Swimming stress in DN 14-3-3 mice triggers maladaptive cardiac remodeling: role of p38 MAPK

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1Department of Clinical Pharmacology, Niigata University of Pharmacy and Applied Life Sciences, Higashijima, Niigata City, Japan; 2Departments of Cell Biology and Physiology, Center for Cardiovascular Research, Washington University School of Medicine, St. Louis, Missouri; and 3First Department of Medicine, Niigata University School of Medicine, Niigata City, Japan

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Swimming stress in DN 14-3-3 mice triggers maladaptive cardiac remodeling: role of p38 MAPK. Am J Physiol Heart Circ Physiol 292: H1269–H1277, 2007. First published October 13, 2006; doi:10.1152/ajpheart.00550.2006.—It is generally believed that a mechanical signal initiates a cascade of biological events leading to coordinated cardiac remodeling. 14-3-3 family members are dimeric phosphoserine-binding proteins that regulate signal transduction, apoptotic, and checkpoint control pathways. To evaluate the molecular mechanism underlying swimming stress-induced cardiac remodeling, we examined the role of 14-3-3 protein and MAPK pathway by pharmacological and genetic means using transgenic mice with cardiac-specific expression of dominant-negative (DN) mutants of 14-3-3 (DN 14-3-3/TG) and p38α/β MAPK (DNp38α and DNp38β) mice. p38 MAPK activation was earlier, more marked, and longer in the myocardium of the TG group compared with that of the nontransgenic (NTG) group after swimming stress, whereas JNK activation was detected on day 5 and decreased afterward. In contrast, ERK1/2 was not activated after swimming stress in either group. Cardiomyocyte apoptosis, cardiac hypertrophy, and fibrosis were greatly increased in the TG group compared with those in the NTG group. Moreover, we found a significant correlation between p38 MAPK activation and apoptosis in the TG group. Furthermore, DN 14-3-3 hearts showed enhanced atrial natriuretic peptide expression. In contrast, DNp38α and DNp38β mice exhibited reduced mortality and increased resistance to cardiac remodeling after 28 days of swimming stress compared with TG and NTG mice. Besides, treatment with a p38 MAPK inhibitor, FR-167653, resulted in regression of cardiac hypertrophy and fibrosis and improvement in the survival rate in the TG group. These results indicate for the first time that 14-3-3 protein along with p38 MAPK plays a crucial role in left ventricular remodeling associated with swimming stress.

14-3-3 protein; intracellular signal transduction; mechanical overload; transferase-mediated dUTP nick-end labeling; cardiac hypertrophy and fibrosis

CARDIAC REMODELING is an adaptive response incurred by mechanical, hemodynamic, or hormonal stimuli, which may be of physiological or pathological origin. The myocardium generally adapts to increased workload through enlargement of individual muscle cells and results in cardiac hypertrophy. Exercise training is mainly related to volume overload-induced cardiac eccentric hypertrophy with predominant longitudinal myocyte growth (12). Previous studies have shown that cardiomyocyte morphology is altered (increase in cell size and myofibrillar reorganization) and that protein synthesis is activated after mechanical stress (9, 24). Kaplan et al. (7) showed that swimming stress in mice causes increased heart weight-to-body weight ratio as well as cardiac hypertrophy. In addition, Kazakov and Mikhailov (8) showed that swimming stress induces cardiomyocyte apoptosis in mice.

14-3-3 proteins are intracellular, dimeric, phosphoserine-binding molecules that have been identified in many eukaryotic organisms and are found primarily in the cytoplasmic compartment of cells and play critical roles in signal transduction, apoptotic, and checkpoint control pathways (15). We previously demonstrated that 14-3-3 protein regulates MAPK family members and plays an important role in cell fate decisions (5, 23). Transgenic mice generated with cardiac-specific overexpression of a dominant-negative (DN) mutant of 14-3-3 protein (DN 14-3-3/TG) are normal at baseline but are unable to compensate for pressure overload (23).

It is generally believed that mechanical signals initiate a cascade of biological events leading to coordinated cardiac remodeling. If that is true, then signals for pressure and volume overload may result in different patterns of cardiac growth. The molecular mechanisms underlying exercise-induced physiological cardiac remodeling are not yet well understood. In the present study, we examined the role of the 14-3-3 protein and MAPK pathway in swimming stress-induced cardiac remodeling using DN 14-3-3/TG mice.

MATERIALS AND METHODS

Generation of transgenic mice. DN 14-3-3/TG and p38α/β MAPK (DNp38α and DNp38β) mice were generated as described previously (23, 29) at the Neuroscience Transgenic Facility of Washington University School of Medicine and were analyzed by polymerase chain reaction to detect transgene integration using mouse-tail DNA as template. Nine-week-old C57BL/6 JAX mice (Charles River) were used as nontransgenic (NTG) controls.

Swimming stress. NTG, TG, DNp38α, and DNp38β mice were subjected to swimming stress in tanks containing water maintained at 30–32°C to avoid thermal stress induced by cold water and were constantly monitored. These mice were made to swim for a period of
90 min twice a day, separated by a 4-h break (7, 14a), and were euthanized at various time courses, i.e., after 0, 1, 5, 7, 14, 21, or 28 days of swimming stress. Throughout the study, animals were reviewed carefully in accordance with the Guidelines for Animal Experiments of our institute. All animals were handled according to the approved protocols and animal welfare regulations of the Institutional Review Board at Niigata University of Pharmacy and Applied Life Sciences.

Animal study protocol. Kaplan-Meier survival analysis was carried out using the NTG group without swimming stress (n = 30), TG group without swimming stress (n = 30), DNp38α group without swimming stress (n = 15), DNp38β group without swimming stress (n = 15), NTG group with swimming stress (n = 40), TG group with swimming stress (n = 46), DNp38α group with swimming stress (n = 15), DNp38β group with swimming stress (n = 15), DN 14-3-3 mice treated with FR-167653 (60 mg/kg ip) group (n = 6), and DN 14-3-3 mice treated with saline vehicle group (n = 6). FR-167653 (p38 MAPK inhibitor) was obtained from Fujisawa Pharmaceutical (Osaka, Japan). Hearts were isolated for analysis after anesthetizing mice with a single injection of pentobarbital sodium (50 mg/kg ip). Heart weight (HW) and the ratio of HW-to-body weight (HW/BW) were determined for each animal. The left ventricle was quickly dissected and cut into two parts. One part was immediately transferred into liquid nitrogen and then stored at ~80°C for protein analysis. Another part was stored in formalin for studying apoptosis, cardiac hypertrophy, and fibrosis.

Protein analysis. Cytosolic extracts of left ventricular (LV) tissue were separated by SDS-PAGE and electrophoretically transferred to nitrocellulose filters. Filters were blocked with milk, washed, and then incubated with primary antibody. The primary antibodies employed included rabbit polyclonal anti-phospho-p38 MAPK, anti-p38 MAPK, anti-phospho-JNK, anti-JNK, anti-phospho-ERK1/2, and anti-ERK1/2 antibodies (Cell Signaling Technology, Beverly, MA) and goat polyclonal anti-GAPDH and rabbit polyclonal anti-atrial natriuretic peptide (ANP) (Santa Cruz Biotechnology, CA) antibodies. Filters were washed and then incubated with horseradish peroxidase-coupled secondary antibodies (Santa Cruz Biotechnology), and bands were visualized with the enhanced chemiluminescence system (ECL Plus, Amersham, Piscataway, NJ). The mean value on day 0 in the NTG group was taken as 1 arbitrary unit. Data were expressed as the ratio to the values on day 0 in the NTG group.

Terminal transferase-mediated dUTP nick-end labeling assays. The transferase-mediated dUTP nick-end labeling (TUNEL) assay was performed as specified in the instructions for the in situ apoptosis detection kit (TaKaRa Bio, Shiga, Japan). Sections embedded in paraffin were mounted and examined using light microscopy. Digital photomicrographs were obtained by using a color image analyzer (CAI-102, Olympus) at ×400 magnification, and 25 random fields from each heart were chosen and the signals therein were quantified in a blinded manner. For each animal, three sections were scored for apoptotic nuclei. Only nuclei that were clearly located in cardiac myocytes were considered. The mean value on day 0 in the NTG group was taken as 1 arbitrary unit. Data were expressed as the ratio to the values on day 0 in the NTG group.

Histological analysis. Cardiomyocytes from LV cross sections were stained with hematoxylin-eosin, and the diameters of 500 cardiomyocytes were measured in each animal using light microscopy at ×400 magnification. Azan-Mallory-stained sections were quantitatively analyzed using light microscopy at ×100 magnification to evaluate myocardial fibrosis using the difference in color (blue fibrotic area as opposed to red myocardium). Digital photographs were obtained by using a color image analyzer (CAI-102, Olympus).

Statistical analysis. Data are shown as means ± SE. Statistical analysis was performed by one-way analysis of variance followed by Tukey’s method. Correlations between groups of values were evaluated, calculating the best fit based on least squares regression analysis, and the coefficient of correlation (r) was estimated. A value of P < 0.05 was considered to be statistically significant.

RESULTS

Swimming stress promotes p38 MAPK pathway activation in DN 14-3-3 mice. As shown in Fig. 1, A–C, there was earlier, higher, and longer activation of p38 MAPK in the TG group than in the NTG group. Basal day 0 p38 MAPK activation was 1.3 ± 0.5-fold higher in the TG group than in the NTG group. One day after swimming stress, p38 MAPK activation was markedly increased, by 1.5 ± 0.4- and 3.9 ± 0.3-fold in the NTG and TG groups, respectively, relative to the basal p38 MAPK activity in the NTG group. In addition, 5 and 7 days after swimming stress, p38 MAPK activation was greatly increased in both the NTG and TG groups; i.e., 5 days after swimming stress, p38 MAPK activation was 2.6 ± 0.8 (P < 0.05) and 3.7 ± 1.0-fold higher in the NTG and TG groups, respectively. On day 7 after swimming stress, p38 MAPK activation was 3.2 ± 0.8 (P < 0.01) and 5.0 ± 0.6-fold (P < 0.01) higher in the NTG and TG groups, respectively, compared with the basal p38 MAPK activity in the NTG group.

The basal JNK activation in the TG group was 1.1 ± 0.2-fold higher compared with that in the NTG group. Peak JNK activation was found on day 5 after swimming stress, at which time JNK activation was 5.5 ± 1.8-fold (P < 0.01) increased in the TG group compared with the basal JNK activation in the NTG group. On the other hand, ERK1/2 was not activated during the time course of swimming stress examined in either the NTG or TG groups. Membranes were also analyzed in parallel with anti-ERK1/2 antibody, and identical levels of total ERK1/2 were found in all the samples.

Increased myocardial cell death in DN 14-3-3 mice after swimming stress. To establish the relationship between the activation of MAPK pathways and apoptosis during swimming stress, the TUNEL assay was performed on LV tissue sections obtained from both the NTG and TG groups. A small percentage of TUNEL-positive cells were detected in basal myocardium on day 0 of both the NTG and TG groups; however, at this time, the percentage of TUNEL-positive cardiomyocytes in the basal TG group (2.5 ± 0.6-fold vs. basal NTG group) was higher than that in the NTG group (Fig. 1D). A significant (P < 0.01) increase in apoptosis was found 1 day after swimming stress in the TG group (11.6 ± 0.7-fold vs. basal NTG group); in addition, on day 5, the TG group showed a peak of apoptosis (12.2 ± 1.4-fold vs. basal NTG group). Moreover, the number of apoptotic cells in the TG group was higher compared with that in the NTG group throughout the time course examined in the present study. A significant (P < 0.0001) and positive correlation (r = 0.853) was found between the activation of p38 MAPK (Fig. 1E) and apoptosis in the TG group after swimming stress, whereas there was no significant correlation between the activation of JNK (Fig. 1F) and apoptosis.

Increased hypertrophic response to swimming stress in DN 14-3-3 mice. Cardiomyocyte size before and after swimming stress in both the NTG and TG groups is shown in Table 1 and Fig. 2. On day 0, there were no significant differences in cardiomyocyte size between the NTG (11.6 ± 0.3 μm) and TG (11.7 ± 0.3 μm) groups. As depicted in Table 1, both HW as
well as HW/BW were found to be increased after 21 days of swimming stress in both NTG and TG mice, confirming that stress caused by swimming is detected in the myocardium of mice after a period of 21 days. The TG group had a significant increase of cardiomyocyte size (16.9 ± 0.4 μm) compared with the NTG group (13.6 ± 0.3 μm; \( P < 0.01 \), Fig. 2A).

Cardiomyocyte diameter and HW/BW were found to be significantly larger in the TG group at 28 days after swimming stress (Fig. 3, A and C) compared with those in NTG mice on the same day. Azan-Mallory staining revealed that long-term swimming stress caused development of fibrosis in the myocardium of the TG group (Figs. 2B and 3B and D).

Survival probability in DN 14-3-3 mice after swimming stress. To determine whether modulation of the 14-3-3 phenotype has any effect on survival probability after swimming stress, we performed Kaplan-Meier survival analysis. As depicted in Fig. 4, no significant difference in survival rate was found between the NTG and TG groups; however, the survival rate in the TG group was lower than that in the NTG group during swimming stress.

Table 1. Changes in HW, HW/BW, and cardiomyocyte size during the course of swimming stress in the NTG and TG groups

<table>
<thead>
<tr>
<th>Day</th>
<th>NTG</th>
<th>TG</th>
<th>NTG</th>
<th>TG</th>
<th>NTG</th>
<th>TG</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>HW, mg</td>
<td>HW/BW, mg/g</td>
<td>Cardiomyocyte Size, μm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>88 ± 1</td>
<td>97.5 ± 5</td>
<td>4.58 ± 0.08</td>
<td>3.85 ± 0.09†‡</td>
<td>11.6 ± 0.3</td>
<td>11.7 ± 0.3</td>
</tr>
<tr>
<td>1</td>
<td>86 ± 5</td>
<td>99 ± 9</td>
<td>4.85 ± 0.13</td>
<td>4.28 ± 0.42</td>
<td>11.6 ± 0.3</td>
<td>11.7 ± 0.3</td>
</tr>
<tr>
<td>5</td>
<td>94 ± 1</td>
<td>124 ± 5†‡</td>
<td>5.23 ± 0.06†</td>
<td>4.69 ± 0.07</td>
<td>11.7 ± 0.3</td>
<td>13.1 ± 0.3†</td>
</tr>
<tr>
<td>7</td>
<td>102 ± 6</td>
<td>127 ± 8†‡</td>
<td>5.15 ± 0.32</td>
<td>5.04 ± 0.08†</td>
<td>11.8 ± 0.2</td>
<td>15.1 ± 0.8‡†</td>
</tr>
<tr>
<td>14</td>
<td>109 ± 2†</td>
<td>129 ± 2†‡</td>
<td>5.10 ± 0.11†</td>
<td>4.97 ± 0.11†</td>
<td>11.9 ± 0.3</td>
<td>15.3 ± 0.3 †</td>
</tr>
<tr>
<td>21</td>
<td>124 ± 3†</td>
<td>141 ± 5†‡</td>
<td>5.17 ± 0.17†</td>
<td>5.47 ± 0.13†</td>
<td>11.6 ± 0.3</td>
<td>11.7 ± 0.3</td>
</tr>
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Values are means ± SE. HW/BW, heart weight-to-body weight ratio; NTG, nontransgenic; TG, transgenic. * \( P < 0.05 \) and †\( P < 0.01 \) vs. NTG-0 day; ††\( P < 0.01 \) vs. same time period in NTG group.
Involvement of p38 MAPK in swimming stress induced cardiac remodeling. To further clarify the role of p38 MAPK in swimming stress, we subjected DNp38α and DNp38β mice to a 28-day swimming stress protocol and compared them with NTG and TG mice. We found that expression of phospho-p38 MAPK at 28 days of swimming stress (Fig. 5) was clearly reduced in DNp38α and DNp38β mice. In contrast, the higher p38 MAPK in DN 14-3-3 hearts was also in accord with increased expression of hypertrophy marker protein ANP compared with the expression levels in the NTG, DNp38α, and DNp38β mice. Histological analysis of LV tissue sections (Fig. 6) further revealed that in contrast to DN 14-3-3 hearts (which displayed significant myocyte enlargement as well as increased extracellular matrix content) and NTG hearts (which developed prominent cardiac hypertrophy after 28 days of swimming stress), DNp38α and DNp38β hearts displayed relatively preserved myofibrillar architecture with modest myocyte enlargement. To test whether DNp38α and DNp38β mice are resistant to the development of fibrosis after 28 days of swimming stress, cardiac tissue was examined by Azan-Mallory staining, and the results showed that both DNp38α and DNp38β mice exhibited little or no fibrosis.

To examine whether there was a direct relationship between 14-3-3 protein and p38 MAPK activity, we tried to determine whether inhibition of the p38 MAPK activity could block the DN 14-3-3 phenotype and salvage DN 14-3-3 mice after swimming stress. We treated DN 14-3-3 mice with FR-167653 during the 28-day swimming stress protocol. As expected, we

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Fig. 2. DN 14-3-3 mice show accelerated progression of cardiac remodeling after volume overload. Representative photomicrographs of left ventricular cross sections from the NTG and TG group are shown. A: sections were stained with hematoxylin-eosin. Photographs are shown at ×400 magnification. Bar = 10 μm. B: sections were stained with Azan-Mallory. Blue indicates area of fibrosis. Photographs are shown at ×200 magnification. C: sections were stained with the TUNEL method. Arrows indicate apoptotic nuclei that were stained with brown.
observed that FR-167653-treated DN 14-3-3 hearts displayed regression of cardiac hypertrophy with insignificant fibrosis (Fig. 6), which was comparable with that of DNp38α and DNp38β mice.

Survival probability in DNp38α/β and DN 14-3-3 mice treated with FR-167653 after swimming stress. Significantly reduced mortality after 28 days of swimming stress was observed in DNp38α and DNp38β mice compared with that in TG mice (Fig. 7A). FR-167653-treated DN 14-3-3 mice, on the other hand, developed tolerance and displayed improved survival after the swimming stress protocol (Fig. 7B).

DISCUSSION

Recognition of molecular targets associated with cardiac remodeling may provide novel therapeutic strategies for
diverse cardiac ailments. In this study, we examined the role of 14-3-3 protein and its relationship with three major MAPK pathways to evaluate the molecular mechanism underlying swimming stress-induced cardiac remodeling using transgenic mice with cardiac-specific overexpression of DN 14-3-3β. In addition, we also investigated cardiac-specific DNP38α and DNP38β MAPK mice to dissect out the role of p38 MAPK in swimming stress.

Fig. 5. Effect of DN 14-3-3 and p38α/β MAPK (DNP38α and DNP38β) mice on hypertrophic response after swimming stress. Representative Western immunoblots of p-p38 MAPK and atrial natriuretic peptide (ANP) after 28 days of swimming stress. Protein lysates were prepared from left ventricular tissue. These immunoblots are representative of 3 separate experiments.

Fig. 6. Histological analysis of left ventricular tissue after 28 days of swimming stress. A and B: representative photomicrographs of left ventricular cross sections from control and 28-day swimming-stressed in NTG and TG DNP38α, DNP38β, and FR-167653 (FR)-treated DN 14-3-3 mice stained with hematoxylin-eosin (A) and Azan-Mallory (B). C and D: quantification of cardiac hypertrophy and fibrosis in control and 28-day-swimming-stressed hearts. DN 14-3-3/TG mice were treated with FR-167653 (60 mg·kg⁻¹·day⁻¹) for 28 days. FR-167653 was administered for 3 days before swimming stress and was continued for 28 days after swimming stress. Original magnification was ×400 for hematoxylin-eosin-stained sections and ×100 for Azan-Mallory-stained sections. Note the preserved cardiac morphology, resistance to cardiomyocyte enlargement, and reduced extracellular matrix content after p38 MAPK modulation. Data are expressed as the ratio to the value at day 0 in the NTG group.
DN-14-3-3 increases the sensitivity of cardiac tissue to proapoptotic stimuli (23). We found that both the level of activation of p38 MAPK and the percentage of cardiomyocytes undergoing apoptosis were severalfold higher in the DN 14-3-3 group than in the NTG group. Moreover, activation of p38 MAPK and apoptosis in the swimming stress myocardium were significantly correlated ($P < 0.0001$, $r = 0.853$). These results in the DN 14-3-3 group indicate for the first time that 14-3-3 plays an important antiapoptotic role in swimming stress-induced cardiomyocyte apoptosis, at least in part through limiting p38 MAPK activation. The antiapoptotic role of 14-3-3 observed in the present study can be explained through its ability to bind with Ask1 (apoptosis signal regulating kinase 1), BAD (proapototic member of the Bcl-2 family), and FKHRL1 (forkhead family transcription factor) (1, 23, 25).

The adaptive response of the heart to enhanced myocardial apoptosis generally results in cardiac hypertrophy. On further analysis, our results showed that NTG mice developed myocyte enlargement but were resistant to fibrosis. In contrast, DN 14-3-3 hearts displayed exacerbated cardiac hypertrophy and fibrosis after swimming stress. The fact that the level of activation of p38 MAPK was lower in NTG hearts compared with DN 14-3-3 hearts suggests that intact 14-3-3 function aids NTG hearts in limiting to a considerable extent p38 MAPK-mediated cardiac remodeling events associated with swimming stress. Therefore, it may be inferred from the present findings that endogenous 14-3-3 protein controls the transition from the adaptive response of the heart, as observed in NTG mice, to the maladaptive response, observed in DN 14-3-3 mice, through p38 MAPK after swimming stress.

Documented evidence suggests that p38 MAPK-deficient cultured cardiomyocytes are resistant to apoptosis (16). The main isoform expressed in the heart is p38$\alpha$. p38$\beta$ and p38$\gamma$ are expressed at low levels, and p38$\delta$ is not expressed in the heart. p38$\alpha$ and p38$\beta$ exhibit extensive sequence similarity (3, 10, 11, 22). To explore whether p38 MAPK is essential in swimming-stressed myocardium, we subjected DNp38$\alpha$ and DNp38$\beta$ mice to a 28-day swimming stress protocol. Our results revealed that DNp38$\alpha$ and DNp38$\beta$ hearts were resistant to swimming stress-induced cardiac remodeling. These findings are in contrast to our previous report, where we observed that DNp38$\alpha$ and DNp38$\beta$ mice develop cardiac hypertrophy in response to pressure overload (29). This dis-
crepancy may perhaps be explained by the possibility that p38 MAPK behaves differently in response to varying degrees of pressure or volume overload. Since DNp38α and DNp38β hearts are resistant to cardiac remodeling in our model of swimming stress and since TUNEL-positive cells in DN 14-3-3 hearts were correlated highly with p38 MAPK activity compared with other MAPKs, it is very likely that p38 MAPK is a key player in mediating cardiac remodeling-associated events after swimming stress, given that overstimulation of p38 MAPK, as demonstrated in DN 14-3-3 hearts in particular, has more deleterious consequences.

On the one hand, p38 MAPK is well known to play a proapoptotic role in several cell types (16, 19), whereas, on the other hand, studies also indicate that activation of p38 MAPK may have an antiapoptotic effect (13). This discrepancy in the role of p38 MAPK may be partly explained by the difference in experimental models, the presence of multiple p38 MAPK isoforms (p38α, p38β, p38γ, and p38δ), and the existence of several upstream MAPK kinases (MKKs), e.g., MKK3 and MKK6, which activate p38 MAPK.

In addition, we demonstrated a marked increase in ANP expression, which corresponded with increased p38 MAPK activity in the DN 14-3-3 hearts compared with NTG, DNp38α, and DNp38β hearts after 28 days of swimming stress. The fact that p38 MAPK activation may mediate the activation of GATA-4 and ETS-like gene-1 (Elk-1) and that GATA-4 is a major nuclear factor that mediates induction of hypertrophic patterns of genes, including B-type natriuretic peptide during hemodynamic loading (2, 14, 18), is in accordance with the findings of the present study.

We have shown previously that compound transgenic DNp38α and DNp38β mice display resistance to myocardial injury after pressure overload (28), demonstrating that the enhanced survival observed in these mice is a consequence of p38 MAPK inhibition that is 14-3-3 mediated. To establish whether inhibition of p38 MAPK can reverse the DN 14-3-3 phenotype after swimming stress, we treated DN 14-3-3 mice with FR-167653 (p38 MAPK inhibitor) in the present study. We found that treatment with FR-167653 resulted not only in regression of cardiac hypertrophy and decreased extracellular matrix content but also in a markedly improved survival rate in DN 14-3-3 mice. FR-167653 is a pyridinyl-imidazole, structurally similar to SB-203580, a well-known inhibitor of p38 MAPK. FR-167653 does not affect the activation of other MAPKs, such as JNK and ERK (6), and forms an inhibitory complex with p38 MAPK (Fig. 8A). As shown in an immune complex kinase assay, the inhibitory potential of FR-167653 toward p38α MAPK is comparable with that of SB-203580 (20). The fact that DN 14-3-3 mice were salvaged by treatment with FR-167653 substantially supports the notion that p38 MAPK contributes to cardiac remodeling after swimming stress. Since myocyte enlargement was apparently absent in DNp38α and DNp38β as well as FR-167653-treated DN 14-3-3 mice indicates that direct modulation of p38 MAPK may result in tolerance to cardiac hypertrophic response after volume overload.

In addition, we investigated the role of the JNK pathway in swimming-stress myocardium. JNK activation increased; however, we could not observe any significant correlation between JNK activity and apoptosis. On the other hand, ERK1/2 was not activated during the course of swimming stress studied in the NTG or TG groups. Zhang et al. (26) have reported that activated p38 MAPK directly interacts with ERK1/2 and blocks its phosphorylation by MAPK/ERK kinase 1 (MEK1).

Taken together, these findings substantially support the idea that cardiac remodeling associated with swimming stress is dependent on a signal transduction pathway that involves 14-3-3 protein and p38 MAPK. Modulation of p38 MAPK activity by DN mutation as well as pharmacological inhibition of this activity appears to result in regression of cardiac hypertrophy and increased tolerance to swimming stress. As shown in Fig. 8B, intact 14-3-3 protein function plays a critical role in controlling p38 MAPK action and thereby restricts the transition of adaptive physiological to maladaptive pathological cardiac remodeling.

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