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SHORT COMMUNICATION

The effects of exercise training on myocardial adrenergic and muscarinic receptors

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Abstract We investigated the effects of exercise training on heart rate variability (HRV) and myocardial adrenergic and muscarinic receptors in rats. Exercise training induced a decrease in body mass while ventricular size remained unchanged, a development we considered as a relative cardiac hypertrophy. In addition, there was a reduction in the density of myocardial β_1-adrenergic receptors. These structural changes were associated with functional adaptations, as illustrated by the increased response of the sinus node to sympathetic blockade.

Key words heart rate variability · myocardial receptors · physiological cardiac hypertrophy · exercise training

Introduction

Morphological and functional adaptations of the cardiovascular system have a significant role in the aerobic performance improvement during exercise training [13]. Chronic exercise induces cardiac hypertrophy [10]. It is associated with an improvement of diastolic function, stroke volume, maximal cardiac output, and thus maximal oxygen uptake [13]. These beneficial effects may, at least in part, be explained by changes in autonomic cardiovascular control [20, 25].

Heart rate responses to autonomic stimulation can be measured by determining heart rate variability (HRV) [14]. Spontaneous and permanent heart rate fluctuations mainly depend on the sinus node responses to the interaction of parasympathetic and sympathetic activity. Exercise training decreases intrinsic heart rate, increases parasympathetic HRV and decreases sympathetic HRV [5, 21]. It has been proposed that altered sensitivity and density of β-adrenergic (β AR) and muscarinic (M AchR) receptors may underlie these changes in HRV [12, 16, 25].

The aim of this study was to investigate the effects of exercise training on HRV and determine the associated changes in adrenergic and muscarinic receptors in the myocardium of rats.

Materials and methods

Experimental models

Twenty-four (12 controls, C, and 12 trained, T) 15-month old (middle aged) male Wistar rats were studied. All the procedures were carried out following the French Ministry of Agricultures Guide for the Care and Use of Laboratory Animals.

Training protocol

Physical training was performed on a home-made motor driven treadmill according to a progressive 12-week protocol, previously validated by our laboratory [1].

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HRV study

After the last exercise session, a radio ECG transmitter was implanted in the abdominal area of the anesthetized rat. Two days later, a placebo intraperitoneal (ip) saline injection (PBO; 2 ml; 2 mg · kg^{-1}) was given to establish a baseline, followed by injections of atropine sulfate (A; 0.15 ml; 2 mg · kg^{-1}) at 90 min intervals. A day later, ip propranolol (P; 2 ml; 4 mg · kg^{-1}), then ip atropine and propanolol (A + P; 2.15 ml) was administered following the same protocol. Thirty minutes after each injection, the ECG was recorded for ten minutes while the rats were housed in their own cages [18]. ECG data analysis was performed as already described [11, 22]. HRV spectral analysis was performed to distinguish parasympathetic and sympathetic HRV components [14].

Biological and western blot analysis

The rats were sacrificed 24 h after the last ECG recording. The heart was rapidly removed and dissected to separate the right and left atria and ventricles. The β_1 AR, β_2 AR and M_2 AchR densities were determined in left atria and ventricles. Membrane protein preparation and western blot analysis were performed as previously described [1].

Statistical analysis

All values are expressed as mean ± standard deviation. Mann-Whitney U test was performed to assess the differences between C and T groups. Wilcoxon test was performed to compare HRV during placebo and injection, i. e., to determine the effects the drugs on HRV within groups. Student *t*-tests were used to compare morphological data and receptor densities between C and T groups. $p < 0.05$ was considered significant.

Results

HRV results

In both groups RR interval was decreased after A ($p < 0.01$), increased after P ($p < 0.01$) and A + P injections ($p < 0.05$ in controls and $p < 0.01$ in trained). All absolute HRV indexes were decreased after A and A + P injections. P injection alone did not alter the HF component, but decreased the LF component. In the trained group, P injection decreased the LF/HF ratio ($p < 0.01$).

There was no difference in HRV indices between the trained and controlled groups after placebo, A alone or A + P. However, after P alone, the LF component and LF/HF ratio were smaller in the trained rats compared to the controls ($p < 0.05$). These results are summarized in Table 1.

Table 1 Comparison of mean RR and HRV indexes in control (C) and trained (T) rats after pharmacological injections and after training session. Placebo (PBO) injection was used as the basal state, atropine (A), propranolol (P), atropine + propranolol (A + P)

	Injection	RR (ms)	HF (ms^2)	LF (ms^2)	LF/HF
C	PBO	178 ± 1	1.96 ± 0.31	1.93 ± 0.41	0.93 ± 0.6
	A	152 ± 7[b]	0.55 ± 0.10[b]	0.14 ± 0.01[b]	0.35 ± 0.07[a]
	P	202 ± 12[a]	1.56 ± 0.30	0.93 ± 0.18[a]	0.67 ± 0.13
	A + P	191 ± 6[a]	0.53 ± 0.09[b]	0.19 ± 0.07[b]	0.50 ± 0.24
T	PBO	169 ± 5	2.33 ± 0.36	2.43 ± 0.49	1.07 ± 0.15
	A	145 ± 4[b]	0.99 ± 0.36[a]	0.30 ± 0.08[b]	0.51 ± 0.11[a]
	P	205 ± 4[b]	1.93 ± 0.12	0.40 ± 0.06[b*]	0.35 ± 0.04[b*]
	A + P	189 ± 3[b]	0.70 ± 0.16[b]	0.39 ± 0.10[b]	0.75 ± 0.17

[a] $p < 0.05$; [b] $p < 0.01$ between PBO and drugs; * $p < 0.05$ between C and T

Morphological and anatomical data

At the end of the study body weight was higher in the control group compared to the trained group ($p < 0.001$). Absolute ventricle or atria weights were not different between groups. However, left ventricular weight/body weight (11 %, $p < 0.05$), right ventricular weight/body weight (13 %, $p < 0.05$) and atria weight/body weight (23 %, $p < 0.01$) ratios were increased in trained rats. These results are summarized in Table 2.

Densities of β AR and M_2 AchR

In the trained rats, the density of β_1 AR was reduced by 20 % in left ventricle (80 ± 14 % in T *vs* 100 ± 10 % in C, $p < 0.01$) and by 33 % in atria (67 ± 18 % in T *vs* 100 ± 9 % in C, $p < 0.01$). β_2 AR densities were not altered in the left ventricle (100 ± 17 % in T *vs* 102 ± 12 % in C) nor in the atria (100 ± 10 % in T *vs* 90 ± 21 % in C). M_2 AchR densities were also unchanged in the left ventricle (98 ± 21 % in T *vs* 100 ± 12 % in C) and left atria (98 ± 20 % in T *vs* 100 ± 16 % in C). These findings are summarized in Fig. 1.

Table 2 Morphological and physiological data observed in control (C) and trained (T) groups

	C	T
BW (g)	648 ± 15	553 ± 30***
AW (mg)	63.0 ± 0.6	66.0 ± 0.2
AW/BW (mg · g^{-1})	0.09 ± 0.01	0.12 ± 0.01**
LVW (mg)	754 ± 7	769 ± 3
LVW/BW (mg · g^{-1})	1.16 ± 0.60	1.39 ± 0.50*
RVW (mg)	195 ± 5	201 ± 2
RVW/BW (mg · g^{-1})	0.30 ± 0.07	0.36 ± 0.06*

BW Body weight; *AW* Atria weight; *AW/BW* Atria weight/body weight ratio; *LVW* Left ventricle weight; *LVW/BW* Left ventricle weight/body weight ratio; *RVW* Right ventricle weight; *RVW/BW* Right ventricle weight/body weight ratio.
* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ between C and T

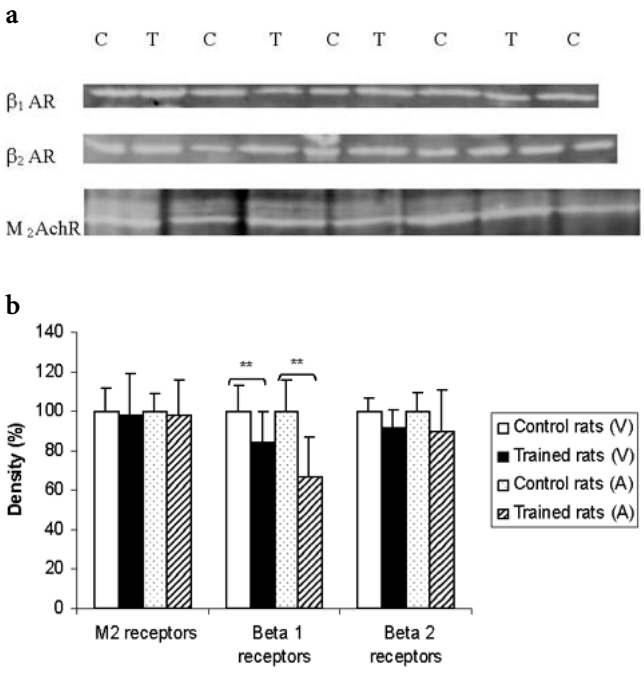

Fig. 1 **a** Identification of subtypes of left ventricle β-adrenergic (β AR) and muscarinic (M_2 AchR) receptors in control (C) and trained (T) rats. **b** Comparison of β_1, β_2 AR and M_2 AchR densities observed from C and T left ventricle (V) and atria (A). ** $p < 0.01$: between C and T left ventricle densities; ** $p < 0.01$: between C and T atria densities

Discussion

This study showed that in middle-aged Wistar male rats exercise training induced a decrease in body mass while ventricular size remained unchanged, a development we considered as a relative cardiac hypertrophy (i. e., weight of the ventricles in relation to the body mass), and specific modifications of atria and left ventricle ANS receptors. Moreover, these structural adaptations are associated with altered cardiac responses, demonstrated by an increased sensitivity of the sinus node to a sympathetic antagonist.

ANS myocardial receptors density

Left ventricle and atria M_2 AchR densities were not altered in the trained rats. These results are in agreement with previous data from young [1, 9] and senescent rats [4] subjected to exercise training. In thyrotoxicosis or aortic stenosis, cardiac hypertrophy was associated with a decrease in M AchR density [6]. The difference in M AchR density associated with cardiac hypertrophy during disease states and exercise suggests that pathological conditions have disease-induced specific effect on the heart muscle and its receptors [3, 6].

β AR densities are increased or decreased depending on the experimental model used to induce cardiac hypertrophy [6, 9, 23]. The β_1 AR density decrease we observed in middle-aged exercise trained rats was similar to our previous finding in younger trained rats [1]. Studies have shown that global β AR densities are unaltered by exercise training [4, 19]. Several factors, including the training protocol and strain of rats, could explain this discrepancy [4, 9, 12, 19]. The decrease in β_1 AR density seen in our protocol may be due to the exposure of the myocardium to catecholamines during exercise. Indeed, this exposure is associated with a down-regulation, or desensitization, of β AR [6, 23]. During acute exercise, norepinephrine levels increase more than epinephrine. As the β_1 AR are more sensitive to norepinephrine, they may be selectively down-regulated [1, 23]. Conversely, as the β_2 AR are less sensitive to norepinephrine, they may be unaffected by exercise training [1, 23].

Heart rate variability

We observed no significant effect of exercise training upon basal or intrinsic heart rate values, which is in agreement with similar studies in Wistar rats [6, 21]. Exercise training occasionally results in resting bradycardia in young rats [2, 8, 21]. The cause of this is multifactorial; involving changes in autonomic control [8] and/or intrinsic heart rate modifications [1]. An intrinsic heart rate decrease has been described in the case of a strong bradycardia [21]. The absence of such bradycardia in our rats could be explained by their older age. Indeed, in humans, the effect of exercise training on resting heart rate decreases with age [17, 25]. Moreover, as specific heart rate adaptations depend on the training protocol [5], discrepancies in the result may have arisen from the difference forms of exercise training. Finally, in our studies heart rate recordings were measured in awake and freely moving rats, and this may also account for some of the difference.

HRV spectral analysis reflects the sinus node responses to altered cardiovascular control [14]. After the injection of atropine and propanolol, separately and combined, the HF component, a reflection of the parasympathetic tone, was altered in the same manner in trained and untrained rats. In this study, exercise-induced cardiac hypertrophy was not associated with an alteration in parasympathetic HRV indexes, a similar finding has been shown in pathological cardiac hypertrophy in rats [6, 24]. However, in humans with physiological and pathological cardiac hypertrophy, HRV parasympathetic components are increased and decreased, respectively [15, 25]. Thus, a species specific and/or pathological origin might explain the divergence of this data.

LF component is linked to the baroreflex control [14]

and reflects the sinus node responses to sympathetic and parasympathetic activity. As expected, the LF component was decreased in both groups in response to A, P and A + P injections. However, after sympathetic blockade (i. e., P injection), the LF component decrease was greater in the trained rats; moreover, the LF/HF ratio was decreased only in the trained rats. Therefore, it would seem that in trained middle-aged rats the sinus node response to sympathetic blockade is improved. These results are in agreement with the increase of the LF component described in older people following physical training [7, 25].

In trained rats the unaltered M_2 AchR and decreased β_1 AR density, in atria and left ventricle, resulted in an increase in the M_2 AchR/β_1 AR ratio. This cellular adaptation was associated with altered cardiac function, as demonstrated by the increase in the sinus node response to sympathetic blockade. This finding is in agreement with the decrease in the LF component and decrease in the M AchR/β AR ratio previously reported in thyrotoxicosis cardiac hypertrophy [6]. Therefore, it may be hypothesized that the β_1 AR density decrease, as observed in physiological cardiac hypertrophy, may be compensated for by an increase in β_1 AR sensitivity, as previously suggested [9]. However, other underlying mechanisms, such as a modification of the Gi-adenylate cyclase system [4], may also be involved.

The use of propranolol, a global β AR-blockade drug, did not allow the investigation of the specific β_1 response, and thus is a potential limitation of this study. However, the myocardial β_2 AR may be less important in the regulation of heart rate [18].

Conclusion

In our experimental model, exercise training induced a decrease in body mass while ventricular size remained unchanged, a development we considered as a relative cardiac hypertrophy (i. e., weight of the ventricles in relation to the body mass). There was also a decrease in β_1-adrenergic density, and a consequent increase in the M_2 AchR/β_1 AR density ratio. These structural changes were associated with functional adaptations, as illustrated by the increased response of the sinus node to sympathetic blockade.

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