The Influence of Cold Stress on the Myosin Heavy Chain Expression of Cardiac and Smooth Muscle in Normotensive and Spontaneously Hypertensive Female Rats

Katrin Adler, Piet Boels, Ursula Ganten, Detlev Ganten, and Ingo Morano

Cold exposure (6 weeks at 4°C) of normotensive (Wistar-Kyoto) and stroke-prone spontaneously hypertensive female rats led to cardiac hypertrophy (in stroke-prone spontaneously hypertensive rats), increased the level of plasma thyroxine, and increased the \( \alpha \)-myosin heavy chain expression in the left ventricle. In contrast, myosin heavy chain expression of both main mesenteric artery and uterus was not affected by cold stress and chronic hypertension, suggesting different regulation of myosin heavy chain expression in smooth and cardiac muscle in vivo. (Circulation Research 1991;69:1640–1644)

In both the cardiac and smooth muscle, two different tissue-specific myosin heavy chains (MHCs) are expressed. In the ventricle of the rat heart, these two MHCs are designated as \( \alpha \)- and \( \beta \)-MHC located on chromosome 14 in tandem 4 kb apart.\(^1\,^2\) Change in the expression of cardiac MHC isoenzymes is a common feature of cardiac adaptation to different environmental demands and is regulated by multiple hormonal stimuli (for review see Reference 3). Cardiac adaptation by changing the MHC isoenzyme transcription pattern is directly mediated by the different kinetic properties of the MHC isoenzymes, thus regulating both contractile and energetical parameters of the heart.\(^4\,^5\) During cold adaptation, basal metabolic rate increases; this increase is probably due to elevated thyroxine secretion and turnover.\(^6\) The thyroid hormone directly regulates transcription of cardiac MHCs: it increases the transcriptional rate of \( \alpha \)-MHC and decreases transcription of \( \beta \)-MHC.\(^9\,^10\) Therefore, we studied expression of MHC isoenzymes of the ventricle of cold-adapted normotensive (Wistar-Kyoto [WKY]) rats and spontaneously hypertensive rats of the stroke-prone strain (SHRSP). To contribute to an understanding of the regulation of smooth muscle MHC (SM-MHC) expression, we investigated both mesenteric artery and uterus of cold-adapted and control SHRSP and WKY rats (e.g., during physiologically changed thyroxine levels). This is of particular interest, since little is known about the regulation of SM-MHC gene expression. Two different SM-MHC isoenzymes have been characterized on the protein level\(^11\,^12\) and by cDNA cloning.\(^13\) In the chicken, two SM-MHC isoenzymes are produced by an alternative splicing mechanism.\(^14\)

Materials and Methods

Animal Model

We used 30-week-old female SHRSP and age-matched female WKY rats as the proper control (SHRSP are bred from WKY rats). SHRSP have been bred in Heidelberg since 1975 in the laboratory of Prof. Ganten, University of Heidelberg, FRG. The rats were kept for cold adaptation at a temperature of 4±1°C, a relative humidity of 30–50%, a wind speed of 1 m/sec, and a 12-hour day/night photoperiod for 6 weeks; control rats were kept at a temperature of 25±2°C and a 12-hour day/night photoperiod.

Tissue Preparation

The rats were killed by cervical dislocation after 6 weeks of cold exposure and weighed. The hearts were excised, blotted, and weighed, and the left ventricle was prepared and quickly frozen in liquid nitrogen. Main mesenteric artery and uterus also were prepared and frozen in liquid nitrogen. Tissues were stored at −80°C.
Ventricular Myosin Isoenzymes

Ventricular isoenzymes were investigated by pyrophosphate–polyacrylamide gel electrophoresis. In short, whole tissues were crushed in liquid nitrogen and extracted for 20 minutes at 4°C with 3.4 μl/mg muscle wet weight of a modified Guba-Straub solution containing (mM) NaCl 300, NaPO₄ 100, MgCl₂ 1, Na₂HPO₄ 10, and EDTA 10, leupeptine 0.1, along with 1% NaN₃ and 1% β-mercaptoethanol, pH 6.5. The extract was centrifuged (20,000 rpm for 20 minutes), and the supernatant was mixed 1:1 with glycerol and stored at −20°C. Electrophoresis was performed in 4% acrylamide gels for 20 hours at 2±4°C (80 V constant). Gels were stained for 30 minutes with Coomassie blue and destained overnight. Gels were scanned densitometrically, and the peak areas were evaluated geometrically (peak height). The relative proportions of the myosin isoenzymes were expressed as percentage of total myosin determined from the entire tracing.

SM-MHC Isoenzymes

MHC isoenzymes of crude myosin extracts of rat main mesenteric artery and uterus were prepared as described above for ventricular myosin isoenzymes and analyzed using sodium dodecyl sulfate–polyacrylamide gel electrophoresis (4% stacking gel, 5% separation gel, and 25% glycerol). Gels were run for 6 hours (40 mA/gel) at 15°C, stained for protein with Coomassie blue, destained overnight, and evaluated densitometrically as described above.

Western Blot Analysis

Myosin and filamin were identified by a semidry Western blot analysis with antibodies against chicken gizzard MHC raised in rabbit (kindly provided by Prof. Gröschel-Stewart, Darmstadt, FRG) and antibodies against chicken gizzard filamin raised in goat (BioYeda, Israel) (first antibodies). Proteins were transferred to FluoroTrans membranes (Pall, FRG) for 25 minutes (5 mA/cm²) and incubated with the first antibody for 2 hours at room temperature and subsequently with the second biotinylated antibody (anti-rabbit [MHC] or anti-goat [filamin]) for 1 hour at room temperature. Proteins were visualized by the streptavidin (biotinylated horseradish peroxidase complex)/chloronaphthol reaction.

Triiodothyronine Levels

Serum triiodothyronine (T₃) levels were determined from blood samples taken from the inferior vena cava by a commercially available enzyme immunoassay (Boehringer Mannheim, FRG).

Statistical Analysis

Values are expressed as mean±SD. For determination of statistical significance, the t test was used.

| Table 1. Heart Weight, Body Weight, and the Heart Weight/Body Weight Ratio in 36-Week-Old Hypertensive and Normotensive Female Rats Kept at 25°C or 4°C for 6 Weeks |
|-----------------|-------|-------|-----------------|-------|-----------------|
|                 | 25°C  | 4°C   | 25°C            | 4°C   |
| HW (g)          | 0.934±0.037 | 1.034±0.035 | 1.078±0.045 | 1.24±0.075* |
| BW (g)          | 263±7.3   | 262±12 | 208±8.4       | 202±10 |
| HW/BW           | 3.55±0.24 | 3.76±0.35 | 4.79±0.17     | 5.93±0.2* |

Values are mean±SD; n=6 rats per group. WKY, normotensive Wistar-Kyoto female rats; SHRSP, stroke-prone spontaneously hypertensive female rats; HW, heart weight; BW, body weight.

*p<0.01 vs. SHRSP at 25°C.

Results

Cardiac Hypertrophy

Absolute heart weight of WKY rats increased with cold exposure, but this was not statistically significant (p>0.05) (Table 1). In SHRSP, however, cold stress induced a statistically significant increase of the heart weight (p<0.01). Body weight did not change with cold exposure in either rat strain studied, but WKY rats weighed significantly more than SHRSP (p<0.01). Relative heart weight (heart weight/body weight relation) was hardly affected in WKY rats, but there was a statistically significant (p<0.01) increase in SHRSP after cold exposure (Table 1). Relative heart weights of SHRSP were always significantly higher (p<0.01) than those of the corresponding WKY groups.

Ventricular Myosin Isoenzymes

Expression of the V₁ myosin isoenzyme in control SHRSP kept at 25°C and in SHRSP kept at 4°C was statistically lower than the V₁ expression of the corresponding WKY group (p<0.001) (Figure 1). In both rat strains studied, 6 weeks' exposure to 4°C increased the expression of the V₁ isoenzyme of ventricular myosin: in SHRSP the relation V₁/V₃ changed from 29.8/38.5 in control rats to 50.2/19.5 in rats kept at 4°C. The control V₁/V₃ relation of WKY rats changed from 49.8/22.4 to 70/8.1 after cold exposure. The effects of cold exposure in both rat strains were statistically significant at p<0.001.

SM-MHC Isoenzymes

SM-MHCs were analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and characterized by Western blot analysis using polyclonal antibodies against SM-MHCs and filamin antibodies. As demonstrated in Figure 2, two SM-MHCs could be detected in the rat uterus by sodium dodecyl sulfate–polyacrylamide gel electrophoresis, protein staining, and Western blotting; these SM-MHCs were designated, with increasing mobility (decreasing molecular weight), as SM-1 and SM-2. The MHC antibody did not react with cardiac MHC (not shown). The filamin antibody reacted with protein bands having higher molecular weights than SM-MHCs.

The influence of cold stress on the expression of SM-MHC isoenzymes was analyzed by densitometrically scanning the Coomassie-stained SM-MHCs. In all
FIGURE 1. Bar graph showing effects of cold stress on ventricular myosin heavy chain isoenzyme distribution in normotensive female rats (Wistar-Kyoto [WKY]) and spontaneously hypertensive female rats of the stroke-prone strain (SHRSP) (36 weeks old). The rats were kept at 4°C or 25°C. Insert: Myosin isoenzymes after pyrophosphate–polyacrylamide gel electrophoresis and Coomassie staining. V₁, V₂, and V₃ refer to the αα-, αβ-, and ββ-myosin heavy chain isoenzymes, respectively. Values are mean±SD. There were six rats per group. ***p<0.001, comparing 25°C with 4°C of the corresponding groups.

Discussion

Because of the development of high blood pressure, SHRSP expressed a lower amount of the V₁ isoenzyme than normotensive rats from the WKY strain (compare with Reference 17). The main finding of our

TABLE 2. Distribution of Myosin Heavy Chain Isoenzymes in Mesenteric Artery and Uterus of Cold-Acclimatized (4°C) and Control (25°C) Normotensive and Stroke-Prone Spontaneously Hypertensive Female Rats

<table>
<thead>
<tr>
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<th>25°C</th>
<th>4°C</th>
<th>25°C</th>
<th>4°C</th>
</tr>
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<tbody>
<tr>
<td>WKY</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Uterus (%)</td>
<td>56.9/44.1</td>
<td>55.1/45.8</td>
<td>57.5/43.2</td>
<td>55.7/45.1</td>
</tr>
<tr>
<td>Artery (%)</td>
<td>58.5/42.3</td>
<td>60.9/40.4</td>
<td>55.5/45.2</td>
<td>54.9/46.3</td>
</tr>
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Values are mean±SD; n=6 rats per group; SD was always <7% of the mean value. WKY, normotensive Wistar-Kyoto female rats; SHRSP, stroke-prone spontaneously hypertensive female rats. No statistical differences were detected between the groups.

rat groups studied (SHRSP at 25°C and 4°C, WKY rats at 25°C and 4°C), expression of SM-MHCs in both main mesenteric artery and uterus was unchanged (Table 2). Furthermore, there were no differences in the expression of SM-1/SM-2 in the two smooth muscle types studied; when taking all groups and smooth muscle types together, the SM-1/SM-2 relation was 56±2/44±2 (mean±SD of 48 rats) (Table 2 and Figure 3). Results in Figure 3 also demonstrate that even chronic hypertension had no influence on SM-MHC expression in both the uterus and the blood vessel.

Triiodothyronine Levels

There was no difference in the T₃ levels of SHRSP and WKY rats. However, when cold-acclimatized rats were compared with controls, increased T₃ levels could be observed in rats kept at 4°C; T₃ increased from the control level of 1.85±0.4 to 2.4±0.12 nmol/l in cold-acclimatized rats (p<0.05, four rats per group).
involves the cold enhanced degree of cardiac hypertrophy of SHRSP while the expression of the α-MHC in the left ventricle increased. A similar finding with a less pronounced effect on the heart weight was observed in normotensive rats. These observations could be mediated by the thyroid hormone: during cold exposure, the steady-state level of T₃ increased, which is in accordance with previous studies (for review see Reference 8). Indeed, it could be demonstrated that the thyroid hormone elevated the transcriptional rate of the α-MHC gene and attenuated the transcription of the β-MHC gene in the rat ventricle by directly interacting with responsive elements in the promoter regions of both genes.⁹,¹⁰ Inversely, warm stress decreases the serum thyroxine level and decreased the expression of the α-MHC in favor of the β-MHC.¹⁸ The effects of cold exposure of SHRSP on the heart and MHC expression is interesting, since in this model two opposing factors exist: on the one hand, hypertension and hypertrophy, which enhance β-MHC expression, and, on the other hand, elevated thyroxine levels, which increase α-MHC and decrease β-MHC expression and have an additional hypertrophying effect. The enhanced α-MHC expression under these conditions could mean that thyroxine is a dominant factor of MHC transcription regulation. A similar observation was reported with the steroid hormone testosterone, which, similar to thyroxine, overrides the hemodynamic stimuli present in SHRSP and increases the α-MHC expression.¹⁹ Expression of SM-MHC depends on the tissue type¹¹,²⁰,²¹ and the physiological state (e.g., there is increased SM-1 expression in the uterus of late pregnant rats).²² In addition, it could be demonstrated that large arteries revealed a higher SM-2 expression than

**Figure 2.** Western blot analysis of rat uterus crude myosin extracts after sodium dodecyl sulfate–polyacrylamide gel electrophoresis. SM, smooth muscle; MHC, myosin heavy chain; F, filamin; SM₁ and SM₂, two SM-MHCs detected in the rat uterus and designated with increasing mobility (decreasing molecular weight). Left panel: Coomassie stain of the sodium dodecyl sulfate–gel (protein). Middle panel: Reaction with the SM-MHC antibody (anti-SM-MHC). Right panel: Reaction with the filamin antibody (anti-Filamin).

**Figure 3.** Sodium dodecyl sulfate–polyacrylamide gel electrophoresis showing smooth muscle (SM) myosin heavy chain isoforms in the main mesenteric artery and uterus of 36-week-old control (kept at 25°C) and cold-exposed (kept at 4°C) normotensive rats (Wistar-Kyoto [WKY]) and stroke-prone spontaneously hypertensive rats (SHRSP). F, filamin; SM₁ and SM₂, two SM myosin heavy chains detected in the rat and designated with increasing mobility (decreasing molecular weight). The relation between SM₁ and SM₂ was approximately 55/45 (mean±SD <5%) in all groups and smooth muscle types investigated.
small arteries (resistance vessels).23 We found in the uterus a relation between SM-1 and SM-2 of approximately 55:45 of both main mesenteric artery and uterus in all rat groups studied. These findings are not in disagreement with the studies of Schlimmeyer and Seidel20 and Sparrow et al.,24 who reported 65:35 and 68.6:31.4 relations, respectively. In this report, rats were rather old (36 weeks), suggesting that SM-MHC expression is regulated developmentally. Even chronic hypertension did not change SM-MHC expression, which is in accordance with our previous study.11 Interestingly, in smooth muscle cells of both main mesenteric artery and uterus, MHC expression was not changed by the cold stress applied, either in SHRSP or WKY rats. This suggests that the MHC genes of cardiac and smooth muscle cells are regulated in a different manner; the heart, but not the arteries and the uterus, respond to physiologically enhanced thyroxine levels. It has been shown previously that not all MHC genes respond to changes in thyroxine levels. Thus, the α-MHC gene, which is also expressed in the atrium of the rat heart,25 is not responsive to hyperthyroidism or hypothyroidism.26

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References


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