β-Adrenoceptor adenylate cyclase system adaptation to physical training in rat ventricular tissue

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PLOURDE, G., S. ROUSSEAU-MIGNERON, AND A. NADEAU. β-Adrenoceptor adenylate cyclase system adaptation to physical training in rat ventricular tissue. J. Appl. Physiol. 70(4): 1633-1638, 1991.—The β-adrenergic receptor adenylate cyclase system of ventricular tissue was evaluated in a group of rats submitted to a progressive 10-wk running program on a treadmill and compared with that in a group of rats maintained sedentary during the same period. Adequate training was confirmed by a 46% increase in the gastrocnemius isocitrate dehydrogenase activity in the trained group [1.50 ± 0.04 vs. 1.03 ± 0.06 (SE) pmol·g⁻¹·min⁻¹; P < 0.01]. Binding studies with [125I]iodocyanopindolol showed a 13% reduction in the density of β-adrenergic receptors in trained rats (42.6 ± 2.1 vs. 49.0 ± 2.1 fmol/mg; P < 0.05) without any significant modification in the dissociation constant. The amount of [125I]iodocyanopindolol bound to β-adrenoceptors in the high-affinity state was reduced by 16.6% in trained rats (12.5 ± 0.9 vs. 15.0 ± 0.5 fmol/mg; P < 0.05) without any significant changes for those in the low-affinity state, indicating a decrease in the coupling between the β-adrenoceptors and the guanine stimulatory binding protein. Furthermore, although the basal and sodium fluoride-stimulated adenylate cyclase activities were similar in the two groups of rats, the response of adenylate cyclase maximally stimulated by 10⁻⁵ M isoproterenol was reduced by 16% in trained rats (29.7 ± 1.4 vs. 36.3 ± 1.3 pmol·mg⁻¹·min⁻¹; P < 0.05). Overall, these data suggest that the well-known manifestations of decreased sympathetic activity present in the hearts of trained animals could be related to a diminution in the number of β-adrenergic receptors and probably more importantly to a decrease in the coupling between those receptors and the guanine stimulatory binding protein.

physical training; β-adrenergic receptor; guanine stimulatory protein; adenylate cyclase activity; rat ventricular tissue

MATERIALS AND METHODS

Drugs and chemicals. (−)-Propranolol hydrochloride, (−)-isoproterenol hydrochloride, ascorbic acid, isobutylmethylxanthine, sodium fluoride, bovine serum albumin, and sheep gamma globulin were purchased from Sigma Chemical (St. Louis, MO). [125I]iodocyanopindolol (ICYP) (2,200 Ci/mmol), [8-³H]adenosine 3',5'-cyclic monophosphate (cAMP), and [α²²P]ATP (30 Ci/mmol) were obtained from New England Nuclear (Boston, MA). Polyethylene glycol 8000 was purchased from Biotech Scientifique (Lachine, Quebec). Creatine phosphate, creatine phosphokinase, dithiothreitol, ATP, guanosine triphosphate, and 5'-guanylylimidodiphosphate [Gpp(NH)P] were obtained from Boehringer Mannheim (Dorval, Quebec).

Training program. Male Wistar rats were randomly assigned to two groups: sedentary (n = 17) and trained (n = 17) rats. Rats were individually housed at 23°C under standard lighting (0500-1900 h) and fed with Purina rat chow and tap water ad libitum. Training was done by having the rats run on a motor-driven treadmill (Quinton Instrument, model 42-15) set at 8° incline, according to a program adapted from Pattengale and Holloszy (20). The animals were exercised twice a day, 4 h apart, 5 days a week for 10 wk in a progressive fashion; they initially ran for 10 min at 22 m/min for 3 wk, then for 40 min at 28 m/min for 3 wk, and finally for 60 min at 31 m/min for 4 wk. Although electrical shock was used at the beginning of the training program to promote conditioning, it should be noted that care was taken later on to avoid such reinforcement to evaluate more specifically

PHYSICAL TRAINING is associated with cardiovascular adaptations (23) such as bradycardia at rest (26) and lower heart rate at any given submaximal exercise work load (3, 8). These manifestations seem to be explainable at least in part by a decrease in the activity of the sympathetic nervous system (23). From a theoretical standpoint, such an adaptation could be mediated by one or more of the following changes: a decrease in the number or affinity of cardiac β-adrenergic receptors, a reduction in the coupling between the β-adrenergic receptor and the guanine stimulatory binding protein (Gs), and/or a diminution in the activity of the postreceptor adenylate cyclase. Up to now, no study has simultaneously reported the impact of a physical training program on these three specific levels. Moreover, studies published so far have shown controversial results. Although we have previously reported a diminution in the number of ventricular β-adrenergic receptors in rats submitted to physical training (25), other studies have failed to observe this phenomenon (11, 18, 29). Disagreement also exists concerning the impact of exercise training on the response of adenylate cyclase to β-agonist stimulation, because either a decrease (5), no modification (18), or even an increase (31) has been reported. Moreover, the effect of training on the coupling between the receptor and the Gs has not still been elucidated. The present study was thus conducted to better clarify the effects of physical training on 1) the number and affinity of rat ventricular β-adrenergic receptors; 2) the coupling between the β-adrenergic receptor and the Gs; and 3) the adenylate cyclase activity, either in the basal state or after stimulation with sodium fluoride or isoproterenol.

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1633
the response to physical conditioning alone and that each exercise session was supervised by an attendee who gently pushed the animals on the back with a rod if necessary. Sedentary rats were hand manipulated in the same fashion as the trained animals with each exercise session. At the end of the 10-wk training period the rats were killed by decapitation 64 h after the last session of exercise to dissociate as much as possible between a training effect and a postexercise event. The heart and the gastrocnemius muscles were rapidly excised, washed in saline solution, weighed, and then frozen in liquid nitrogen and stored at −80°C. Gastrocnemius NAD-linked isocitrate dehydrogenase (NAD-ICDH) activity was measured according to the method of Vaughan and Newsholme (27).

**Preparation of ventricular membranes.** We prepared ventricular membranes by first mincing the right and left ventricles with scissors in a 0.32 M sucrose solution at 4°C, followed by two 10-s bursts with a Polytron homogenizer set at high speed. The homogenates were filtered through a triple layer of cheesecloth and centrifuged at 40,000 g for 20 min. Finally, the pellets were suspended and diluted in incubation buffer, 50 mM Tris [tris(hydroxymethyl)aminomethane]·HCl, 1.1 mM ascorbic acid pH 7.4 at 4°C, and homogenized with a glass-Teflon Potter-Elvehjem driven at high speed. The homogenates were filtered and centrifuged at high speed to give a protein concentration corresponding approximately to 0.30 mg/ml. Protein concentration was measured according to Lowry et al. (16), using bovine serum albumin as standard.

**Binding assay.** For binding assay, 300 μl of ventricular membrane preparations were incubated with increasing concentrations (5–250 μM) of (±)-ICYP in a total volume of 500 μl of incubation buffer. Preliminary experiments revealed that the range of ICYP concentrations used was sufficient to demonstrate saturation in radioligand binding isotherm plots and that binding of ICYP to ventricular cardiac membranes reached equilibrium within 40 min and remained stable thereafter for at least 120 min at 37°C. Therefore, the membranes were incubated for 60 min at 37°C during the course of this study. Incubation was stopped by the addition of 100 μl of 1% sodium lauryl sulfate, 40 mM ATP, 1.4 mM cAMP, and [3H]cAMP containing ~30,000 cpm (pH 7.5), followed by 3 min of boiling. Adenylate cyclase activity was measured in the absence or presence of 10−8 M sodium fluoride (NaF) or 10−5 M (−)-isoproterenol (ISO). This concentration of ISO was selected because it has been previously shown to give maximal stimulation in a similar membrane preparation (13). [32P]cAMP generated in a similar membrane preparation (13). Statistical analysis. The results are given as means ± SE. Statistical comparisons between the sedentary and trained groups were performed by the Student’s t test. The variables not normally distributed were log-transformed to reduce skewing. The number of receptors and the dissociation constant were determined according to the method of Scatchard (22). All the binding curves were analyzed with the use of the iterative program LIGAND (17).

**RESULTS**

Some characteristics of the two groups of rats are shown in Table 1. The rate of growth was less in the trained group, with a 14% reduction in body weight relative to their sedentary counterparts at the end of the program. This was not reflected by any significant change in heart weight. The heart weight-to-body weight ratio was significantly higher in the trained rats. The protein content in the ventricular membrane preparations from the two groups of rats was not different. The gastrocnemius weight was significantly lower in trained rats. The gastrocnemius NAD-ICDH activity was significantly higher in trained than in sedentary rats.

Figure 1 shows the amount of ICYP specifically bound to ventricular membranes from the sedentary and the trained rats at the different concentrations of ICYP used. It can be seen that saturation of β-adrenergic receptors was achieved in both groups of animals. Moreover, there appears to be a difference in the β-adrenergic receptor density between the two groups. This was confirmed by computer analysis of the binding data in membranes from each rat. As shown in Fig. 2, the density of ventricular β-adrenergic receptors was 13% lower in the trained than in the sedentary group (42.6 ± 2.1 fmol/mg of protein; P < 0.05). Kd values were similar in the trained (64.6 ± 9.3 pM) and in the sedentary (65.7 ± 9.4) rats.

The results of the competitive binding study between ICYP and ISO on ventricular membranes in the absence
or presence of $10^{-4}$ M Gpp(NH)p are shown in Table 2. In the sedentary rats, 43.6 ± 1.6% of the β-adrenergic receptors were in the high-affinity state. In the trained group, the proportion of β-adrenergic receptors in the high-affinity state was 10.8% lower than in the sedentary rats, but the difference was not statistically significant. No significant difference was observed between the two groups in the $K_d$ for the receptors in high- or low-affinity states. With the addition of Gpp(NH)p, there was an affinity shift transforming the receptors in the high-affinity state into receptors in the low-affinity state. There were no differences in the number of β-adrenergic receptors in the low-affinity state or in the corresponding dissociation constant ($K_d$) between sedentary and trained rats.

The amount of ICYP bound to the receptors existing in the high- or low-affinity state is further shown in Fig. 3. The concentration of receptors in the high-affinity state was significantly reduced by 16.6% in trained rats relative to the sedentary group (12.5 ± 0.9 vs. 15.0 ± 0.5 fmol/mg protein; $P < 0.05$). No difference was observed for the $K_d$ between sedentary (19.9 ± 1.5) and trained (20.8 ± 2.0) rats.

The basal activity of ventricular adenylate cyclase was similar in the two groups of rats (Fig. 4). The NaF-stimulated adenylate cyclase activity was also not altered in trained animals compared with sedentary controls. However, the maximal ISO-stimulated adenylate cyclase activity was significantly reduced by 16% in the trained rats (29.7 ± 1.4 vs. 35.3 ± 1.3 pmol cAMP·min⁻¹·mg⁻¹ protein; $P < 0.05$).

**DISCUSSION**

This study is the first to report the effect of endurance training on the entire ventricular β-adrenoceptor-adenylate cyclase system. Our results indicate that exercise conditioning in the rat is associated with a significant reduction in the maximal ISO-stimulated adenylate cyclase activity. This seems to be related to a significant reduction in the total number of β-adrenergic receptors and, more specially, of those existing in the high-affinity state, suggesting that training induced a diminution in the coupling between the β-adrenergic receptor and the Gs.

From previous studies in the literature, conflicting data exist concerning the effect of physical training on β-adrenergic receptor density. Although some studies have not observed any significant difference in the number of ventricular β-adrenergic receptors between sedentary and trained animals when $[^3H]$dihydroalprenolol was used as the ligand (11, 18, 28), a significant (21%) reduction in β-adrenergic receptor density has been previously reported by our laboratory (25). The results of the present study show that a treadmill training program in the rat induced a 13% decrease in the concentration of

**TABLE 1. Effect of physical training program**

<table>
<thead>
<tr>
<th></th>
<th>Sedentary (n = 16)</th>
<th>Trained (n = 17)</th>
<th>$P$</th>
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<tbody>
<tr>
<td>Body wt, g</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Initial</td>
<td>206±2</td>
<td>207±1</td>
<td>NS</td>
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<tr>
<td>Final</td>
<td>348±7</td>
<td>298±7</td>
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<tr>
<td>Heart wt, g</td>
<td>1.02±0.02</td>
<td>1.04±0.03</td>
<td>NS</td>
</tr>
<tr>
<td>Heart wt/body wt, mg/g</td>
<td>2.87±0.05</td>
<td>3.49±0.06</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Protein content in membra</td>
<td>45.75±0.93</td>
<td>48.45±0.78</td>
<td>NS</td>
</tr>
<tr>
<td>n preparations, mg</td>
<td>2.73±0.06</td>
<td>2.42±0.08</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Gastrocnemius wt, g</td>
<td>1.03±0.06</td>
<td>1.50±0.04</td>
<td>&lt;0.001</td>
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</tbody>
</table>

Values are means ± SE; n, no. of rats. NAD-ICDH, gastrocnemius NAD-linked isocitrate dehydrogenase activity.

![FIG. 1. Amount of $[^3H]$iodocyanopindolol (ICYP) specifically bound to ventricular membranes from sedentary (n = 16) and trained (n = 17) rats at different concentrations of ICYP used. Data are means ± SE.](image)

![FIG. 2. Concentration of β-adrenergic receptors (Bmax) and corresponding affinities ($K_d$) in ventricular membranes from sedentary (n = 16) and trained (n = 17) rats. *$P < 0.05$.](image)
TABLE 2. Percentage of RH and RL and KH and KL in ventricular membranes

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>RH, %</th>
<th>RL, %</th>
<th>KH, log nM</th>
<th>KL, log nM</th>
<th>RH, %</th>
<th>KL, log nM</th>
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<tbody>
<tr>
<td>Without Gpp(NH)p</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Sedentary</td>
<td>12</td>
<td>43.6±1.6</td>
<td>56.4±1.6</td>
<td>1.30±0.08</td>
<td>2.75±0.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trained</td>
<td>14</td>
<td>38.9±2.5</td>
<td>61.1±2.5</td>
<td>1.25±0.12</td>
<td>2.89±0.09</td>
<td>100</td>
<td>3.12±0.21</td>
</tr>
<tr>
<td>With Gpp(NH)p (10^-4 M)</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Values are means ± SE; n, no. of rats. RH and RL, high- and low-affinity receptors, respectively; KH and KL, high- and low-affinity dissociation constants, respectively; Gpp(NH)p, S'-guanylylimidodiphosphate.

in downregulation of β-adrenergic receptors. Because during exercise the concentration of plasma catecholamines increases exponentially with work intensity (9), the intensity of training may well be of prime importance in the development of such adaptive changes and further studies will be needed to address this point.

In humans, conflicting data also exist for the relationship between the level of physical training and the status of lymphocyte β-adreceptors in the same subjects. Butler et al. (2) have suggested that the density of β-adrenergic receptors on lymphocytes was inversely correlated with the degree of physical fitness in athletes. In contrast, Lehmann et al. (15) failed to observe this relationship. Frey et al. (7) have recently reported that the density of β-adrenergic receptors on lymphocyte membranes measured at rest or at peak exercise did not correlate with the maximal \( O_2 \) consumption. On the other hand, Ohman et al. (19) have found a significant (25%) reduction in the number of lymphocyte β-adrenergic receptors with training, suggesting that change in β-adreceptor density is an important component in the adaptation of trained human subjects.

The results of the present study showed a 16.6% reduction in the number of receptors in the high-affinity state in the trained rats as opposed to those in the low ventricular β-adrenergic receptors, without any significant change in the binding affinity. The approach utilized in the present study differed somewhat from those used by prior investigators (11, 18, 25, 28). A potential source of difference between the present study and three of the previous studies (18, 25, 28) is the ligand used to characterize the binding parameters. In the present study, the density and affinity of β-adrenergic receptors on ventricular membranes was measured with ICYP, whereas three (18, 25, 28) of the four studies presented above have used \(^[3]H\)dihydroalprenolol (DHA). ICYP possesses an affinity 40 times higher and a specific radioactivity 45 times higher than DHA (6). The only study (11) that used ICYP to examine the effect of physical training on β-adreceptor number in heart tissue showed a significant decrease in the membranes from right atria but no changes in those from the left ventricle. Other potential sources of difference between the present study and three of the studies cited above (11, 18, 25, 28) include the animal species used, the type of exercise programs utilized, and the degree of physical fitness induced by these programs. In the present study, the activity of gastrocnemius NAD-ICDH, a mitochondrial enzyme well known to increase with physical training (1, 12), was shown to be significantly higher in trained rats, thus confirming that our program was of a sufficiently high intensity. It may be interesting to add that the training program utilized in the present study was exactly the same as the program used in our previous study (25) and our two studies using either DHA (25) or ICYP (present study) have disclosed a significant reduction in the number of β-adrenergic receptors. It is well known that the chronic increase in ambient catecholamine levels results
erved that the maximal ISO-stimulated heart rate was decreased with training, suggesting downregulation of β-adrenergic receptors. In our study we did not measure the heart rate, but we observed that the maximal ISO-stimulated adenylyl cyclase activity was significantly reduced in trained rats; this also suggested a downregulation of β-adrenergic receptors in response to physical training. It is well known that the proportion of β-adrenergic receptors in the high-affinity state (which reflects receptor-Gs coupling) is related to activation of adenylyl cyclase (4, 24). Therefore, decreases in maximal responses of both adenylyl cyclase activity and heart rate to ISO may be related in part to a reduction in the number of β-adrenoceptors as well as to a decrease in coupling between these receptors and Gs. Alternatively, a reduction in Gs with training could also be postulated. Surprisingly, the only study in which this issue has been examined (10) showed 42% and 76% increases in the amount of immunoassayable α, portion of the Gs in atrial and ventricular membranes, respectively. Obviously, further work will be needed to clarify this apparent discrepancy between our results and those of Hammond et al. (10).

The basal and NaF-stimulated adenylyl cyclase activities measured in the present study were not modified in response to training. This agrees well with the report of Wyatt et al. (31) in trained cats. By contrast, the adenylyl cyclase response to ISO stimulation was reduced by 16% in ventricular tissues of trained animals. These results are also in agreement with the reduction in the epinephrine-stimulated adenylyl cyclase activity previously reported by Dohm et al. (5) in rats but differ from the results obtained by Wyatt et al. (31), who found an increase in the response of myocardial adenylyl cyclase activity to catecholamine stimulation in cats submitted to a longterm swimming program. Our results also differ from those obtained by Moore et al. (18), who have reported that the basal and ISO-stimulated adenylyl cyclase activities were not modified by endurance training in the female rat heart. Here again, the disparities between these results might well be due to differences in the species or sex of animals studied or in the training protocol itself.

In conclusion, our data indicate that the total number of ventricular β-adrenoceptors, and more specifically of those existing in the high-affinity state, was significantly reduced by training in the rat. Furthermore, whereas the basal and NaF-stimulated adenylyl cyclase activities were not modified by training, the maximal ISO-stimulated adenylyl cyclase activity was significantly reduced in trained rats. Overall, it would appear that exercise training acts by diminishing the coupling between the β-adrenergic receptors and Gs in heart tissue. Thus the results of the present study provide some new insight on the mechanism whereby exercise conditioning may alter the myocardial response to cardiac sympathetic activity.

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REFERENCES

1638 HEART β ADRENOCEPTORS IN TRAINED RATS
