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Research Article

Minoxidil-induced cardiac hypertrophy in guinea pigs

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Abstract. To investigate whether during cardiac hypertrophy changes occur in contractile protein composition and in mechanical and energetic properties of the myocardium, contractile protein composition, isometric force and adenosine triphosphate (ATP) consumption were studied in control and hypertrophied guinea-pig hearts. Cardiac hypertrophy was induced by adding minoxidil (120 or 200 mg/l) to the drinking water. Protein analysis was performed by one-dimensional gel electrophoresis. The myosin heavy-chain (MHC) composition was determined in an enzyme-linked immunosorbent assay (ELISA). ATP consumption and force development were simultaneously measured during isometric contraction in chemically skinned trabeculae. Histochemical analysis of cross-sectional area of cardiomyocytes and interstitial space was performed on the left ventricular tissue of 200 mg/l minoxidil-treated and control guinea pigs. Minoxidil treatment (120 and 200 mg/l) significantly increased left ventricular dry weight normalized for body weight by 19 ± 4 and $24 \pm 4\%$, respectively. No significant differences were found in the cellular cross-sectional area, while interstitial space was slightly decreased in minoxidil-treated hearts. In left ventricular trabeculae of 200 mg/l minoxidil-treated guinea pigs, ATPase activity was slightly less than in those of control guinea pigs, whereas force did not differ significantly. Calcium sensitivity of force and ATPase activity were not affected by minoxidil treatment. Gel electrophoresis revealed no difference in contractile protein composition, but a tendency towards a lower amount of α-MHC in the minoxidil-treated hearts was found in ELISA.

Key words. Guinea pig; cardiac hypertrophy; ATPase activity; force development; calcium sensitivity; contractile proteins; myosin heavy chains.

During various cardiac pathologies the expression of different contractile proteins changes [1], which has profound consequences for the performance of the heart. In this study we investigated whether the contractile protein composition was altered during mild cardiac hypertrophy induced by volume overload.

During cardiac hypertrophy in rats, the fast α-myosin heavy-chain isoform (α-MHC) is replaced by the slow β-myosin heavy-chain isoform (β-MHC), which results in a reduced adenosine triphosphatase (ATPase) activity [2] and a decrease in maximal velocity of shortening [3–5]. Until now, studies using immunohistochemistry and gel electrophoresis have indicated that in the adult human heart the β-MHC predominates in the ventricles, whereas α-MHC is present in only small amounts [6–8]. Based on these studies, it was thought that a similar shift in MHC composition in response to cardiac overload does not occur in humans, and other factors need to be responsible for the decrease in myofibrillar ATPase activity found in human heart failure [9, 10]. Anderson et al. [11] correlated the decrease in

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myofibrillar ATPase activity with reexpression of the fetal troponin T4 isoform. However, Solaro et al. [12] have found the fetal troponin T4 to be present in only 1 out of 10 failing ventricles, whereas Mesnard et al. [13] did not find a difference in TnT composition between control and failing human ventricles at the messenger RNA (mRNA) level.

Recently, Nakao et al. [14] found a substantial amount of α-MHC mRNA in nonfailing human ventricular tissue, which was significantly decreased in failing human hearts. They argue that, because of the large homology between the α- and β-MHC in human hearts, which makes it difficult to separate them on protein level, such a change in MHC composition may have gone undetected in the past. This might explain the decrease in myofibrillar ATPase activity observed in human cardiac failure [9].

To investigate whether MHCs and other contractile proteins are altered during mild hypertrophy, contractile protein composition and energetic and mechanical myocardial properties were studied in control and hypertrophied ventricular myocardium of guinea pig. Guinea pigs were used because, as in human ventricular myocardium, β-MHC predominates in guinea pig ventricles. To investigate the MHC protein composition, two monoclonal antibodies were used directed specifically against α- and β-MHC in an enzyme-linked immunosorbent assay (ELISA). To relate contractile protein composition with energetic and mechanical myocardial properties, isometric force and ATP consumption were measured in chemically skinned trabeculae isolated from right and left ventricles. The chemical skinning procedure removed all membranes (sarcoplasmic reticulum, mitochondria), whereas the contractile apparatus remained intact. In this way, the intracellular environment could be accurately controlled and manipulated, allowing a direct comparison between protein composition and contractile function. The ATPase activity was measured by means of an enzyme-coupled assay in which the resynthesis of ATP was coupled to the breakdown of nicotinamide adenine dinucleotide (NADH). The breakdown of NADH can be quantified photometrically, since NADH absorbs near-ultraviolet (UV) light (340 nm). An advantage of this method is that it allows determination of force development and ATPase activity simultaneously, which enables investigation of the relationship between energy turnover and mechanical performance (i.e. tension cost).

To induce cardiac hypertrophy, the arterial vasodilator minoxidil was added to the drinking water of the guinea pigs. In previous studies on rats, prolonged minoxidil treatment resulted in eccentric left ventricular hypertrophy by volume overload [15, 16]. This method was chosen because inducing volume overload surgically by means of an arteriovenous shunt [17] proved to be very difficult in guinea pigs. Furthermore, previous studies using acute aortic constriction to induce hypertrophy in guinea pigs by pressure overload resulted in high mortality rates [18, 19]. Since minoxidil-induced volume overload develops gradually by activation of the renin-angiotensin system and subsequent water and sodium retention, mortality rates are low.

The aim of this study was to characterize the effects of minoxidil treatment on guinea-pig hearts and to investigate the correlations between cardiac hypertrophy, contractile protein composition and contractile parameters such as isometric force, isometric ATP consumption and their ratio tension cost.

Materials and methods

Minoxidil treatment. Guinea pigs (Dunkin Hartley, female) were divided into two groups and were treated with 120 or 200 mg minoxidil (Pharmacia/Upjohn, Puurs, Belgium) per liter of drinking water. The first group of 17 guinea pigs was treated with 120 mg/l of minoxidil, while 11 control guinea pigs did not receive minoxidil. The second group consisted of 5 guinea pigs treated with 200 mg/l of minoxidil, while 7 age-matched guinea pigs served as controls. The weight of the animals was measured regularly. Body weight from both treated and control animals was determined on the day of heart removal. In control animals, body weight is a reliable index of age because the growth curves of individual animals show little variation. To determine if minoxidil treatment influenced growth, body weight of the minoxidil-treated guinea pigs was compared with the previously obtained growth curve for guinea pigs [20]. The treatment period in both groups was 5–10 weeks (mean treatment period was 44 ± 2 and 58 ± 6 days in 120 and 200 mg/l minoxidil-treated guinea pigs, respectively). This period was based on minoxidil treatment of rats, in which no further increase in ventricular weight was observed between 5 and 10 weeks of minoxidil treatment [15]. Fluid intake was monitored regularly in treated and control groups.

Preparation. After the treatment period guinea pigs were anaesthetized with xylazine [5 mg/kg intramuscularly (i.m.)], ketamine (60 mg/kg i.m.) and pentobarbitone [50 mg/kg intraperitoneally (i.p.)]. Hearts were rapidly excised, and trabeculae were dissected from right and left ventricles (RVs and LVs, respectively). Thereafter, both atria, right ventricle and left ventricle (left ventricular free wall and septum) were separated, freeze-dried and weighed. After dissection, the trabeculae were transferred to a dish containing standard relaxing solution to which 1% (v/v) Triton X-100 was added for 2 h to remove all membranous structures (i.e. mitochondria, sarcoplasmatic reticulum, sarcolemma). The composition of this standard relaxing solution was (in

Table 1. Composition of solutions for force and ATPase activity measurements.

Solution	$MgCl_2$	Na_2ATP	EGTA	HDTA	CaEGTA	Kprop
Relaxing	8.13	5.82	20	–	–	39.4
Preactivating	7.69	5.82	0.5	19.5	–	40.3
Activating	7.57	5.94	–	–	20.0	40.0

Concentrations are given in millimolar quantities. In addition, all solutions contained 0.9 mM NADH, 100 mM BES, 5 mM Na-azide, 10 mM phosphoenolpyruvate, 4 mg/ml of pyruvate kinase (298 U/mg), 0.24 mg/ml of lactate dehydrogenase (800 U/mg), 10 μM oligomycin B, and 0.2 mM p^1,p^5-di(adenosine-5′)pentaphosphate. The free Mg^{2+} and MgATP concentrations were 1 and 5 mM, respectively. CaEGTA was made by dissolving equimolar amounts of $CaCO_3$ and EGTA. The pH was adjusted to 7.0 with KOH. Potassium propionate (Kprop) was added to adjust ionic strength to 200 mM.

mM): Na_2ATP 6.13, $MgCl_2$ 6.56, ethylene glycol-bis-(amino-ethylether)N,N,N′,N′-tetra acetic acid (EGTA) 20, PCr 10, N,N-bis[2-hydroxyethyl]-2-amino-ethanesulphonic acid (BES) 100; pH 7.1, adjusted with KOH; ionic strength 200 mM, adjusted with potassium propionate.

Experimental protocol. Isometric force development and ATPase activity were measured as described previously [20]. In short, the trabecula was mounted between a force transducer and a fixed hook by means of aluminium T-clips. The muscle preparation could be transferred manually between several baths to expose the trabecula to various solutions. Isometric force and ATPase activity were measured at different free calcium concentrations at 20 °C. During the experiments, the preparations were incubated in relaxing solution for at least 4 min, in the preactivating solution with low calcium-buffering capacity for at least 3 min, in the measuring bath containing activating solution for about 1.5 min, and then transferred back into the relaxing solution. The composition of the solutions were calculated as described by Fabiato [21] and is given in table 1.

Before the first activation-relaxation cycle, the sarcomere length of the preparation, as measured in relaxing solution, was adjusted to 2.2 μm. Then, following a first activation at saturating calcium concentration (pCa 4.5; $pCa/-_{10}log[Ca^{2+}]$), the sarcomere length was readjusted to 2.2 μm, if necessary, and the length of the trabecula between the clips as well as the width and the thickness were measured (at × 50 magnification). It was found that after this readjustment, the resting sarcomere length remained stable throughout the experiment. The second measurement at pCa 4.5 was used to calculate maximal force per cross-sectional area and ATPase activity per volume. The next two to three contractions were carried out at different pCa values (> pCa 4.5). These measurements were followed by a control measurement at saturating Ca^{2+} concentration. Isometric force and ATPase activity were corrected for muscle deterioration by linear interpolation between control values obtained at pCa 4.5. The intermediate isometric results, obtained at higher pCa values, were normalized to the interpolated values. Measurements were continued until full force-pCa and ATPase-pCa curves were obtained or until the isometric force of a control measurement ≤ 80% of the first control contraction at maximal Ca^{2+} concentration. The usual number of force measurements per trabecula amounted to 9, of which at least 3 were performed at maximal calcium concentration. Typically, force decline, between the first and final maximal activation was 15%.

ATP consumption of the skinned trabeculae was measured by an enzyme-coupled assay in which the resynthesis of ATP is coupled to the breakdown of NADH [20, 22]. The measuring bath had thin glass windows to allow transmission of near-UV light (340 nm) for the measurement of NADH absorbance. ATPase activity was derived from linear regression analysis of the NADH absorbance signal. The Ca^{2+}-activated ATPase

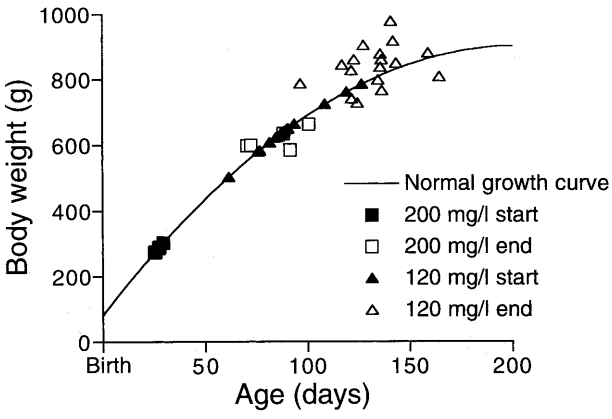

Figure 1. Growth in minoxidil-treated guinea pigs is compared with the growth curve of control guinea pigs. The body weight of guinea pigs at birth amounted to 82 g (data obtained from Harlan, Horst, the Netherlands). Start refers to the body weight of the animals at the start of minoxidil treatment. The age of the animals at the start of minoxidil treatment was calculated from their body weight and the growth curve, end, body weight and age on the day the hearts were excised.

Table 2. Absolute heart weights.

Group	HW (mg)	RV (mg)	LV (mg)
Control ($n = 11$)	405 ± 25	74 ± 7	253 ± 11
120 mg/l minoxidil ($n = 17$)	482 ± 10*	87 ± 2	321 ± 8*
Control ($n = 7$)	290 ± 12	57 ± 3	181 ± 9
200 mg/l minoxidil ($n = 5$)	372 ± 8*	71 ± 5*	237 ± 8*

HW, total heart weight; RV, right ventricular heart weight; LV, left ventricular heart weight (septum included); n = number of animals. Values are given $\pm$ SEM. Differences between control and minoxidil-treated guinea pigs are significant at the level of 0.05 (*).

activity was corrected for basal ATPase activity measured in relaxing solution (pCa 9). Basal activity amounted to $18.4 \pm 1.5\%$ of the maximal ATPase activity.

Contractile protein analysis. Quantification of the α- and β-MHCs in guinea-pig ventricular tissue was performed using two monoclonal antibodies directed specifically against α-MHC (mAb 2495A4) and β-MHC (mAb 1691D5) in ELISA, as described in detail previously [20]. Calibration of the ELISA was performed using hypothyroid and euthyroid rat ventricles, in which the fast (α) and slow (β) myosin heavy-chain isoforms could be separated by SDS-polyacrylamide gel electrophoresis (PAGE) and quantified by subsequent laser densitometry. Hypothyroid rat myocardium proved to be 100% β-MHC. The euthyroid control animals contained a mixture of 95% α-MHC and 5% β-MHC. The calibration curves for the ELISA were obtained from the optical density readings from hypothyroid and euthyroid tissue and from mixtures of these two samples. Using these calibration curves, the amounts of α- and β-MHC present in the guinea-pig ventricles were calculated.

SDS-PAGE was performed as described previously by Giulian et al. [23] using an acrylamide to bis-acrylamide ratio of 200:1 in the separating gel (12% total acrylamide; pH 9.3) and of 20:1 in the stacking gel (3.5% acrylamide; pH 6.8). On each gel 25 samples were loaded. Silver staining was performed as described by Giulian et al. [23]. Protein composition was quantified by measuring the area underneath each peak obtained with laser densitometry. Contractile proteins were identified by Western immunoblotting using specific antibodies and/or molecular weight standards (Bio-Rad, high range: 161-0303 and low range: 161-0304). The following antibodies were used: α-MHC antibody (mAb 249-5A4, 1:50), β-MHC antibody (mAb 169-1D5, 1:100), α-actinin antibody (clone EA-53, 1:200, Sigma), actin antibody (clone C4, 1:100, Boehringer Mannheim), troponin T antibody (clone JLT-12, 1:200, Sigma), tropomyosin antibody (clone TM311, 1:400, Sigma), troponin I antibody (mAb 1691, 1:200, Chemicon) and myosin light chain antibody (clone MY-21, 1:200, Sigma).

Histochemistry. To determine the cross-sectional area of myocytes and interstitial space tissue sections of left ventricular myocardium of minoxidil-treated (200 mg/l, $n = 5$) and control ($n = 5$) guinea pigs were cut and stained with hematoxylin and eosine (HE). The histochemistry was performed as described previously by Lee-de Groot et al. [24]. In short, freeze-dried tissue samples were embedded in 15% (w/v) gelatin (Merck) in 0.1 M Tris-maleate (containing 15 mM EGTA, pH 7.5), and frozen in liquid nitrogen. Sections (10 μm thick) were cut in a cryostat at -22 °C, collected on slides, and air-dried for ~ 10 min at room temperature. Subsequently, the tissue was fixed for 5 min in 4% formalin and stained with hematoxylin for 1 min (Sigma, 2% dissolved in ethanol) and eosin for 5 min (Merck, 1% dissolved in water). All steps were performed at room temperature. Before mounting in Entallan (Merck), the tissue was dehydrated (70–97% ethanol). To obtain insight into the effect of freeze-drying prior to embedding of tissue for histochemical analysis, freeze-dried tissue was compared with fresh tissue. One part of a rat heart was embedded directly after Langendorff perfusion in 15% (w/v) gelatin in Tyrode solution, while another part was embedded after freeze-drying as described above.

For the determination of cross-sectional area and interstitial space, regions in the tissue sections were randomly chosen in which the myocardial cells were cut perpendicularly to their longitudinal axis. The area analysed contained approximately 20 cardiomyocytes. The interstitial space (i.e. the area not occupied by cardiomyocytes) was expressed relative to the total area (cardiomyocytes and interstitial space) investigated. The cross-sectional area of the cardiomyocytes was corrected for average sarcomere length, which was measured in regions where the cells were cut along their longitudinal axis. The average sarcomere length was calculated from 5 cardiomyocytes in different regions from each section.

Data analysis. Sigmoidal force-pCa and ATPase-pCa relations were fit by a nonlinear fit procedure to a modified Hill equation:

$$P(Ca^{2+})/P_0 = [Ca^{2+}]^{nHill}/(K^{nHill} + [Ca^{2+}]^{nHill})$$

where P is steady-state force (or ATPase activity). P_0 denotes the steady isometric force (or ATPase activity) at saturating Ca^{2+} concentration, nHill represents the steepness of the relationship and K (or pK) represents the Ca^{2+} concentration (or pCa) at which force (or ATPase activity) $= 0.5 \times P_0$, that is the midpoint of the force-pCa (ATPase-pCa) relationship. P_0 was deter-

mined from the first reference activation at a pCa of 4.5. The SEM values were used as a weight factor for the average force and ATPase activity values.

Values are given as means ± SEM of n experiments. Differences were tested by means of Student's t-test at an 0.05 level of significance ($P < 0.05$).

Results

Minoxidil intake. Water uptake did not differ between control and minoxidil-treated guinea pigs and amounted to, respectively, 130 ± 4 and 143 ± 8 ml per animal per day in the control and 120 mg/l minoxidil-treated groups and, respectively, 127 ± 4 and 115 ± 7 ml per animal per day in the control and 200 mg/l minoxidil-treated groups. The mean minoxidil intake during the treatment period amounted to 23 and 52 mg/kg body weight per day in the 120 and 200 mg/l minoxidil-treated groups, respectively.

Cardiac hypertrophy. Minoxidil treatment did not affect growth of the guinea pigs, since growth of the minoxidil-treated guinea pigs did not differ from the growth curve obtained from guinea pigs studied previously [20] (fig. 1). In this figure, body weight of the minoxidil-treated guinea pigs at the start and at the end of treatment are included. The age of the minoxidil-treated animals at the start of treatment was calculated from their body weight and the growth curve. Nevertheless, a small, but significant difference was present between body weight (i.e. age), measured before excision of the heart, of control (765 ± 14 g) and 120 mg/l-treated (841 ± 16 g) guinea pigs. This small difference in age was presumably already present when the animals arrived in the laboratory and minoxidil treatment was started. Body weight between control (585 ± 20 g) and 200 mg/l-treated (616 ± 15 g) guinea pigs at the end of the minoxidil treatment did not differ.

Minoxidil treatment increased absolute heart weight in both 120 and 200 mg/l minoxidil-treated groups compared with the control groups (table 2). A significant increase in heart weight to body weight ratio was found in both minoxidil-treated groups, which was mainly due to the increase in left ventricular weight, since the right ventricular weight to body weight ratio did not differ significantly between treated and untreated groups (fig. 2). The left ventricular weight normalized to body weight ratio increased by 19 ± 4% and 24 ± 4% in the 120 and 200 mg/l minoxidil-treated groups compared with the control groups, respectively. The left ventricular weight normalized to body weight ratio did not differ significantly between the 120 and 200 mg/l minoxidil-treated groups (0.38 ± 0.01 for both groups). There was no tendency towards higher heart weight/body weight ratios after > 5 weeks of minoxidil treatment.

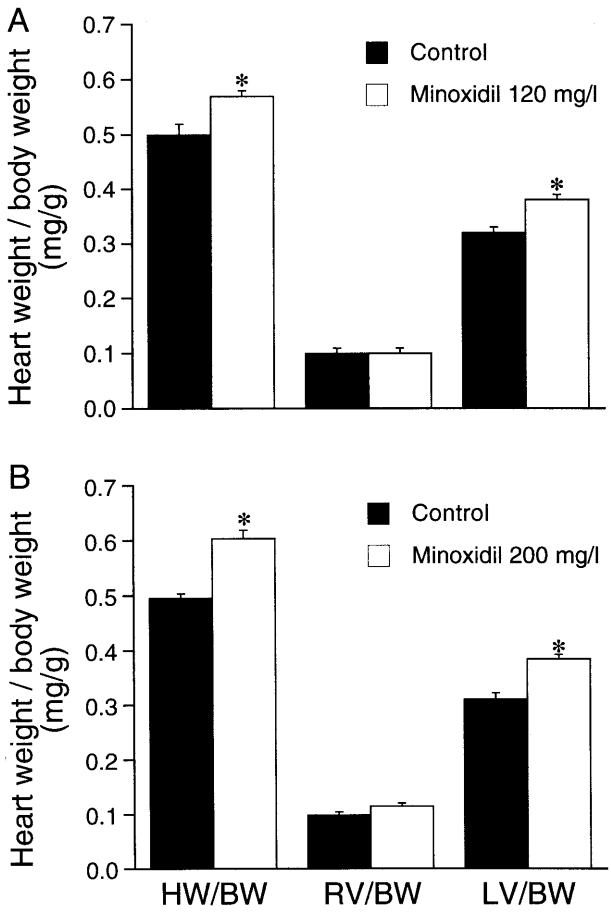

Figure 2. Heart weights (mg) normalized for body weights (g) from control and 120 mg/l (A) and 200 mg/l (B) minoxidil-treated guinea pigs. HW, total heart weight; BW, body weight; RV, right ventricular weight; LV, left ventricular weight. *$P < 0.05$.

To determine whether the increase in left ventricular weight could be attributed to an increase in cross-sectional area or an increase in length of the cardiac myocytes, histochemical analysis was performed on left ventricular tissue of 200 mg/l minoxidil-treated and control guinea pigs. Sarcomere length, cross-sectional area of cardiomyocytes and interstitial space were determined in hematoxylin-eosine (HE)-stained tissue sections. The results are given in table 3. HE-stained tissue sections of a control and minoxidil-treated guinea-pig heart are shown in figure 3A and B, respectively. Sarcomere length measured in regions where the cells were cut along their longitudinal axis did not differ between control and minoxidil-treated samples (1.52 ± 0.08 and 1.51 ± 0.11 μm, respectively). A minor increase in the average cross-sectional area normalized on sarcomere length (13.6%) was observed in the minoxidil-treated versus control hearts (table 3), but the difference be-

tween the two groups was not significant. The relative amount of interstitial space (i.e. the area occupied by interstitial space divided by the total area investigated) was slightly lower in minoxidil-treated hearts in comparison with control hearts. To determine whether freeze-drying of the samples affected cross-sectional area and/or interstitial space, a comparison was made between fresh and freeze-dried tissue of rat heart. Freeze-drying of the tissue did not affect the relative amount of interstitial space, whereas cross-sectional area of the cells was significantly decreased by 39% in the freeze-dried tissue compared with fresh tissue (table 3). This implies that caution should be exerted when comparing the values for the cross-sectional area of the cardiomyocytes of the freeze-dried guinea-pig hearts with those of intact cardiomyocytes.

Force and ATP consumption. ATP consumption and force production were measured in a total of 128 chemically permeabilized trabeculae during isometric contraction. From each heart about 3 suitable trabeculae were obtained. Dimensions of the preparations (means $\pm$ SEM) from control animals were 1.87 ± 0.17 and 1.65 ± 0.12 mm in length, 203 ± 13 and 202 ± 14 μm in width, and 195 ± 13 and 192 ± 14 μm in thickness for respectively right ($n = 17$) and left ($n = 19$) ventricle, measured at a resting sarcomere length of 2.2 μm. The dimensions of the right ($n = 44$) and left ($n = 48$) ventricular preparations from minoxidil-treated animals were, respectively, 1.96 ± 0.08 and 1.69 ± 0.07 mm in length, 226 ± 11 and 205 ± 10 μm in width, and 223 ± 11 and 196 ± 9 μm in thickness.

For all trabeculae measured in this study, maximum force per cross-sectional area and ATP consumption per trabecula volume were calculated. The mean values from right and left ventricular trabeculae of control and minoxidil-treated groups are given in table 4. The ATP consumption per volume of left ventricular trabeculae from 200 mg/l minoxidil-treated guinea pigs was slightly

Table 3. Histochemical data.

Tissue	Cross-sectional area (μm^2)	Interstitial space (%)
Control ($n = 5$)	59 ± 6	21 ± 1
200 mg/l minoxidil ($n = 5$)	67 ± 10	16 ± 1*
Fresh rat tissue	167 ± 11	21 ± 3
Freeze-dried rat tissue	102 ± 6**	23 ± 3

Cross-sectional area of myocytes at a sarcomere length of 2.0 μm. Interstitial space was determined relative to total tissue region analyzed. n = number of left ventricles; mean values are given $\pm$ SEM. Differences are significant at the level of 0.05 (*) and 0.001 (**).

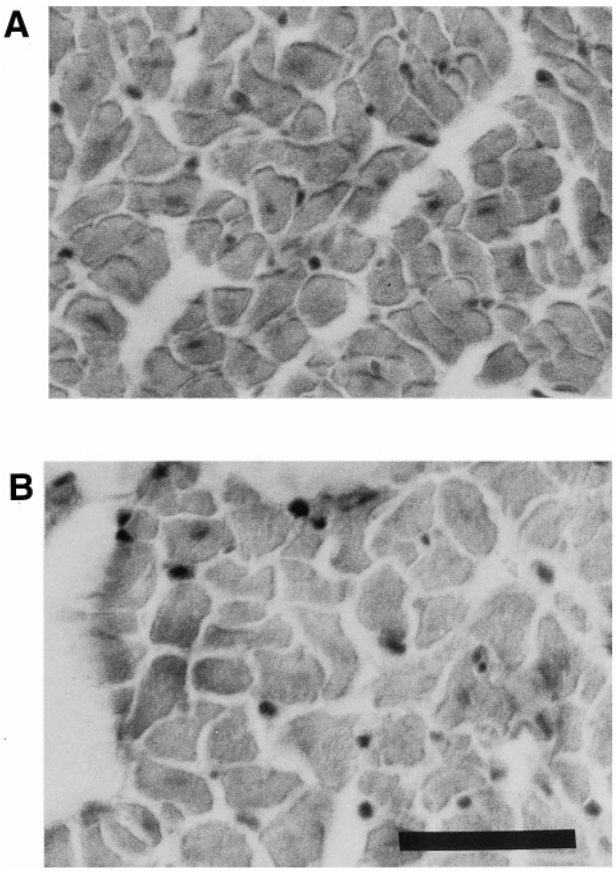

Figure 3. Hematoxylin-eosin-stained transverse sections of cardiomyocytes of the left ventricle of a control (*A*) and minoxidil (200 mg/l)-treated (*B*) guinea pigs. Although the cross-sectional area of cells from the minoxidil-treated heart seems to be larger than that from the control heart, the average values from the two groups did not differ significantly. Bar, 20 μm.

($\sim 25\%$), but significantly lower compared with the untreated control animals ($P < 0.05$), whereas isometric force development did not differ. When ATP consumption per volume is divided by force per cross-sectional area, tension cost is obtained, which is a measure of muscle economy. Values are included in table 4. Tension cost did not differ significantly between control and minoxidil-treated animals. No significant differences were present in force, ATP consumption and tension cost between right and left ventricular trabeculae from the same group.

Calcium sensitivity of force and ATP consumption. The calcium sensitivity of force and ATP consumption was determined by exposing the trabeculae to different Ca^{2+} concentrations. The mean force-pCa and ATPase activity-pCa curves of control and 200 mg/l minoxidil-treated animals are shown in figure 4A and B, respectively. No significant differences were observed between

the steepness (nHill) and midpoint (pK) of the mean force-pCa and ATPase activity-pCa relationships of control and minoxidil-treated groups. Furthermore, no differences were present in these parameters between right and left ventricular trabeculae.

Contractile protein composition. Contractile protein composition of all right and left ventricular samples was analyzed by means of SDS-PAGE. In figure 5A a silver-stained polyacrylamide gel of electrophoretically separated proteins from left ventricular control and minoxidil-treated (200 mg/l) myocardium are shown. All contractile protein bands identified, and the intense unidentified protein bands 1–6 as indicated in figure 5A, were quantified by laser densitometric analysis. The laser densitometric scans from typical control and 200 mg/l minoxidil-treated tissue (lanes C and M in fig. 5A) are shown in figure 6A and B, respectively. To allow a comparison of the densitometric scans the minoxidil scan was subtracted from the control scan (included in fig. 6B). This difference tracing illustrates that the minoxidil scan is very similar to the control scan. To quantify protein composition, the area underneath the peaks indicated in figure 5 was determined in all right and left ventricular samples. It was verified by applying different amounts of tissue that only the actin and MHC bands were saturated when this amount of sample was used. Accordingly, the actin and MHC bands were not taken into account in the evaluation. No significant differences were found between the protein areas of right and left ventricular samples. Moreover, the areas underneath the peaks did not differ between control and minoxidil samples. This indicates that the protein contents of control and minoxidil-treated hearts were the same.

Since the α- and β-MHC isoforms from guinea pig could not be separated by SDS-PAGE, the MHC composition of the ventricular tissues was determined with an ELISA. The percentages of α-MHC present in right and left ventricular tissue are given in table 5. A small but significant difference was present between the fractions of α-MHC present in the left ventricles of control and 120 mg/l minoxidil-treated guinea pigs, but not in the 200 mg/l group. The total $(\alpha+\beta)$-MHC content in the ventricles, expressed relative to the total MHC content in the rat samples used to calibrate the assay, did not differ significantly between control and minoxidil-treated ventricles as indicated by the MHC ratios given in table 5.

Although the α- and β-MHCs were not separated by SDS-PAGE in guinea-pig ventricular muscle, the presence of α-MHC and β-MHC could be visualized with Western immunoblotting using two monoclonal antibodies directed specifically against α-MHC and β-MHC (fig. 5B).

Discussion

Minoxidil-induced cardiac hypertrophy. Minoxidil treatment (120 mg/l for 5 weeks) of normotensive rats induced enlargement of the left ventricle by 30% [25]. In our study, minoxidil treatment (120 and 200 mg/l) of guinea pigs increased left ventricular weight significantly by 19% and 24%, respectively. The cross-sectional area of the left ventricular cardiac myocytes of 200 mg/l minoxidil-treated hearts was $\sim$13.6% larger than in control ventricles, but this difference did not reach statistical significance. Nevertheless, this might explain part of the increase in left ventricular weight. However, since it was shown in rats that minoxidil induces dilated left ventricular hypertrophy, we consider it more likely that the increase in left ventricular weight observed in the guinea pigs was mainly due to an increase in length of the cardiac myocytes. In general, these findings are similar to the effects observed in rat. However, in rat hearts minoxidil induced concentric right ventricular hypertrophy [15, 26], which was not observed in our study because in guinea pigs right ventricular weight was not affected by minoxidil.

Table 4. Isometric force, ATPase activity and tension cost in control and minoxidil-treated guinea pigs.

Group	ATP RV	ATP LV	F RV	F LV	TC RV	TC LV
Control	0.10 ± 0.01 (11)	0.12 ± 0.0 (8)	141 ± 3 (11)	45 ± 4 (8)	2.4 ± 0.2 (11)	2.8 ± 0.3 (8)
120 mg/l Minoxidil	0.10 ± 0.01 (37)	0.12 ± 0.01 (42)	41 ± 2 (37)	46 ± 2 (42)	2.7 ± 0.2 (37)	2.8 ± 0.2 (42)
Control	0.10 ± 0.01 (6)	0.11 ± 0.01 (11)	40 ± 3 (6)	43 ± 3 (11)	2.5 ± 0.3 (6)	2.7 ± 0.2 (11)
200 mg/l Minoxidil	0.09 ± 0.01 (7)	0.08 ± 0.01* (6)	42 ± 4 (7)	37 ± 6 (6)	2.2 ± 0.1 (7)	2.6 ± 0.3 (6)

ATP, ATPase activity/volume (mM/s); F, force/cross-sectional area (kN/m^2); TC, tension cost ($mmol/kN \cdot m \cdot s$); RV, right ventricular trabeculae; LV, left ventricular trabeculae. Average values are given/SEM. *$P<0.05$, significantly different from control group. Values between brackets denote the number of trabeculae.

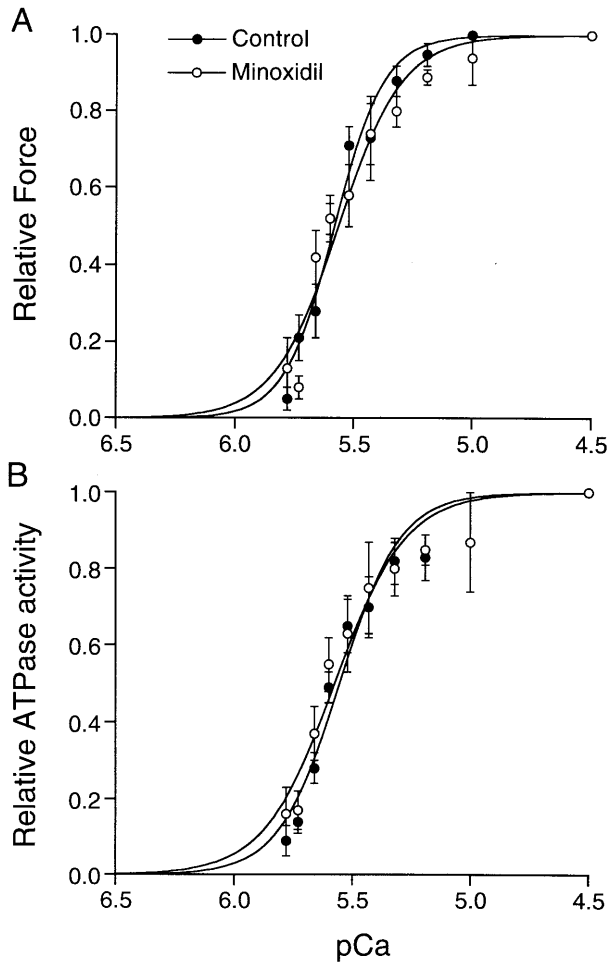

Figure 4. Calcium sensitivity of force and ATPase activity. Isometric force and ATPase activity at different free calcium concentrations in trabeculae from control and 200 mg/l minoxidil-treated hearts. Isometric force (*A*) and ATPase activity (*B*) for each trabecula are normalized to the control force and ATPase activity found at saturating calcium concentration. Mean values are shown $\pm$ SEM. Hill curves were fitted to the data points as indicated in 'Materials and methods'. The steepness (nHill) of the force-pCa and ATPase-pCa relations was, respectively, in control guinea pigs: 4.13 ± 0.49 and 3.37 ± 0.49, and in minoxidil-treated guinea pigs: 3.20 ± 0.50 and 2.89 ± 0.43. The midpoint (pK) of the force-pCa and ATPase-pCa relations was, respectively, in control guinea pigs: 5.58 ± 0.01 and 5.55 ± 0.02, and in minoxidil-treated guinea pigs: 5.57 ± 0.02 and 5.58 ± 0.02.

The decrease in interstitial space found in minoxidil-treated hearts differs from previous data on cardiac hypertrophy induced in rats by monocrotaline [24]. However, it may be noted that the difference in interstitial space does not interfere with our quantification of hypertrophy based on the comparison of dry heart weights between control and minoxidil-treated animals. It can be noted that the minoxidil intake in the 120 and 200 mg/l minoxidil groups was respectively 4–8 times higher than in the rats [25] because guinea pigs drink more. Previous studies on dogs [27, 28] and rats [29] have shown that similar doses of minoxidil, under certain conditions, may have cardiotoxic effects. However, macroscopic inspection of the guinea-pig hearts and histochemical analysis did not reveal any lesions or necrosis. Furthermore, fluid intake and body weight were not affected.

Energetic and contractile properties of the heart. The ATPase activity per volume of left ventricular trabeculae from 200 mg/l minoxidil-treated guinea pigs was significantly lower compared with the untreated control animals, whereas isometric force development was not affected. However, no significant differences were present in tension cost between control and minoxidil-treated guinea pigs, indicating that economy of contraction was similar in control and hypertrophied hearts. Furthermore, no significant differences were found between calcium sensitivity of force and ATP consumption between control and minoxidil-treated guinea-pig hearts, indicating that minoxidil treatment induces left ventricular hypertrophy without affecting mechanical and energetic properties of the heart.

Contractile protein composition. In normotensive rats minoxidil treatment increased the duration of both contraction and relaxation of left ventricular papillary muscle [26], which may be explained by a shift from the α-MHC isoform to the β-MHC isoform observed in minoxidil-treated spontaneously hypertensive rats [30]. In our study, some α-MHC protein was found in the ventricles of guinea pigs using ELISA (~ 2–7% in control myocardium) and Western immunoblotting (fig. 5B). In the 120 mg/l minoxidil group, the α-MHC fraction was significantly lower compared with the controls, in left ventricular tissue only ($P < 0.05$). This can be explained by a difference in average age (i.e. body weight) between the control (765 ± 14 g) and minoxidil-treated (841 ± 16 g) animals before excision of the hearts. This age difference may have been present at the

Table 5. Percentages of α-MHC.

Group	α-MHC RV	α-MHC LV	MHC ratio
Control	3.6 ± 1.2	5.3 ± 1.6	0.85 ± 0.05
120 mg/l minoxidil	1.6 ± 0.5	1.3 ± 0.7*	0.91 ± 0.02
Control	7.1 ± 1.7	5.0 ± 2.7	0.83 ± 0.08
200 mg/l minoxidil	3.4 ± 2.1	1.2 ± 0.8	0.80 ± 0.05

Percentage of α-MHC, expressed relative to total ($\alpha+\beta$)-MHC content, present in right (RV) and left (LV) ventricular heart tissue. MHC ratio is total ($\alpha+\beta$)-MHC content expressed relative to the total MHC content in the rat samples used to calibrate the assay. Values are given/SEM. *$P<0.05$, significantly different from control group.

start of the minoxidil treatment, since the animals were not exactly age-matched at this point. Previously, we showed that with aging the α-MHC was replaced by the β-MHC, which consequently altered the energetic properties of the myocardium [20]. In the present study the α-MHC fraction was lower in the older minoxidil-treated animals compared with the younger control animals, which is in agreement with our previous findings.

A tendency towards a lower percentage of α-MHC was observed in minoxidil-treated hearts (both RV and LV, table 5). The total MHC content did not differ significantly between control and minoxidil-treated hearts, suggesting that the changes in α- and β-MHC are complementary. The small decrease in ATPase activity observed in left ventricular trabeculae from 200 mg/l minoxidil-treated guinea pigs could be due to a shift from the α-MHC isoform to the β-MHC isoform [20]. However, the difference in α-MHC present in the left ventricles of control ($5.0 \pm 2.7\%$) and 200 mg/l treated ($1.2 \pm 0.8\%$) animals was small and did not reach the level of significance.

The decrease in myofibrillar ATPase activity observed in failing human hearts was explained by the reexpression of a fetal TnT isoform (TnT4) by Anderson et al. [11]. Examination of the four different TnT isoforms in our guinea-pig samples, denoted TnT1 to TnT4, did not reveal any differences between control

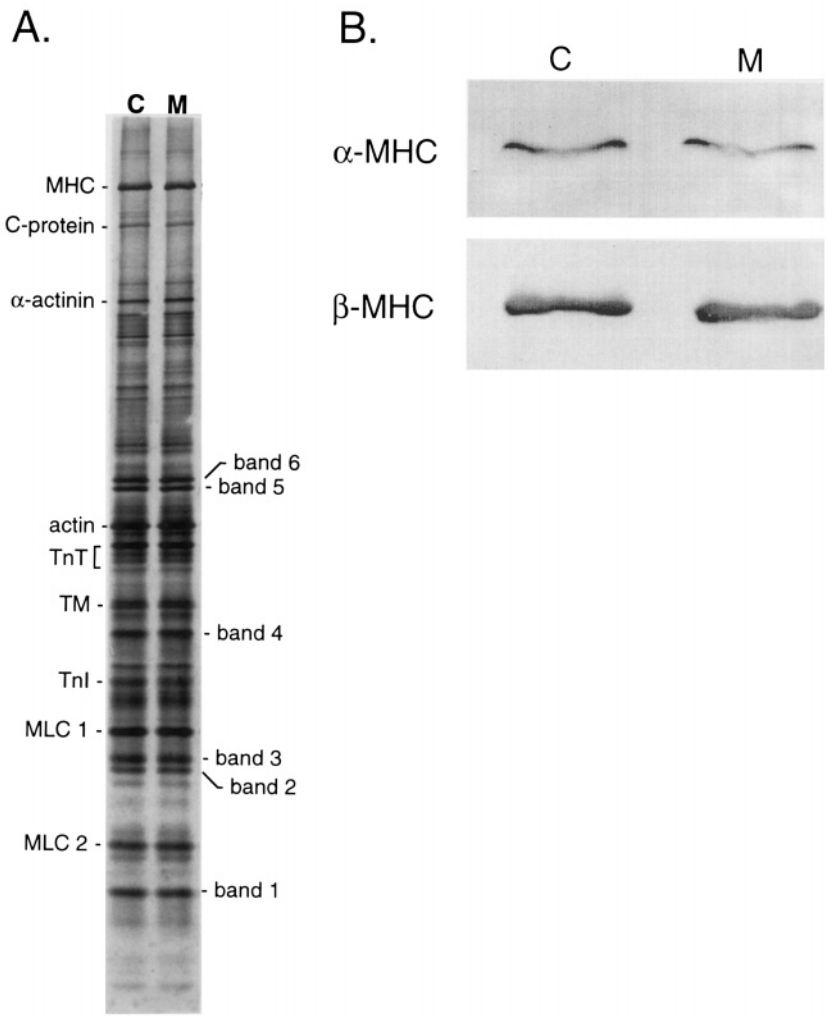

Figure 5. Silver-stained SDS-PAGE (*A*) of left ventricular myocardial contractile proteins present in control (lane C) and minoxidil (lane M) (200 mg/l)-treated hearts (0.6-μg sample was loaded in each lane). Contractile proteins: MHC, myosin heavy chain; TnT, troponin T; TM, tropomyosin; TnI, troponin I; MLC-1, myosin light chain 1; and MLC-2, myosin light chain 2. Bands 1–6: Intense unidentified protein bands. (*B*) Western immunoblots of α- and β-MHC present in control (C) and minoxidil (M)-treated ventricular tissue (1.0-μg sample was loaded in each lane).

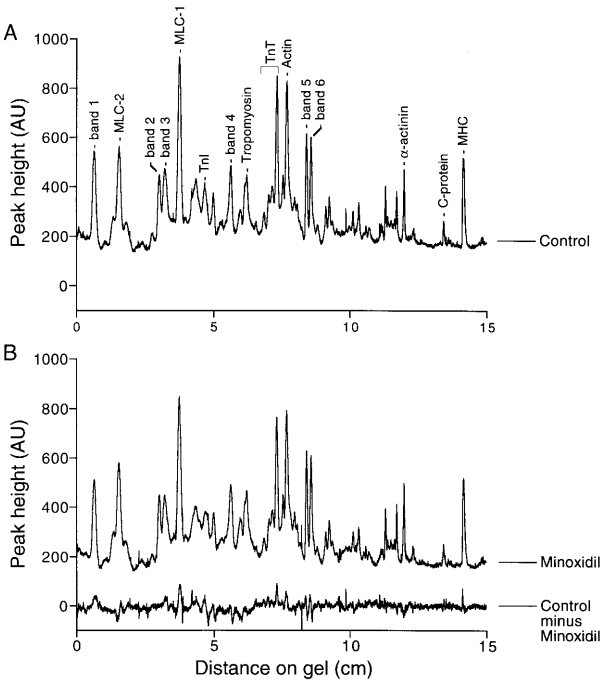

Figure 6. Laser densitometric scans of control (*A*) and minoxidil-treated (*B*) heart tissue, corresponding to lane C and M in figure 5A, respectively. All protein bands indicated in figure 5A were scanned and quantified. The distance on the gel indicates distance relative to the starting point of scanning. The lower line in figure 6B denotes the difference between control and minoxidil-treated tissue (control scan minus minoxidil scan). AU = arbitrary units.

and minoxidil hearts. Thus, the decrease in ATP consumption observed in 200 mg/l minoxidil-treated left ventricles cannot be explained by a change in TnT composition. Protein analysis by SDS-PAGE and subsequent laser densitometric scanning did not reveal any quantitative differences between the contractile protein bands identified and nonidentified protein bands (bands 1–6) in control and hypertrophied tissue. The results indicate that minoxidil treatment tends to induce a reduction in the α-MHC content, but does not cause discernable alterations in the major protein bands studied.

Conclusion

Our results indicate that minoxidil treatment induces mild left ventricular hypertrophy without major changes in contractile protein composition. A tendency towards a lower α-MHC fraction was observed in the 200 mg/l minoxidil-treated group, which may explain the slight decrease in ATPase activity.

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