Exercise training alters the effect of chronic hypoxia on myocardial adrenergic and muscarinic receptor number

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Exercise training alters the effect of chronic hypoxia on myocardial adrenergic and muscarinic receptor number. J Appl Physiol 91: 1283–1288, 2001.—Chronic hypoxic exposure results in elevated sympathetic activity leading to downregulation of myocardial α1- and β-adrenoceptors (α1-AR, β-AR). On the other hand, it has been shown that sympathetic activity is reduced by exercise training. The objective of this study was to determine whether exercise training could modify the changes in receptor expression associated with acclimatization. Four groups of rats were studied: normoxic sedentary rats (NS), rats living and training in normoxia (NTN), sedentary rats living in hypoxia (HS, inspired P O2 = 110 Torr), and rats living and training in hypoxia (HTH, inspired P O2 = 110 Torr). Training consisted of running in a treadmill at 80% of maximal O2 uptake during 10 wk. Myocardial receptor density was measured by radioactive ligand binding. Right ventricular (RV) hypertrophy occurred in HS but not in HTH. No effect of exercise was detected in RV weight of normoxic rats. Acclimatization to hypoxia (HS vs. NS) resulted in a decrease in both α1- and β-AR density, whereas muscarinic receptor (M-Ach) expression increased. Hypoxic exercise training (HS vs. HTH) modulated β-AR downregulation and M-Ach upregulation and prevented the fall in α1-AR density. Normoxic training (NS vs. NTN) did not change β-AR density. On the other hand, densities of α1-AR in both ventricles as well as RV M-Ach increased in NTN vs. NS. The data show that exercise training in hypoxia 1) prevents RV hypertrophy, 2) suppresses the downregulation of α1-AR in the left ventricle (LV) and RV, and 3) attenuates the changes in both β-AR and M-Ach receptor density in LV and RV. Exercise training in normoxia increases M-Ach receptor expression in the RV.

α1-adrenoceptor; β-adrenoceptor; M-Ach receptor; right ventricular hypertrophy; moderate hypoxia

CHRONIC EXPOSURE TO SEVERE hypoxia [inspired P O2 (P I O2) = 70 Torr for 3 wk] results in elevated sympathetic activity leading to downregulation of myocardial α1- and β-adrenoceptors (9, 14, 29) as a consequence of the prolonged agonist stimulation (1, 27). These changes are accompanied by an increase in muscarinic M2 (M-Ach) myocardial receptor expression (9, 15, 33).

Exercise training, on the other hand, results in a decrease in sympathetic activity (6, 19, 23, 32) and in an increase in parasympathetic tone at rest and during exercise (6, 23). As a consequence of these effects, heart rate at rest and during submaximal exercise is reduced after exercise training (6, 23). These effects of exercise training on cardiac autonomic control were estimated from the modifications in heart rate after successive blockade of β-adrenoceptors by propranolol and cholinergic receptors by atropine (19, 23). Whether these changes in sympathetic and parasympathetic activity are accompanied by variations in myocardial adrenergic and muscarinic receptor expression under normoxic conditions is disputed. Both decreases and no change in myocardial β-adrenoceptor density have been reported after exercise training (21, 24, 28, 30, 31), whereas a lack of effect on α1-adrenoceptors and muscarinic receptor expression has been observed (18, 31).

Because acclimatization to hypoxia and exercise training appear to have opposing effects on autonomic control of cardiac function, we hypothesized that exercise training could influence the effect of acclimatization to hypoxia on myocardial adrenergic and cholinergic receptor density. This is a significant problem because one of the effects of β-adrenoceptor downregulation of chronic hypoxia is a reduced chronotropic response to β-adrenergic agonists (1, 7, 9, 12, 27) and a decrease in maximal heart rate (1, 7, 11, 25, 26). The reduced maximal heart rate is one of the factors responsible for the limitation in maximal exercise capacity observed after acclimatization (5, 10).

The objective of these experiments was to determine whether exercise training modifies the effect of hypoxia on myocardial adrenergic and muscarinic receptor expression. Myocardial receptor characteristics were determined in rats in which the effect of living and training in hypoxia on exercise performance and sys-
temic O$_2$ transport was studied. The exercise data have been published separately (12).

**METHODS**

**Animal Model and Training Protocol**

All procedures were carried out following the regulations for laboratory animal care and use of the French Ministère de l’agriculture and the Guide for the Care and Use of Laboratory Animals. Seven-week-old male Sprague-Dawley rats were randomly assigned to live in normoxia (PIO$_2$ = 147 Torr) or in moderate hypoxia (PIO$_2$ = 110 Torr; 2,300 m). Each group was then subdivided into sedentary and trained subgroups. This resulted in four experimental groups of seven to eight rats each: normoxic sedentary (NS), normoxic trained in normoxia (NTN), hypoxic sedentary (HS), and hypoxic trained in hypoxia (HTH). All four groups were housed in the same room with the hypoxic groups placed in hypobaric chambers set to PIO$_2$ = 110 Torr. Training lasted 10 wk and was performed in an eight-lane treadmill through which the appropriate O$_2$-N$_2$ gas mixtures could be circulated; NTN trained at PIO$_2$ = 147 Torr and HTH at PIO$_2$ = 110 Torr. Absolute training intensity was the same for NTN and HTH, starting at ~80% of the maximal O$_2$ uptake of normoxic sedentary animals. Equal training intensity for hypoxic and normoxic groups was selected because at work rates O$_2$ needs are the same, independent of the P O$_2$ and of O$_2$ fluxes. This approach eliminates work intensity as a confounding variable. Work rate was increased gradually over 6 wk until it reached 30 m/min on a 10° incline, 1 h/day, 5 days/wk. This work rate was maintained for the last 4 wk of the training protocol.

**Studies of Myocardial Autonomic Receptors**

At the end of training, the animals exercised maximally. These data on exercise capacity and O$_2$ transport have been published elsewhere (12). After the exercise bout, the animals were killed with an overdose of pentobarbital sodium (60 mg/kg iv), and the heart was rapidly removed. The left ventricle with septum was separated from the right ventricular free wall, and both ventricles were immediately frozen in liquid nitrogen.

**Myocardial cell membrane isolation.** The procedure used was a slightly modified version of the method of Kacimi et al. (14). The ventricles were weighed and immediately homogenized in 6 ml of buffer (30 mM Tris•HCl, 100 mM NaCl, 5 mM MgCl$_2$, 1 mM EGTA, 1 mM trypsin inhibitor, 1 mg/ml leupeptin; pH 7.5) with a polytron tissue homogenizer. The suspension was centrifuged at 1,000 g for 10 min at 4°C. The supernatant was transferred to another tube and centrifuged at 50,000 g for 30 min at 4°C. The supernatant was discarded, and the pellet was resuspended with 6 ml of buffer and centrifuged at 50,000 g for 30 min at 4°C. Finally, the pellet was suspended with incubation buffer (50 mM Tris•HCl, 5 mM MgCl$_2$; pH 7.5) and stored at ~80°C. Protein content was measured with a dye-binding assay using a commercial kit (Bio-Rad; Ref. 4) and using bovine serum albumin as standard.

**$\alpha_1$-Adrenoceptor-binding assay.** [3H]prazosin, an $\alpha_1$-adrenoceptor antagonist, was used to label the receptors. Eight different concentrations of [3H]prazosin (Amersham Pharmacia Biotech; specific activity of 75 Ci/mmol) ranging from 0.02 to 1.5 nM were used in each assay. Unlabeled prazosin (1 $\mu$M) was added to determine nonspecific binding. Protein concentration of each sample was adjusted to 40–80 $\mu$g/100 $\mu$l on the day of the assay.

Duplicate samples of the membrane preparations were incubated for 1 h at 25°C in the incubation buffer (final volume of 200 $\mu$l). Incubation was terminated by rapid vacuum filtration (Skatron) through 1-$\mu$m filters. The titration plaques were rinsed 10 times with ice-cold incubation buffer. The radioactivity retained on the filters was determined by liquid scintillation spectrometry. The binding assays were carried out in duplicate. Average nonspecific binding was 11% of the total binding.

**$\beta$-Adrenoceptor-binding assay.** The procedure used was the same as that described for the $\alpha_1$-adrenoceptor binding assay, except for the following: [3H]labeled CGP-12177 ((-)4-(3-t-butyl amino-2-hydroxy-propoxy) benzimidazole-2-one, Amersham Pharmacia Biotech; specific activity of 45 Ci/mmol), a $\beta$-adrenoceptor antagonist, was used to label the receptors. Eight different concentrations of [3H]labeled CGP-12177 ranging from 0.06 to 4 nM were used in each assay. Unlabeled propranolol (10 $\mu$M) was added to determine nonspecific binding. The protein concentration was adjusted to 30–60 $\mu$g/100 $\mu$l on the day of the assay. Duplicate samples of the membrane preparations were incubated for 1 h at 37°C. Average nonspecific binding was 9% of the total binding.

**M-Ach receptor-binding assay.** The procedure used was the same as the ones described above, except for the following: [3H](quinuclidinyl benzilate (Amersham Pharmacia Biotech; specific activity of 45 Ci/mmol), a M-ACh antagonist, was used to label the receptors. Eight different concentrations of [3H](quinuclidinyl benzilate, ranging from 0.01 to 0.8 nM, were used in each assay. Unlabeled atropine (10 $\mu$M) was added to determine nonspecific binding. The protein concentration was adjusted to 25–60 $\mu$g/100 $\mu$l on the day of the assay. Duplicate samples of the membrane preparations were incubated for 1 h at 25°C. Average nonspecific binding was 7.5% of the total binding.

**Data Analysis**

Radio ligand binding data were analyzed with Ligand, a weighed, nonlinear, least-square curve-fitting computer program (22). For saturation experiments, equilibrium dissociation constants (receptor apparent affinity) and maximum numbers of binding sites were determined by nonlinear regression fitting.

**Statistical Analysis**

The data are expressed as means ± SE. Statistical analysis was carried out by using a one-way analysis of variance. The effect of acclimatization was evaluated by comparing NS vs. HS. The effect of training in normoxia and in hypoxia was assessed by comparing NS vs. NTN and HS vs. HTH, respectively. Finally, comparison of NTN vs. HTH provided an estimate of the effects of living and training in normoxia vs. living and training in hypoxia. Significance was established with the t-test using the Bonferroni correction for multiple comparisons. A P value <0.05 was considered to indicate a significant difference.

**RESULTS**

**Body and Ventricular Weights**

There were no significant differences in body weight (g) among the four groups (NS: 379.2 ± 5.8, NTN:
Acclimatization to hypoxia resulted in the expected increase in right ventricular weight (HS vs. NS, \( P < 0.05 \), Fig. 1A). Exercise training, on the other hand, significantly moderated the right ventricular hypertrophy, with right ventricular weight of HTH being significantly lower than that of HS (Fig. 1A, \( P < 0.05 \)). Normoxic exercise training did not influence right ventricular weight (NS vs. NTN, \( P > 0.05 \), Fig. 1A). No significant effects of acclimatization or of exercise training were detected on left ventricular weight (Fig. 1B).

Density and Affinity of Myocardial Receptors

Acclimatization to moderate hypoxia induced a decrease in \( \beta \)-adrenoceptor density in the left (Fig. 2A) and the right (Fig. 3A) ventricles in untrained rats (NS vs. HS, \( P < 0.05 \)). This reduction was attenuated in both ventricles by exercise training during acclimatization: \( \beta \)-adrenoceptor density was lowest in HS, inter-

Fig. 1. Right and left ventricular weight. A: right ventricular free wall weight. B: left ventricular plus septum weight. \( *P < 0.05 \) or better, hypoxic sedentary (HS) vs. normoxic sedentary (NS). \( #P < 0.05 \) or better, rats living and training in hypoxia (HTH) vs. HS. Values are means ± SE.

336.5 ± 5.4, HS: 380.7 ± 7.5, HTH: 334.0 ± 10.2). Acclimatization to hypoxia resulted in the expected increase in right ventricular weight (HS vs. NS, \( P < 0.05 \), Fig. 1A). Exercise training, on the other hand, significantly moderated the right ventricular hypertrophy, with right ventricular weight of HTH being significantly lower than that of HS (Fig. 1A, \( P < 0.05 \)). Normoxic exercise training did not influence right ventricular weight (NS vs. NTN, \( P > 0.05 \), Fig. 1A). No significant effects of acclimatization or of exercise training were detected on left ventricular weight (Fig. 1B).

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Fig. 2. Density of \( \alpha_1 \)- and \( \beta \)-adrenergic and muscarinic receptors (fmol/mg protein) in the left ventricle. A: \( \beta \)-adrenergic receptor density. B: \( \alpha_1 \)-adrenoceptor density. C: muscarinic receptor (M-Ach) receptor density. \( *P < 0.05 \) or better, HS vs. NS. \( #P < 0.05 \) or better, HTH vs. HS and rats living and training in normoxia (NTN) vs. NS. \&P < 0.05 \) or better, HTH vs. NTN. Values are means ± SE.
mediate in HTH, and highest in NTN and NS (Figs. 2A and 3A). No difference was observed between NS and NTN, indicating that normoxic exercise training does not influence β-adrenergic receptor density in either ventricle.

α1-Adrenoceptor density decreased significantly in both ventricles during the course of acclimatization to hypoxia (NS vs. HS; \( P < 0.05 \), Figs. 2B and 3B). Hypoxic exercise training partially moderated this fall in both left and right ventricles (HS vs. HTH, Figs. 2B and 3B). On the other hand, normoxic exercise led to an increase in α1-adrenoceptor in both ventricles (NTN vs. NS).

M-Ach receptor density was significantly increased during acclimatization in both ventricles (NS vs. HS, Figs. 2C and 3C). This elevation was attenuated by exercise training in hypoxia, with M-Ach receptor density being significantly lower in HTH than in HS (Fig. 2C and 3C). In addition, exercise training in normoxia also resulted in a significant increase in M-Ach receptor density; however, this occurred only in the right ventricle (Fig. 3C, NS vs. NTN).

No significant effect of hypoxia or training was observed in the affinity of adrenergic or cholinergic receptors for the respective ligand.

DISCUSSION

This study represents the first characterization of the effect of exercise training on the response of myocardial autonomic receptors to prolonged hypoxia. The major findings are:

1. The protocol of moderate hypoxia used in these studies produced changes in myocardial autonomic receptors that are similar in direction and magnitude to those produced by more severe hypoxia.
2. Exercise training profoundly influenced the effects of moderate hypoxia on myocardial autonomic receptors.
3. Exercise training prevented the hypoxia-induced right ventricular hypertrophy.

Effects of Moderate Hypoxia on Myocardial Autonomic Receptors

The present study shows that the effect of moderate hypoxia (\( \text{PIO}_2 \sim 110 \) Torr) on myocardial adrenergic and cholinergic receptors is similar to that produced by severe hypoxia (\( \text{PIO}_2 \sim 70 \) Torr). Density of both α1- and β-adrenoceptors of the sedentary rats living in hypoxia decreased to values that were not different from those observed in sedentary rats acclimatized to severe hypoxia (9). Key features of the response to prolonged severe hypoxia are elevated sympathetic activity (1, 27), decreased myocardial response to β-adrenergic agonists (27), and downregulation of myocardial α1- and β-adrenoceptors (9, 14). The downregulation of myocardial adrenergic receptors in severe hypoxia may be the result of the prolonged increased agonist stimulation secondary to the elevated sympathetic activity (1). The fact that moderate hypoxia also results in a decrease in myocardial adrenergic receptor density of an extent similar to that of severe hypoxia suggests that sympathetic activity is also increased in moderate...
Hypoxia. However, because the duration of the acclimatization period was longer in the present study than in those investigating the effects of severe hypoxia (9, 14), it is not possible to determine whether sympathetic activity increases to similar levels in both conditions. Nevertheless, the fact remains that this hypoxic exposure protocol does result in substantial modification of myocardial adrenergic receptor density.

Upregulation of myocardial M-Ach receptors was also of similar magnitude to that observed in severe hypoxia (9). The mechanisms responsible for the increased density of myocardial M-Ach receptors either in moderate or severe hypoxia are not clear. M-Ach receptor stimulation mediates the negative chronotropic and inotropic effects of acetylcholine. It is not known whether acclimatization to hypoxia is associated with changes in vagal output to the heart that could explain the increase in receptor density.

**Effect of Exercise Training on Myocardial Autonomic Function**

The animals that lived and trained in hypoxia showed a smaller reduction in density of α₁- and β-adrenoceptors in both ventricles than that observed in the sedentary hypoxic rats. Because exercise training is known to decrease sympathetic activity (19, 23, 32), a possible explanation for the attenuation of hypoxia-induced adrenoceptor downregulation is that exercise training results in a lower level of sympathetic activity during hypoxia. This would lead to a lower degree of adrenoceptor stimulation and attenuation of receptor downregulation.

The functional significance of the changes in myocardial adrenergic receptor density associated with training and hypoxia cannot be surmised from the present data. One characteristic feature of acclimatization to more severe hypoxia (P_O₂ ~70 Torr) is the decrease in maximal exercise heart rate (5, 11), which contributes to limit maximal cardiac output and exercise capacity (2, 25). Maximal heart rate correlates tightly with ventricular β-adrenoceptor and M-Ach receptor density during acclimatization to severe hypoxia (9). The decrease in chronotropic response to β-adrenergic agonists observed in humans (27) and rats (7) acclimatized to severe hypoxia is consistent with the downregulation of ventricular β-adrenoceptors seen in this condition.

In the present study, however, acclimatization to moderate hypoxia did not result in the decrease in maximal heart rate characteristic of more severe hypoxia: maximal heart rate values observed during exercise were 536 ± 5, 530 ± 2, 535 ± 9, and 534 ± 9 beats/min in NS, HS, NTN, and HTH, respectively (12). A lack of effect of acclimatization to moderate hypoxia on maximal heart rate has also been observed in humans (26). One possible explanation for the lack of correlation between maximal heart rate values and ventricular β-adrenoceptor density is that ventricular receptor density in moderate hypoxia does not reflect atrial receptor density. Atrial β-adrenoceptor density, particularly in or near the sinoatrial node, is likely to have a larger influence in heart rate responses to adrenergic agonists than ventricular β-adrenoceptor density. In severe hypoxia, the strong correlation between ventricular β-adrenoceptor density, maximal heart rate, and chronotropic responses to isoproterenol observed across several studies (7, 9, 14, 15, 26, 27) suggests that ventricular receptor density changes parallel changes in atrial receptors. However, several interventions (3, 20), including moderate hypoxia (8), have been shown to influence atrial and ventricular adrenoceptors to different extents. Doshi et al. (8) showed in the newborn lamb that moderate hypoxia resulted in downregulation of ventricular β-adrenoceptors without changes in atrial β-adrenoceptor density. A similar effect of moderate hypoxia in the present experiments could explain the lack of effect of acclimatization to hypoxia on maximal heart rate of sedentary rats in the presence of ventricular β-adrenoceptor downregulation, and, by extension, the lack of effect of exercise training on heart rate of hypoxic rats. Thus whether hypoxia influences atrial or ventricular receptors may depend on its severity; this could explain the lack of correlation between changes in β-adrenoceptor density and maximal heart rate observed in moderate hypoxia and the good correlation between these variables seen in more severe hypoxia.

M-Ach receptor density increased in moderate hypoxia, and exercise training attenuated this increase. Maximal exercise in trained as well as untrained subjects is accompanied by decreased vagal output; accordingly, a change in M-Ach receptor density should have only limited inotropic and chronotropic effects during maximal exercise.

**Effect of Exercise Training on Right Ventricular Hypertrophy**

Prolonged hypoxia results in right ventricular hypertrophy as a result of pulmonary hypertension due, in part, to hypoxic pulmonary vasoconstriction. In the present study, the rats living and training in hypoxia showed no significant increase in right ventricular weight, in contrast with the hypoxic sedentary rats. The lack of increase in right ventricular weight in the exercise-trained rats observed in the present study was accompanied by a substantial moderation of hypoxic pulmonary hypertension determined by direct measurement of pulmonary arterial pressure at rest and during maximal exercise (12). The mechanism responsible for the lower pulmonary arterial pressure in the exercise-trained rats living in hypoxia has not been determined; however, it has been shown previously that exercise training prevents hypoxic pulmonary vasoconstriction (16) and attenuates the response to pulmonary vasoconstrictors (17). The effect of exercise training on hypoxic pulmonary vasoconstriction, in turn, could be related to the increase in endothelium-dependent pulmonary vasodilation observed after exercise training (13).

In summary, the results of this study show that moderate hypoxia maintained for 10 wk leads to...
changes in myocardial autonomic receptors similar to those observed in more severe hypoxia and that these changes are substantially attenuated by exercise training in hypoxic conditions. The effect of exercise training extends to the pulmonary circulation by preventing the hypertension-induced right ventricular hypertrophy. The mechanisms underlying these changes, as well as their functional implications, remain to be determined.

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