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Adenylyl Cyclase System Is Affected Differently by Endurance Physical Training in Heart and Adipose Tissue

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ABSTRACT. Adaptive changes in the β-adrenergic adenylyl cyclase (AC) system in response to endurance training were studied in heart and adipose tissue. Training was performed by making male Wistar rats run on a motor-driven treadmill. The changes following exercise training were opposite in the two tissues studied. The density of β-adrenergic receptors in left ventricular membranes of trained rats showed a marked decrease. Comparison of AC activities in cardiac membranes prepared from trained and sedentary rats revealed a depressing effect of endurance training on: 1. the β-adrenergic stimulatory pathway and the inhibition of AC *via* receptor; 2. the G_s component and the G_s-adenylyl cyclase coupling, as shown by the response of adenylyl cyclase to GppNHp and NaF; and 3. the enzyme catalytic activity in the presence of Mn^{2+} or forskolin. The levels of $G_{s\alpha}$ subunits in the left ventricle, as measured in terms of ADP-ribosylated and immunologically reactive proteins, were decreased by endurance exercise, whereas immunodetectable levels of $G_{i\alpha 2}$ increased in the membranes of trained myocardium. In contrast to the diminished sensitivity that characterizes the behavior of the cardiac β-adrenergic-AC system, endurance physical training increased sensitivity of this signal transduction system in adipose tissue. Thus, the density of β-ARs as well as AC activity and the β-adrenergic stimulatory pathway were increased in adipose membranes of trained rats compared with the corresponding sedentary controls. In addition, the levels of $G_{s\alpha}$ subunits were higher in the adipose plasma membranes of trained rats. However, immunodetectable levels of $G_{i1\alpha}$ and $G_{i3\alpha}$ increased with training, whereas the amount of $G_{i2\alpha}$ decreased in membranes of trained rats. In conclusion, the present study shows that chronic exercise is associated with a tissue-specific adaptation of the β-adrenergic AC system. BIOCHEM PHARMACOL 51;10:1321–1329, 1996.

KEY WORDS. β-adrenoceptors; G-proteins; adenylyl cyclase; exercise

The most important control processes during exercise involve the large increase in the flow of blood, O_2, and nutrients to muscle, and the mobilization of fuels for oxidative purposes [1]. The acute adjustments of the heart to dynamic exercise are met by marked increases in mechanical and metabolic activity [2]. The release of fatty acids from adipose tissue through lipolysis in fat cells is essential to the energy supply during prolonged physical exercise [3]. Physical training, from moderate to high intensity, markedly activates sympathoadrenal mechanisms [4]. Catecholamines have powerful regulatory properties that exert control over a number of critical physiological and metabolic functions. These responses are specific to the target tissue involved, many being mediated *via* the AC† transmembrane signaling system [4]. This transduction system comprises membrane-bound receptors, the effector enzyme AC, and several members of a family of guanine nucleotide-binding regulatory proteins (G-proteins) [5]. The stimulatory G_s couples β-AR with activation of AC. The inhibitory G_i couples inhibitory receptors such as the muscarinic and adenosine and prostaglandin E_2 receptors, with inhibition of the effector enzyme [5].

Endurance training results in adaptations that are tissue specific, enhance the maintenance of exercise energetics, and improve myocardial contractile function [1]. The AC system might be an important site where alterations induced by exercise training may occur. From previous studies in the literature, it is evident that there is much controversy concerning the effect of endurance training on the AC system in the myocardium [2]. Several groups have reported that physical training increases the lipolytic effects of catecholamines in both laboratory animals [6, 7] and humans [8, 9]. The biochemical mechanisms of the adaptive phenomena responsible for enhancing adipose tissue lipolytic capacity, however, are still not fully understood.

The aim of the present study was to investigate the ef-

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† *Abbreviations:* AC, adenylyl cyclase; β-AR, β-adrenergic receptor; cAMP, cyclic AMP; G_s, GTP-binding protein coupled to stimulation of adenylyl cyclase; G_i, GTP-binding protein coupled to inhibition of adenylyl cyclase; GppNHp, guanosine 5′-[βγ-imido] triphosphate; ISO, isoproterenol; FK, forskolin; CHA, cyclohexyladenosine.

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fects of endurance physical training on: 1. the number and affinity of β-AR; 2. the regulation of AC activity; and 3. the levels of G_s- and G_i-proteins in the left ventricular tissue and adipose tissue of rats.

MATERIALS AND METHODS

Animal Care and Training Protocol

Forty male Wistar rats initially weighing 110–120 g were used in this study. All animals were fed a standard rat chow and tap water *ad lib.*, and were maintained on a 12-hr light–12 hr dark cycle in a temperature-controlled room. All procedures were performed in accordance with the legal requirements of the Complutense University and the NIH guidelines for the use of experimental animals. Rats were randomly assigned to one of two groups, the sedentary control group or the exercise-trained group (n = 20 each). The training program was performed on a motor-driven rodent treadmill (Columbus Instruments) and initiated by a two-week adaptation period, during which the running speed was increased progressively from 15 $m \cdot min^{-1}$ for 10 $min \cdot day^{-1}$ to 30 $m \cdot min^{-1}$ for 60 $min \cdot day^{-1}$, respectively. The adaptation period was followed by the training period, which was extended to 10 weeks, 6 $days \cdot week^{-1}$ at a running speed of 30 $m \cdot min^{-1}$ for 60 $min \cdot day^{-1}$. In training sessions, treadmill inclination was kept constant at 0%. At the end of the training period, the animals were killed by decapitation 64 hr after the last training session to dissociate the effects of training from postexercise events as much as possible. Body weight was determined immediately before death.

Myocardial Membrane Preparation

Hearts were quickly removed, weighed, and the left ventricles dissected free from the atria and fat, cut in quarters, frozen in liquid nitrogen, and stored at −80°C. Approximately 40 mg of left ventricle were thawed and the myocardium chopped and rinsed in 50 mM Tris-HCl, pH 7.5 (25°C) buffer, supplemented with 3 mM EDTA, 0.1 mM phenylmethanesulphonyl fluoride (PMSF) and 5 $\mu g \cdot mL^{-1}$ of soybean trypsin inhibitor (buffer A). Ventricles were subsequently homogenized in a Polytron tissue homogenizer (Brinkmann Instruments) for 15 sec at half maximal speed. The homogenate was filtered through 4 layers of cheesecloth and centrifuged twice at 14,000 *g* for 20 min. The resulting pellet was resuspended at approximately 6 mg protein $\cdot mL^{-1}$ and stored at −80°C until use.

White Adipose Tissue Membrane Preparation

The membrane preparation used in this study was obtained according to the method previously described [10]. Briefly, the rat epididymal fat tissue was homogenized with a Polytron PT-20 tissue homogenizer (Brinkmann) for 15 sec at position 3 in buffer A. The crude extract was centrifuged at 1000 *g* for 3 min. The infranatant was removed by syringe from between the fat plug and cell debris, and centrifuged at 30,000 *g* for 30 min. The resulting pellet was resuspended and stored at −80°C until use.

β-Adrenoreceptor Binding Assay

β-ARs were identified using the radioligand [^{125}I]cyanopindolol (ICYP) in saturation isotherm experiments conducted on plasma membrane preparations as previously described [11]. Nonspecific binding, as assayed in the presence of 10^{-5} M propranolol, accounted for approximately 10–15% of total binding. The maximal density (B_{max}) and apparent affinity (K_d) of receptors were obtained through linearization of the specific binding curve according to Scatchard [12]. Assays were carried out in triplicate and the values presented represent the mean ± SD of 3 different experiments with different membrane batches.

Adenylyl Cyclase Assay

AC activity was measured in a fraction of left ventricular cardiac membranes or adipose membranes (50 μg of protein) according to the method previously described [13]. Activation experiments were performed in the presence of 10 mM Mg^{2+}. Inhibition experiments were referred to the enzyme activity in the presence of 3 mM Mg^{2+}, 10^{-6} M GTP, and 10^{-6} M ISO. Assays were run in triplicate and the values given in the figures and tables represent the mean ± SD of 3 different experiments.

Bacterial Toxin-Catalyzed Labelling

Pertussis and cholera toxin catalysed ADP ribosylation, sample process, and electrophoretical separation of ADP-ribosylated substrates were performed as previously described [14]. The experiments reported were repeated 3 times with different membrane batches.

Western Blotting

G-proteins from membranes were resolved on 10% (30:1) SDS-polyacrylamide gels and subsequently transferred to nitrocellulose and immunoblotted as described before [11]. The antibodies used in this study i.e. anti-$G_{s\alpha}$ (1/200), anti-$G_{i1,2\alpha}$ (1/200), and anti-G_{i3} (1/250), were obtained against the peptides RMHLRQYELL, KNNLKDCGLF, and KNNLKECGLY from the carboxy-terminal sequences of the vertebrate $G_{s\alpha}$-, $G_{i1,2\alpha}$-, and G_{i3}-proteins respectively, whereas the anti-$G_{i1\alpha}$ (1/250) was obtained against the internal sequence DLDRIAQPNY.

Protein Determination

Protein concentration was determined by the method of Lowry *et al.* [15] using BSA as a standard.

Statistical Analysis

All values shown in tables and figures are expressed as mean ± SD. Differences between exercised and control rats were analyzed using an unpaired Student's *t*-test.

Materials

[α-^{32}P]ATP and [8-^{3}H]AMPc were purchased from Amersham International. [^{125}I]cyanopindolol, [α-^{32}P]NAD and [^{125}I]protein A were from New England Nuclear Corp. Cholera toxin, BSA, isoproterenol, glucagon, nucleotides and other reagents were from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Forskolin, pertussis toxin and the anti-$G_{i1\alpha}$ and anti-$G_{i3\alpha}$ antibodies were purchased from Calbiochem. The anti-$G_{s\alpha}$ and anti-$G_{i1,2\alpha}$ were kindly provided by Dr. V. Homburger (CCIPE, Montepellier, France).

RESULTS

Response to Exercise

Although the initial body weights were similar in both groups of rats (110–120 g), body weights at the end of the treadmill training program were significantly lower ($P < 0.005$) in trained rats than in sedentary controls (384 ± 25 vs 451 ± 23 g, respectively). However, there was no significant difference in heart weight in trained animals relative to their sedentary counterparts (969 ± 100 vs 1032 ± 85 mg, respectively). Thus, the exercise training program used in the present study did not induce cardiac hypertrophy because it is generally accepted that myocardial hypertrophy does not occur unless there is an absolute increase in cardiac mass [16]. As expected, the relative heart weight ($mg \cdot g^{-1}$ body weight) of trained rats significantly increased ($P < 0.05$) with respect to their sedentary controls (2.54 ± 0.10 vs 2.32 ± 0.09, respectively). Most [17] training studies with rats running on a motor-driven treadmill have reported significantly lower body weights in exercise-trained animals than in sedentary controls, with heart weights similar in both animal groups. However, Scarpace *et al.* observed an increase and a decrease in ventricle weights of young and senescent rats, respectively, when runners were compared with sedentary controls [18].

β-Adrenergic Receptors

The binding of ICYP to membrane preparations was saturable and highly specific. Nonspecific binding accounted for 15–20% of total binding. Scatchard analysis of ICYP binding data was linear, indicating a single type of antagonist binding site (data not shown). Table 1 summarizes the results of radioligand binding studies. The density (B_{max}) of β-AR in ventricular membranes of trained rats showed an important decrease (30%) compared to controls. However, the number of adipose β-AR increased in membranes of trained rats. There was no difference in receptor affinity for the antagonist either in myocardium or in adipose tissues in trained versus sedentary rats.

A training-induced decrease in the number of β-ARs without any significant effect on binding affinity has been shown by some authors in rat ventricles [19]. However, others have reported no significant effects of endurance exercise training with regard to either β-AR density or affinity in rat heart [20, 21]. In treadmill-trained pigs (Yucatan miniswine) a decrease in right atrial β-AR density with no change in left ventricular density has been described [22]. Few data are available concerning β-AR adaptation to exercise in adipose tissue, either in density or in affinity. A training-induced increase in the number of β-AR without any significant effect on binding affinity has been shown in the adipose tissue of human bicyclers [9]. However, others have reported no significant effect of endurance exercise training on either β-AR density or affinity in rat white adipose tissue of swimming rats [6] or human runners [8]. Although the use of various ligands with different affinities of β-AR could be considered a potential source of discrepancies with previous studies, other factors, such as animal species and training protocol could also play a role.

Adenylyl Cyclase Regulation

To estimate which components of the AC system were altered with exercise, AC activity in response to β-AR, G proteins, and catalytic subunit modulators was measured (Table 2). As shown in Fig. 1A, β-adrenergic-induced

TABLE 1. Number and affinity of β-adrenoceptors as determined by ICYP binding in left ventricular membranes and white adipose tissue from control and exercised rats

	Myocardium		Adipose	
	Control	Trained	Control	Trained
B_{max} (fmol/mg)	47.3 ± 5.9 (100%)	32.9 ± 3.1* (69.7 ± 6.5%)	117.6 ± 5.8 (100%)	147.9 ± 1.1* (125.6 ± 0.9%)
K_d (pM)	117.3 ± 0.7	115.9 ± 0.1	17.0 ± 3.4	18.5 ± 3.7

Values are means of 3 different experiments ± SD. The values given in parentheses correspond to the % of variation with respect to the group of control rats.*

stimulation of cardiac AC activity underwent desensitization with training, as indicated by a rightward shift in the K_a for ISO-induced stimulation of AC activity with no variation in the maximal response. In other studies, only AC activation at the maximal concentration of β-AR agonists has been determined, and the results obtained are conflicting. Indeed, some authors [21] have observed no variation in the maximum activation of AC, whereas others [23] have reported a decrease; Böhm *et al.* [20] even reported an increase. In contrast, β-adrenergic-mediated stimulation of white adipose tissue AC activity underwent sensitization with training (Table 2), indicated by a leftward shift in the K_a for ISO-induced stimulation of AC activity with no variation in the maximal response (data not shown).

Muscarinic cholinergic receptors mediate parasympathetic control of heart function [24] through inhibition of AC activity. Fig. 2B shows the concentration-dependent inhibition induced by the muscarinic agonist carbachol on isoproterenol-stimulated AC. The lesser effect of carbachol in trained rats appears to be associated with a decreased V_{max} and not with an increase in K_i. Adenosine is a potent inhibitor of lypolisis and AC activity in rat adipocytes [3] and promotes antiadrenergic effects on the force of cardiac contraction [24]. We assayed here the enzyme activity in the presence of cyclohexiladenosine (CHA), an adenosine analog. There was no significant difference in CHA-promoted inhibition of adipose AC activity between trained and sedentary animals (Table 2). However, AC inhibition by CHA and PGE_2 (through A_1-adenosine receptors and PGE_2 receptors, respectively) was decreased in ventricular membranes with exercise (Table 2). Thus, a diminished inhibition of AC appears to be a common feature in cardiac membranes from trained animals.

To assess the functionality of AC modulated by G protein, we studied AC activation by GppNHp, a non-hydrolysable GTP analogue, or fluoride, which activates AC by interacting with the G_s-protein. GppNHp appears to be able to dissociate and activate not only G_s but also G_i, and GppNHp-dependent AC activity may, therefore, represent a balance between the two regulatory pathways. In contrast, fluoride presumably activates AC without dissociating the G_s heterotrimer, and fluoride-dependent AC activity might, then, be insensitive to changes in the inhibitory pathway [25]. Fig. 1C and Table 2 show that a decrease in fluoride and GppNHp-stimulated AC activity in cardiac membranes is induced by exercise, whereas it remains unchanged in adipose tissue. Previous studies have not observed any significant difference in basal or NaF-stimulated cardiac AC activity in response to training [19], whereas others have reported an increase in basal and GppNHp-stimulated AC activity [20], as well as a decrease in both basal AC activity [16] and GppNHp and forskolin stimulation of enzyme activity [26] in rat heart with training. Results shown in Table 2 suggest that physical training does not affect AC stimulation *via* G_s in adipose tissue. Similar results have been observed in previous studies [8].

Nonreceptor-mediated activation of AC was also studied. The effect of training on the catalytic unit of the AC system was examined by measuring enzyme activity in the presence of either Mn^{2+} (Fig. 1D) or FK (Table 2). Manganese ions are known to uncouple the AC catalytic subunit from G_s and G_i [27]; thus, under these conditions, the activity of the catalyst itself can be studied. The diterpene FK can activate the catalytic entity directly through a low-affinity site, but can also interact with a high-affinity site, which promotes coupling between G_s and the catalytic subunit [28]. The results obtained in the present study when AC activity was assayed in cardiac membranes in the presence of Mn^{2+} (Fig. 2D) or Mg^{2+} + FK (Table 2) suggest that the levels or functional state of the catalytic unit were specifically depressed by training. In adipose tissue, how-

TABLE 2. Activation and inhibition of adenylyl cyclase activity (pmol/min/mg protein) by different modulators

	Myocardium				Adipose			
	Control (pmol/min/mg)	(%)	Trained (pmol/min/mg)	(%)	Control (pmol/min/mg)	(%)	Trained (pmol/min/mg)	(%)
Activation:								
Basal (Mg^{2+} 10 mM)	9.8 ± 0.2	100	9.1 ± 0.5	100	10.4 ± 0.5	100	13.4 ± 0.4*	100
Mn^{2+} 5 mM	67.2 ± 0.4	–	48.7 ± 8.2‡	–	25.1 ± 0.8	–	28.1 ± 0.2†	–
FK 3.5×10^{-5} M	83.6 ± 1.4	848	71.2 ± 2.1	779‡	82.5 ± 9.5	791	105.8 ± 5.4	790
GppNHp 10^{-5} M	54.2 ± 0.5	550	44.4 ± 0.8	486‡	42.5 ± 0.7	408	54.2 ± 0.9	405
F^- 7 mM	75.3 ± 1.9	764	59.0 ± 0.0	645‡	129.2 ± 5.7	1239	171.4 ± 7.2	1280
ISO 10^{-7} M	19.2 ± 0.9	195	14.8 ± 0.5	162‡	15.7 ± 0.1	151	21.5 ± 0.6	161‡
Inhibition:								
Control (ISO 10^{-6} M)	14.3 ± 0.5	100	13.0 ± 0.3	100	9.7 ± 0.5	100	10.9 ± 0.6	100
CB 10^{-4} M	10.6 ± 0.0	74	10.9 ± 0.2	84‡	–		–	
CHA 10^{-6} M	10.1 ± 1.6	71	11.5 ± 0.1	88‡	6.9 ± 0.6	71	8.5 ± 0.4	78*
PGE_2 10^{-6} M	11.2 ± 0.4	78	10.9 ± 0.3	84†	–		–	

FK, forskolin; GTP, guanosine 5′-triphosphate; F, fluoride; GppNHp, guanylylinidodiphosphate; ISO, isoproterenol; CB, carbachol; CHA, cyclohexyladenosine; and PGE_2, prostaglandin E_2. In the inhibitory experiments, the Mg^{2+} concentration was 3 mM and the GTP concentration 10^{-6} M. Values are means of 3 different experiments ± SD.
*$P < 0.05$; †$P < 0.01$; ‡$P < 0.005$ (trained vs control).

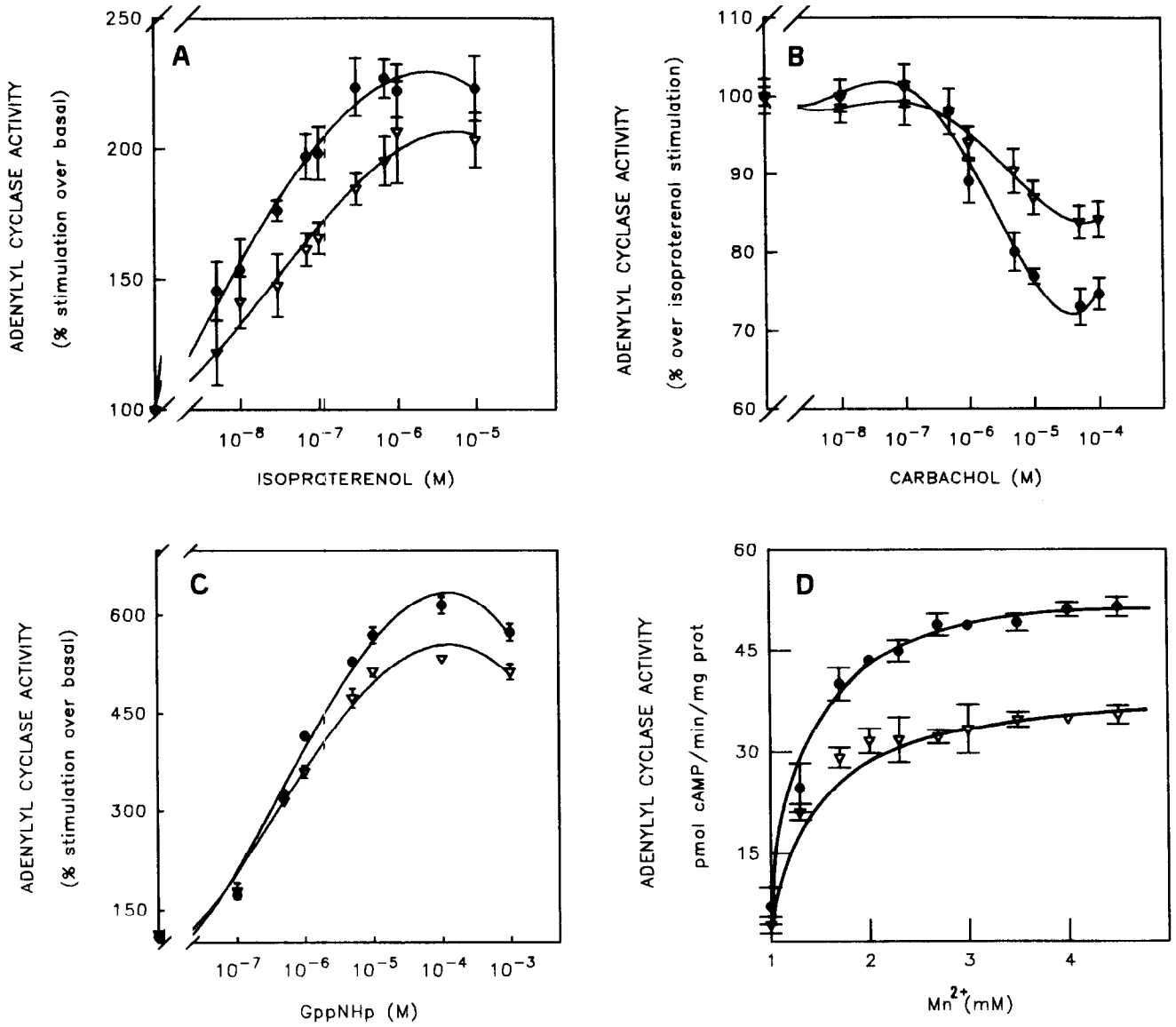

FIG. 1. Activation of adenylyl cyclase in myocardial membranes of control (●—●) and trained (▽—▽) rats by different modulators. Values are expressed as means of 3 different experiments. (A) isoproterenol. The basal activities (pmol cAMP · min^{-1} · mg^{-1} of protein) were: control, 11 ± 1.5 and trained, 13.8 ± 1.8. The differences observed in the interval 5 × 10^{-8}–7 × 10^{-7} M were stastically significant ($P < 0.005$). (B) carbachol. The basal activities (pmol cAMP · min^{-1} · mg^{-1} of protein) were: control, 17.2 ± 1.1, and trained, 14 ± 0.2. The differences observed in the interval 10^{-5}–10^{-4} M were statistically significant ($P < 0.005$). (C) GppNHp. The basal activities (pmol cAMP · min^{-1} · mg^{-1} of protein) were: control, 16.6 ± 0.7 and trained, 15.3 ± 1.9. The differences observed in the interval 10^{-5}–10^{-3} M were statistically significant ($P < 0.005$). (D) Mn^{2+}. The differences observed in the interval 2–5 M were statistically significant ($P < 0.005$).

ever, AC activity in the presence of Mn^{2+} or Mg^{2+} + FK was increased in trained animals (Table 2).

G-proteins

In an attempt to determine whether G-protein levels were altered as a consequence of exercise, we performed ADP-ribosylation and immunoblotting studies in cardiac and adipose plasma membranes from trained and sedentary rats. When cardiac membranes were incubated with [α-^{32}P]NAD in the presence of activated cholera toxin, and proteins were subsequently separated by SDS-PAGE, the autoradiogram of the gel showed two major radioactive bands at 42 and 53 KDa (data not shown). A polyclonal antibody raised against the carboxy-terminal sequence of the α subunit of the G_s-protein ($G_{s\alpha}$) was able to cross-react on Western blots with only two proteins in plasma membranes at the same molecular masses as the cholera toxin substrates (Fig. 2). These results are in agreement with those previously described for the two forms of the $G_{s\alpha}$-protein in the heart [29]. The levels of each form of G_s were quantified with [^{125}I]protein A; results shown in Fig. 2

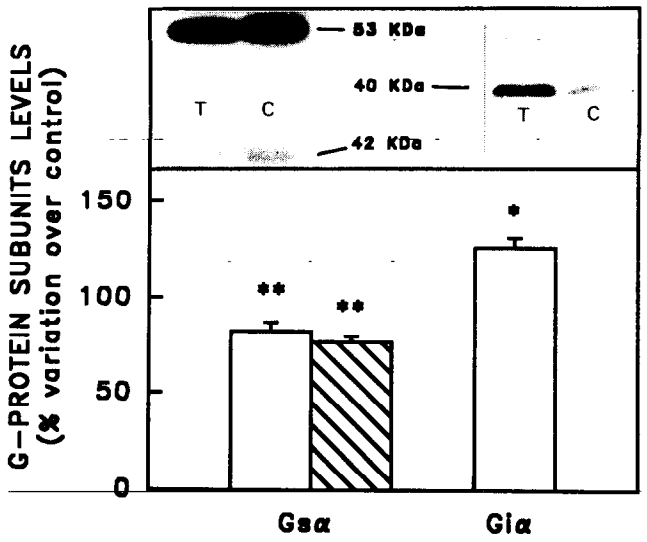

FIG. 2. Immunodetectable $G_{s\alpha}$ and $G_{i2\alpha}$ content in myocardial membranes. Rat ventricular myocardium contains two $G_{s\alpha}$ subunit isoforms of 42 (□) and 53 KDa (▨). The radioactivity incorporated into the 53 KDa sheet of control group was 2586 ± 60 cpm, and 271.6 ± 10.2 cpm in the 42 KDa. A single protein of approximately 40 KDa is immunodetected with anti-$G_{i12\alpha}$ antibody and the radioactivity incorporated in that band in the control group was 257.5 ± 33.5 cpm. C, control; T, training. Values correspond to a significant experiment. * $P < 0.01$; ** $P < 0.005$ (trained vs control).

indicate that levels of both $G_{s\alpha}$ isoforms were 20% lower in trained rats than in sedentary controls. Similar results were obtained when we determined the ADP-ribosylation levels in plasma membranes from trained and sedentary controls.

On the other hand, cardiac plasma membrane preparations used in our study contained only two substrates, at 40 and 41 KDa, which were ADP-ribosylated by pertussis toxin (data not shown). The antibodies obtained against the internal sequence of G_{i1} ($G_{i1\alpha}$) and against the carboxy-terminal segment of the α subunit of G_{i3} ($G_{i3\alpha}$) were not able to cross-react with any band, whereas the antibody anti-$G_{i1,2\alpha}$ raised against the carboxy-terminal peptide common to the α subunits of G_{i1} ($G_{i1\alpha}$) and G_{i2} ($G_{i2\alpha}$) recognized a single band at 40 KDa identified as $G_{i2\alpha}$. As shown in Fig. 2, the levels of $G_{i2\alpha}$ increased in the heart of trained rats compared with control animals.

Cholera toxin-catalyzed ADP ribosylation of adipocyte plasma membranes showed two substrates at 42 and 46 kDa. The antibody against $G_{s\alpha}$ also detected these two bands (Fig. 3). These results are in agreement with those previously described for the two forms of the $G_{s\alpha}$-protein in adipocyte membranes [30]. Quantification of the two bands with ^{125}I-protein A showed an overexpression of both $G_{s\alpha}$ species in adipocyte membranes of trained rats. Similar results were obtained with cholera toxin-catalyzed ADP ribosylation (data not shown). ADP ribosylation of adipose membranes in the presence of pertussis toxin showed two substrates at 40 and 41 kDa. However, the antibodies anti $G_{i1\alpha}$ and $G_{i3\alpha}$ cross-react only with one band at 41 kDa (Fig. 4). In contrast, the antibody anti $G_{i1,2\alpha}$ recognized two bands at 40 and 41 KDa, identified as $G_{i2\alpha}$ and $G_{i1\alpha}$, respectively. Because these results are in accordance with those previously described for the different forms of the $G_{i\alpha}$ in adipocyte membranes [31], the antibodies used in our investigation are a valid tool for examining the expression of $G_{i1\alpha}$, $G_{i2\alpha}$, and $G_{i3\alpha}$ in adipose tissue. As shown in Fig. 4, levels of $G_{i1\alpha}$ and $G_{i3\alpha}$ increased in adipose tissue from trained rats as compared to control animals. The results obtained with the anti-$G_{i1,2\alpha}$ antibody suggest that levels of $G_{i2\alpha}$ (40 KDa band) decreased with exercise, whereas levels of $G_{i1\alpha}$ (41 KDa) increased in a fashion similar to that observed with the anti-$G_{i1\alpha}$ antibody.

DISCUSSION

Adaptive changes in the AC system in response to endurance exercise training were studied in heart and adipose tissues. Catecholamines, either as sympathetic neurotransmitters or hormones, play an essential role in the activation of the cardiovascular system, as well as in the regulation of energy metabolism during exercise [4]. Many of these effects are mediated *via* β-AR. A decrease in receptor density in a system with many spare receptors (as β-AR in rat ventricular myocardium) is more likely to produce a shift in the apparent K_a than in the V_{max} for hormone-sensitive stimulation [32]. Alternatively, the observed change in K_a could be explained by a reduction in G_s with training because the proportion of β-AR in the high-affinity state (which reflects receptor-G_s coupling) is related to activation of AC. Plourde *et al.* described β-AR in the high-affinity state as being significantly reduced by training in the rat [19].

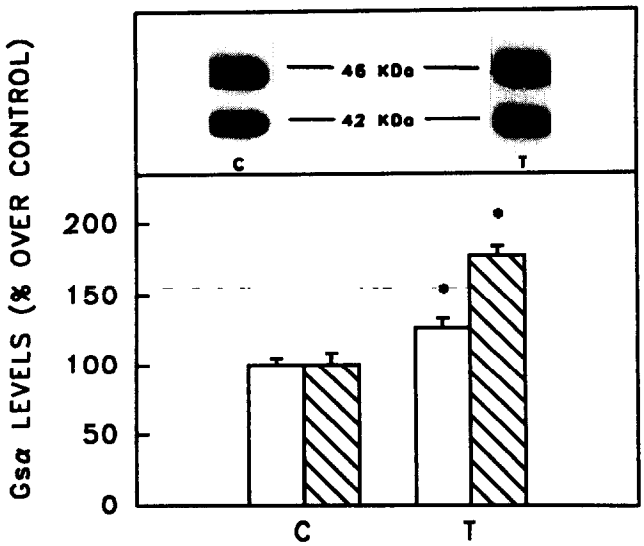

FIG. 3. Immunodetectable $G_{s\alpha}$ content in adipose plasma membranes. Rat white adipose tissue contains two $G_{s\alpha}$ subunit isoforms of 42 (▨) and 46 KDa (□). The radioactivity incorporated into the 46 KDa sheet of control group was 1775 ± 80 cpm, and 1195 ± 100 cpm in the 42 KDa band. C, control; T, training. * $P < 0.005$ (trained vs control).

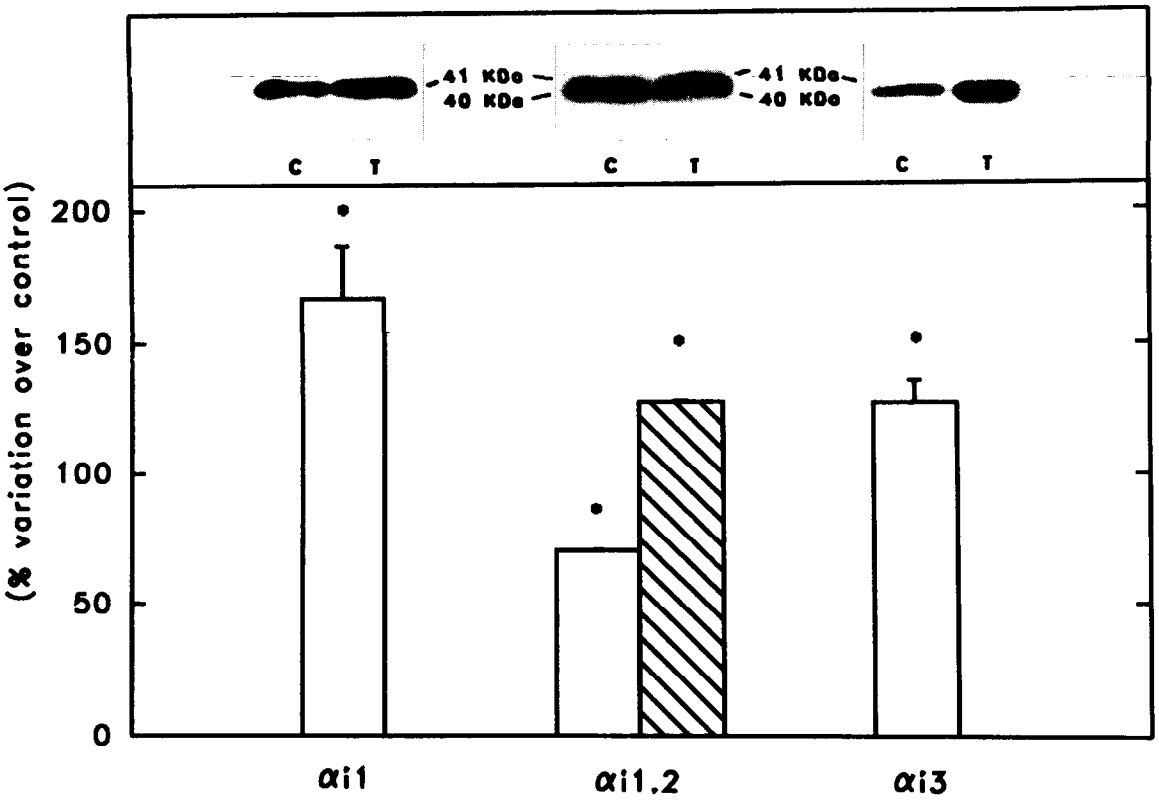

FIG. 4. Immunodetectable $G_{i1\alpha}$, $G_{i2\alpha}$, and $G_{i3\alpha}$ content in adipose plasma membranes. A single protein of approximately 41 KDa was immunodetected with anti-$G_{i1\alpha}$ and anti-$G_{i3\alpha}$ antibodies and the radioactivity incorporated in these bands of the control group is 2182.6 ± 261.6 cpm and 1211.9 ± 145.5 cpm, respectively. The anti-$G_{i1,2\alpha}$ antibody cross-reacted with two proteins of 40 (□) and 41 KDa (▨), in the control group; the radioactivity incorporated into the 40 KDa and 41 KDa band was 6969.9 ± 724.2 and 4788.4 ± 734.25 cpm, respectively. C, control; T, training. * $P < 0.005$ (trained vs control).

The sympathetic and parasympathetic mechanisms for controlling heart function are opposed [24]. The lesser effect of CB in trained rats might be explained by a decrease in the number of muscarinic receptors coupled to AC, although several reports indicate that the density of muscarinic receptors is unchanged in the heart of trained rats [20]. It has been observed that chronic β-blockade transregulates inhibitory A_1-adenosine and muscarinic M_2-receptors of the cardiac AC system by a new regulatory mechanism that involves several inhibitory receptor systems [33].

Nonreceptor-mediated regulation of cardiac AC was also studied. AC activity was also reduced in trained rats when AC was stimulated at the level of G proteins. The decrease in fluoride- and GppNHp-stimulated AC activity could be related to a reduction in the levels of G_s or in its functional state.

Regulation of AC activity is accomplished by two distinct guanine nucleotide-binding proteins, G_s and G_i, which mediate enzyme activation and inhibition, respectively [5]. There are four forms of G_s, which represent splice variants originating from a single gene, although the functional significance of this diversity has not been clearly established. Several lines of evidence suggest that stimulated AC activity is a function, not only of the amount of $G_{s\alpha}$ present in the membrane, but also of the isoform that is predominantly expressed. Three forms of G_i have been identified to date (G_{i1}, G_{i2}, and G_{i3}). The mechanisms whereby G_i elicits inhibition of AC have yet to be fully elucidated. Furthermore, the heterogeneity of AC suggests that there may be several mechanisms by which both stimulation and inhibition can be accomplished, depending on the enzyme involved. The levels of $G_{s\alpha}$ in myocardium as measured in terms of ADP-ribosylated and immunologically reactive proteins, were decreased in trained rats. These findings are in line with those obtained for AC activation by ISO, GppNHp, and fluoride in our study. They also are in agreement with the results previously reported by Plourde *et al.* [4]. In other studies, Böhm *et al.* have shown that physical training had no effect on the amount of immunodetectable $G_{s\alpha}$ [20], and Hammond *et al.* described a noncoordinated regulation of cardiac G_s-protein and β-AR in pigs in response to chronic dynamic exercise, in addition to an increase in the amount of immunoassayable $G_{s\alpha}$ with training without any significant effect on ventricular β-AR [22].

The predominant G_i-protein in heart is G_{i2}, which, although not unequivocally established, seems to be the principal subtype responsible for inhibition of AC [34]. Our results are in accordance with those previously described for the different forms of $G_{i\alpha}$ in cardiac membranes [29]. The

increase in this isoform is in apparent contradiction with results obtained for AC inhibition, because an increase in $G_{i2\alpha}$ would suggest sensitization of the inhibitory AC pathway. It is not clear whether a difference in $G_{i\alpha}$-protein levels must necessarily modify the effects of $G_{i\alpha}$-coupled receptors. Thus, as has been previously reported [33], the effects of A_1-adenosine receptor agonists and muscarinic agonists on AC in failing human myocardium are unchanged even in the presence of increased $G_{i\alpha}$.

When Mn^{2+} was used to assess uncoupled AC activity, it is noteworthy that a significant increase in basal enzyme activity in myocardium occurred. This could be attributed to uncoupling of the tonic inhibitory influence of G_i. If this were the case, then Mn^{2+}-stimulated activity might be dependent upon the extent of uncoupling from G_i, because it is possible that G_i still elicits inhibition of AC in membranes from exercised rats, in accordance with the increased levels of G_i.

In conclusion, the present study shows that endurance training by running is associated with a desensitization of the cardiac β-AR-AC transduction system. This desensitization mechanism involves a coordinated reduction of β-AR density, $G_{s\alpha}$-protein levels, and AC catalytic unit activity, as well as an upregulation of $G_{i2\alpha}$-protein, which does not appear to be accompanied by an increase in G_i functional activity. This desensitization process might play a protective role in a tissue exposed to high concentrations of catecholamines during exercise, suggesting that such changes in the AC system are an important component of the physiological responses to exercise training.

By contrast, the present study also shows that endurance training by running is associated with a sensitization of the adipose β-AR-AC transduction system. However, in the analysis of relative values, the decline in the cardiac β-AR-AC system with exercise was more pronounced than the increase in β-AR-AC observed in adipose tissue. As far as we know, our investigation is the first to thoroughly examine this aspect in white adipose tissue. A substantial β-AR reserve exists in fat cells approximately 50% of which are spare receptors [35]. The results obtained here could be explained by an increase in receptor density in a system with many spare receptors or, alternatively, by an increase in G_s levels with training because the proportion of β-AR in the high-affinity state (which reflects receptor-G_s coupling) is related to activation of AC. Williams and Bishop [6] described that β-AR in the high-affinity state was increased in swimming rats, but did not observe any variation in β-AR density. The results of other studies [36] could also indicate either an increased capacity of β-AR to interact with G_s in adipose tissue of training rats or the existence of raised G_s levels. Other studies have also reported an increase in the V_{max} for hormone-sensitive stimulation with training [6, 7, 36, 37]. Williams and Bishop [6] also observed that the degree of inhibition of AC activity tended to be greater in adipocytes of sedentary rats than in those of swimming rats, although the differences were small and did not reach statistical significance. However, Izawa *et al.* [37] reported that GTP inhibition of FK-stimulated cyclase activity was significantly reduced in membranes from trained rat adipocytes, and attributed these observations to a specific decrease in the amount of inhibitory regulatory proteins.

The sensitization mechanism in adipose tissue involves a coordinated elevation of β-AR density, $G_{s\alpha}$-protein levels, and AC catalytic unit activity, as well as a downregulation of $G_{i2\alpha}$-protein that does not appear to be accompanied by a decrease in G_i functional activity. The increased levels of both $G_{s\alpha}$ species in adipocyte membranes from trained rats are in line with results obtained for AC activation by ISO in this study, as well as with the findings of previous studies [8, 23]. In another study, Izawa *et al.* [37], however, did not report any variation with exercise in the unique radioactive substrate (45 KDa) obtained in cholera toxin-catalysed ADP-ribosylation experiments.

The decrease in $G_{i2\alpha}$ in adipose tissue is in apparent contradiction with the results obtained for AC inhibition by CHA, because this G_i isoform is the one principally responsible for AC inhibition. However, it has been previously suggested that white adipose tissue possesses an excess of G_i, termed as spare G_i, thereby accounting for a similar discrepancy between AC inhibition and the levels of immunodetectable G_i [38].

In agreement with the physiological role of white adipose tissue, this sensitization process might play an important role in the adaptation of energy metabolism in the trained state. Thus, it should be pointed out that adaptations of the AC system to training seem to be complex and tissue-specific, and cannot be considered a single unified mechanism. Our results may give new insights into the biochemical bases of physiological adaptations to physical exercise and reveal an interesting aspect of the tissue-specific adaptation of the AC system that underlies adaptation of many membrane receptor-linked processes.

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References

1. Portmans JR. Ed. *Principles of Exercise Biochemistry*, 2nd Edition, Karger, Basel, 1993.
2. Moore RL and Horzick DH, Cellular adaptations of the myocardium to chronic exercise. *Prog Cardiovasc Dis* **37:** 371–396, 1995.
3. Coppack SW, Jensen MD and Miles JM, In vivo regulation of lipolysis in humans. *J Lipid Res* **35:** 177–193,
4. Svedenhag J, Influence of physical training on plasma catecholamine concentrations at rest and during exercise. *Acta Physiol Scand* **125:** 33–37, 1985.
5. Taussig R and Gilman AG, Mammalian membrane-bound adenylyl cyclases. *J Biol Chem* **270:** 1–4, 1995.

6. Williams RS and Bishop T, Enhanced receptor-cyclase coupling and augmented catecholamine-stimulated lipolysis in exercising rats. *Am J Physiol* **243:** E345–E351, 1982.
7. Izawa T, Komabayashi T, Mochizuki T, Suda K and Tsuboi M, Enhanced coupling of adenylate cyclase to lipolysis in permeabilized adipocytes from trained rats. *J Appl Physiol* **71:** 23–29, 1991.
8. Wahrenberg H, Bolinder J and Arner P, Adrenergic regulation of lipolysis in human fat cells during exercise. *Eur J Clin Invest* **21:** 534–541, 1991.
9. Lönnqvist F, Wahrenberg H, Hellström L, Reynisdottir S and Arner P, Lipolytic catecholamine resistance due to decreased β_2-adrenoceptor expression in fat cells. *J Clin Invest* **90:** 2175–2186, 1992.
10. Belsham GJ, Denton RM and Tanner MJA, Use of a novel rapid preparation of fat-cell plasma membranes employing Percoll to investigate the effects of insulin and adrenaline on membrane protein phosphorylation within intact fat-cells. *Biochem J* **192:** 457–467, 1980.
11. Nieto JL, Díaz-Laviada I, Guillén A, García-Barreno P and Haro A, Cardiac β-adrenoceptors, G-proteins and adenylate cyclase regulation during myocardial hypertrophy. *Cell Signalling* **5:** 169–179, 1993.
12. Scatchard G, The attraction of proteins for small molecules and ions. *Ann NY Acad Sci* **51:** 660–672, 1949.
13. Díaz-Laviada I, Larrodera P, Nieto JL, Cornet ME, Díaz-Meco MT, Sánchez MJ, Guddal PH, Johansen T, Haro A and Moscat J, Mechanism of inhibition of adenylate cyclase by phospholipase C-catalyzes hydrolysis of phosphatidylcholine. Involvement of a pertussis toxin-sensitive G protein and protein kinase C. *J Biol Chem* **266:** 1170–1176, 1991.
14. Guillén A, Jallon J-M, Fehrentz J-A, Pantaloni C, Bockaert J and Homburger V, A Go-like protein in *Drosophila melanogaster* and its expression in memory mutants. *EMBO J* **9:** 1449–1455, 1990.
15. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with Folin phenol reagent. *J Biol Chem* **193:** 265–275, 1951.
16. Bugaisky L and Zak R, Biological mechanisms of hypertrophy. In: *The Heart and Cardiovascular System* (Eds. Fozzard H, Haber E, Katz A, Jennings R and Morgan HE), pp. 1491–1506. Raven Press, New York, 1986.
17. Sylvestre-Gervais L, Nadeau A, Nguyen MH, Tancrede G and Rousseau-Migneron S, Effects of physical training on beta-adrenergic receptors in rat myocardial tissue. *Cardiovasc Res* **16:** 530–534, 1982.
18. Scarpace PJ, Lowenthal DT and Tumer N, Influence of exercise and age on myocardial β-adrenergic receptor properties. *Exp Gerontol* **27:** 169–177, 1992.
19. Plourde G, Rousseau-Migneron S and Nadeau A, β-adrenoceptor adenylate cyclase system adaptation to physical training in rat ventricular tissue. *J Appl Physiol* **70:** 1633–1638, 1991.
20. Böhm M, Dorner H, Htun P, Lensche H, Platt D and Erdmann E, Effects of exercise on myocardial adenylate cyclase and $G_{i\alpha}$ expression in senescence. *Am J Physiol (Heart Circ Physiol* 33) **264:** H805–H814, 1993.
21. Moore RL, Riedy M and Gollnick PD, Effect of training on β-adrenergic receptor number in rat heart. *J Appl Physiol* **52:** 1133–1137, 1982.
22. Hammond HK, Ransnas LA and Insel PA, Noncoordinate regulation of cardiac Gs protein on β-adrenergic receptors by a physiological stimulus, chronic dynamic exercise. *J Clin Invest* **82:** 2168–2171, 1988.
23. Döhm GL, Pennington SN and Barakat H, Effect of exercise training on adenylyl cyclase and phosphodiesterase in skeletal muscle, heart and liver. *Biochem Med* **16:** 138–142, 1976.
24. Pappano AJ and Mubagwa K, Actions of muscarinic agents and adenosine on the heart. In: *The Heart and Cardiovascular System*. 2nd Ed. (Eds. Fozzard HA *et al.*) pp. 1765–1776, 1992.
25. Feldman AM, Modulation of adrenergic receptors and G-transduction proteins in failing human ventricular myocardium. *Circulation* **87** [suppl IV]: IV-27–IV-34, 1993.
26. Scarpace PJ, Shu Y and Tumer N, Influence of exercise training on myocardial beta-adrenergic signal transduction: differential regulation with age. *J Appl Physiol* **77:** 737–741, 1994.
27. Limbird LE, Hickey AR and Lefkowitz RJ, Unique uncoupling of the frog erythrocyte adenylate cyclase system by manganese. Loss of hormone and guanine nucleotide-sensitive enzyme activities without loss of nucleotide-sensitive, high affinity agonist binding. *J Biol Chem* **254:** 2677–2683, 1979.
28. Hubbard JW, Conway PG, Nordstrom LC, Hartman HB, Lebedinsky Y, O'Malley GJ and Kosley RW, Cardiac adenylate cyclase activity, positive chronotropic and inotropic effects of forskolin analogs with either low, medium or high binding site affinity. *J Pharmacol Exp Ther* **256:** 621–627, 1991.
29. Eschenhagen T, G proteins and the heart. *Cell Biol International* **17:** 723–749, 1993.
30. Milligan G and Saggerson ED, Concurrent up-regulation of guanine-nucleotide-binding proteins $G_{i1\alpha}$, $G_{i2\alpha}$ and $G_{i3\alpha}$ in adipocytes of hypothyroid rats. *Biochem J* **270:** 765–769, 1990.
31. Green A, Johnson JL and Milligan G, Down-regulation of Gi sub-types by prolonged incubation of adipocytes with an A1 adenosine receptor agonist. *J Biol Chem* **265:** 5206–5210, 1990.
32. Bristow MR, Kantrowitz NE, Ginsburg R and Fowler MB, β-adrenergic function in heart muscle disease and heart failure. *J Mol Cell Cardiol* **17:** 41–52, 1985.
33. Marquetant R, Brehm B and Strasser RH, Chronic β-blockade transregulates inhibitory A1 adenosine and muscarininc M2 receptors of the adenylyl cyclase system. *J Mol Cell Cardiol* **24:** 535–548, 1992.
34. Remaury A, Larrouy D, Daviaud D, Rouot B and Paris H, Coupling of the α_2-adrenergic receptor to the inhibitory G-protein G_i and adenylate cyclase in HT29 cells. *Biochem J* **292:** 283–288, 1993.
35. Arner P, Adrenergic receptor function in fat cells. *Am J Clin Nutr* **55:** 228 S–236 S, 1992.
36. Izawa T, Komabayashi T, Tsuboi M, Koshimizu E and Suda K, Augmentation of catecholamine-stimulated [^{3}H]GDP release in adipocyte membranes from exercise-trained rats. *Jpn J Physiol* **36:** 1039–1045, 1986.
37. Izawa T, Komabayashi T, Shinoda S, Suda K, Tsuboi M and Koshimizu E, Possible mechanism of regulating adenylate cyclase activity in adipocyte membranes from exercise-trained male rats. *Biochem Biophys Res Commun* **151:** 1262–1268, 1988.
38. Begin-Heick N, α-subunits of G(s) and G(i) in adipocyte plasma membranes of genetically diabetic (db/db) mice. *Am J Physiol* **263:** C121–C129, 1992.