshold for muscle contraction) by using a pair of closely spaced platinum electrodes (0.5 mm diameter) that were placed on the base of the papillary muscle and connected to an isolated current source. Transducer output was displayed on a digital storage oscilloscope.

**Protocol.** After the preparation was equilibrated for 60 min, a length-tension curve was generated in a control state by stretching the muscle in 100-μm increments starting at the length just beyond slack length associated with the first development of passive tension (L0). Developed tension resulting from electrical stimulation was recorded at each increment of stretch. Resting length that resulted in maximum developed tension (Tmax) was defined as Lmax. The muscle was stretched beyond Lmax until developed tension decreased to 0.8 Tmax. After the initial curve was obtained, the muscle was reequilibrated to zero passive tension at L0, and a second curve was acquired in identical fashion to assure stability of the preparation. The muscle was again equilibrated back to zero passive tension and was then set to one of three resting lengths associated with 0.2 Tmax; physiological stretch, as normalized values, and pooled values represent means ± SE.

Gd³⁺ was added to the bath in some experiments to inhibit SA channels. Gadolinium chloride hexahydrate (Aldrich Chemical, Milwaukee, WI) was dissolved in 100 ml of Krebs solution using several drops of diluted hydrochloric acid and was added to the total perfusate volume to yield a 20 μM solution (306 mosmol/l). Nifedipine, a DHP (Sigma, St. Louis, MO) antagonist of L-type calcium channels, was added to the perfusate in other experiments. Nifedipine was dissolved in DMSO (200 μl/l) to yield a 2.8 μM solution (307 mosmol/l). In these experiments, Gd³⁺ or nifedipine was added to the bath before collection of control data.

Some muscles were exposed to modified hypoxia-reoxygenation (MHR) after collection of control data. These muscles were superfused for 40 min with an “ischemic” bath (13) containing the following (in mM): 123.0 NaCl, 10.0 KCl, 0.9 NaH₂PO₄, 6.0 NaHCO₃, 2.5 CaCl₂, 0.5 MgSO₄, and 20.0 sodium lactate. This solution was bubbled with a 90% N₂-10% CO₂ gas mixture. At 37°C, the Po₂ in the bath was ~45 mmHg and had a pH of 6.8. The solution contained 293 mosmol/l. After exposure to ischemic solution, the muscle was superfused with standard, oxygenated solution for 45 min.

**Data analysis.** Analog papillary muscle tensions were automatically digitized and recorded. Developed tension (Td) at each increment of stretch was recorded as

\[ T_d = T_t - T_p \]

where Tt is total tension and Tp is passive tension. Tmax observed at 85 min was normalized to Tmax observed at control. Normalized values were pooled within each group, and means were compared among groups by ANOVA. Where indicated, statistical significance was determined by the Student-Newman-Keuls test. Significance was defined by a P value <0.05. All developed tensions are presented as normalized values, and pooled values represent means ± SE.

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**Fig. 1.** Representative length-developed tension curve from an isolated guinea pig papillary muscle. Resting length at maximum developed tension (Tmax) is defined as Lmax. Unloaded and physiological stretch refer to the resting lengths resulting in 20 and 80% of Tmax respectively. Overstretched refers to the resting length on the descending limb of the curve resulting in a 20% decrease in Tmax.
RESULTS

Effects of stretch on developed tension. In muscles superfused with standard Krebs solution for 85 min (Fig. 2A) neither physiological stretch \((n = 12)\) nor mechanical unloading \((n = 12)\) was associated with any changes in \(T_{\text{max}}\) compared with control \((T_{\text{max}} = 0.93 \pm 0.05\) and \(1.03 \pm 0.07\), respectively). Overstretching, however, \((n = 18)\), resulted in a decrease in \(T_{\text{max}}\) to \(0.77 \pm 0.03\) \((P < 0.05\) vs. physiological). Overstretching also exacerbated the changes observed in developed tension when muscles were exposed to MHR (Fig. 2B).

Effects of \(Gd^{3+}\) and nifedipine. In muscles superfused with standard buffer only (Fig. 3A), the adverse effect of overstretching on developed tension was pre-

Fig. 2. Changes in \(T_{\text{max}}\) (means \(\pm\) SE) in isolated guinea pig papillary muscles held at different resting lengths during superfusion with oxygenated buffer for 85 min (A) and exposure to modified hypoxia-reoxygenation (MHR; B). See text for definitions of resting length of each group. Overstretch causes a reduction in contractile tension in muscles superfused with oxygenated buffer and exacerbates the reduction in contractile tension after exposure to MHR. *\(P < 0.05\) vs. physiological stretch.

Muscles held at physiological stretch during exposure to MHR \((n = 8)\) exhibited a decrease in \(T_{\text{max}}\) to \(0.63 \pm 0.07\), whereas muscles that were overstrretched \((n = 9)\) sustained a further decrease to \(0.44 \pm 0.04\) \((P < 0.05\) vs. physiological stretch). Muscles that were mechanically unloaded during exposure to MHR \((n = 8)\) exhibited no change in \(T_{\text{max}}\) \((0.98 \pm 0.07; P < 0.05\) vs. physiological stretch).
vented with 20 μM Gd3+ (n = 12; T_max = 0.98 ± 0.06; P < 0.05 vs. overstretch alone) but not with 2.8 μM nifedipine (DHP) [n = 12; T_max = 0.82 ± 0.04; P = not significant (NS) vs. overstretch alone]. In muscles exposed to MHR, Gd3+ (n = 8) prevented the exacerbation of contractile dysfunction induced by overstretching (T_max = 0.64 ± 0.05; P < 0.05 vs. overstretch), but again DHP (n = 6) had no effect (T_max = 0.48 ± 0.04; P = NS vs. overstretch). Importantly, addition of up to 100 μM Gd3+ did not further enhance the effect of 20 μM Gd3+ in muscles that were overstretched during MHR. Similarly, Gd3+ in concentrations of 20 μM (n = 4) or 40 μM (n = 9) did not prevent contractile dysfunction in muscles held at physiological stretch during MHR (T_max = 0.59 ± 0.02 and 0.77 ± 0.04, respectively). Treatment with DHP also failed to prevent contractile dysfunction during exposure to MHR at physiological stretch (n = 5; T_max = 0.48 ± 0.07).

The effects of Gd3+ and DHP were further compared in other experiments in which length-tension data were acquired before and after addition of the drugs to the perfusate (Fig. 4). Addition of 20 μM Gd3+ (n = 3)
resulted in only a minimal decrease in maximal developed tension \(T_{\text{max}} = 0.91 \pm 0.02\), whereas addition of DHP \(n = 3\) resulted in a marked decrease \(T_{\text{max}} = 0.42 \pm 0.04\).

**DISCUSSION**

The salutary effects of physiological myocardial stretch on contractile function are well described (27, 28, 32), yet little has been written about the potential role of overstretch in the etiology and/or progression of cardiac contractile problems, many of which are characterized by abnormal stretch (12, 26, 31). Downing et al. (11) reported that overdistention of the potassium-arrested (i.e., nonischemic) left ventricle induces a global contractile defect consistent with myocardial stunning (11). Others have reported that mechanical relief of stretch, using a left ventricular assist device for cardiac decompression, enhances recovery of contractile function in both regionally stunned myocardium (15, 26) and dilated cardiomyopathy (10). The present data clearly demonstrate that overstretching induces contractile dysfunction in normal guinea pig papillary muscles and exacerbates contractile dysfunction resulting from exposure to MHR.
We hypothesized that contractile dysfunction resulting from overstretch might involve specific SA ion channels, which have been identified in cardiac myocytes (6–8, 19, 20, 34). Persistent, exaggerated membrane stretch could result in abnormal accumulation of intracellular ions passing through these channels. Intracellular overload of certain ions, particularly calcium, is a characteristic feature of both stunned and chronically failing myocardium (1, 14, 16, 25, 29). Stretch-induced accumulation of intracellular calcium (24, 32, 34) could thus play a role in the pathophysiology of contractile problems. Our observations using Gd3+ in the present study suggest that SA channels are indeed involved in overstretch-induced changes in contractile function. Both the decrease in developed tension and the exacerbation of contractile dysfunction associated with hypoxia-reoxygenation that was observed with overstretch were prevented by Gd3+ (Fig. 3). Further investigation is needed, however, to determine which SA channels are involved in mediating stretch-induced contractile dysfunction, because Gd3+ is a nonselective SA channel antagonist.

The extent to which SA channels are involved in the etiology and/or progression of contractile dysfunction, particularly with ischemia-reperfusion, remains unclear. We noticed that muscles held at physiological stretch exhibited a decrease in developed tension when exposed to MHR but not when superfused in standard buffer. We hypothesized therefore that the sensitivity of SA channels to stretch and/or Gd3+ might be altered by MHR. We observed, however, in muscles exposed to MHR that Gd3+ was unable to prevent the decrease in developed tension in muscles held at physiological stretch and that increasing the concentration of Gd3+ failed to enhance the effect of the 20 μM solution with overstretching. Myocardial stunning is clearly a multifactorial process, the mechanisms of which are yet to be fully defined (21), and can occur in the absence of stretch. The fact, however, that abnormal stretch is a characteristic feature of stunned myocardium in vivo supports the idea that inhibition of SA channels may be important as part of a clinical strategy for treating postischemic contractile dysfunction.

One potential problem in the present study is evidence that Gd3+ may exert nonspecific effects on voltage-gated ion channels, particularly L-type calcium channels (5). This issue is particularly important in light of the fact that nifedipine has been shown to exert partial block of stretch-induced calcium currents in vascular smooth muscle, suggesting that L-type calcium channels can be activated by mechanical stretch (9). To account for these potential nonspecific actions of either Gd3+ or stretch on L-type calcium channels, we also tested the effects of nifedipine in our model. Our results indicate that Gd3+ modifies contractile function in the guinea pig heart via a mechanism that does not involve L-type calcium channels. Nifedipine failed to alter the decrease in developed tension induced by overstretch (Fig. 3) and exhibited inherent negative inotropic effects that were not observed with Gd3+ (Fig. 4). These results agree with those recently published by Bode et al. (2), who demonstrated very different effects of Gd3+ and verapamil on stretch-induced vulnerability of the isolated rabbit heart to atrial fibrillation.

Another potential problem in the present study is that overstretch may cause contractile dysfunction through mechanisms unrelated to ion channels. It may for instance induce tissue hypoxia through an increase in resting muscle tension. Stretching a muscle of constant volume, however, would also result in muscle thinning and would thus facilitate delivery of dissolved oxygen to core fibers in an isolated papillary muscle preparation. Furthermore, studies in the intact heart suggest that the effects of stretch on contractile force (32) and the effects of destretching on recovery of contractile function in stunned myocardium (26) occur independent of myocardial blood flow (i.e., oxygen supply). The fact that Gd3+, a channel antagonist, reversed the effect of stretch on contractile function also argues against oxygen supply/demand as an important factor. Another potential alternate mechanism underlying the adverse effects of overstretch is malalignment or literal tearing apart of contractile elements, but, again, it is unlikely that Gd3+ could modulate such a phenomenon.

The magnitude of overstretch placed on the papillary muscles in the present study is a fair representation of the degree of stretch observed in the intact heart under pathological conditions. As noted above, overstretch was defined as the length beyond Lmax associated with a 20% decrease in maximal developed tension. This required an ~30% increment of stretch beyond Lmax and was associated with an ~50% increase in passive muscle tension. We recently reported that the x-axis intercept of the regional preload recruitable stroke work relation (a theoretical measure of unstressed fiber length) increased by 50% and that left atrial pressure increased by 50% in a canine model of regional ischemia-reperfusion (26). Clemo et al. (6) have similarly reported that end-diastolic volume increases by 50% in a pacing-induced model of dilated cardiomyopathy and both end-diastolic pressure and volume may increase by >100% over normal in humans with advanced dilated cardiomyopathy.

In summary, physical tissue overstretch may be an important factor in the etiology and/or progression of cardiac contractile problems. The adverse effect of overstretch appears to be mediated, at least in part, via SA ion channels. Further investigation is required to define the precise channels involved, but the present results suggest novel approaches to therapy of contractile dysfunction.

REFERENCES


