

Swimming training improves the vasodilator effect of angiotensin-(1–7) in the aorta of spontaneously hypertensive rat

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Silva DM, Gomes-Filho A, Olivon VC, Santos TM, Becker LK, Santos RAS, Lemos VS. Swimming training improves the vasodilator effect of angiotensin-(1–7) in the aorta of spontaneously hypertensive rat. *J Appl Physiol* 111: 1272–1277, 2011. First published September 8, 2011; doi:10.1152/jappphysiol.00034.2011.—**Introduction:** endothelial dysfunction plays a critical role in the pathogenesis of hypertension. It is well established that physical training has beneficial effects on the cardiovascular system. We recently reported that angiotensin-(1–7) [Ang-(1–7)] concentration and the *Mas* receptor expression is increased in the left ventricle of trained spontaneous hypertensive rats (SHR). The vascular effects of Ang-(1–7) in trained animals remain so far unknown. In the present study we investigated the effects of physical training on the vasodilator effect of Ang-(1–7) in the aorta of SHR. **Methodology:** normotensive Wistar rats and SHR were subjected to an 8-wk period of 5% overload of body weight swimming training. Changes in isometric tension were recorded on myograph. Western blot was used to investigate Ang-(1–7) receptors expression. **Results:** in aortas from normotensive rats Ang-(1–7) and ACh induced a concentration-dependent vasodilator effect, which was not modified by the physical training. Vessels from SHR had an impaired vasodilator response to Ang-(1–7) and ACh. The swimming training strongly potentiated the vasodilator effect induced by Ang-(1–7) in SHR, but did not modify the effect of ACh. Interestingly, *Mas* receptor protein expression was substantially increased by physical training in SHR. In trained SHR, the vasodilator effect of Ang-(1–7) was abrogated by removal of the endothelium and by the selective Ang-(1–7) receptor antagonists A-779 and D-Pro⁷-Ang-(1–7). L-NAME decreased Ang-(1–7) vasodilator response and indomethacin abolished the remaining dilatory response. **Conclusion:** physical training increased *Mas* receptors expression in SHR aortas, thereby improving the vasodilator effect of Ang-(1–7) through an endothelium-dependent mechanism involving nitric oxide and prostacyclin.

hypertension; *Mas* receptor; swimming training; endothelial dysfunction

ANGIOTENSIN-(1–7) [ANG-(1–7)] is an important peptide of the renin angiotensin system (RAS) formed from angiotensin I and from angiotensin II (Ang II) by the action of tissue peptidases (34, 36). Although several enzymes can participate in the synthesis of Ang-(1–7), ACE2 (angiotensin-converting enzyme 2) seems to play an important role in the formation of this peptide through the hydrolysis of Ang II (41, 46).

Several studies have shown that Ang-(1–7) has important physiological effects, including inhibition of vascular smooth

muscle cells proliferation (11, 38, 39), antithrombotic (10, 18) and antiarrhythmic effect (6), natriuretic and diuretic action (5) and in most vascular beds studied vasodilator effects (17, 23, 28, 31, 37). A growing number of studies indicate that the biological actions of Ang-(1–7) are mediated by the activation of the *Mas* receptor (20, 21, 23, 27, 35, 42). Moreover, several authors suggest the existence of an axis formed by ACE2-Ang-(1–7) and *Mas* receptor. This axis would have an important counter-regulatory role over the actions of the main axis of RAS, ACE-Ang II-AT₁ receptor (4, 7).

Because of its actions contrary to those of Ang II, especially with regard to the cardiovascular system, many researchers have studied the effects of Ang-(1–7) in experimental models of hypertension (8, 13, 33, 43). Spontaneous hypertensive rats (SHR), which most closely resemble primary hypertension in humans (40), are one of the most widely used models. In SHR, it has been shown that the plasma concentration of Ang-(1–7) is greater compared with control normotensive rats (16, 45). Furthermore, the infusion of Ang-(1–7) decreases blood pressure (2) and this effect is inhibited by coinfusion of A-779, an Ang-(1–7) *Mas* receptor antagonist (35), suggesting that the hypotensive effect induced by Ang-(1–7) is mediated by stimulation of the *Mas* receptor (44).

In addition to the beneficial actions mediated by Ang-(1–7), it has also been shown that physical activity has beneficial effects on the cardiovascular system, in both animals and humans. Several studies have shown that beneficial effects of physical activity are related to RAS. Braith et al. in 1999 (3) demonstrated that physical training of low-moderate intensity, in humans with heart failure, induced 26% reduction in plasma levels of angiotensin II. Hayashi et al. in 2000 (15) showed that training reduces plasma concentration and renin activity in SHR. Moreover, chronic treatment with perindopril (an ACE inhibitor) enhances exercise capacity and promotes adaptive changes of skeletal muscle in response to exercise such as increases in capillary density and percentage of type I fiber (25). More recently, Gomes-Filho et al. in 2008 (14) demonstrated that swimming training increases Ang-(1–7) concentration and the *Mas* receptor expression in the left ventricle of SHR.

Impaired vascular dilatation is a hallmark of cardiovascular risk factors and has been largely implicated in the pathophysiological processes of hypertension. The effect of physical training in the vascular effects of Ang-(1–7) remains to be characterized. This work was undertaken to evaluate the vascular effects of Ang-(1–7) in trained hypertensive rats. We

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hypothesize that physical training improves the vasodilator effect of Ang-(1-7) in SHR.

MATERIALS AND METHODS

Animals. Four-month-old male SHR and Wistar rats 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 12–12 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 program. 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 ($32 \pm 2^\circ\text{C}$) swimming pools during 1 h/day for 5 days/wk during 8 wk. Overload swimming training (5% of the body wt) was achieved by fixing weights on the tail of the animals. The swimming training reduced heart rate (24) but did not modify blood pressure in either strain, according to our previous report (14). Mean arterial pressure values were 108 ± 4 before vs. 105 ± 3 mmHg at the end of training program for Wistar and 163 ± 3 vs. 160 ± 2 mmHg for SHR.

Rat aortic rings preparation and mounting. At the end of the swimming training (48 h after the last session), the rats were killed by decapitation and tissues were rapidly removed. Rings (2–3 mm) from the descending thoracic aorta, free of adipose and connective tissue, were set up in gassed (95% O_2 –5% CO_2) Krebs-Henseleit solution (in M): 110.8 NaCl, 5.9 KCl, 25.0 NaHCO_3 , 1.07 MgSO_4 , 2.49 CaCl_2 , 2.33 NaH_2PO_4 , and 11.51 glucose, at 37°C , under a tension of 1.0 g, for 1 h to equilibrate (37). The presence of a functional endothelium was assessed by the ability of ACh (1 μM ; Sigma, St. Louis, MO) to induce more than 70% relaxation of vessels precontracted with phenylephrine (10^{-7} M; Sigma). When necessary, the endothelium was removed by rubbing the intimal surface with a wooden stick. Mechanical activity was recorded isometrically, as previously described (22).

Experimental protocol. Ang-(1-7) (Bachem, Torrance, CA) and ACh were added in increasing cumulative concentrations (10^{-10} to 3×10^{-7} M and 10^{-9} to 3×10^{-5} M, respectively) in vessels precontracted to the same tension level (~ 1.5 g of tension) with phenylephrine (3×10^{-8} – 10^{-7} M). To verify the participation of endothelium-derived products in the relaxant effect of Ang-(1-7), experiments were performed in vessels without a functional endothelium or in the presence of N^G -nitro-L-arginine methyl ester (L-NAME; 100 μM ; Sigma) or indomethacin (10 μM Sigma) or L-NAME (100 μM) plus indomethacin (10 μM). In experiments

performed in endothelium-denuded vessels or in the presence of L-NAME, vessels were precontracted with 3×10^{-8} M of phenylephrine, to achieve the same tension level as the others. In an attempt to determine the participation of the Mas receptor in the vasorelaxant response induced by Ang-(1-7), experiments were performed in the presence of the selective Ang-(1-7) antagonists A-779 (0.1 μM ; Bachem) or D-Pro⁷-Ang-(1-7) (0.1 μM ; ByoSynthan, Berlin-Bush, Germany). L-NAME, indomethacin, and the Mas receptor antagonists were added to the bath 20 min prior to the addition of phenylephrine. As a control for the above-mentioned protocol, another vessel segment from each rat was simultaneously monitored for Ang-(1-7) effect alone.

Western blot analysis. Approximately 40 μg of protein from trained and untrained SHR aortas were homogenized in RIPA buffer (Trizma 50 mM; NaCl 150 mM; EDTA 1 mM; plus NP-40 1% and sodium deoxycholate 0.25%) and were separate on a 10% gel via SDS-PAGE. Proteins were transferred onto a polyvinylidene fluoride membrane (Immobilon P; Millipore, MA) and blocked with 5% non-fat dry milk and 0.3% BSA in Tween-Tris-buffered saline for 24 h. The membrane was incubated (4°C) overnight in 5% non-fat dry milk in Tween-Tris-buffered saline with antibodies anti-Mas receptor (1:500; Alomone laboratories, Jerusalem, Israel) and anti- β -actina (1:1,000; Santa Cruz, Biotechnology, Santa Cruz, CA). After 1 h incubation, the membranes were probed with a secondary antibody (anti-rabbit IgG, 1:1,000; Millipore) raised in goat. Blots were then washed and subjected to enhanced chemiluminescence with Luminol (Millipore).

Statistical analysis. Results are shown as means \pm SE. Regular two-way ANOVA was used to compare concentration-response curves obtained in aortic rings. Student's *t*-test was used to compare maximal values (E_{max}) and Western plot results. The vasodilator effects of Ang-(1-7) and ACh were expressed as percentage decrease in maximal contraction induced by phenylephrine. All statistical analyses were considered significant when $P < 0.05$.

RESULTS

Vasorelaxant effect of ACh and Ang-(1-7) in endothelium-intact aortic rings from trained and untrained normotensive and hypertensive rats. In endothelium-containing aortic rings from untrained normotensive rats, ACh induced a concentration-dependent vasodilator effect (Fig. 1A), which was not changed by the physical training (Fig. 1B). Aortas from untrained SHR showed an impaired vasorelaxant response to ACh (Fig. 1A), E_{max} values being 96.7 ± 0.8 and 47.3 ± 4.0 for normotensive and hypertensive animals, respectively ($P <$

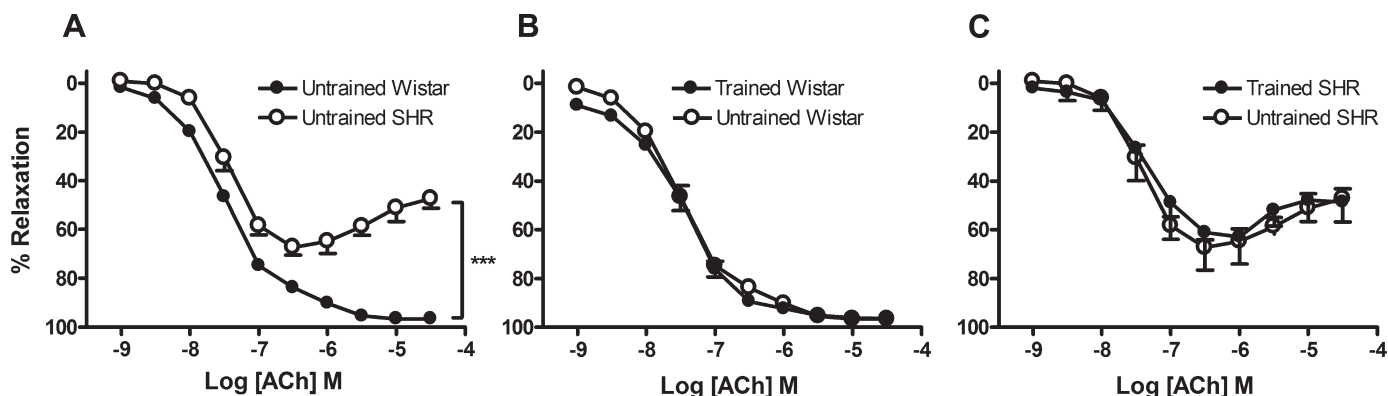


Fig. 1. Vasodilator effect of ACh in endothelium-containing aortic rings from untrained and trained spontaneously hypertensive rats (SHR) and Wistar rats. Each point represents the mean \pm SE generated from 5–10 separate experiments. *** $P < 0.001$.

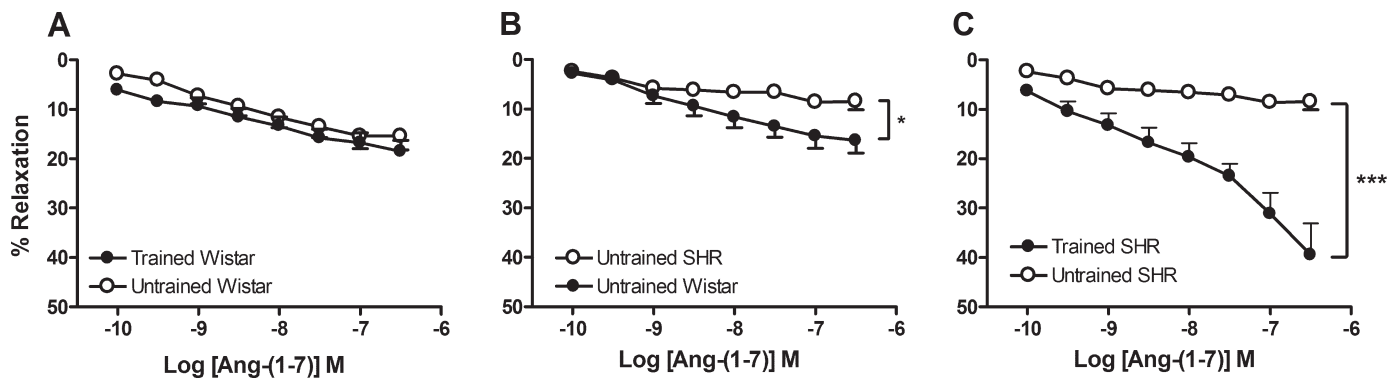


Fig. 2. Vasodilator effect of Ang-(1-7) in endothelium-intact aortic rings from untrained and trained SHR and Wistar rats. Each point represents the mean \pm SE generated from at least 7 separate experiments. * $P < 0.05$; *** $P < 0.001$.

0.001). The vasodilator effect induced by ACh was not different between trained and untrained animals (Fig. 1C). As shown in Fig. 2, Ang-(1-7) also induced a concentration-dependent vasorelaxant effect in the aortas ($E_{\max} = 16.4 \pm 2.5$). Physical training did not modify the vasorelaxant effect of Ang-(1-7) in normotensive animals ($E_{\max} 16.9 \pm 2.5$; Fig. 2A). Aortas from untrained SHR had an impaired vasorelaxant response to Ang-(1-7) (Fig. 2B), E_{\max} being 9.9 ± 1.2 ($P < 0.05$, compared with Wistar). However, interestingly, the vasodilator effect of Ang-(1-7) was greatly improved in aortas from SHR by the physical training (Fig. 2C), E_{\max} value being 43.3 ± 8.7 ($P < 0.001$, compared with untrained SHR).

Mechanism of the vasodilator effect of Ang-(1-7) in aortas from trained SHR. In an attempt to understand the mechanism by which swimming training improved the vasodilator effect induced by Ang-(1-7) in SHR, a series of experimental protocols was performed. The participation of the endothelium on the vasodilator response induced by Ang-(1-7) was evaluated in vessels with and without a functional endothelium. In endothelium-denuded vessels from trained SHR the vasorelaxant effect induced by Ang-(1-7) was abolished (Fig. 3). Inhibition of NO synthase with L-NAME (100 μ M) decreased but not abolished the endothelium-dependent relaxation induced by Ang-(1-7) in trained SHR. Indomethacin (10 μ M) that inhibits cyclooxygenase-derivatives formation only partially

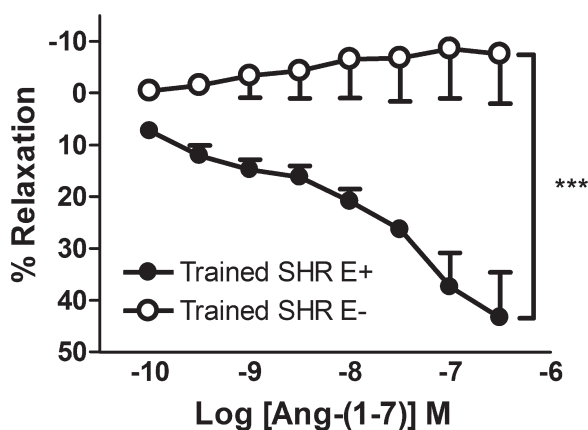


Fig. 3. Vasodilator effect of Ang-(1-7) in aortic rings from trained SHR in endothelium-intact (E+) and endothelium-denuded (E-) vessels. Each point represents the mean \pm SE generated from at least seven separate experiments. *** $P < 0.001$.

inhibited vasodilator response. However, double blockade with indomethacin plus L-NAME abrogated vasodilation produced by Ang-(1-7) in aortas from trained SHR (Fig. 4). To verify the involvement of the *Mas* receptor in the vasorelaxant effect induced by Ang-(1-7), aortic rings were preincubated with two Ang-(1-7) *Mas* receptor antagonists A-779 or D-Pro⁷-Ang-(1-7). Both antagonists completely inhibited the vasodilator effect of Ang-(1-7) (Fig. 5).

Mas receptor expression. Western blot analysis showed a significant increase in the level of *Mas* receptor expression in trained compared with sedentary SHR (Fig. 6).

DISCUSSION

Several studies have shown a significant relationship between physical activity and RAS both in humans (1) and in animals (15). Although Ang II is the major mediator of RAS, increasing evidence indicates that Ang-(1-7) has important actions that are often opposed to those of Ang II. Recently, experiments performed in our laboratory suggested that Ang-(1-7) and its specific *Mas* receptor are involved in the cardiac beneficial effects of physical training in SHR (14). In the present work we show for the first time that physical training increases *Mas* receptor expression in aortas from SHR and improves the vasodilator effect of Ang-(1-7) through an endothelium-dependent mechanism involving nitric oxide and prostacyclin.

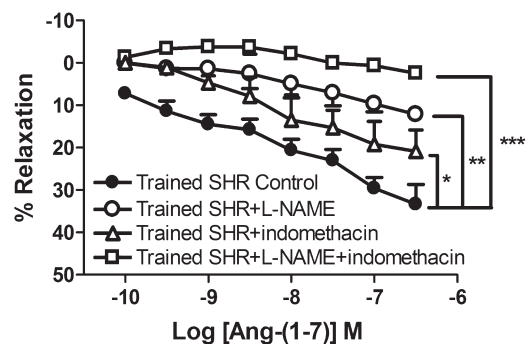


Fig. 4. Vasodilator effect of Ang-(1-7) in endothelium-intact aortic rings from trained SHR, in the presence or in the absence of L-NAME, indomethacin, and L-NAME plus indomethacin. Each point represents the mean \pm SE generated from at least 5 separate experiments. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

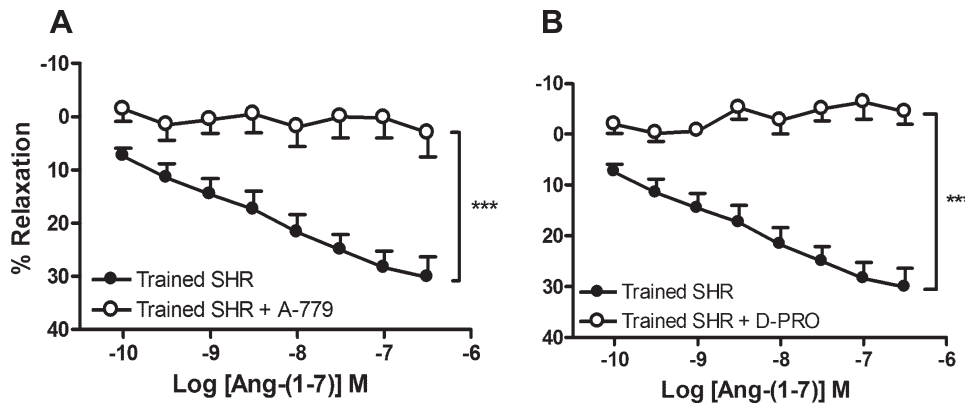


Fig. 5. Vasodilator effect of Ang-(1-7) in endothelium-intact aortic rings from trained SHR in the presence or absence of A-779 (A) and in the presence or absence of D-Pro⁷-Ang-(1-7) (D-PRO; B). Each point represents the mean ± SE generated from at least 5 separate experiments. ****P* < 0.001.

Endothelial dysfunction characterized by impaired endothelium-dependent vasodilatation has been linked to each of the known risk conditions, such as hypertension, diabetes mellitus, dyslipidemia, obesity, cigarette smoking, and aging. The more impaired vascular endothelium, the more severe the risk for cardiovascular diseases. In keeping with these data we found in the present work, an impaired endothelium-dependent vasodilator response in SHR. Interestingly, physical training importantly improved the endothelium-dependent vasodilator effect in response to Ang-(1-7). However, the impaired vasodilator response to ACh was unchanged by the swimming training. The mechanism for the specific increase in the vasodilator response to Ang-(1-7) is probably due to the increase in *Mas* receptor expression in the aorta from SHR, as shown in our Western blot experiments.

We sought to better understand the mechanism mediating the vasodilator response to Ang-(1-7) in trained hypertensive animals. Removal of the vascular endothelium abolished the vasorelaxant response to Ang-(1-7). These data agree with many studies using normotensive animals, which showed that

the vasodilator effect of Ang-(1-7) is endothelium-dependent (19, 31, 37).

The endothelium-dependent vasodilator effect mediated by Ang-(1-7) occurs through NO or prostaglandin production (9, 19, 29–31). In this study, inhibition of NO synthesis by L-NAME promoted a decrease on the vasodilator effect of Ang-(1-7), showing that NO contributes to the endothelium-dependent vascular response to Ang-(1-7) in trained hypertensive animals. Blockade of cyclooxygenase with indomethacin also partially inhibited vasodilation to Ang-(1-7); and double blockade with L-NAME plus indomethacin abolished the vasodilator response in trained SHR. Together, these data indicate that physical training improves Ang-(1-7)-induced relaxation in aorta from SHR through a pathway that involves either NO and prostacyclin.

Although some studies suggest the involvement of AT₁ and AT₂ receptors in the effects mediated by Ang-(1-7) (12, 26, 28) several studies have demonstrated that the *Mas* receptor is a major binding site for Ang-(1-7) (23, 32, 35). To evaluate the participation of *Mas* receptor on the effect of Ang-(1-7) in the aorta of trained SHR, we tested two selective antagonists for this receptor: A-779 and D-Pro⁷-Ang-(1-7). In the presence of both antagonists, the vasodilator effect of Ang-(1-7) was abolished. This result strongly suggests that the potentiation of the vasodilator effect produced by Ang-(1-7) in the aorta of trained SHR is mediated via *Mas* receptor.

In conclusion, our results show that physical training induces enhancement of *Mas* receptor expression and Ang-(1-7)-mediated vascular function in the aorta of SHR. This effect is endothelium dependent, mediated by stimulation of *Mas* receptor and involves increased production of NO and prostacyclin. Therefore, association of physical training and stimulation of the vasodepressor axis composed of Ang-(1-7)/*Mas* receptor emerges as a potential therapeutic strategy in the treatment of vascular complications in cardiovascular diseases.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

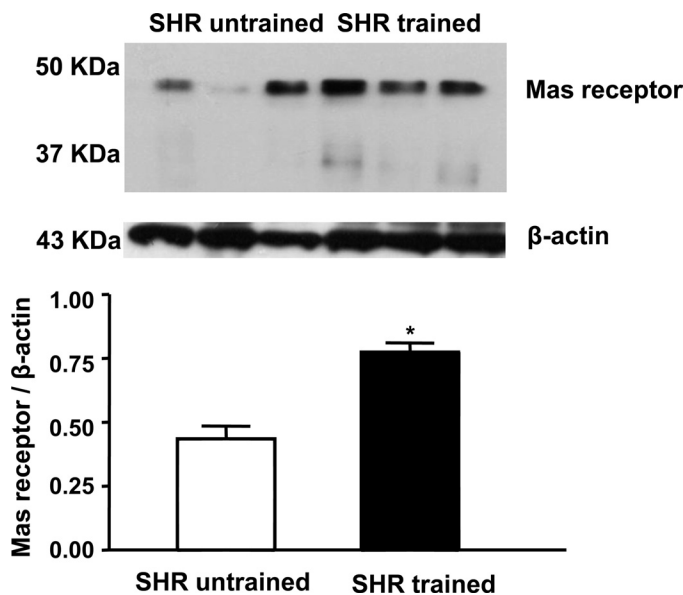


Fig. 6. Representative immunoblots showing the presence of *Mas* receptor in untrained and trained SHR aortas. Top shows representative Western blot signals. Bar graph represent *Mas* receptor protein expression in untrained (*n* = 6) and trained SHR (*n* = 6; densitometric mean values normalized to β-actin protein levels). **P* < 0.05 untrained vs. trained SHR.

AUTHOR CONTRIBUTIONS

Author contributions: D.M.R.S., A.G.-F., V.C.O., T.M.S., and L.K.B. performed experiments; D.M.R.S., A.G.-F., and V.C.O. analyzed data; D.M.R.S. and V.S.L. interpreted results of experiments; D.M.R.S. and V.C.O. prepared figures; D.M.R.S. and V.S.L. drafted manuscript; D.M.R.S., A.G.-F., V.C.O., T.M.S., L.K.B., R.A.S.S., and V.S.L. approved final version of manuscript; R.A.S.S. and V.S.L. conception and design of research; V.S.L. edited and revised manuscript.

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