Augmentation of Catecholamine-stimulated [^3H]GDP Release in Adipocyte Membranes from Exercise-trained Rats

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Summary The effects of exercise training on the catecholamine-stimulated [^3H]GDP release in rat adipocyte membranes prelabeled with [^3H]GTP and the adenylate cyclase activity were investigated. Exercise training significantly increased the release of [^3H]GDP in response to (–)isoproterenol. The adenylate cyclase activity induced by a nonhydrolyzable guanine nucleotide analogue, Gpp(NH)p, was significantly greater in exercise-trained rats.

Key words: exercise, [^3H]GDP release, adenylate cyclase.

Free fatty acids mobilization from adipose tissue serve as the major metabolic fuel for working muscle during long-term exercise (FRIEDBERG *et al.*, 1963; AHLBORG *et al.*, 1974); adipose tissue is generally considered to play a fundamental role in metabolic adaptation to exercise training. In this regard, for the past few years, some evidence has been obtained that exercise training enhances the lipolytic response of adipocytes to catecholamines (ASKEW *et al.*, 1975; BUKOWIECKI *et al.*, 1980; WILLIAMS and BISHOP, 1982). Among these studies, ASKEW *et al.* (1975) and WILLIAMS and BISHOP (1982) have noted the enhanced adrenaline-stimulated adenylate cyclase activity in epididymal fat pad and in adipocyte membranes from exercise-trained rats, respectively. Although adenylate cyclase is activated through the initial occupation of β -adrenergic receptors (β -AR) by catecholamines in the cell membrane (FAIN, 1973), the enhanced adenylate cyclase activity in adipocyte membranes from exercise-trained rats could not be explained by the increase in β -AR number or in receptor affinity for antagonist binding (BUKOWIECKI *et al.*, 1980; WILLIAMS and BISHOP, 1982; IZAWA *et al.*, 1984). On the other hands, WILLIAMS and BISHOP (1982) have observed that exercise training significantly increased the formation of the high-affinity state of β -AR for agonists in rat adipocyte membranes by an assay of agonist-competition curves using a computerized curve

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modeling method, suggesting that the enhanced adenylate cyclase activity in adipocyte membranes from exercise-trained rats may be due to the improvement of the coupling of β -AR-cyclase. We have also observed the same phenomenon (unpublished). However, more direct evidence of the effect of exercise training on the coupling of β -AR-cyclase has not been obtained for the present time, and it would be premature to ascribe the enhanced adenylate cyclase activity in adipocyte membranes from exercise-trained rats to the enhanced high-affinity agonist binding.

Much information about the mechanism of the receptor-mediated activation of adenylate cyclase has accumulated during the past few years (RODBELL, 1980; ROSS and GILMAN, 1980; LEFKOWITZ *et al.*, 1983). Stimulation of the membrane receptor by an agonist leads to release guanosine 5'-diphosphate (GDP) from the binding sites on the stimulatory guanine nucleotide regulatory protein (N_s), and guanosine 5'-triphosphate (GTP) subsequently binds to N_s . This reaction is called "turn-on" reaction, since the GTP-bound form of N_s activates the catalytic unit (C) of adenylate cyclase. Thus, receptor agonists may activate adenylate cyclase by increasing the rate of release of GDP from N_s . Therefore, the agonist-induced release of GDP from the binding sites on N_s appears to be a good index for the "coupling" of the receptor to N_s .

The present study was designed more directly to clarify the effect of exercise-training on the coupling conditions between the β -AR and N_s in rat adipocyte membranes by means of an assay of the agonist-induced [3 H]GDP release.

[3 H]GTP (10 Ci/mmol) was obtained from New England Nuclear Corp. Other compounds used in this report were: (–)isoproterenol bitartrate (IPR), ATP, phosphocreatine, creatine phosphokinase, 5'-guanylylimidodiphosphate (Gpp(NH)p), and bovine serum albumin (fraction V, Sigma); 3-isobutyl-1-methylxanthine (IBMX; Aldrich); crude collagenase (Type I, Worthington); dithiothreitol (Wako); ethylene glycol-bis-(β -aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA; Wako).

Male Wistar rats with the initial body weights of 110–120 g were used for the experiments. Animals were divided into two groups; control and the exercise-trained group. The exercise-trained rats ran on a rodent treadmill 5 days per week. The treadmill was set at an angle of 8°. The speed and duration of each training session were progressively increased over 8 weeks until the rats were able to run continuously for 90 min at 25–27 m/min. The exercise-trained rats were killed by decapitation at least 24 h after their last bout of exercise. Adipocytes were isolated from the epididymal adipose tissue by the modified method of RODBELL (1964). Crude adipocyte membranes were prepared by the method of WILLIAMS *et al.* (1976).

The labeling of adipocyte membranes with [3 H]GTP and the subsequent [3 H]GDP release assay were performed according to the procedure (MURAYAMA and UI, 1984) based on the method of CASSEL and SELINGER (1978). Membranes (1–2 mg of protein) were suspended in 200 μ l of buffer A (25 mM Tris, 5 mM $MgCl_2$, 1 mM EGTA, 0.5 mM dithiothreitol, 1 mM ATP, 6.7 mM phosphocreatine, and 30 units/ml

of creatine phosphokinase, pH 7.4). The labeling of membranes was initiated by the addition of $0.5\ \mu\text{M}$ [^3H]GTP and $50\ \mu\text{M}$ (–)IPR to the membrane suspension followed by a 3 min incubation at 30°C . The reaction was terminated by the addition of $100\ \mu\text{M}$ unlabeled GTP and $20\ \mu\text{M}$ propranolol, and the reaction mixture was immediately cooled to 4°C . Membranes thus labeled with [^3H]GTP were washed three times with buffer B (25 mM Tris, 5 mM MgCl_2 , pH 7.4) by repeating centrifugation at $10,000\ g$ for 10 min and dilution, and then suspended in $200\ \mu\text{l}$ of buffer A further supplemented with $100\ \mu\text{M}$ GTP. The suspension was incubated for 10 min at 37°C . This second incubation was designed to reduce the nonspecific background of nucleotide release during the subsequent [^3H]GDP release assay. The labeled membranes (1–2 mg of protein) were finally suspended in 1 ml of buffer A. The labeled membrane suspension ($50\ \mu\text{l}$) was combined with $150\ \mu\text{l}$ of buffer A containing 0.3 mM Gpp(NH)p and with various concentrations of (–)IPR. The mixture was incubated at 30°C for 5 min. The assay was terminated by the addition of 1 ml of ice-cold buffer B, and then 1 ml of the supernatant obtained by a 3 min centrifugation at $25,000\ g$ was counted for tritium. More than 90% of the [^3H]guanine nucleotides released specifically in response to (–)IPR were found to be GDP resulting from hydrolysis of the bound [^3H]GTP to [^3H]GDP (MURAYAMA and UI, 1984).

Adenylate cyclase activity induced by Gpp(NH)p was determined as follows. Membranes (20–30 μg of protein) were incubated for 10 min at 37°C in the presence

Fig. 1. Release of [^3H]GDP in adipocyte membranes from exercise-trained (closed symbols) and control rats (open symbols) in response to various concentrations of (–)isoproterenol. Data presents the mean $\pm$ S.E. of triplicate determinations in four or five separate experiments. Significantly different from control values: * $p < 0.05$, ** $p < 0.01$. Other experimental conditions were described in the text.

of various concentrations of Gpp(NH)p in the total volume of 200 μ l of buffer B further supplemented with 1 mM EGTA, 0.3 mM dithiothreitol, 0.5 mM IBMX, 6.7 mM phosphocreatine, 30 units/ml of creatine phosphokinase, 0.5 mM ATP, and 1 mg/ml of bovine serum albumin. Cyclic AMP generated during incubation was measured by a protein binding method (GILMAN, 1970; BROWN *et al.*, 1971). Protein was determined according to the method of LOWRY *et al.* (1951). The concentration of an agonist required to produce a half-maximal response (EC_{50}) was derived from a four-parameter logistic equation (DE LEAN *et al.*, 1978). The significance of the difference within an experiment was estimated using Student's *t*-test.

As shown in Fig. 1, the release of [3 H]GDP was increased by (–)IPR in a dose-dependent manner and exercise training tended to increase the [3 H]GDP release at all concentrations of (–)IPR. The EC_{50} for the (–)IPR-stimulated [3 H]GDP release was not significantly different between the exercising and control group (1.2 and 0.9 μ M, respectively). However, maximal [3 H]GDP release was significantly greater in the exercising group than in control. The activation of adenylate cyclase by a nonhydrolyzable guanine nucleotide analogue, Gpp(NH)p, was also studied in this report (Fig. 2). Basal adenylate cyclase activity was not significantly different between the two groups. The activity induced by Gpp(NH)p was increased in a dose-dependent manner and the activities at 10^{-5} and 10^{-4} M Gpp(NH)p were

Fig. 2. Adenylate cyclase activities in adipocyte membranes from exercise-trained (closed symbols) and control rats (open symbols) in response to various concentrations of Gpp(NH)p. Data presents the mean $\pm$ S.E. of triplicate determinations in three separate experiments. Significantly different from control values: * $p < 0.05$, ** $p < 0.01$. Other experimental conditions were described in the text.

significantly greater in the exercising group than in control. There was no significant difference in EC_{50} ($1.7 \mu M$; control vs. $1.6 \mu M$; the exercising group).

The result shown in Fig. 1 demonstrates that the efficacy was increased in adipocyte membranes from exercise-trained rats, strongly suggesting that exercise training may enhance the ability of the β -AR and N_s to interact effectively. In other words, exercise training may enhance the efficiency of coupling between β -AR and N_s in rat adipocyte membranes.

The receptor affinity for hormonal agonists can be promoted by coupling of the receptor to N_s , and the exchange of GTP for GDP on N_s is facilitated through the formation of the high-affinity hormone-receptor- N_s (HRN) complex (CASSEL and SELINGER, 1978; LEFKOWITZ *et al.*, 1983). In this regard, the result in Fig. 1 also appears to directly exhibit the increase in the formation of the HRN complex in adipocyte membranes from exercise-trained rats, and since exercise training could not increase the number of β -AR as previously discussed, the enhanced coupling of β -AR to N_s in adipocyte membranes from exercise-trained rats may result from the possible change(s) of N_s . Although, to definitely establish this possibility, the actual amount and/or the quantity of N_s present in the membrane must be determined, the result shown in Fig. 2 supplies us with an attractive suggestion. That is, since Gpp(NH)p may exclusively occupy the guanine nucleotide binding sites, the fact that peak Gpp(NH)p-stimulated adenylate cyclase activity was significantly higher in adipocyte membranes from exercise-trained rats without the change of EC_{50} suggests that the coupling of β -AR to N_s in rat adipocyte membranes may be enhanced by exercise training, at least in part, due to the possible increase in the amount of functional N_s rather than the change of the affinity of N_s for guanine nucleotides.

There is another possible mechanism that the increased Gpp(NH)p-stimulated adenylate cyclase activity in adipocyte membranes from exercise-trained rats may result from the enhanced coupling of N_s to C. However, ARAD *et al.* (1984) recently observed that both the native enzyme $N_s \cdot (GDP) \cdot C$ and Gpp(NH)p-preactivated species $N_s' \cdot Gpp(NH)p \cdot C'$ were found to possess the same molecular mass and support the view of the tight association between N_s and C. Furthermore, they strongly support that the activation of adenylate cyclase by β -AR occurs by the "collision coupling" mechanism. If so, the possibility of the enhanced coupling of N_s to C appears to be unlikely. Preferably, a possible change in membrane fluidity may result in the enhanced coupling of β -AR to N_s . In fact, the changes in membrane fluidity have been shown to affect hormone-receptor-cyclase interactions (LEVITZKI and HELMREICH, 1979; HYSLOP and YORK, 1980).

In any case, this report suggests the possibility that the increased adenylate cyclase activity in adipocyte membranes from exercise-trained rats may result from, at least in part, the enhanced coupling efficiency between β -AR and N_s .

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