Nonreceptor-Mediated Responses of Adenylate Cyclase in Membranes from Liver, Muscle, and White and Brown Adipose Tissue of Obese (*fa/fa*) and Lean (*Fa/*) Zucker Rats (43157)

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Abstract. **Adenylate cyclase activity was determined in membranes of liver, muscle, white adipose tissue, and brown adipose tissue (BAT) of lean (*Fa/*) and obese (*fa/fa*) Zucker rats. Responses were monitored following β-adrenergic receptor stimulation and addition of GTP, GTPγS, or forskolin. β-Adrenergic responses in liver, white adipose tissue, and BAT were lower in obese than in lean animals. No such difference was observed in muscle membranes. Production of cAMP after addition of guanine nucleotides was lower in liver and white adipose tissue membranes from obese rats compared with their lean littermates. Synthesis of cAMP in muscle membranes of obese animals after addition of GTP was either not different, or slightly higher, than that observed in muscle membranes from lean animals. Furthermore, production of cAMP after forskolin addition to muscle membranes of obese rats was significantly higher than that observed from lean rats under the same conditions. Interestingly, BAT membranes of obese rats were significantly more sensitive to guanine nucleotide activation than those of lean animals. The results confirm recent findings indicating inferior function of G proteins in liver plasma membranes of obese Zucker rats, and extend this observation to adipose tissue. The present results further suggest that the "nonreceptor" components (e.g., G proteins) responsible for the activation of adenylate cyclase in BAT membranes of obese rats are more responsive to stimulation than those of lean animals. Such sensitivity may be related to and perhaps compensate for the reduced thermogenic activity in the obese Zucker rat during the development of obesity.** [P.S.E.B.M. 1990, Vol 195]

In genetic models of obesity, such as the *ob/ob* mouse or the Zucker (*fa/fa*) rat, excess fat deposition is accompanied by a positive energy balance, characterized mainly by impaired energy expenditure (1). The resultant high metabolic efficiency of these animals can only be slightly modified by diet (2, 3) and, therefore, attention has been focused on the metabolic profile of this syndrome. Obese animals manifest hyperinsulinemia, leading to insulin resistance and metabolic abnormalities, that are apparently modulated by the sympatho-adrenal system (1, 4, 5). The impaired responses to catecholamines result in a failure to stimulate thermogenesis following cold exposure or feeding (4). These impaired responses may partly explain the high metabolic efficiency in genetically obese rodents.

Catecholamine stimulation normally involves β-adrenergic activation of adenylate cyclase with increased production of cAMP (6). However, lower activation of adenylate cyclase in response to catecholamine stimulation has been observed in brown adipose tissue (BAT) of both *ob/ob* mouse and the *fa/fa* Zucker rats (7, 8). These reduced responses to adrenergic stimuli in BAT are compatible with decreased thermogenic activity which, along with a reduction in lipolysis in

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white adipose tissue, may contribute to the maintenance of obesity in these animals. Impaired adenylate cyclase activity was also found in the liver and white adipose tissue of *ob/ob* mice in response to adrenergic stimuli (9, 10). A reduced response of glucagon-induced adenylate cyclase activity in the liver (11) and impaired isoprenaline- and NaF-stimulated adenylate cyclase in the BAT (12) have been recently reported in the *fa/fa* Zucker rat.

Recent studies have shown that both the receptor and "nonreceptor" components (G proteins [guanine nucleotide binding proteins] and the catalytic subunit) of the adenylate cyclase cascade can be modified by hormonal and dietary changes (13–16). For example, cold exposure and sucrose feeding increased the forskolin-stimulated adenylate cyclase activity in BAT of rats (15), and changes in receptor number did not account fully for alterations of adrenergic responsive adenylate cyclase with age and food restriction (16). Thus, in addition to mechanisms such as receptor desensitization, these changes may affect G proteins and/or the catalytic unit of the adenylate cyclase. Studies in *ob/ob* mice have led to the suggestion that the white adipose tissue in this model lacks an active G_i (9). However, the assignment of the defect to one of the regulatory proteins, G_i (17), and its physiologic significance in reduced lipolysis (18) are still controversial. Liver plasma membranes of both lean (*Fa/*) and obese (*fa/fa*) Zucker rats were found to contain an immunohistochemically identifiable G_i protein, but only in liver plasma membranes from lean individuals was a functionally active G_i detected (11). These lesions in G protein may lead to reduced coupling between glucagon receptors and adenylate cyclase (11). To explore whether the impaired adenylate cyclase activity in the obese Zucker rat is tissue specific, the present study was designed to evaluate the responses of adenylate cyclase in plasma membranes from liver, white adipose, brown adipose, and muscle tissues to stimulation of the G proteins by GTP and by the nonhydrolyzable analog of GTP, GTPγS, and to stimulation of the catalytic subunit by forskolin.

Materials and Methods

Two sets, each containing 17 female lean (*Fa/*) and 17 female obese (*fa/fa*) Zucker rats (53–55 days old) were purchased from Charles River, Wilmington, MA. Animals were kept in individual cages (25 × 25 × 25 cm) for 10 days at 23 ± 2°C with a 12-hr dark/light cycle starting at 18:00 hr. During this period they were offered food (Purina Rat Chow) and tap water *ad libitum*. Animals were then sacrificed by decapitation between 08:00 and 11:00 hr. Whole liver was dissected and about 2 g from each animal were cut into small pieces, rinsed with a cold isotonic 30 m*M* Tris-HCl buffer (pH 7.5) containing 150 m*M* NaCl, 2 m*M* EDTA, and 1 m*M* phenylmethyl sulfonyl fluoride, and kept in this buffer until homogenized. Retroperitoneal fat pads (0.6–0.8 g), femoral skeletal muscle (1 g), and interscapular brown adipose tissue (BAT, *in toto*) were dissected into the same buffer. This and all subsequent operations were carried out at 0–4°C.

A partial membrane fraction was prepared in a two-step procedure essentially as described previously (19, 20) from all four tissues sampled. Pooled samples containing the same tissue from three rats were transferred into hypotonic (lysis buffer) 30 m*M* Tris-HCl (pH 7.5) containing a 2 m*M* EDTA and 1 m*M* phenylmethyl sulfonyl fluoride and homogenized (200–300 mg/ml) with a Brinkman polytron homogenizer (30 sec, speed setting 6).

Following the removal of tissue pieces, intact cells, and nuclei by centrifugation at 1100*g* for 15 min, the supernatant was centrifuged at 27,000*g* for 30 min. The sedimented membranes were resuspended and recentrifuged for 20 min under the same conditions. The resulted sedimented membranes were resuspended in the lysis buffer at 500 μg of protein/ml, aliquoted, frozen in liquid nitrogen, and stored at −70°C. Samples were thawed only once prior to determination of adenylate cyclase activity.

Adenylate cyclase activity was evaluated by assaying the production of cAMP derived from ATP (21) in the presence of 3-isobutyl-1-methyl xanthin. Membrane concentrations (in 100 μl of reaction mixture) were in the range of 10–20, 4–10, 2–4, and 3–7 μg of protein for liver, muscle, white adipose tissue, and BAT, respectively. cAMP was determined by a protein binding assay using a [^{3}H]cAMP Kit (Diagnostic Products Corp., Los Angeles, CA). ATP, creatine phosphate, creatine phosphokinase, DL-dithiothreitol, bovine serum albumin, GTP, GTPγS, forskolin, 3-isobutyl-1-methyl xanthin, norepinephrine (+(−)-arternol) and (−)-isoproterenol were purchased from Sigma Chemical Co. (St. Louis, MO). The protein content was measured according to the method of Bradford (22) using bovine serum albumin as a standard. 5′-Nucleotidase was used as a plasma membrane enzyme marker (23) and its activity was determined (24) using a standard reagent kit from Sigma Chemical Co.

Statistical significance was determined by the use of one-way analysis of variance, and Duncan's multiple range test was used to determine the difference among the means.

Results

The body weights of obese Zucker rats were 44% higher than those of the lean animals (Table I). Actual weights of livers of the obese rats were 42% higher than those of the lean rats (10.94 ± 0.17 vs 7.73 ± 0.11 g, respectively) but no difference in liver weight was evident when expressed per 100 g body wt (Table I). Actual weights of BAT were 722 ± 63 and 293 ± 23 mg for

Table I. Body, Liver, and BAT Weight and 5′-Nucleotidase Activity in Liver, White Adipose Tissue, and BAT Membranes[a]

Tissue	Lean	Obese
Body weight (g)	199 ± 2.6	286 ± 3.5[b]
Liver weight (g/100 g body wt)	3.91 ± 0.06	3.83 ± 0.06
BAT (mg/100 g body wt)	150 ± 12	251 ± 20[b]
5′-nucleotidase activity (μg phosphorus/mg protein/min)		
Liver	10.47 ± 2.41	10.03 ± 2.36
White adipose	3.32 ± 0.58	3.83 ± 0.27
BAT	1.83 ± 0.25	1.74 ± 0.27

[a] Weight values are the mean ± SE of 15–17 rats for each group. Enzyme activity values are the mean ± SE of five samples, each derived from tissue pools of three rats.
[b] Indicates significant differences ($P < 0.01$) between lean and obese animals.

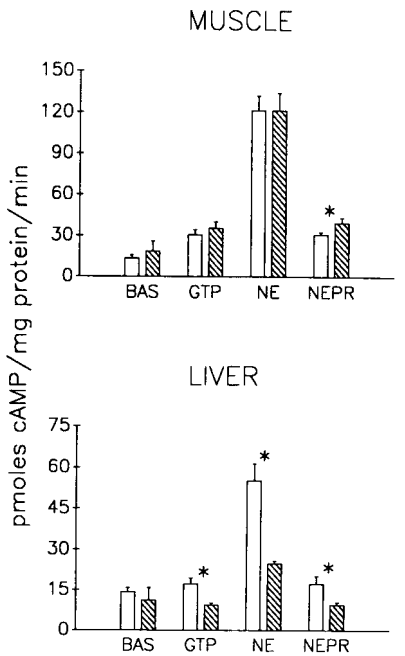

Figure 1. Adenylate cyclase activity in muscle and liver membranes from lean (open bars) and obese (hatched bars) Zucker rats. Conditions are basal activity (BAS), 1 μM GTP; 10 μM norepinephrine in the presence of 1 μM GTP (NE); and norepinephrine in the presence of 1 μM GTP and 100 μM propranolol (NEPR). Values are mean and SE of five samples, each derived from tissues pooled from three rats. * Indicates significant difference ($P < 0.05$) between lean and obese animals.

obese and lean rats, respectively (a 2.5-fold difference), while less of a difference (but still significant) was observed when BAT weights were expressed per 100 g body wt (Table I).

Liver membranes from obese Zucker rats exhibited lower adenylate cyclase activity in response to stimulation by norepinephrine than did membranes from lean animals (Fig. 1), in line with recent findings of lower glucagon-stimulated adenylate cyclase responses in hepatocyte membranes of obese Zucker rats (11). The cyclase response to norepinephrine was blocked by propranolol, suggesting the involvement of β-adrenergic receptors (Fig. 1). This difference in activity between liver membranes of obese and lean rats was also observed with isoproterenol (data not shown). There was no difference, however, in the adenylate cyclase activity between muscle membranes of obese and lean animals in response to norepinephrine (Fig. 1). The inferior function of nonreceptor components of adenylate cyclase in liver membranes of obese rats may be one reason for lower accumulation of cAMP in these animals in response to β-adrenergic stimulation compared with lean rats. Responses to increasing concentrations of GTP, GTPγS, and forskolin were significantly higher in membranes from lean than in those from obese animals (Fig. 2). These differences occurred without a discernible difference in 5′-nucleotidase activity (a plasma membrane marker enzyme) of the liver membranes between obese and lean animals (Table I). In contrast to the effects with GTPγS and forskolin, there was no clear elevation in adenylate cyclase activity in response to increasing concentrations of GTP, suggesting the presence of some endogenous GTP in our liver membrane preparation. There was, however, a concentration effect of GTP on the muscle membrane preparation (Fig. 2). In contrast to the membranes from liver, muscle membranes from obese animals showed a trend toward higher adenylate cyclase activity with added GTP and a significantly higher cyclase response to forskolin compared with membranes from lean rats.

The response of adenylate cyclase to isoproterenol and norepinephrine stimulation in white adipose tissue and in BAT is shown in Figure 3. As in the liver, β-adrenergic response in white adipose tissue membranes were lower in obese rats than in lean animals. Similar (although less pronounced) differences were found in BAT membranes, but no change was observed in the norepinephrine-evoked response. However, it was evident that in contrast to liver and white adipose tissue membranes, the basal activity of adenylate cyclase and the responses to GTP in BAT membranes from obese rats were higher than those in lean animals. This phenomenon is illustrated across effector concentrations in Figure 4. The adenylate cyclase dose-response curves of both GTP and GTPγS were significantly higher in BAT membranes from obese than in those from lean animals. Only slight differences in the forskolin response between obese and lean BAT membranes were found (Fig. 4). The overall responses of adenylate cyclase to stimulation by GTP, GTPγS, and forskolin in white adipose tissue (Fig. 4) were similar to those in liver (Fig. 2), except that there was a positive slope in the GTP dose-response curve in white adipose membranes but not in liver.

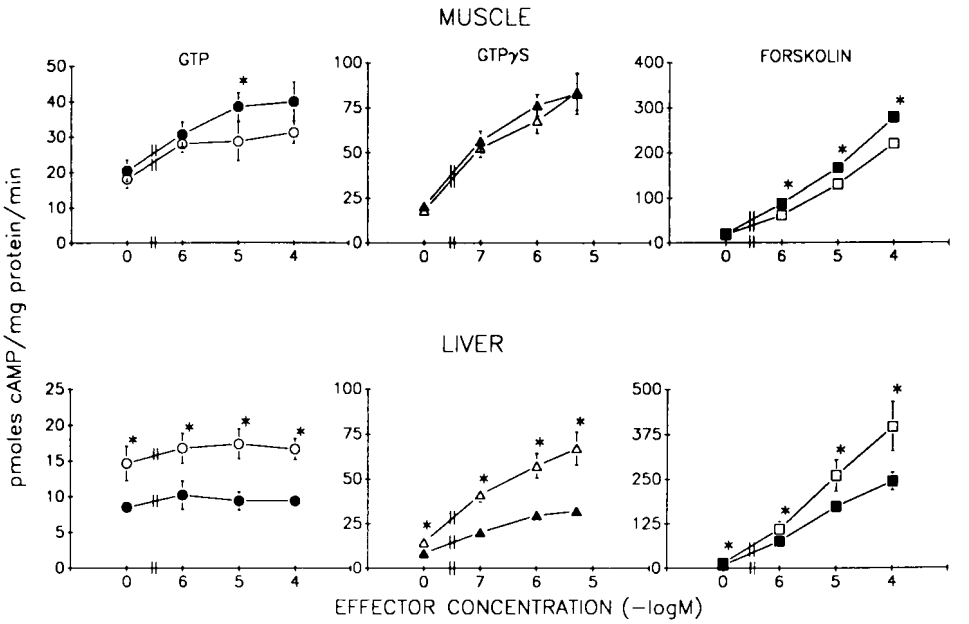

Figure 2. Production of cAMP as a function of concentration of GTP, GTPγS, and forskolin in muscle and liver membranes from lean (open symbols) and obese (filled symbols) Zucker rats. Values are mean ± SE of five samples, each derived from tissues pooled from three rats. * Indicates significant difference ($P < 0.05$) between lean and obese animals.

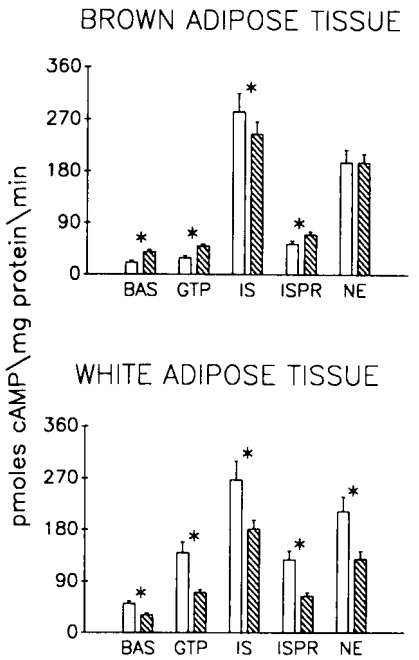

Figure 3. Adenylate cyclase activity in membranes of BAT and white adipose tissue of lean (open bars) and obese (hatched bars) Zucker rats. Conditions are basal activity (BAS), 1 μM GTP; 10 μM isoproterenol in the presence of 1 μM GTP (IS); isoproterenol in the presence of 1 μM GTP and 100 μM propranolol (ISPR); and norepinephrine in the presence of 1 μM GTP (NE). Values are mean and SE of five samples, each derived from tissues pooled from three rats. * Indicates significant difference ($P < 0.05$) between lean and obese animals.

Discussion

A variety of both β-adrenergic- and guanine nucleotide-stimulated responses of adenylate cyclase were found in membranes of four tissues from obese and lean Zucker rats. These differences in level of stimulation were observed with no difference in 5′-nucleotidase activity (used as a plasma membrane marker enzyme) in tissues from both obese and lean animals, indicating that the observed results indeed occurred in the plasma membrane, component of the partial membrane fraction. As found for glucagon stimulation (11), adenylate cyclase activity in response to β-adrenergic stimulation was lower in liver membranes from obese Zucker rats than in those from lean animals (Fig. 1). Since binding experiments were not conducted in this study, it is not evident whether changes in nonreceptor components were the only reason for the reduced adrenergic response. It is likely, however, that the lower response of cyclase in obese animals is mainly due to defects in nonreceptor components of the adenylate cyclase cascade, as recently suggested (11). The dose-response curves for nucleotide- and forskolin-stimulated cyclase activity were significantly higher in lean than in obese rats (Fig. 2). According to Houslay *et al.* (11), defects occur in both G_s and G_i proteins of hepatocytes from obese rats. They further suggested that such reduced G_i function in liver is characteristic of the insulin-resistant state. Since only a minimal increase in cAMP formation was noted with increasing concentrations of GTP in liver membranes (Fig. 2), we are almost certain that our liver membrane preparations contained some endogenous GTP. If so, the present data cannot address the question of the enzyme itself being inferior in obese rats, even though both basal and forskolin responses were lower in liver membranes from obese compared with those of their lean littermates.

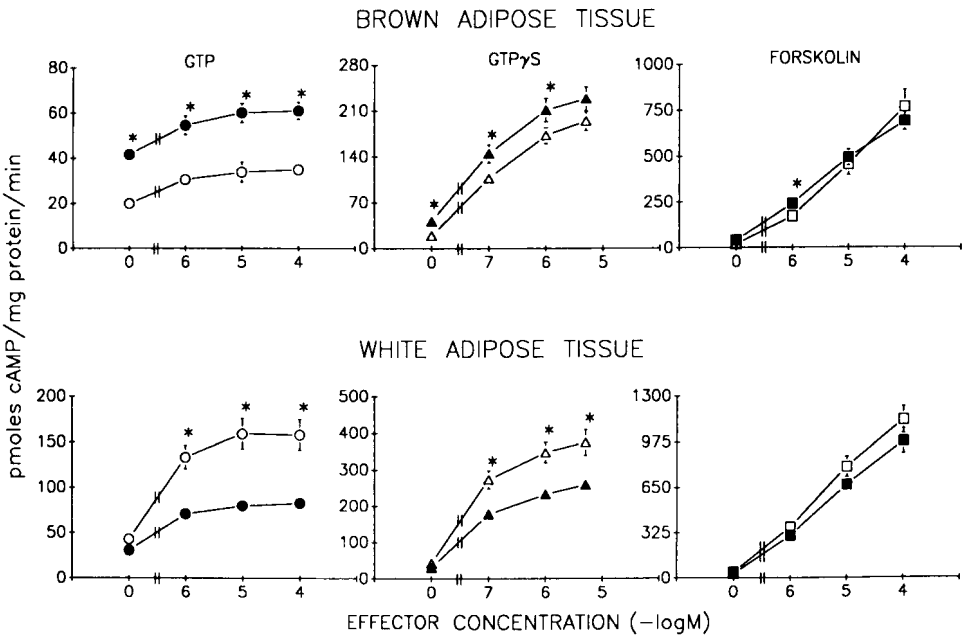

Figure 4. Production of cAMP as a function of increasing concentrations of GTP, GTPγS, and forskolin in brown adipose tissue and white adipose tissue membranes from lean (open symbols) and obese (filled symbols) Zucker rats. Values are mean ± SE of five samples, each derived from tissues pooled from three rats. * Indicates significant difference ($P < 0.05$) between lean and obese animals.

The observed alterations in nonreceptor components of the adenylate cyclase cascade are certainly not present in every cell membrane of the obese Zucker rats. In muscle membranes, adenylate cyclase activities following stimulation by norepinephrine were equal for membranes from both obese and lean animals (Fig. 1). Yet according to our data (Fig. 2), in contrast to the response of liver membranes to adenylate stimulation, stimulation with GTP and forskolin leads to higher production of cAMP in muscle membranes from obese than from lean animals. It remains to be determined if the number of β-adrenergic receptors is reduced in muscle membranes of obese animals (likely due to hormone resistance). If so, it is then possible that the enhanced nonreceptor responses account for the lack of expected reduced hormone-evoked responses in muscle membranes of obese rats.

Responses of adenylate cyclase of white adipose tissue membranes to exogenous stimulation were similar to those found in liver, again indicating lower cyclase activity in obese Zucker rats in response to β-adrenergic stimulation (Fig. 3). Furthermore, the dose-response relationships of GTP- and GTPγS-stimulated production of cAMP from obese animals (Fig. 4) were lower than those from lean animals, while the basal activities and forskolin-stimulated responses of adenylate cyclase were similar in the white adipose tissue membranes from both obese and lean rats. These findings may indicate that the impairment in production of cAMP in white adipose tissue membranes of obese animals is, as in the liver, related to the function of G proteins rather than to the enzyme itself. Such a phenomenon is compatible with the concept of reduced lipolysis contributing to obesity.

Most interesting are the results of adenylate cyclase activity in BAT. Adenylate cyclase activity in response to β-adrenergic stimulation with isoproterenol was slightly lower in obese than in lean rats (Fig. 3), but the dose-response curves following stimulations by GTP were about 2-fold higher in BAT membranes from obese animals (Fig. 4). Production of cAMP following GTPγS stimulation was also higher in the obese group, but not to the same extent as seen with GTP. The present results indicating impaired isoproterenol-stimulated adenylate activity in obese Zucker rats agree with those reported recently by Muzzin *et al.* (12). However, the overall adenylate cyclase capacity may be higher in obese rats, as their BAT weights were 67% higher than those of their lean littermates (Table I). Although the overall thermogenic activity in BAT, which is mediated by catecholamine-stimulated adrenergic receptors, is impaired in obese Zucker rats (8), it would appear from our data that the sensitivity of nonreceptor components in BAT of obese rats is enhanced.

It is possible that the enhanced G protein responses in BAT membranes of obese rats may be compensating for the reduced thermogenic activity of these animals. Heat production in BAT and shivering thermogenesis in skeletal muscle can both be modified by β-adrenergic blockade (25). However, the density of α- but not of β-adrenergic receptors is lower in the BAT of obese Zucker rats than in lean rats (26). The enhanced stimulation of production of cAMP by nonreceptor components of adenylate cyclase upon exogenous addition

of GTP, GTPγS, and forskolin to membranes from BAT (Fig. 4) (and smaller enhancement in muscle, Fig. 2) leads one to speculate that this heightened sensitivity of the nonreceptor components is linked to the important role these tissues play in thermogenesis.

The reported defect in G protein function in the white adipose tissue and liver of obese Zucker rat may be a primary contributor to the development of obesity, while the hypersensitivity of nonreceptor components of adenylate cyclase in BAT is a secondary defensive process. This hypothesis remains to be tested.

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