Selective increase of angiotensin(1–7) and its receptor in hearts of spontaneously hypertensive rats subjected to physical training

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In the present study we investigated the effects of physical training on plasma and cardiac angiotensin(1–7) [Ang(1–7)] levels. In addition, possible changes in expression of the Ang(1–7) Mas receptor in the heart were also evaluated. Normotensive Wistar rats and spontaneously hypertensive rats (SHR) were subjected to an 8 week period of 5% overload swimming training. Blood pressure was determined by a tail-cuff system. Heart and left ventricle weights and cardiomyocyte diameter were analysed to evaluate cardiac hypertrophy. Radioimmunoassay was used to measure angiotensin levels. Expression of Mas was determined by semi-quantitative polymerase chain reaction, immunofluorescence and Western blotting. Physical training induced cardiac hypertrophy in Wistar rats and SHR. A significant decrease of plasma angiotensin II (Ang II) levels in both strains was also observed. Strikingly, trained SHR, but not trained Wistar rats, showed a twofold increase in left ventricular Ang(1–7) levels. No significant changes were observed in plasma Ang(1–7) and left ventricular Ang II concentrations in either strain. Furthermore, Mas mRNA and protein expression in left ventricle were substantially increased in trained SHR. The physical training protocol used did not change blood pressure in either strain. These results suggest that the beneficial effects induced by swimming training in hypertensive rats might include an augmentation of Ang(1–7) and its receptor in the heart.

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Clinical and experimental data have indicated a beneficial effect of physical training on cardiovascular function in both normotensive and hypertensive humans and other mammals (Cox et al. 1985; Schaible & Scheuer, 1985, 1986; Friberg et al. 1988; Geenen et al. 1988; Negrão et al. 1992). Although the precise mechanisms by which exercise ameliorates cardiovascular function are not fully understood, putative factors include increased myocardial contractility (Schaible & Scheuer, 1985), improved myosin ATPase activity (Schaible et al. 1986), improved systolic heart function (Schaible et al. 1986), decreased myocardial oxygen consumption (Friberg et al. 1988), elevation of stroke volume (Friberg et al. 1988), and reduction of resting (Geenen et al. 1988; Negrão et al. 1992) and submaximal heart rate (Cox et al. 1985). Accordingly, physical training reduced ventricular failure induced by hypertension (Moreno Júniior et al. 1995), recovered ATPase activity and improved systolic function in spontaneously hypertensive rats (SHR) subjected to swimming training (Scheuer et al. 1982). It has been suggested that a decreased plasma renin activity is also involved in the beneficial effects of physical training in SHR (Hayashi et al. 2000; Zamo et al. 2004) and in humans (Geyssant et al. 1981). Furthermore, exercise training decreased plasma angiotensin (Ang) II levels in humans with chronic heart failure (Brath et al. 1999).

Although Ang II is the major effector of the renin–angiotensin system (RAS), various other angiotensins are now recognized as being biologically active. Angiotensin(1–7) is considered to be an important peptide fragment of the RAS because it has important actions which are often opposite to those of Ang II. Several
beneficial actions have been attributed to Ang(1–7) in the heart. Angiotensin(1–7) induced vasodilatation in porcine (Porsti et al. 1994; Gorelik et al. 1998) and canine coronary artery (Brosnihan et al. 1996) and potentiated the vasodilatory effect of bradykinin in isolated perfused rat hearts (Almeida et al. 2000). With respect to cardiac function, Ang(1–7) decreased the incidence and duration of ischaemia–reperfusion arrhythmias and improved postischaemic contractile function in isolated perfused rat hearts (Ferreira et al. 2001, 2002). Recently, it has been shown that chronic administration of Ang(1–7) or its synthetic analogue, AVE 0991, significantly improved the cardiac function in isolated perfused hearts from SHR treated with L-NAME (Benter et al. 2006) and in infarcted rat hearts (Ferreira et al. 2007). Additionally, an 8 week period of Ang(1–7) infusion improved endothelial aortic function and coronary perfusion and preserved cardiac function in rats with heart failure induced by ligation of the left coronary artery (Loot et al. 2002). Acute infusion of this peptide produced a significant increase in cardiac output and stroke volume in normal anaesthetized rats (Sampaio et al. 2003). Moreover, transgenic rats TGR(A1-7)3292, which present a 2.5-fold increase in plasma Ang(1–7) levels, show an increase in cardiac output (Botelho-Santos et al. 2007) and a slight but significant increase in the first derivative of left ventricular pressure over time (dP/dt), a less pronounced cardiac hypertrophy induced by isoproterenol, a reduced duration of reperfusion arrhythmias, and improved postischaemic function in isolated perfused hearts (Santos et al. 2004). A similar cardioprotective effect of Ang(1–7) was obtained by Grobe and colleagues in rats chronically treated with Ang II (Grobe et al. 2007) and in Deoxycorticosterone acetate (DOCA)–salt-induced hypertensive rats (Grobe et al. 2006).

The mechanisms underlying these beneficial effects could be related to the observations that Ang(1–7) reduces the growth of cardiomyocytes (Tallant et al. 2005) and has an inhibitory effect on collagen synthesis (Iwata et al. 2005). Recently, we have found that hearts from Mas-deficient mice exhibited a marked change of extracellular matrix protein expression to a profibrotic profile, accompanied by an important cardiac dysfunction (Santos et al. 2006). Moreover, Grobe et al. (2006, 2007) reported that Ang(1–7) prevents cardiac fibrosis independent of blood pressure changes. An underlying mechanism that may also be involved in its beneficial effects in the heart is the Ang(1–7)-induced decrease in cardiac Ang II levels (Mendes et al. 2005).

The purpose of the present study was to investigate whether the beneficial effects of physical training could include changes in levels of Ang(1–7) in plasma and heart, as well as of its receptor (Santos et al. 2003). To test this possibility we used normotensive Wistar rats and SHR subjected to swimming training.

**Methods**

**Animals**

Four-month-old male normotensive Wistar rats and SHR were used in this study. All rats were provided by the animal facilities of the Biological Sciences Institute (CEBIO, Federal University of Minas Gerais). Animals were housed in a temperature-controlled room and maintained on a 12 h–12 h light–dark cycle with free access to water and food. All experimental procedures were performed in compliance with the Institutional Animal Care and Use Committee at the Federal University of Minas Gerais, Brazil.

**Training programme**

Animals were randomly assigned to one of the four groups: sedentary Wistar rats; trained Wistar rats; sedentary SHR; and trained SHR. The exercise training was performed in temperature controlled (32 ± 2°C) swimming pools for 1 h day⁻¹, 5 days week⁻¹, over 8 weeks. Overload swimming training (5% of the body weight) was achieved by fixing weights on the tails of the animals (Lancha et al. 1995; Medeiros et al. 2004).

**Arterial pressure measurements**

Arterial pressure was measured indirectly (tail-cuff method) in conscious animals (n = 14–16 animals) before training sessions once a week. In our tail-cuff system, blood pressure measurements correspond to mean arterial pressure (MAP; Britto et al. 1997).

**Analysis of cardiac hypertrophy**

At the end of the swimming training, the rats were killed by decapitation and the hearts were immediately removed. The atria and right ventricles were dissected free from the left ventricles and discarded. Wet weights of the hearts and left ventricles were recorded, normalized for body weight and expressed as muscle mass index (mg g⁻¹; n = 16–25 animals). In addition, four to five left ventricles from each group were left in 10% formalin in 0.1 mol l⁻¹ phosphate buffer, pH 7.4, for 24 h at room temperature. The tissues were dehydrated by sequential washes with 70, 80, 90 and 100% ethanol and embedded in glycidyl methacrylate (JB-4™, Polysciences, St Louis, MO, USA). Transverse sections (2 μm thick) were cut, starting from the base area of the left ventricle, at intervals of 40 μm, and stained with Haematoxylin and Eosin (Santos et al. 2004). Tissue sections (3–4 for each animal) were examined with a light microscope (Axio Plan 2, Zeiss) at ×100 magnification, photographed (Axio Cam Digital Camera, Zeiss), and analysed with Zeiss KS 400 3.0 software. Only digitized images of cardiomyocytes cut longitudinally with nuclei...
and cellular limits visible were used for analysis (an average of 30 cardiomyocytes for each slice). The diameter of each myocyte was measured across the region corresponding to the nucleus. Fifty to one hundred cardiomyocytes were analysed for each animal. Quantification of cardiomyocyte diameters was carried out by an observer who was blinded to the group identity of the animals.

**Angiotensin measurements**

Blood samples were collected into polypropylene tubes containing 1 mmol l⁻¹ p-hydroxymercury benzoate, 30 mmol l⁻¹ 1,10-phenanthroline, 1 mmol l⁻¹ phenylmethylsulphonyl fluoride (PMSF), 1 mmol l⁻¹ pepstatin A and 7.5% EDTA (50 μl/ml blood⁻¹). After centrifugation at 250 g for 10 min, plasma samples were stored at −80°C.

After heart dissection, left ventricles were weighed and immediately frozen in liquid nitrogen. Left ventricles were homogenized with 0.045 N HCl in ethanol (10 ml/g tissue⁻¹) containing 0.90 μmol l⁻¹ p-hydroxymercury benzoate, 131.50 μmol l⁻¹ 1,10-phenanthroline, 0.90 μmol l⁻¹ PMSF, 1.75 μmol l⁻¹ pepstatin A, 0.032% EDTA and 0.0043% protease-free bovine serum albumin (BSA) and evaporated. After evaporation, the samples were dissolved in 0.003% trifluoroacetic acid. Peptides were extracted onto Bond-Elut phenylsilica cartridge (Varian, Inc., Palo Alto, CA, USA) as previously described (Mendes et al. 2005). After evaporation, plasma and cardiac samples were used to measure Ang II and Ang(1–7) immunoreactivity levels using radioimmunoassay (n = 5–20 rats per group) according to Botelho et al. (1994). The polyclonal Ang II antibody cross-reacts 100% with the angiotensin fragments, Ang(2–8), Ang(3–8) and Ang(4–8). Cross-reactivity of less than 0.001% is observed with Ang I and Ang(1–7). In addition, the polyclonal Ang(1–7) antibody cross-reacts less than 0.01% with Ang(2–7) and Ang(3–7), and less than 0.08% with Ang(4–7). The cross-reactivity with Ang I, Ang II and amino-terminal fragments is less than 0.001% (Botelho et al. 1994). Protein concentration in the crude homogenates was determined by the Bradford method (Bradford, 1976). The ratio between Ang(1–7) and Ang II was calculated only for animals in which both measurements were performed.

**Semi-quantitative reverse transcriptase-polymerase chain reaction (RT-PCR)**

An additional set of Wistar rats and SHR (n = 4–5 for each group) was used to analyse Mas mRNA expression. Total RNA was isolated with TRIzol reagent (Invitrogen Life Technologies, Carlsbad, CA, USA) according to the manufacturer’s protocol. Samples of RNA (1 μg) were treated with DNase to eliminate genomic DNA present in the samples. Gene expression was assessed by polymerase chain reaction after reverse transcription of RNA (RT-PCR). Total RNA treated with DNase was reverse transcribed using random primers. A 310 bp fragment of single-stranded cDNA was amplified by PCR using 35 cycles (30 s, 95°C; 30 s, 60°C; 60 s, 72°C) and the following primers: forward 5′-ACTGCCGGCCGTCATCATC-3′ and reverse 5′-GTTGGAAGAAACGCAAGAGA-3′. The same samples were employed for β-actin cDNA amplification as an internal control (fragment size of 400 bp) using the forward primer 5′-GCCCTGAGGACACCTGTG-3′ and the reverse primer 5′-AGGAAGAGATGCGCCAGTGG-3′ to ensure that equal amounts of RNA were reverse transcribed. The amplified PCR products were electrophoresed on 1.5% agarose gels, stained with ethidium bromide, visualized by an ultraviolet transilluminator and photographed. The results were quantified by densitometry, dividing the Mas by β-actin values, and reported as the ratio of Mas to β-actin.

**Western blotting analysis**

Additional left ventricles (n = 4 for each group) were minced in homogenization buffer consisting of 50 mmol l⁻¹ Tris-HCl, 5 mmol l⁻¹ EDTA, 150 mmol l⁻¹ NaCl and MgCl₂ containing protease inhibitors (PMSF (20 mmol l⁻¹), aprotinin (10 mg ml⁻¹), benzamidine (20 mmol l⁻¹), pepstatin (10 μmol l⁻¹), leupeptin (12 mmol l⁻¹)) and centrifuged at 7000 g, 4°C for 12 min. Protein concentration was determined by the Lowry method. Forty micrograms of protein were loaded onto a 10% polyacrylamide gel for electrophoresis. After electrophoresis, proteins were transferred to a nitrocellulose membrane, blocked with 5% non-fat milk solution, and washed in phosphate-buffered saline (PBS) containing 0.1% Tween 20. Membranes were incubated with a polyclonal Mas antibody (1:1000 dilution) raised in Mas-knockout mice produced in our laboratory (Sampaio et al. 2007; Becker et al. 2007) or a mouse anti-rat glyceraldehyde 3-phosphate dehydrogenase (GAPDH) monoclonal antibody (1:10 000 dilution; Sigma-Aldrich) overnight at 4°C, followed by incubation with an anti-mouse immunoglobulin G secondary antibody conjugated with peroxidase (1:2000 dilution; Sigma-Aldrich). Antibody binding was visualized using the ECL system (Amersham Pharmacia Biotech, UK) and the amount of Mas was expressed relative to the amount of GAPDH in each sample.

**Immunohistochemical analysis**

In order to substantiate the data obtained with Western blotting, changes in Mas in the left ventricles of trained and untrained Wistar rats and SHR were evaluated by
imunofluorescence using a polyclonal Mas antibody produced in our laboratory (Sampaio et al. 2007; Becker et al. 2007). For immunofluorescence (n = 2 for each group) the dilution of the primary antibody was 1:100 in 1% BSA. The secondary antibody used was an Alexa 594 goat anti-mouse (Molecular Probes, Carlsbad, CA, USA), diluted 1:200 in 1% BSA. Cryostat-cut sections (10 μm thick) of left ventricles prefixed in 4% paraformaldehyde and frozen at −80°C in Optimal cutting temperature (OCT; Electron Microscopy Sciences, Hatfield, PA, USA) embedding medium were rehydrated in 0.02 mol l−1 PBS for 1 h. Sections were subsequently incubated with a permeating buffer containing 0.2% Triton X-100 in PBS for 30 min, followed by a blocking step of 30 min with 3% BSA in PBS. Primary antibody incubation was performed at 4°C overnight. Sections were washed three times (5 min) in PBS and then incubated with the secondary antibody at room temperature for 90 min. Afterwards, sections were washed three times (5 min) in PBS and mounted on slides with 90% glycerol in 50 mmol l−1 Tris-HCl, pH 7.4. To validate the staining procedure, heart sections were incubated with the secondary antibody alone without the primary antibody. The immunostaining was detected using a Zeiss LSM 510 Meta Confocal microscope equipped with an oil-immersion objective lens (×63). The images were created using Adobe Photoshop 7.0 software.

### Statistical analysis

The data are presented as means ± s.e.m. Differences between groups were analysed using Student’s unpaired t test or two-way ANOVA followed by Bonferroni post hoc test. The criterion for statistical significance was set at P < 0.05.

### Results

As expected, sedentary SHR showed a significantly higher tail blood pressure compared with sedentary Wistar rats (values at the end of training programme, 162 ± 7 versus 104 ± 3 mmHg, respectively, P < 0.0001). The swimming training protocol used did not change tail blood pressure in either strain.

As shown in Table 1, sedentary and trained hypertensive rats showed a reduced body weight compared with the normotensive control animals. Physical training did not change the body weight in either strain. The SHR also presented higher heart/body weight ratio and left ventricle/body weight ratio compared with sedentary Wistar rats. Accordingly, cardiomyocyte diameters of sedentary SHR were significantly elevated in comparison with those of Wistar rats (14.83 ± 0.38 versus 16.81 ± 0.62 μm in SHR, an increase of 13.35%, P < 0.05, Student’s unpaired t test). Physical training induced significant cardiac hypertrophy in Wistar rats and SHR as assessed by cardiac and left ventricle weight normalized to body weight. In addition, it induced an increase of 12.75% in cardiomyocyte diameters of Wistar rats, but only an increment of 4.1% in cardiomyocyte diameters of SHR, resulting in a decrease of the difference observed in cardiomyocyte diameters between both strains (13.35% difference in sedentary rats versus 4.66% in trained rats). Owing to the differential change in cardiomyocytes, the cardiomyocyte cross-sectional area was increased in trained Wistar rats, but not in trained SHR, compared with sedentary rats (n.s., two-way ANOVA).

In basal conditions, SHR presented a significant increase in cardiac Ang II concentrations and a decrease in plasma Ang(1–7) levels compared with Wistar rats (Figs 1B and 2B). Physical exercise produced a significant decrease in plasma Ang II levels in both strains (72.7 ± 6.5 pg ml−1 in sedentary versus 50.7 ± 5.9 pg ml−1 in trained Wistar rats and 59.9 ± 6.4 pg ml−1 in sedentary versus 44.1 ± 4.8 pg ml−1 in trained SHR, Fig. 1A). Interestingly, left ventricular Ang(1–7) concentration was substantially increased in trained SHR, but not in trained Wistar rats (1.4 ± 0.1 pg (mg protein)−1 in

### Table 1. Cardiac morphological parameters in sedentary and trained Wistar rats and SHR

<table>
<thead>
<tr>
<th>Parameters</th>
<th>SWR</th>
<th>TWR</th>
<th>SSHR</th>
<th>TSHR</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW (g)</td>
<td>380.4 ± 8.7 (26)</td>
<td>368.2 ± 8.2 (25)</td>
<td>259.4 ± 2.5 (25)</td>
<td>260.0 ± 4.5* (22)</td>
</tr>
<tr>
<td>HW/BW (mg g−1)</td>
<td>0.30 ± 0.005 (25)</td>
<td>0.32 ± 0.004† (25)</td>
<td>0.40 ± 0.006* (23)</td>
<td>0.45 ± 0.005†† (19)</td>
</tr>
<tr>
<td>LVW/BW (mg g−1)</td>
<td>0.20 ± 0.003† (19)</td>
<td>0.21 ± 0.003 (19)</td>
<td>0.30 ± 0.004* (23)</td>
<td>0.32 ± 0.004†† (16)</td>
</tr>
<tr>
<td>MD (μm)</td>
<td>14.83 ± 0.38 (4)</td>
<td>16.72 ± 0.54 (5)</td>
<td>16.81 ± 0.62 (5)</td>
<td>17.5 ± 0.59 (5)</td>
</tr>
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</table>

Values are means ± s.e.m. *P < 0.001 compared with Wistar rats in the same conditions (sedentary or trained; two-way ANOVA followed by Bonferroni post hoc test). †P < 0.01, ††P < 0.001 compared with sedentary animals from the same strain. Numbers in parentheses indicate number of animals used in each group. Abbreviations: SWR, sedentary Wistar rats; TWR, trained Wistar rats; SSHR, sedentary SHR; TSHR, trained SHR; BW, body weight; HW, heart weight; LVW, left ventricle weight; and MD, myocyte diameter.
Figure 1. Plasma Ang II levels in sedentary and trained Wistar rats and SHR (A) and basal left ventricular and plasma Ang II levels in both strains (B). The data represent the means ± s.e.m. *P < 0.05, **P < 0.01 compared with sedentary rats and #P < 0.05 compared with sedentary Wistar rats (Student’s unpaired t test). Abbreviations: SWR, sedentary Wistar rats; TWR, trained Wistar rats; SSHR, sedentary SHR; and TSHR, trained SHR.

Figure 2. Left ventricle Ang(1–7) levels in sedentary and trained Wistar rats and SHR (A) and basal left ventricular and plasma Ang(1–7) levels in both strains (B). The data represent the means ± s.e.m. **P < 0.001 compared with sedentary rats and #P < 0.05 compared with sedentary Wistar rats (Student’s unpaired t test). Abbreviations as in legend to Fig. 1.

Expression of the Mas gene was assessed by measuring Mas mRNA levels by means of the semi-quantitative RT-PCR technique. As observed for the left ventricular Ang(1–7) concentration, Mas mRNA expression was significantly increased in trained SHR, but not in trained Wistar rats (6.46 ± 1.25 in sedentary versus 12.84 ± 0.73 in trained SHR, Fig. 3). In order to ascertain whether the increase in mRNA would be reflected in the protein levels in the left ventricle of trained SHR, we evaluated Mas protein levels using Western blotting and confocal immunofluorescence techniques. Although both protein analysis methods clearly indicated a higher expression of Mas protein in left ventricle of trained SHR (Fig. 4), only Western blotting results were analysed quantitatively. Western blotting analysis also revealed a slight, but not
significant, increase of Mas protein expression in left ventricles of trained Wistar rats.

Discussion

The present study was carried out to examine the effects of physical exercise on cardiac and plasma angiotensin levels in normotensive (Wistar) and hypertensive rats (SHR) subjected to a 5% overload swimming programme. The major finding of this study is the observation of a selective and marked increase of Ang(1–7) concentration accompanied by a significant increase of Mas expression in the left ventricles of trained SHR. These data raise the possibility that changes in Ang(1–7) and Mas might be involved in the beneficial effects of physical training in hypertension.

Physical training effects on blood pressure in SHR depend on the intensity of the exercise. Véras-Silva et al. (1997) and Tipton (1991) observed a decrease in blood pressure only after low-intensity treadmill training (55% of the maximal oxygen uptake). In swimming training, decreases in blood pressure seem to be induced when the animals are subjected to training with no overload (Ghaemmaghami et al. 1991; Ikeda et al. 1994; Song et al. 1998). However, Zamo et al. (2004) found that, at the same intensity of training as used in our study, a significant decrease in blood pressure was obtained. Differences in the methodology used to measure blood pressure may account for these divergent findings. In addition, it has been described that physical training induces physiological hypertrophy without altering the blood pressure in Wistar rats (Geenen et al. 1996). In keeping with these data, we did not observe changes in tail blood pressure in our animals.

In basal conditions, cardiac hypertrophy was observed in the left ventricles of SHR. However, when we analysed the four groups (sedentary and trained SHR and sedentary and trained Wistar rats) by two-way ANOVA the difference in cardiomyocyte diameter did not reach statistical significance, probably owing to the relatively young SHR used in the present study (4 months old). In addition, no significant differences in plasma Ang II concentration were observed, although an increase in the Ang II levels in left ventricles of SHR was observed. The normal plasma Ang II levels in SHR are in accordance with previous studies showing that SHR present renin-independent hypertension (Kohara et al. 1993; Campbell et al. 1995; Dang et al. 1999). In addition, our observations agree with studies demonstrating a significant augmentation of Ang II levels (Dang et al. 1999), as well as of ACE mRNA expression (Iemitsu et al. 2001), in the heart of SHR. This increase may be functionally relevant, considering that increases in heart Ang II levels can induce gene reprogramming that leads to an increase in the expression of growth factors and proteins of the extracellular matrix in the heart (Dostal, 2000).

We have found that physical training decreased Ang II concentrations in plasma without changing plasma Ang(1–7) levels in Wistar rats and SHR. In humans with chronic heart failure, physical training also decreased plasma Ang II levels (Braith et al. 1999). Furthermore, Geyssant et al. (1981) reported that physical exercise decreases plasma renin activity in normal humans. This effect was also observed in SHR (Hayashi et al. 2000; Zamo et al. 2004).

A growing body of evidence suggests that Ang(1–7) plays an important cardioprotective role. These effects include a reduction in the incidence and duration of ischaemia–reperfusion-induced arrhythmias (Ferreira et al. 2001; Santos et al. 2004) and an improvement of postischaemia contractile function in isolated perfused
rat hearts (Ferreira et al. 2002; Santos et al. 2004; Benter et al. 2006) and in an experimental rat model of heart failure (Loot et al. 2002). Strikingly, we found a selective twofold increase in left ventricular Ang(1–7) levels in swimming-trained SHR, but not in trained Wistar rats. No significant changes were observed in left ventricular Ang II concentration in either strain. These observations suggest that increased local concentrations of Ang(1–7) in the heart may contribute to the beneficial effects of physical exercise on cardiac function in hypertension. Although we observed cardiac hypertrophy in both strains after physical training, the change in cardiac Ang(1–7) levels was present only in SHR, which developed less hypertrophy. These data suggest that the cardiac Ang(1–7)–Mas axis is particularly activated by exercise in pathological conditions such as hypertension. Apparently, in this case the exercise stimuli turned the Ang(1–7)–Mas axis on, which has many cardioprotective actions. The reason for the absence of changes in left ventricular Ang(1–7) levels in normotensive rats remains to be clarified and may be related to the intensity and/or duration of the exercise.

The mechanisms underlying the increased left ventricular Ang(1–7) levels induced by swimming training in SHR were not investigated in this study. However, our results demonstrated that physical training significantly increased the Ang(1–7)/Ang II ratio, suggesting a higher production of Ang(1–7) and/or a higher degradation of Ang II. In this context, an increased ACE2 activity should be considered, since this novel ACE homologue is highly expressed in the heart (Oudit et al., 2003) and possesses a higher catalytic affinity for Ang II, forming Ang(1–7) (Vickers et al., 2002; Zisman et al., 2003).

Associated with the increase of Ang(1–7) levels in the left ventricles of trained SHR, we observed a simultaneous

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**Figure 4. Mas protein expression in left ventricles of Wistar rats and SHR**

Immunofluorescent localization (A) and protein expression determined by Western blotting (B) of Mas in the left ventricles of Wistar rats and SHR. Expression of Mas is increased in the left ventricles of trained SHR. C, Mas levels were normalized to GAPDH for quantification and represent the mean ± S.E.M. *P < 0.01 compared with sedentary SHR (Student’s unpaired t test). Abbreviations as in legend to Fig. 1. Scale bar represents 20 μm.
increase of both Mas mRNA and Mas protein in this tissue, suggesting that an overall activation of the Ang(1–7)–Mas axis could be involved in the beneficial effects of the physical training in hearts. These data are in agreement with previous studies reporting that Ang(1–7) is an endogenous ligand for the receptor Mas in hearts (Tallant et al. 2005; Santos et al. 2006). Apparently, the increase in Ang(1–7) levels induced an upregulation of Mas in the left ventricle of SHR. A similar observation was described for Ang II and its receptor, the angiotensin type 1 (AT1) receptor, in renal proximal tubule (Cheng et al. 1995). Another possibility is a direct effect of the exercise training on the expression of Mas. Future studies are needed to clarify these possibilities, as well to investigate whether the activation of the Ang(1–7)–Mas axis is related to cardiac functional changes and the less pronounced hypertrophy observed in trained SHR.

The co-ordinated increase in Ang(1–7) levels and Mas expression in the heart after chronic physical training represents strong evidence that activation of the Ang(1–7)–Mas axis could be involved in the cardiovascular beneficial effects induced by physical exercise. However, one limitation of our study is related to the fact that cardiac function was not evaluated. Therefore, future studies should include functional measurements, including pharmacological blockade of the Mas receptor, in order to test the hypothesis that the activation of the Ang(1–7)–Mas axis observed in our experiments could contribute to the improvement of heart function induced by physical training. Further experiments are being prepared to address these issues.

Also, since in certain instances the AT1 and AT2 receptors are involved in Ang(1–7) effects, the role and density of these receptors should be considered in future studies. However, AT1-mediated actions of Ang(1–7) are usually observed at pharmacological concentrations of this peptide, and the affinity of Ang(1–7) for AT2 receptors is very low (Rowe et al. 1995; Gurzu et al. 2005; Santos et al. 2005). In addition, our training protocol should be repeated using other normotensive strains, including Wistar–Kyoto rats, to establish whether the responses observed in the present study are restricted to SHR or not.

In summary, in the present study we have shown that swimming training induced a selective and substantial increase of Ang(1–7) concentration associated with an increase in both Mas mRNA and Mas protein in the left ventricle of trained SHR.

References


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