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Frequency-dependence of the slow force response

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1. ABSTRACT

Stretch induces biphasic inotropic effects in mammalian myocardium. A delayed component (slow force response, SFR) has been demonstrated in various species, however, experimental conditions varied and the underlying mechanisms are controversial. The physiological relevance of the SFR is poorly understood. Experiments were performed in ventricular muscle strips from failing human hearts and non-failing rabbit hearts. Upon stretch, twitch force was assessed at basal conditions (1Hz, 37°C) and after changing stimulation frequency with and without blockade of the Na^+/H^+-exchanger-1 (NHE1) or reverse-mode Na+/Ca+-exchange (NCX). Action potential duration (APD) was assessed using floating electrodes. Low stimulation rates (0.2Hz) potentiated and higher stimulation rates (2 and 3Hz) reduced the SFR. The extent of SFR inhibition by NHE1 or NCX inhibition was not affected by stimulation rate. APD decreased at 0.2Hz but was not altered at higher stimulation rates. The data demonstrate frequency-dependence of the SFR with greater positive inotropic effects at lower stimulation rates. Subcellular mechanisms underlying the SFR are not fundamentally affected by stimulation rate. The SFR may have more pronounced physiological effects at lower heart rates.

2. INTRODUCTION

Increases in ventricular end-diastolic volume caused either by an increase in venous return or a rise in aortic resistance is followed by an increase in force of contraction. It allows the heart to maintain sufficient cardiac output even at elevated pre- and afterload and adapts the output of the right to the left ventricle. In-vitro, stretching cardiac muscle results in an immediate (Frank-Starling mechanism; FSM) followed by a delayed increase in developed force. The latter takes several minutes to develop fully and is thus termed slow force response (SFR). The SFR has been observed in various preparations (isolated myocytes, trabeculae, papillary muscles and whole hearts) of a variety of species including human myocardium (1-7).

The FSM is mediated by an increase in myofilament sensitivity for Ca^{2+} but no changes in Ca^{2+}-transients (8, 9). In contrast, a major part of the SFR is mediated by an increase in intracellular Ca^{2+} due to decreased forward and increased reverse mode of the sarcolemmal Na^+/Ca^{2+} exchanger (NCX). This shift in activity is caused by an increase in intracellular Na^+ due to stretch-dependent activation of the sarcolemmal Na^+/H^+ exchanger-1 (NHE1) (1, 2, 4, 10). Recently, various groups

including ours have investigated signal transduction mechanisms of the SFR. Despite these efforts, the initial part of the signal transduction pathway stimulating the NHE1 remains elusive. The SFR was related to a stretch-dependent autocrine/paracrine release of angiotensin II and endothelin-1 in isolated feline and rat heart muscle (10). However, this was not confirmed in all animal models or in failing human myocardium (1, 2, 6).

Though the SFR is recognized as an intrinsic mechanism of the heart to recruit additional force of contraction at elevated preload, the effect of heart rate on the SFR-dependent stroke work increase is completely unknown. Therefore, we hypothesized that stimulation rate modifies the SFR-related force increase in isolated human and rabbit cardiac muscle.

3. MATERIALS AND METHODS

Experiments were performed in 45 isolated muscle strips (trabeculae) from 18 end-stage failing hearts due to dilated cardiomyopathy (n=8) and ischemic cardiomyopathy (n=10) upon transplantation. The mean age of the heart failure patients was 53.6±3.1 years, 16 were male. The study protocol was approved by the local ethics committee and all patients gave informed consent.

Additional experiments were performed in 99 isolated right ventricular muscle strips from Chinchilla bastard rabbits. This study protocol was approved by the local ethics committee and conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health (NIH Publication No. 85-23, revised 1996).

Immediately after explantation, the heart was stored in ice cold cardioplegic Tyrode´s solution containing (in mmol/L): Na^+ 152, K^+ 3.6, Cl^- 135, HCO_3^- 25, Mg^{2+} 0.6, $H_2PO_4^-$ 1.3, SO_4^{2-} 0.6, Ca^{2+} 0.2, glucose 11.2, Insulin 10 I.U./L and 2,3-butanedione-monoxime (BDM) 30, equilibrated with carbogen (95% O_2, 5% CO_2) to a pH of 7.4 and transported to the laboratory. This solution has been shown to protect the myocardium during transportation and from cutting injury at the time of dissection with full reversibility of the cardioplegic effects upon washout. Small endocardial trabeculae (“muscle strips”, cross-sectional area <0.5 mm2) were dissected with the help of a stereo-microscope. All preparation steps were carried out in the cardioprotective solution, as previously described (11).

Muscles strips were mounted in special chambers between miniature hooks, connected to an isometric force transducer (Scientific Instruments, Germany) and superfused with modified Tyrode´s solution of the composition given above except that BDM was omitted and $(Ca^{2+})_o$ was stepwise increased to 2.5mmol/L. Isometric twitches were evoked through electrical stimulation with a stimulation voltage 25% above threshold (pulse duration 5 ms) at the preload at which maximum steady-state twitch force was achieved (L_{max}).

Experiments (37°C or 23°C, pH 7.4) were performed in ventricular papillary muscles or trabeculae (diameter 0.2-0.7 mm) stretched to L_{max}. Action potentials were recorded at 0.2, 0.5, 1, 2 and 3Hz using 3 M KCl-filled flexible microelectrodes of approximately 10-20 MΩ resistance (12). Impalement was facilitated by gently tapping the micromanipulator holding the electrode. During initial penetration the muscles were not stimulated. When resuming field stimulation stable action potential recordings could be obtained. To minimize stimulation artifacts, the pulse duration was 0.2-0.5 ms.

KB-R 7943 (Tocris, Ballwin, USA) was added from a 10 mmol/L stock (50% DMSO, 50% distilled water) to inhibit reverse mode NCX. HOE642 (Cariporide; Aventis Pharma, Frankfurt, Germany) was dissolved in distilled water to a stock concentration of 10 mmol/L to inhibit NHE1. All other drugs and compounds were of best analytical grade available.

Data are expressed as mean±SEM. Differences were compared by paired student-t-test or two-way ANOVA analysis where appropriate. Statistical significance was taken as p-value of <0.05

4. RESULTS

Stretching muscle strips from 88% (L_{88}) to 98% (L_{98}) of optimal length resulted in a characteristic force response with an immediate (FSM), followed by a delayed increase in developed force (SFR) within 5-10 min. This response was reproducible within the same muscle and we therefore compared changes in stimulation frequency in consecutive stretch protocols as presented in Figure 1A for failing human myocardium. The panel shows an original recording with stretch-dependent force response at the basal stimulation rate of 1 Hz and then, the stretch protocol was repeated at 0.2 Hz. As can be seen absolute developed force at L_{88} is smaller at 0.2 Hz but the relative force increase during SFR increases. Statistical analysis of experiments in failing human myocardium with either lower or higher stimulation frequencies (Figure 1B, upper panel) revealed a significantly larger SFR at 0.2 Hz (increase by 35.2±3.4%; n=14) at 0.2 Hz and a significantly smaller SFR at 2 Hz (increase by 4.4±2.2%; n=8) as compared to the SFR at 1 Hz (19.1±2.3% and 14.4±2.2%, respectively). These results were confirmed in rabbit myocardium at 0.2 and 3 Hz (Figure 1B, lower panel). Basal force of contraction decreased at 0.2 Hz (~60% vs. 1 Hz) and increased at 3 Hz (~70% vs. 1 Hz) due to the positive force-frequency relation in non-failing rabbit myocardium. In contrast, significantly larger SFR were observed at 0.2 Hz (increase by 28.6±2.5%; n=27) and significantly lower SFR at 3 Hz (increase by 10.4±2.4%; n=16) as compared to the SFR at 1 Hz (19.1±1.4% and 22.9±2.3%, respectively).

To test for frequency-dependent modification in signal transduction underlying the SFR we blocked NCX reverse-mode using 5µmol/L KB-R7943 at different stimulation frequencies (0.2, 1 and 3 Hz in rabbit

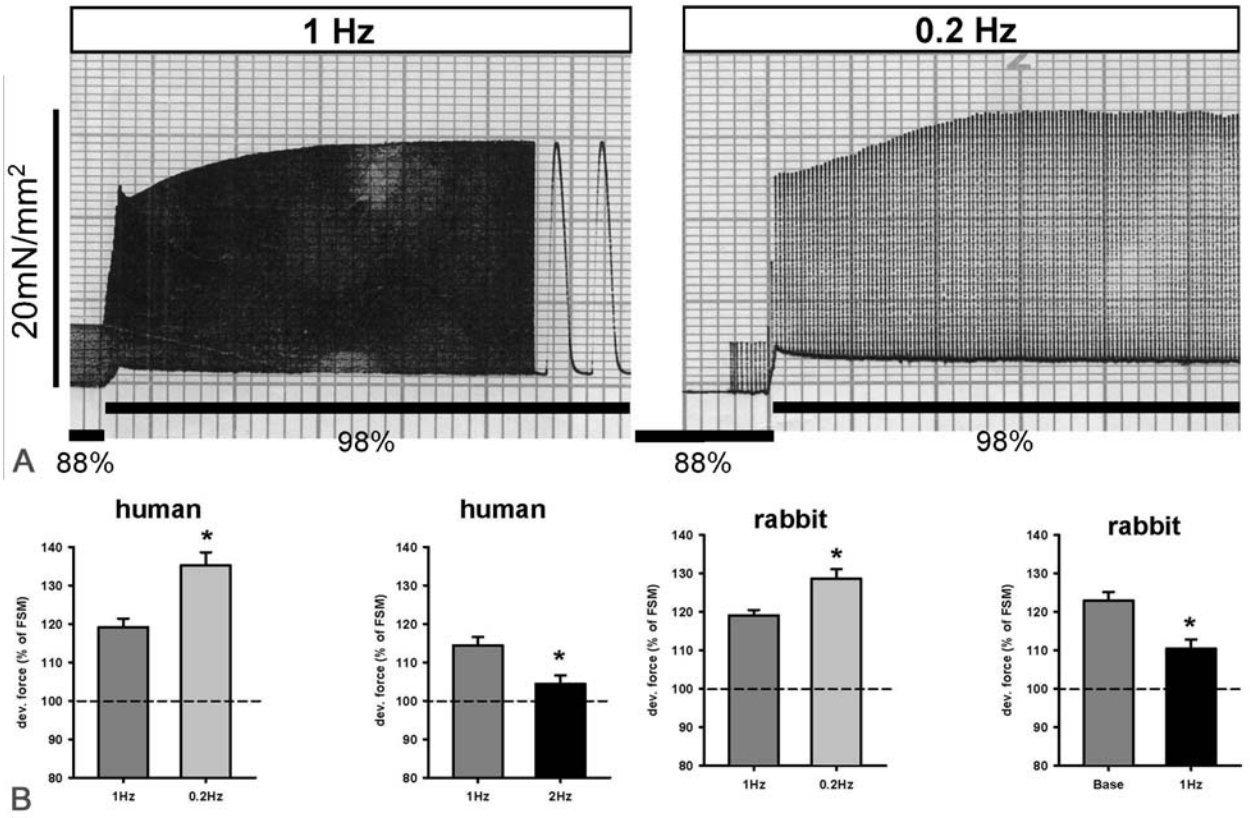

Figure 1. A: Original recording of the stretch protocol at 1 Hz and after reducing stimulation rate to 0.2 Hz. The two consecutive stretch protocols were performed within the same muscle strip. B: Statistic analysis of varying stimulation rates in human (upper graphs) and rabbit (lower graphs) muscle strips. The right graphs depict reduced stimulation rates (0.2 Hz; human n=14, rabbit n=27), the left graphs show the effect of increased stimulation rates (2 Hz in human – n=8 - and 3 Hz in rabbit – n=16 - myocardium). * = $p<0.05$ vs. 1 Hz.

myocardium). Figure 2 summarizes these experiments. As described in Figure 1, the increase in force during the SFR was largest at low stimulation rates. Inhibition of reverse-mode NCX by KB-R7943 reduced the SFR by ~35-50% at each stimulation rate.

We also tested the effect of NHE1-inhibition by HOE642 on the SFR at different stimulation frequencies. Experiments were performed in failing human myocardium and are summarized in Figure 3. Again, the SFR was largest at 0.2 Hz and declined with higher stimulation rates. HOE642 reduced the SFR at each stimulation rate (by ~30-40%): from 132±5% to 121±6% (n=5 from 4 hearts; p=0.09) at 0.2 Hz, 123±2% to 117±2% (n=7 from 6 hearts; $p<0.05$) at 1 Hz and 107±1% to 104±1% (n=11 from 4 hearts, p=0.053) at 2 Hz.

HOE642 did not significantly alter basal force of contraction at L_{88} at each stimulation rate (8±4%, 6±4% and 7±5%, respectively; all n.s.).

To test for a potential relationship between action potential duration, force-frequency response, and the SFR, we simultaneously assessed APs and twitch force in isolated rabbit trabeculae at increasing stimulation rates (0.2 – 3 Hz). Figure 4A shows typical recordings of APs (left) and isometric twitches (right) in one muscle strip. As can be seen, APD showed a biphasic response: It was shortest at 0.2 Hz and increased up to 1 Hz, but shortened again at stimulation rates > 1 Hz. In contrast, there was a continuous increase in twitch force with increasing stimulation rates.

APD50, in average, was shortest at 0.2 Hz (54±6 ms) and tended to increase up to 1 Hz (82±16 ms). At stimulation rates > 1 Hz, APD50 became again shorter (63±2 ms at 3 Hz). Data for APD90 (Figure 4B, right) showed a similar, stimulation-rate dependent behavior.

5. DISCUSSION

1) The SFR is frequency dependent. Its magnitude decreases with increasing stimulation frequencies. 2) NCX "reverse-mode" and NHE1 contribute to the SFR at all frequencies. 3) APD at L_{max} does not correlate with SFR amplitude.

A biphasic response to stretch was first demonstrated in cat papillary muscle (13). In more detailed analyses the initial response to stretch (Frank-Starling

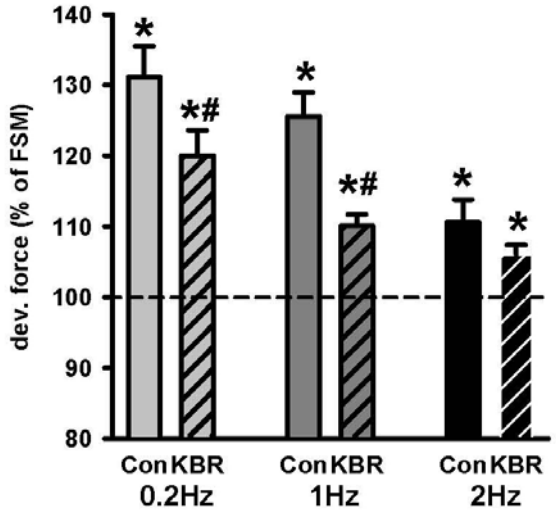

Figure 2. Effect of NCX reverse mode inhibition with KB-R7943 at varying stimulation rates in rabbit myocardium. Controls were performed using the same muscle strip. SFR was significantly reduced from 131±4 to 120±4% (n=10) at 0.2 Hz, 126±3 to 110±2% (n=9) and 111±3 to 106±1% (n=8) n=9 at 3Hz. * = $p<0.05$ vs. developed force at FSM, # = $p<0.05$ vs. 1 Hz.

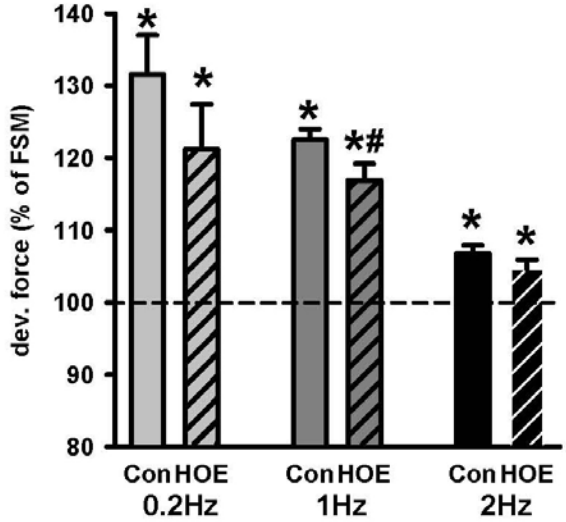

Figure 3. Effect of NHE1 inhibition with HOE642 at varying stimulation rates in human myocardium. Controls were performed using the same muscle strip. n=5 at 0.2Hz, n=7 at 1Hz and n=11 at 2Hz * = $p<0.05$ vs. developed force at FSM, # = $p<0.05$ vs. 1 Hz.

mechanism) was shown to be independent from increases in $(Ca^{2+})_i$ and was related to enhanced responsiveness of the myofilaments for Ca^{2+} (14). In contrast, Allen et al. (15) and others (7, 16) demonstrated slowly rising Ca^{2+} transients as the underlying mechanism for the delayed inotropic response to stretch, but the source of elevated $(Ca^{2+})_i$ remained obscure. During the last years, the subcellular mechanism for the delayed inotropic response to stretch was partially elucidated: Stimulation of the NHE1 with subsequent elevation of $(Na^+)_i$ shifts the NCX activity towards more reverse mode, resulting in elevated Ca^{2+}-transients. SR-Ca^{2+} load increases during the SFR (2), however this mechanism is not a prerequisite for the SFR which is still present after functional inhibition of the SR using thapsigargin and ryanodine (4). The mechanism stimulating the NHE1 is controversial. Using rat and feline myocardium, Cingolani and coworkers reported a stretch-dependent autocrine/paracrine stimulation of angiotensin II and endothelin-1 receptors with subsequent activation of NHE1 (10). In other species, including failing human myocardium, this autocrine/paracrine mode of action could not be confirmed (1, 2).

Early work on the SFR produced evidence that the magnitude of the SFR (relative to the FSM) was dependent upon the level of muscle activation before stretch (13, 17). This concept may give a unifying mechanism for interventions that modulate the amplitude of the SFR and is further supported by our own observation that alpha- and beta-adrenoceptor blockade before stretch potentiates the SFR (unpublished data). It is also supported by a larger SFR at lower stimulation rates in single guinea pig myocytes (18), which show a positive force-frequency relationship, and at higher stimulation rates in rat myocytes (19), which have a negative force-frequency relation. However the "activation concept" may not be the sole predictor of the magnitude of the SFR, since prestimulation with the Na^+/K^+-pump inhibitor strophanthidin (2) or p38MAPK-inhibition using SB203580 (own unpublished data) increased basal force of contraction but did potentiate the SFR after stretch. In addition, the activation concept is also not supported by our data in failing human myocardium (with blunted or negative force-frequency relation), where SFR was maximum at low stimulation rates.

To maintain Ca^{2+}-balance at steady state, cellular Ca^{2+} extrusion via forward mode NCX must match Ca^{2+}-entry via L-type Ca^{2+}-channels and reverse-mode NCX. It can therefore be stated that blocking the reverse mode NCX using KB-R7943 results in a reduction of $(Ca^{2+})_i$ unless L-type Ca^{2+}-current increase or forward mode NCX declines to the same extend. KB-R7943 predominantly blocks NCX reverse mode and has little or no effect on the L-type Ca^{2+}-current (20). It therefore mediates negative inotropic effects in myocardial tissue. In addition, SFR has been shown to be largely mediated by reverse-mode NCX in human and rabbit myocardium (1, 2) as well as in further species (4, 7, 21). It could therefore be speculated that changes in SFR amplitude as seen for bradycardia and tachycardia are mediated by altered NCX function. However, SFR inhibition was comparable at all frequencies tested in this study. Therefore, frequency-dependent changes in (reverse-mode) NCX activity do not underlie frequency-dependency of the SFR.

The sodium dependent transporter, Na^+/H^+-exchanger1 (NHE1) has been shown to be critically involved in mediating the SFR in various species (1, 2, 4, 22). In addition, cariporide, a potent NHE1-inhibitor was capable to restore the negative force-frequency relation in failing rabbit myocardium between 1 and 3 Hz (23),

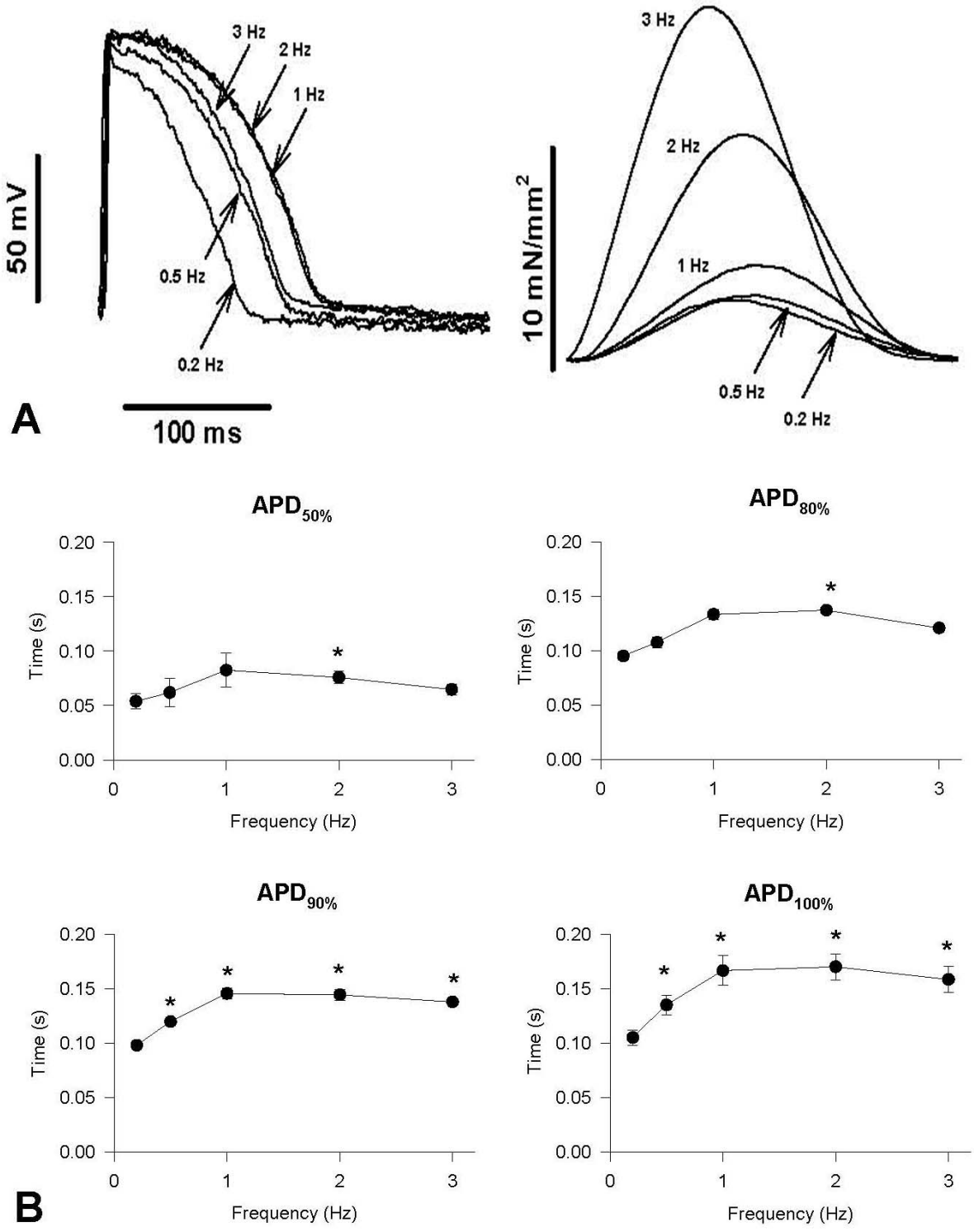

Figure 4. Alteration of action potential duration at varying stimulation rates. A: Original recording of action potential in a rabbit right ventricular muscle strip. Maximum APD is exerted at 1 Hz. APD decreases at lower and at higher stimulation rates. B: Statistic analysis of APD kinetics (n=4). APD_{50} (left), APD_{90} (right) are shown. * = $p<0.05$ vs. 0.2 Hz

indicating a frequency-dependent mode of action which could explain the changes in SFR due to bradycardia and tachycardia in this study. In the above mentioned study, normalisation of the force-frequency-relation (FFR) was

due to a significant reduction of twitch force at 1 Hz, which was not observed at higher stimulation frequencies. In the present study, a significant inhibition of the SFR could only be observed at 1 Hz whereas comparable decreases at 0.2 Hz and 2 Hz did not reach significance, although relative decreases in SFR magnitude have even been stronger at these frequencies. Since basal effects of HOE642 on developed force were comparable at all frequencies tested, contrasting the data in failing rabbit myocardium, and relative inhibition of SFR even tended to be larger at low and higher stimulation frequencies compared to 1 Hz failed significance in theses protocols might be due to data variability and the small control SFR at 2 Hz.

The level of intracellular Na^+ determines frequency-dependent alterations in contractility (24). The elevated basal $(Na^+)_i$ in failing myocardium partially explains the negative force-frequency relation in this tissue due to Na^+-mediated high loading of the SR at low frequencies. Increases in $(Na^+)_i$ result in lesser inotropic effect in failing myocardium than in controls (11, 24), which could either be due to the elevated basal levels of $(Na^+)_i$ in failing myocardium or due to altered sodium-contraction coupling downstream of the initial increase in $(Na^+)_i$. Our data is in line with this observation as basal force decreased in failing human and increased in non-failing rabbit myocardium with higher stimulation frequencies. It could be assumed that the SFR as another positive inotropic effect is reduced in failing human myocardium compared to the non-failing rabbit myocardium, characterized by much lower basal $(Na^+)_i$. However, as can be seen from Figure 1B as well as in recent publications (1, 2) SFR-amplitude is comparable between the two groups indicating an independence of the SFR from basal $(Na^+)_i$ at least in the physiological and pathophysiological range of non-failing and failing myocardium.

$(Na^+)_i$ has also been shown to increase with the stimulation frequency. These alterations are much more acute than with the development of disease and it can be seen from Figure 1B that SFR amplitude significantly drops from 35.2±3.4% and 28.6±2.5% at 0.2 Hz to 4.4±2.2% and 10.4±2.4% at 2 and 3 Hz in human and rabbit myocardium, respectively. These reductions in SFR support the notion that acute but not chronic changes in $(Na^+)_i$ influence the SFR. Although comparable, the frequency-dependent decrease seems to be lesser in non failing rabbit myocardium, and this could still be due to lower basal $(Na^+)_i$ which would result in stronger relative changes at a given increase in sodium after stretch.

Besides regulating other Ca^{2+}-transporting proteins such as ryanodine receptors (25, 26) and L-type Ca^{2+}-channel facilitation (27), cardiac Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) has been implicated in enhanced SR Ca^{2+}-release and frequency-dependent acceleration of relaxation (FDAR) via enhanced SR Ca^{2+}-uptake (28). Although not directly tested in this study, rate-dependent CaMKII activation might contribute to the effects of stimulation rate on SFR. However, since CaMKII activity is increased at higher intracellular Ca^{2+}-concentrations (such as high stimulation rates) one would expect that CaMKII effects on SFR are more pronounced at high (e.g. 2 Hz) as compared to low stimulation rates (e.g. 0.2 Hz). This was apparently not the case in the current study (29).

The slow (I_{Ks}) and rapid (I_{Kr}) delayed rectifier currents play a crucial role for repolarisation of the action potential in cardiac myocytes. Potassium channels KCNQ1 and HERG1 mediate I_{Ks} and I_{Kr}, respectively. It has been shown that both channels are modulated by stimulation frequency and could therefore contribute to changes in APD. However, effects are difficult to predict as current density of the channels is regulated differentially with increased current density of KCNQ1 at higher stimulation frequencies but reduced current density of HERG1 channels (30).

Stretch itself has been described to exert both AP prolongation (12) and AP shortening (31, 32) in mammalian myocardium. A prolongation of APD might underlie or enhance the SFR since prolonged depolaristaion favors reverse-mode NCX Ca^{2+}-entry due to a decrease in driving force to extrude Ca^{2+} from the cytosol during the prolonged plateau of the action potential. In addition, the efflux of Ca^{2+} via the NCX is associated with an inward current that will again tend to prolong the APD (see review (33)). However, these effects do not play a major role in our model as we did not observe any effect of stretch on APD or resting cell membrane potential in isolated rabbit trabeculae in a recent publication (2) and this is in line with previous work in guinea pig myocytes (18) isolated sheep purkinje fibers (34) and Langendorff perfused canine hearts (35).

Although in the present study, action potential duration was reduced at low stimulation rates and tended to decrease at higher stimulation rates compared to 1Hz, these changes did not correlate with the steadily declining SFR amplitude at higher stimulation rates and therefore do not seem to be of particular relevance in mediating frequency-dependent alterations in SFR.

In conclusion, we demonstrated for the first time a frequency-dependence of the SFR in failing human and in non-failing rabbit myocardium with increases in SFR magnitude at lower stimulation frequencies. This increase is associated with a greater increase in Ca^{2+}-transients after stretch at lower stimulation frequencies. APD does not play a significant role in frequency-dependent SFR modulation. Signal transduction is not fundamentally altered. The SFR might be of physiological relevance during hemodynamic overload in species with low heart rates such as humans.

6. ACKNOWLEDGEMENT

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