

Stretch-induced phosphorylation of MAPK and p90^{RSK} in human myocardium

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1. ABSTRACT

Stretch activates various signal transduction pathways including mitogen-activated protein kinases (MAPK). Stretch-induced phosphorylation of MAPK-contribution to contractility in human myocardium is unknown. We tested the effects of stretch on p44/42-, p38-MAPK and p90^{RSK} phosphorylation and the functional relevance for force development in failing (F) and non-failing (NF) human myocardium. Trabeculae were stretched to a diastolic tension of 12mN/mm² for 2.5 to 30 minutes and frozen for Western Blot analysis. Stretch induced a time-dependent increase in phosphorylation of p44/42-, p38-MAPK and p90^{RSK}. For functional analysis, trabeculae from F myocardium were stretched and the immediate (Frank-Starling mechanism; FSM) and delayed (slow force response; SFR) increase in twitch force was assessed before and after blocking the activation of p44/42-MAPK (30 μmol/L U0126) and p38-MAPK (10 μmol/L SB203580). Inhibition of p44/42-MAPK almost completely blocked the SFR (106.7±3.7% vs. 125.4±2.9%), while p38-MAPK-blockade significantly increased the SFR (124.6±1.9% vs. 121.2±2.2%). Stretch induced a time-dependent increase in p44/42-, p38-MAPK and p90^{RSK} phosphorylation in F and NF myocardium. While p44/42-MAPK phosphorylation contributed to the SFR, p38-MAPK activation antagonized the stretch-induced SFR.

2. INTRODUCTION

Stretch is an important regulator of myocardial function. It mediates functional (force development) and trophic (gene expression, protein synthesis) effects via various signal transduction pathways. Changes in force development after acute stretch occur in two phases. First, there is an immediate increase in force (Frank-Starling-mechanism; FSM) followed by a further slow increase in force (slow force response; SFR). While the FSM is based on an increase in myofilament sensitivity for intracellular ([Ca²⁺]_i) (1), the SFR may be associated with an increase in the magnitude of [Ca²⁺]_i transients (2). The mechanisms underlying the increase in [Ca²⁺]_i and the SFR after stretch are not completely understood. Previous studies have shown, that stretch leads to an increase in intracellular Na⁺ ([Na⁺]_i) via stretch activated channels (SAC) or the Na⁺/H⁺ exchanger (NHE), followed by an increase in [Ca²⁺]_i via the Na⁺/Ca²⁺ exchanger (NCX) in its reverse mode (2-8). Furthermore, recently it was shown that stretch activates the NHE1 via p44/42-MAPK/p90^{RSK} phosphorylation and the SFR is inhibited by blockade of MEK1/2, the upstream kinase of p44/42-MAPK and p90^{RSK} (9). This signal transduction cascade, however, was initiated by angiotensin receptor activation, which does not play a role in mediating stretch induced functional effects in human ventricular myocardium (5, 7). In contrast Angiotensin II and

Stretch and MAPK in human myocardium

Endothelin-1 play a role in SFR in human atrial myocardium but without resulting activation of NHE1 and NCX (7). In a small series of patients with and without left ventricular assist devices (LVAD) p44/42-MAPK was reduced after unloading the ventricle whereas p38MAPK was significantly increased (10). Due to tissue specific differences and only very little and indirect data in ventricular human myocardium it is unknown, whether p44/42-, p38-MAPK and p90^{rk} also contribute directly to the functional effects of stretch in human myocardium with consecutive clinical impact.

Various studies have shown, that stretch mediates activation of transcription factors and early genes via multiple signal transduction pathways. Through direct activation of signalling molecules or by inducing neurohumoral factors like Angiotensin II (11) or Endothelin-1 (7, 12). Stretch is converted into intracellular growth signals, resulting in hypertrophy (13, 14). Among the main mediators of stretch-induced hypertrophy are the mitogen-activated protein kinases (MAPK) (12, 14). MAPK are ubiquitous kinases in eukaryotic organisms and control gene transcription via phosphorylation of nuclear substrates (like c-myc, c-jun, ATF-2) and other kinases, like p90^{rk} or MAPKAPK2 (14). Stretch-induced phosphorylation of p44/42-MAPK and its downstream effector p90^{rk} as well as p38-MAPK and JNK1 have been observed in neonatal and adult cardiac myocytes, trabeculae and whole hearts of various animal species (9, 12, 15-19). Therefore, MAPK activation may be also involved in the trophic effects of stretch in mammalian myocardium.

Therefore, we tested, for the first time in human failing and non-failing myocardium, the hypothesis that stretch activates p44/42-, p38-MAPK and p90^{rk} in human myocardium and that stretch-induced phosphorylation of these kinases is directly involved in the changes in force development following acute stretch.

3. MATERIALS AND METHODS

Ventricular trabeculae (n=125) were obtained from 6 non-failing hearts, that could not be transplanted for technical reasons, and 18 end-stage failing hearts due to ischemic (n=5) or dilated cardiomyopathy (n=13). The mean age of the donor heart patients was 33±8 years, 50% were female. The mean age of the heart failure patients was 55±4 years, one of them was female. The mean ejection fraction of failing hearts was 28.8±3.5%, mean cardiac index was 2.1±0.1L/min/m² and mean pulmonary capillary wedge pressure was 19.4±2.1mmHg. The study protocol was approved by the local ethics committee, and the transplant patients gave informed written consent. The investigation conforms to the principles outlined in the Declaration of Helsinki.

3.1. Muscle strip preparation

Immediately after explantation, the heart was stored in ice cold cardioplegic Tyrode's solution equilibrated with carbogen to a pH of 7.4. Small endocardial trabeculae ("muscle strips", cross-sectional area <0.8mm²) were dissected under a stereo-microscope. Muscles strips were mounted in special chambers between miniature hooks,

connected to an isometric force transducer (Scientific Instruments, Germany) and superfused with non-cardioplegic Tyrode's solution. Isometric twitches were evoked through electrical stimulation as described previously (5, 6, 20).

3.2. Experimental protocol

For western blot analysis trabeculae at steady-state contractile conditions were acutely stretched to a diastolic tension of 12 mN/mm². Each trabecula was shock frozen at a particular time (2.5, 5, 10, 15 or 30minutes) after stretch (unrepeated measurements). Control trabeculae were electrically stimulated but remained non-stretched. For functional experiments, trabeculae were gradually stretched to the length at which maximal force development was observed (L_{max}). Afterwards, length was reduced to 88% of L_{max} (L₈₈). After 30 minutes, trabeculae were stretched acutely to 98% of L_{max} (L₉₈). Subsequently, a biphasic increase in developed force was observed, the FSM and the SFR, respectively. To assess the involvement of p44/42- and p38-MAPK, 30 μmol/L U0126 (Calbiochem, 10⁻²mol/L stock solution in DMSO), an inhibitor of MEK1/2 and 10 μmol/L SB203580 (Calbiochem, 10⁻²mol/L stock solution in DMSO), an inhibitor of p38-MAPK were used. All experiments involving U0126 and SB203580 included paired stretch protocols. First, a control stretch protocol without inhibitors was conducted followed by a second stretch protocol in the presence of the respective inhibitor.

3.3. Western blots

Trabeculae were shock frozen in liquid nitrogen and homogenized with homogenization buffer (1% NP40, 10% glycerol, 137mmol/L NaCl, 20mmol/L Tris-HCl, 20mmol/L NaF, 1mmol/L Na₃VO₄, 1mmol/L sodium pyrophosphate, 50mmol/L β-glycerophosphate, 1mmol/L Ethylenedioxy-bis-(ethylenitrilo)-tetraessigsäure (EGTA), 10mmol/L Ethylenediaminetetraessigsäure (EDTA), 1mmol/L Phenylmethylsulfonylfluorid (PMSF), 4μg/ml Aprotinin, 4μg/ml Leupeptin and 4μg/ml Pepstatin-A). The homogenates were centrifuged and protein concentrations in the supernatant were determined using the BCA Protein Assay (Pierce, Rockford, IL, USA). Protein samples were separated using SDS-Page and transferred to nitrocellulose membranes. To examine the protein expression and the phosphorylation of p44/42-MAPK, p38-MAPK and p90^{rk} the membranes were incubated overnight with protein- and phosphoprotein-specific antibodies, followed by incubation for 1h at room temperature with secondary antibody conjugated with horse-radish peroxidase. Immunoreactive bands were visualized by chemoluminescence. In each trabecula we determined basal expression and the phosphorylation state of the respective kinases. The degree of kinase phosphorylation was defined as the ratio of phosphorylated protein to total protein. Non-stretched trabeculae from the same hearts were measured in parallel and served as controls. The stretch-induced alteration in phosphorylation was determined by normalization to the basal phosphorylation state of non-stretched trabeculae.

3.4. Statistical analysis

Data are presented as mean±standard error of the mean (S.E.M.). Difference in time-dependent changes of

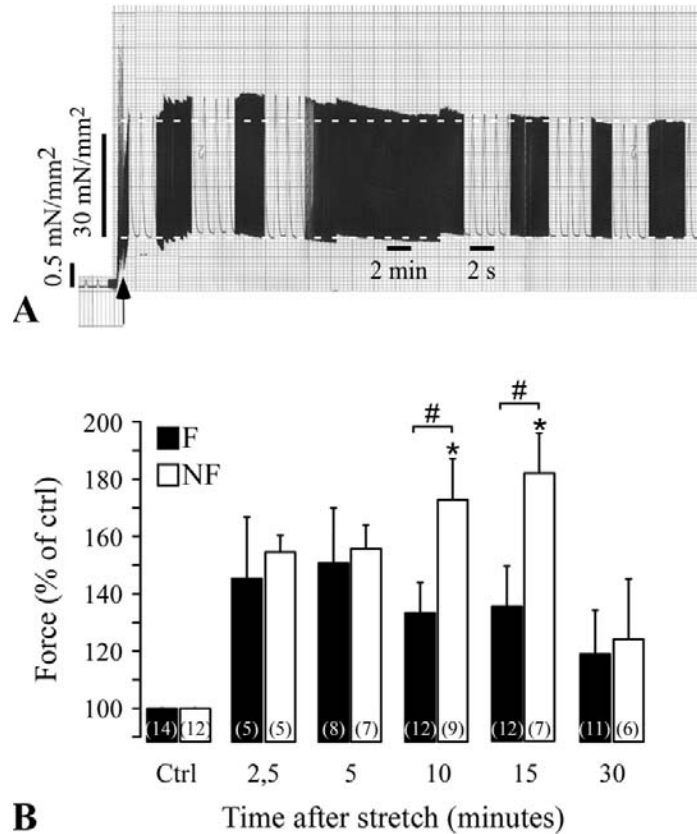


Figure 1. A, Original traces of isometric contractions of a human ventricular trabecula before and during acute stretch. B, Average values (n = 108 trabeculae) of developed force at different time points after acute stretch. */#, $p < 0.05$ vs. control/F vs. NF.

twitch force or protein phosphorylation was tested by a 2-way ANOVA for unrepeated measurements, accounting for nonfailing/failing myocardium. Individual mean values were compared by Tukey post hoc test. Differences were considered statistically significant at $p < 0.05$.

4. RESULTS

4.1. Functional effects of stretch

To measure the effect of acute stretch on the phosphorylation state of p44/42-, p38-MAPK and p90^{rk}, non-stretched trabeculae from failing (F) and non-failing (NF) human myocardium were acutely stretched (within 30 sec) to a diastolic tension of 12mN/mm². Force development was recorded and the each trabeculae was shock frozen at a particular time (2.5, 5, 10, 15 or 30minutes) after stretch for western blot analyses. Figure 1A shows original traces of a muscle strip from a failing human myocardium subjected to this stretch protocol. First, the isometric contractions of the non-stretched muscle strip are shown. Then, the muscle strip was acutely stretched from slack length to a diastolic tension of 12mN/mm². As a result developed force increased immediately (within < 10 beats, FSM) from 0.2 to 26.5mN/mm². During the following minutes, there was an additional increase in developed force to a maximum of 38.8mN/mm² after ~7.5 minutes (SFR). Thirty minutes after acute stretch developed

force amounted to 29.3mN/mm². In the following, we focused on the stretch-induced changes during the SFR. Therefore, changes in twitch force after stretch will be presented as changes normalized to the first inotropic phase (i.e. the FSM) after stretch. Figure 1B shows average data of stretch-induced changes in SFR of trabeculae from failing and non-failing human hearts. Data are normalized to the developed force immediately following stretch (NF: $21.1 \pm 2.5 \text{ mN/mm}^2 = 100\%$, F: $33.2 \pm 3.4 \text{ mN/mm}^2 = 100\%$). The stretch-induced SFR was present in failing and non-failing human myocardium, but the time course was different. In failing human trabeculae the maximum of SFR occurred after 5 min and amounted to $150.7 \pm 19.3\%$ (n=8). After 30 min, developed force had declined to $118.9 \pm 15.2\%$ of the FSM (n=11). In non-failing trabeculae, the SFR was maximal after 15 min. It amounted to $181.8 \pm 14.0\%$ of the FSM (n=7; * $p < 0.05$) and was significantly larger than in failing trabeculae (# $p < 0.05$). Thirty minutes after acute stretch, developed force had decreased to $124.2 \pm 21.1\%$ (n=6).

4.2. Stretch-induced phosphorylation of MAPK and p90^{rk}

To elucidate potential subcellular signal transduction pathways underlying stretch-dependent force development, we directly assessed activation of MAP kinases by Western blot techniques.

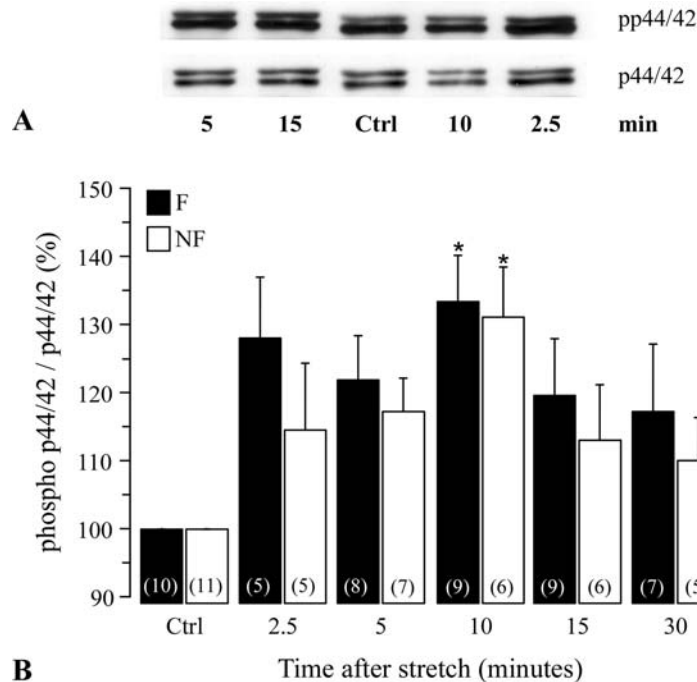


Figure 2. Stretch-dependent p44/42-MAPK phosphorylation in human failing and non-failing myocardium. A, Immunoblots of phosphorylated (pp, top) and total (p, bottom) p44/42-MAPK from human failing ventricular trabeculae. Except for the non-stretched control trabecula (Ctrl), trabeculae were acutely stretched for 2.5, 5, 10 or 15 min. B, Mean ratio of pp44/42-MAPK to p44/42-MAPK in stretched and non-stretched trabeculae in failing (F, black bars) and non-failing (NF, white bars) human myocardium (* $p < 0.05$ vs. control). (NF: white bars; F: black bars; numbers of experiments are indicated in parenthesis).

Figure 2A shows typical western blot experiments for p44/42-MAPK (phosphorylated (pp44/42) and total (p44/42) protein) from failing human trabeculae. In the non-stretched control muscle strip (Ctrl) there was a detectable basal phosphorylation of p44/42-MAPK. In this experiment, four other trabeculae from the same heart were stretched for 2.5, 5, 10 and 15 minutes. Stretch induced an increase in phosphorylation of p44/42-MAPK to 120-130% after 10-15 minutes. The mean values for failing and non-failing human myocardium are shown in Figure 2B. In failing ($n=48$) and non-failing ($n=40$) human myocardium stretch induced a time-dependent increase in p44/42-MAPK phosphorylation. At 2.5 min after stretch there was detectable increase in p44/42-MAPK phosphorylation (NF: $114.6 \pm 9.8\%$, n.s. vs. Ctrl; F: $128.1 \pm 8.9\%$), which reached a maximum after 10 min (NF: $131.1 \pm 7.3\%$, $p < 0.05$; F: $133.4 \pm 6.9\%$, $p < 0.05$). In the subsequent 20 min, there was a reduction of the p44/42-MAPK phosphorylation. There was no significant difference in the magnitude and time course of the stretch-induced p44/42-MAPK phosphorylation between failing and non-failing human myocardium. Hence, the stretch-dependent increase in p44/42 phosphorylation was transient with an increase during the first 10 min after stretch and a slight decline from maximum values at time points > 10 min.

p90^{rsk} colocalizes with p44/42-MAPK and can be phosphorylated and thereby activated by this kinase at Thr⁵⁷³. Therefore, we analyzed the time-dependent phosphorylation of p90^{rsk} after acute stretch in failing

($n=42$) and non-failing ($n=31$) human trabeculae. Figure 3A illustrates the stretch-dependent phosphorylation of p90^{rsk} in failing human trabeculae. Compared to non-stretched controls, stretched trabeculae exhibited an increase in phosphorylation of p90^{rsk}. Figure 3B shows average data for time-dependent p90^{rsk} phosphorylation after stretch. As with p44/42-MAPK, phosphorylation already increased 2.5 min after stretch (NF: $131.1 \pm 23.5\%$, n.s. vs. Ctrl; F: $120.7 \pm 8.8\%$, n.s.). The maximal stretch-dependent phosphorylation of p90^{rsk} was reached after 10 min stretch and was significantly higher in NF than F myocardium (NF: $186.4 \pm 34.3\%$, $p < 0.05$; F: $135.9 \pm 8.8\%$, n.s.; NF vs. F $\# p < 0.05$). As with p44/42-MAPK, the time course of stretch-dependent p90^{rsk} phosphorylation was transient and declined after > 10 min of stretch.

Next, we measured the effect of stretch on p38-MAPK phosphorylation. As evident from the original western blot (Figure 4A) as well as the average data (Figure 4B, F: $n=44$; NF: $n=29$) stretch also induced a time-dependent increase in p38-MAPK phosphorylation. In failing human myocardium the time course was comparable to p90^{rsk} and p44/42-MAPK. Immediately after stretch there was a significant increase in p38-MAPK phosphorylation (2.5 min: $131.6 \pm 11.4\%$, $p < 0.05$ vs. Ctrl). The increase in phosphorylation was slower than for p44/42-MAPK and p90^{rsk} and reached its maximum after 15 min ($175.1 \pm 20.6\%$, n.s.), followed by a gradual decrease in p38-MAPK phosphorylation. 30 minutes after stretch the phosphorylation state amounted to $135.2 \pm 7.3\%$ (n.s.). In

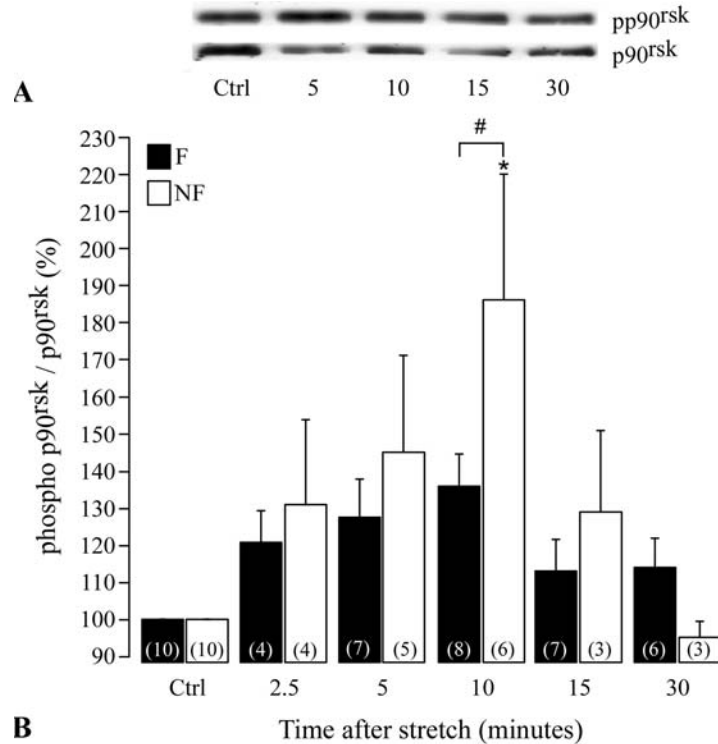


Figure 3. Stretch-dependent p90^{rsk} phosphorylation in human failing and non-failing myocardium. A, Immunoblots of phosphorylated (pp, top) and total (p, bottom) p90^{rsk} from human failing ventricular trabeculae. Except for the control trabecula (Ctrl), all trabeculae were acutely stretched for 5, 10, 15 or 30min. B, Mean ratio of pp90^{rsk} to p90^{rsk} in stretched and non-stretched trabeculae in failing (F) and non-failing (NF) human myocardium (*/# p<0.05 vs. control/F vs. NF, numbers of experiments are indicated in parenthesis).

contrast, trabeculae from non-failing human myocardium responded to acute stretch with an immediate strong increase in p38-MAPK phosphorylation (2.5min: 246.6±32.9%, * p<0.05). This difference was also significantly larger than in failing human myocardium (# p<0.05). The phosphorylation state tended to decrease slightly over the next 20-25 minutes, but was significantly higher than basal p38-MAPK phosphorylation in non-stretched trabeculae (15min: 193.9±36.5%, p<0.05; 30 min: 168.3±24.1%, n.s.).

4.3. Functional relevance of phosphorylation for stretched-induced force alterations

The data shown above indicate that stretch of human myocardium increases phosphorylation of p44/42-, p38-MAPK and p90^{rsk} and elicits a SFR with a similar time course. This may suggest, that activation of these kinases is involved in the functional effects of prolonged stretch. To examine the involvement of these kinase in stretch-induced increases in force development, we employed a stretch protocol previously established to investigate subcellular mechanisms of the immediate and slow force response(5, 6, 20). These experiments were conducted in 17 trabeculae from 9 failing human hearts. Figure 5 illustrates an original recording (A) as well as average results (B) obtained with U0126, a MEK1/2 inhibitor that suppresses activation of p44/42-MAPK and p90^{rsk}. In this experiment (Figure 5A) U0126 markedly suppressed the SFR (108,3%) compared

to the control SFR (137,5%). On average (Figure 5B) U0126 did not affect the basal force development at L₈₈ (U0126: 111.3±15.9% vs control (left)) and the FSM (U0126: 216.1±12.8% vs control: 222.5±10.4% (middle)), but it almost completely reduced the stretch-induced SFR (102.7±4.0%) compared to control (124.0±3.0%, n=10, p<0.01, right).

We also tested the involvement of p38-MAPK in the stretch-induced increase in force development, using the specific p38-MAPK inhibitor SB203580. As evident from the original recording (Figure 6A) as well as from the mean data (Figure 6B, left) SB203580 by itself had a slight positive inotropic effect on basal force development at L₈₈ to 111.9±5.3% of control basal force (n=7, p<0.05). In addition, it significantly prolonged relaxation (RT50 increased from 148±10 to 163±12ms (p<0.05) and RT90 increased from 277±20 to 306±24ms (p<0.05), data are not shown). Inhibition of p38-MAPK had no effect on the FSM (SB203580: 199.4±11.3% vs control: 201.0±9.7%) (Figure 6B, middle), but it significantly augmented the SFR to 124.6±1.9%, n=7, p<0.05) compared to control (121.2±2.2%) (Figure 6B, right).

5. DISCUSSION

MAPK are important mediators of the trophic effects of mechanical stress in the heart (13, 14). Studies on

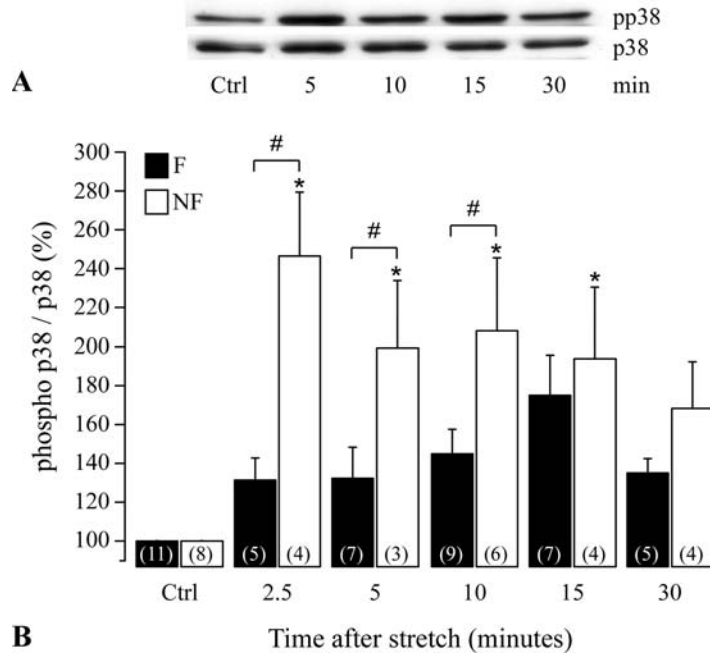


Figure 4. Stretch-dependent p38-MAPK phosphorylation in human failing and non-failing myocardium. A, Immunoblots of phosphorylated (pp, top) and total (p, bottom) p38-MAPK from human failing ventricular trabeculae. Except for the control trabecula (Ctrl), all trabeculae were acutely stretched for 5, 10, 15 or 30min. B, Mean ratio of pp38-MAPK to p38-MAPK in stretched and non-stretched trabeculae in failing (F) and non-failing (NF) human myocardium (*[#] p<0.05 vs. control/F vs. NF, numbers of experiments are indicated in parenthesis).

various animal species have shown that stretch activates MAPK and first small series in patients support these findings (10). In the present study we could show, for the first time, that p44/42-, p38-MAPK and p90^{rsk} are phosphorylated by stretch in a transient time-dependent manner in both failing and non-failing human myocardium and that activation of these kinases directly affects the functional effects of stretch on cardiac contractility.

In various animal species MAPK and p90^{rsk} are activated by mechanical stress. In isolated neonatal and adult myocytes as well as in isolated or perfused hearts mechanical stress induces a time-dependent increase in phosphorylation of p44/42-MAPK (15, 19), p38-MAPK (15, 21) and p90^{rsk} (19, 22). In a small set of patients with or without LVAD these findings could be confirmed for p44/42-MAPK but levels of p38-MAPK were significantly lower in patients with unloaded ventricles (10) whereas Haq *et al.* (23) reported an increase of all MAPK (including p38MAPK) in failing human hearts and Lemke *et al.* (24) demonstrated reduced p38-MAPK-activity in patients with end stage heart failure. It is well recognized that these kinases mediate the trophic effects of mechanical stress by phosphorylation/activation of transcription factors and early genes, which leads to hypertrophy and heart failure. Despite extensive evidence for the involvement of MAPK and p90^{rsk} in stress-induced hypertrophy in animal hearts, however, much less and conflicting data is published about the role of these kinases for the trophic response to mechanical stress in human myocardium and no data are available on stretch induced functional effects.

Furthermore, there are no data available comparing stretch-induced activation of MAPK and p90^{rsk} in failing versus non-failing human myocardium. To investigate these issues, we have established an experimental model that enabled us to apply defined stretch to multicellular human trabeculae. A further advantage of this model is that it allowed us to combine functional data (force development) with molecular biology data (phosphorylation of MAPK and p90^{rsk}) from the same preparation. The results revealed that stretch induces a transient time-dependent increase in p44/42-MAPK and p90^{rsk} as well as p38-MAPK phosphorylation. This response was observed in failing and non-failing myocardium suggesting that in both normal and diseased hearts activation of these kinases might be involved in the regulation of growth signals. Another important finding of our study is that these kinases are not only involved in the long-term regulation of gene expression but also in the short-term regulation of contractility. The latter mechanism could be clinically important since ventricular contractility needs to adapt to increased load rapidly to avoid pulmonary or peripheral congestion.

A biphasic increase in developed force upon stretch composed of immediate FSM and delayed SFR has been described both in atrial and ventricular myocardium and many species. Recently this concept has been further developed to a triphasic functional response to stretch, where the FSM and SFR are followed by a decline in developed force. Despite a number of studies dealing with the underlying mechanisms signalling pathways and

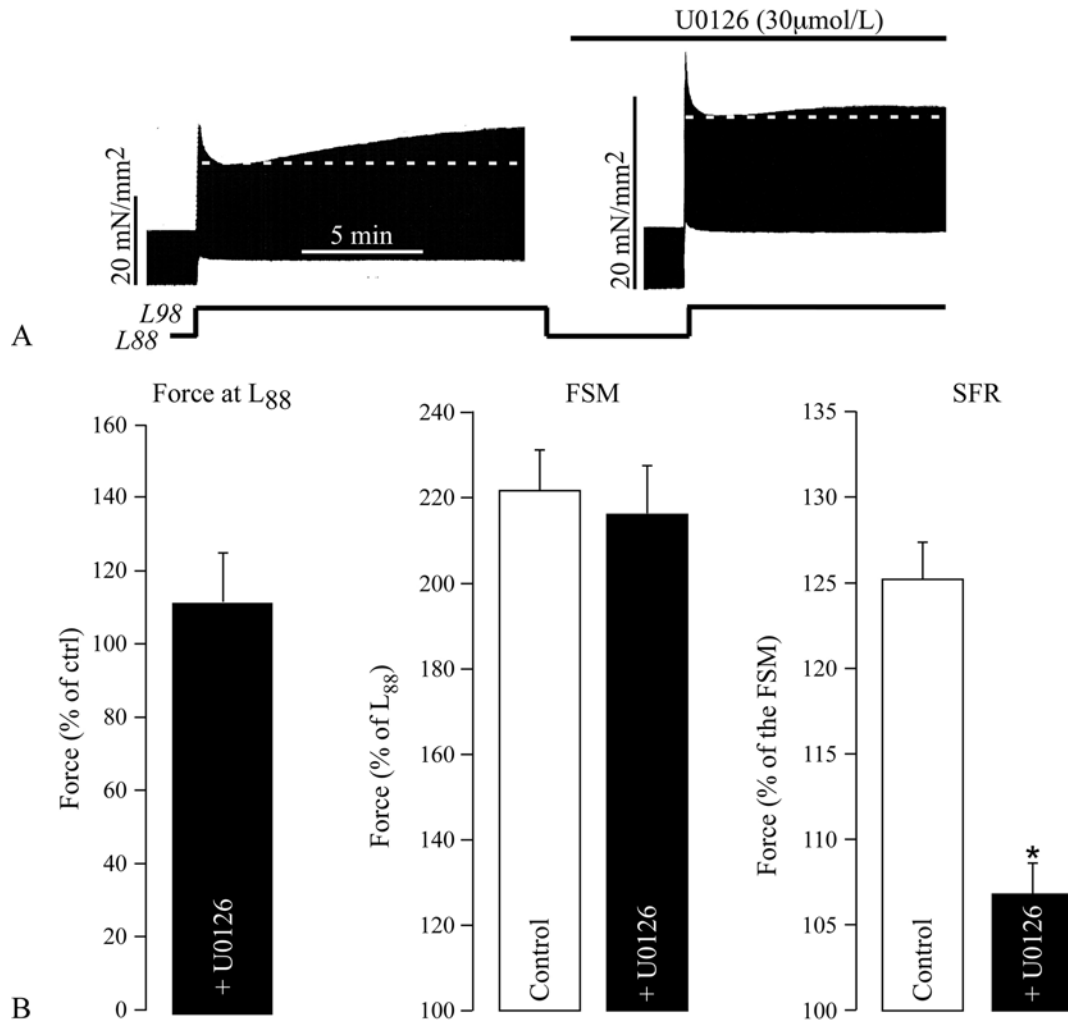


Figure 5. Effect of U0126 on the stretch-induced increases in developed force. A, Original recording of stretch-induced alterations in isometric twitch force of a ventricular muscle strip during stretch from 88% to 98% of optimal length before and following exposure to 30 μ mol/L U0126. B, Effects of 30 μ mol/L U0126 (n=10) on basal twitch force at L88, FSM, and SFR (* $p < 0.05$ vs. control).

cellular mechanisms of the SFR are still not completely understood. A large body of evidence indicates that stretch-induced inotropy is mediated by an increase in $[Na^+]_i$, which in turn alters NCX activity to elevate SR Ca^{2+} load and $[Ca^{2+}]_i$ transients. The stretch-induced $[Na^+]_i$ increase is thought to be caused by stimulation of the NHE1 (5-8). Although in animal myocardium NHE1 stimulation may be brought about by release and autocrine/paracrine actions of angiotensin II and endothelin-1 (25, 26), such a mechanism has been excluded to underlie the SFR in human ventricular myocardium (5). Furthermore, recently it was shown in feline papillary trabeculae that stretch activates the NHE1 via p44/42-MAPK/p90^{rsk} phosphorylation and the SFR was inhibited by p44/42-MAPK blockade (PD98059) (9). The present study has identified p44/42-, p38-MAPK and p90^{rsk} as novel regulators of the stretch-induced SFR in failing human myocardium. Inhibition of p44/42-MAPK and p90^{rsk} (by means of inhibition of the upstream kinase MEK1/2) almost completely suppressed the stretch-induced

SFR. This finding is noteworthy since it points to the MEK1/2-p44/42-p90^{rsk} cascade as a direct and powerful regulator of stretch-induced cardiac inotropy. Because p44/42-MAPK and p90^{rsk} are able to phosphorylate the NHE at its regulatory domain (27, 28), it is possible that these kinases are direct activators of the NHE1 and that they might represent the missing link between myocardial stretch and NHE1 stimulation in human myocardium. In addition, the time course of the p44/42-MAPK and p90^{rsk} phosphorylation after acute stretch fits well to the time course of the SFR after stretch from L88 to L98, supporting the notion of a causal link between p44/42-MAPK-p90^{rsk} signalling and the SFR. In contrast to p44/42-p90^{rsk}, p38-MAPK seems to be a negative regulator of force development that is activated by stretch. Inhibition of p38-MAPK by SB203580 increased basal force development and, in addition, elevated the SFR, suggesting that p38-MAPK regulates contractility under basal conditions as well as following stretch. Thus, stretch not only activates

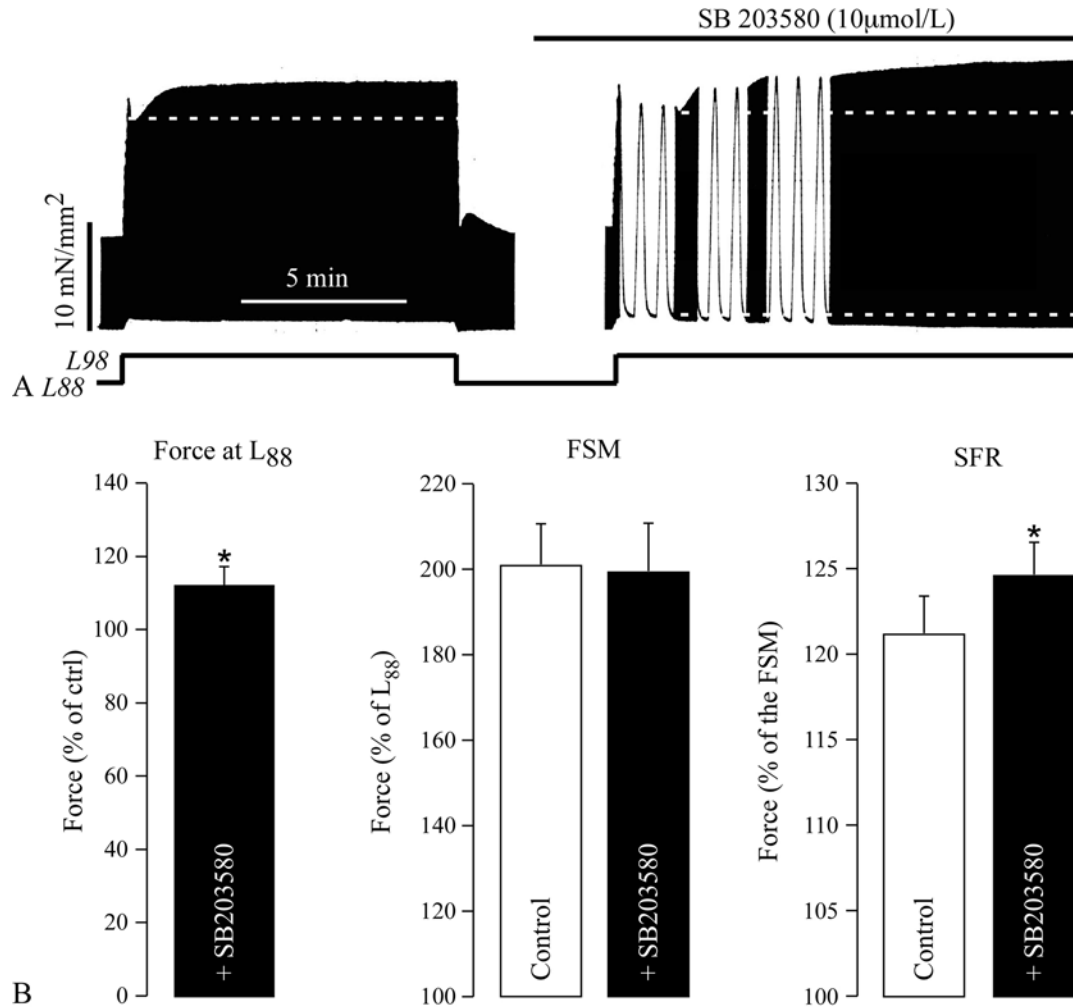


Figure 6. Effect of SB203580 on the stretch-induced increases in developed force. A, Original recording of stretch-induced alterations in isometric twitch force of a ventricular muscle strip during stretch from 88% to 98% of optimal length before and following exposure to 10 μmol/L SB203580. B, Effects of 10 μmol/L SB203580 (n=7) on basal twitch force at L88, FSM, and SFR (* p<0.05 vs. control).

signalling pathways with positive inotropic effects but also at least one pathway with a negative inotropic effect, i.e. p38-MAPK. The precise mechanism underlying the negative inotropic effect of p38-MAPK is not known at present. However, recent evidence from our laboratory suggests that p38-MAPK might act via a decrease of the Ca^{2+} sensitivity of the myofilaments. Typically, an intervention that results in an increased myofilament Ca^{2+} -responsiveness prolongs, while an intervention that reduces Ca^{2+} myofilament sensitivity abbreviates twitch kinetics. In line with that notion the positive inotropic effect of p38-MAPK inhibition was associated with an increase in relaxation.

The stretch-dependent increase in developed force after acute stretch was higher in NF than in F myocardium as observed previously for the SFR (20). Acute stretch led to an increase of p44/42-, p38-MAPK and p90^{rsk} in both F and NF myocardium. The time course was similar for p44/42-MAPK and p90^{rsk}. Unlike p44/42-p90^{rsk}, however,

the phosphorylation of p38-MAPK after stretch was faster and higher in NF than in F myocardium. Therefore, this mechanism would be less effective in the F hearts suggesting that heart failure patients are less vulnerable to the negative inotropic effects following acute stretch. This also means, that differences in the magnitude of the SFR between F and NF myocardium can not be explained by phosphorylation of p38-MAPK, which was higher in the NF hearts despite the larger SFR, indicating that further mechanisms have to contribute to the reduced SFR in F myocardium. One such mechanism might be impaired Na^{+} -contraction coupling (20).

Besides long-term regulation of MAPK in chronic heart failure, the acute stretch-dependent phosphorylation/activation of MAPK during the SFR might be of clinical relevance. For example, during acute increases in right ventricular load as seen in pulmonary embolism the SFR would be beneficial to readapt the right ventricular ejection to that of the left ventricle. Inhibition of

p38-MAPK might potentiate this adaptive mechanism and could therefore be a potential therapeutic tool in pulmonary embolism, which is still characterized by high mortality, especially in patients with right ventricular dilatation and dysfunction (29).

6. ACKNOWLEDGEMENTS

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Abbreviations: F: failing, FSM: Frank-Starling Mechanism, MAPK: mitogen-activated protein kinase,

NCX: Na⁺/Ca²⁺ exchanger, NF: non-failing, NHE: Na⁺/H⁺ exchanger, RSK: ribosomal S6 kinase, SFR: slow force response

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