Journal of Cellular Biochemistry 58:464–473 (1995)

β_3-Adrenergic Activation of Adenylyl Cyclase in Mouse White Adipocytes: Modulation by GTP and Effect of Obesity

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Abstract Lipolysis and adenylyl cyclase (AC) activation in response to β-adrenergic agents are abnormally low in white epididymal adipose tissue (WAT) of the ob/ob mouse. The abundance of G-proteins ($G_s\alpha$ and $G_i\alpha$) linked to AC is also abnormally low. By contrast, β-adrenergic receptor (β-AR) levels were previously found to be normal in WAT and elevated in liver. The relative importance of various forms of the β-AR in mouse WAT was reassessed in view of the discovery of the β_3-AR. The results show that (1) the β_3-AR is mainly responsible for AC activation in lean-mouse WAT; (2) the β_3-AR is only partly responsible for AC activation in obese mouse WAT; and (3) GTP modulates β_3—but not β_1—or β_2-AR activation of AC in a biphasic manner. Therefore, the β_3-AR appears responsible for the well-known bimodal effect of GTP on β-adrenergic receptor-mediated AC activity in WAT. © 1995 Wiley-Liss, Inc.

Key words: fat cells, adipose tissue, β-adrenergic receptors, guanine nucleotides, β-adrenergic agonists, β-adrenergic antagonists, adrenoceptors, ob/ob mouse, guinea pig

β-Adrenergic receptor function is an important modulator of lipolysis in white adipose tissue and of thermogenesis in brown adipose tissue. The adipocyte plays a major role in the maintenance of normal energy balance; much research has been done on the hormonal and biochemical control of lipogenesis and lipolysis. The discovery of a β-adrenergic receptor specific to adipose tissues, which had long been suggested based on pharmacological studies [Arch et al., 1984, 1991], has renewed interest in the subject of adrenergic regulation of lipolysis. White and brown adipose tissue of many species possess this receptor, now termed the β_3-adrenergic receptor. The receptor has been cloned in humans [Emorine et al., 1989], mouse [Nahmias et al., 1991], and rat [Granneman et al., 1991; Muzzin et al., 1991]; its structural properties [Fève et al., 1991; Granneman et al., 1991, 1993; Thomas et al., 1992], and function [Arch et al., 1991; Bojanic et al., 1985; Carpéné et al., 1993; Fève et al., 1991, 1992; Van Leifde et al., 1993; Van Spronsen et al., 1993; Wheeldon et al., 1993] are currently being vigorously investigated. Specific agonists have been developed that ease the study of the receptor's properties [Arch et al., 1991]. Interestingly, species differ in the ability of adipose tissue lipolysis to respond to activation by β_3-adrenergic agonists; thus rat, hamster, and garden dormouse are classified as hyperresponders, rabbit and dog as hyporesponders, and humans and guinea pig as nonresponders, on the basis to the activity of nonselective and selective drugs to promote lipolysis [Lafontan and Berlan, 1993]. The expression of the β_3-adrenergic receptor mRNA is depressed in white and brown adipose tissue of the fa/fa rat compared to lean controls [Muzzin et al., 1991] and in the white and brown adipose tissue of the ob/ob mouse [Collins et al., 1994], suggesting a mechanism for the propensity of these tissues to accumulate lipids. It was therefore of interest to assess the modulation of white epididymal adipose tissue receptor activity in the ob/ob mouse.

The data presented in this paper show that (1) despite the presence of mRNA corresponding to the three β-adrenergic receptor subtypes in the white epididymal adipocytes of the mouse, the β_3-adrenergic receptor is the major subtype responsible for the activation of AC in the lean

Received December 15, 1994; accepted January 19, 1995.

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mouse; (2) β-adrenergic activation of AC is considerably lower in the ob/ob than in the +/+ mouse and both β_1/β_2 and β_3-adrenergic receptor play a role; (3) despite the very low mRNA expression of the β_3-adrenergic receptor in the adipose tissue of the ob/ob mouse, that receptor can activate AC to a significant degree; and (4) the ability of the β_3-adrenergic receptor to stimulate adenylyl cyclase in white epididymal adipose tissue is regulated by GTP in a bimodal manner.

MATERIALS AND METHODS

Materials

[α-^{32}P]-ATP, [^{3}H]-cAMP, and Formula-989 scintillation fluid were obtained from NEN-DuPont Canada (Mississauga, ON). Caffeine, ATP (cat. # A-2383, prepared by the phosphorylation of adenosine), cAMP, creatine phosphate (Tris salt), adenosine, adenosine deaminase, creatine phosphokinase, myokinase, 1(−)isoproterenol, salbutamol, and propranolol were from Sigma Chemical Co. (St. Louis, MO). GTP was from PL Biochemicals (Milwaukee, WI). CL 316,243 was a gift from Dr. Thomas Claus, Lederle Laboratories (Pearl River, NY) [Bloom et al., 1992]; BRL 37344 was from Dr. M.A. Cawthorne, SmithKline Beecham Pharmaceuticals (Great Burgs, Epsom, Surrey, UK) [Arch et al., 1991].

Animals

Six-week-old male C57B1/6J lean (+/+) and obese (ob/ob) mice were obtained from Jackson Laboratories (Bar Harbor, ME). They were fed ad libitum on a pelleted rat diet and water and used for the preparation of tissue fractions at 8–10 weeks.

Preparation of Membranes

The epididymal fat pad was dissected and processed as previously described for the preparation of adipocytes by collagenase digestion, followed by the preparation of membranes from the adipocytes according to method B described in detail previously [Bégin-Heick, 1990].

Adenylyl Cyclase Assay

This assay was done as described in detail previously [Bégin-Heick, 1990]. The ATP concentration was 0.1 mM and $MgSO_4$, 4 mM. The concentration of agonists, antagonists, and GTP was as indicated in the legends to the tables and figures.

Pertussis Toxin Treatment

Pertussis toxin treatment was done as described previously [Bégin-Heick, 1985], except that 1 μg pertussis toxin (List Biochemicals, Campbell, CA) was injected intraperitoneally.

Protein Determination

The protein content of membrane fractions was assessed by the Coomassie blue technique, using serum albumin as the standard.

cDNA Probes

cDNA for hamster β-adrenergic receptor subtypes β_1 and β_2 was obtained from Dr. M. Caron, Duke University; *Sca*I and *Eco*RI fragments, respectively, were used as probes. The mouse β_3 probe was from Dr. A.D. Strosberg [Fève et al., 1991]. The *Pst*I fragments were isolated and used as the probe.

RNA Preparation

Total RNA was isolated from isolated adipocytes and lung tissue of lean and obese mice by acid guanidium thiocyanate phenol–chloroform extraction. PolyA$^+$ RNA was isolated with the Polytract mRNA isolation system (Promega). The samples were size-fractionated by electrophoresis on 1.5% agarose gels containing 6% (v/v) formaldehyde. The RNA was transferred to Zetabind membranes and subjected to Northern blot analysis as described below.

Hybridization

cDNA probes were labeled with Prime-it II Random Primer Labeling kit (Stratagen) to a specific activity of approx 10^9 dpm/μg. Tubulin cDNA was used to standardize the amount of RNA applied to each lane. Hybridization was carried out at 42°C. For the β_1 and β_2 probes, the membranes were washed at 42°C for 30 min in 0.6M Na Cl/0.06 M Na citrate, pH 7.0 (2 × SSC) and 0.2% sodium dodecyl sulfate (SDS) and then two successive washes at 55°C for 45 min with 0.5 × SSC and 0.1 × SSC, respectively, containing 0.1% SDS. For the β_3 and tubulin probes, the second and third washes were at 65°C.

Statistical Analyses

Concentration–response curves were analyzed with a nonlinear regression curve-fitting

program (InPlot, GraphPad Software, San Diego, CA). Differences between means were evaluated by analysis of variance (ANOVA). Data were considered statistically significant with a *P*-value of < 0.05.

RESULTS

The data shown in Table I show that basal and β-adrenergic agonist-stimulated activity are significantly lower in obese than in lean mouse membranes (in this respect, note different scales used in Figs. 2–4) and that GTP inhibits both basal and adrenergic effector-stimulated AC activity in lean- but not in obese mouse membranes.

To establish the specificity of isoproterenol and CL 316,243, full agonist activation curves both without and with inhibition by propranolol were generated for these agonists. The results are shown in Figure 1. Essentially, they demonstrate that, at 1 μM GTP (a concentration that allows considerable stimulation in lean mouse membranes and maximal stimulation by all agonists in obese mouse membranes (cf. Fig. 2), the effects of isoproterenol in lean mouse membranes (Fig. 1A) could be ascribed to the effect of an agonist acting at a single binding site with low affinity ($K_D = 2.0 \times 10^{-5}$ M), whereas in the obese mouse membranes, the data are best represented by a two-component fit with 25% of the sites having high affinity ($K_D = 1.9 \times 10^{-7}$ M) and 75%, low affinity ($K_D = 1.4 \times 10^{-4}$ M) (Fig. 1B). In the presence of 1 μM propranolol, the affinity for isoproterenol remained substantially the same in lean mouse membranes ($K_D = 2.9 \times 10^{-5}$ M), whereas in the obese mouse membranes, the high-affinity component (presumably representing β_1/β_2 activity) disappeared to reveal only one, low-affinity site (presumably representing β_3 activity) with $K_D = 3.6 \times 10^{-5}$ M (solid symbols in Fig. 1A, and B, respectively). With CL 316,243, only one site was revealed in both lean- (Fig. 1C) and obese mouse (Fig. 1D) membranes, and the K_D were not significantly different in the absence and the presence of propranolol (3.0 and 2.9×10^{-6} M in lean and 7.7×10^{-6} and 1.3×10^{-7} M in obese, respectively, for assays without and with propranolol). At high (100-μM) GTP with isoproterenol, there appeared to be a small ($< 10\%$) high-affinity component in lean mouse membranes that was abolished by propranolol, leaving only the low affinity component, again with $K_D = 1.7 \times 10^{-7}$ M (Fig. 1E) that was not modified on addition of propranolol. In obese mouse membranes, the data best fit a one-component model, with $K_D = 3.2 \times 10^{-6}$ M, the addition of propranolol produced a rightward shift in the concentration–response curve (to $K_D = 2.9 \times 10^{-5}$, Fig. 1F), indicating that, at high GTP, activation occurred via β_1/β_2-receptor subtypes.

To probe the effect of GTP further, the concentration of GTP giving maximal cyclase stimulation in the presence of agonists was assessed. The data are described in Figure 2. GTP alone at concentrations greater than 10 nM inhibited basal cyclase activity in lean mouse membranes. By contrast, concentrations of 10 nM and higher stimulated basal activity in obese mouse membranes (Fig. 2A).

With agonists present, maximal stimulation was attained at 1–100 nM GTP, and concentrations of GTP greater than 100 nM progressively diminished the activity in lean mouse membranes (Fig. 2B). In obese mouse membranes, all the agonists tested stimulated cyclase significantly less than they did in lean mouse membranes; moreover, the effect of GTP on cyclase activity depended on the agonist. With isoproterenol, a nonselective β-agonist, and salbutamol, considered a selective β_2-agonist, concentrations of GTP greater than 1 nM stimulated cyclase

TABLE I. Maximal Activity of β-Adrenergic Agonists Relative to Isoproterenol in Mouse Adipocyte Membranes*

	+/+		ob/ob	
	100 nM	100 μM	100 nM	100 μM
[GTP]	Activity (pmol/min/mg protein)			
Basal	68.5 ± 0.8		29.0 ± 0.32	
GTP alone	65.1 ± 3.0	49.5 ± 1.9	44.5 ± 0.8	41.3 ± 1.0
+ isoproterenol	516 ± 10.3	350 ± 8.7	123 ± 1.4	121 ± 1.6

*Membranes from lean (+/+) and obese mice were incubated with 100 μM agonist and the indicated concentrations of GTP. Adenylyl cyclase activity was determined from the amount of cAMP produced, as described under Materials and Methods. The data are means ±SE of 12 or more observations with different membrane preparations.

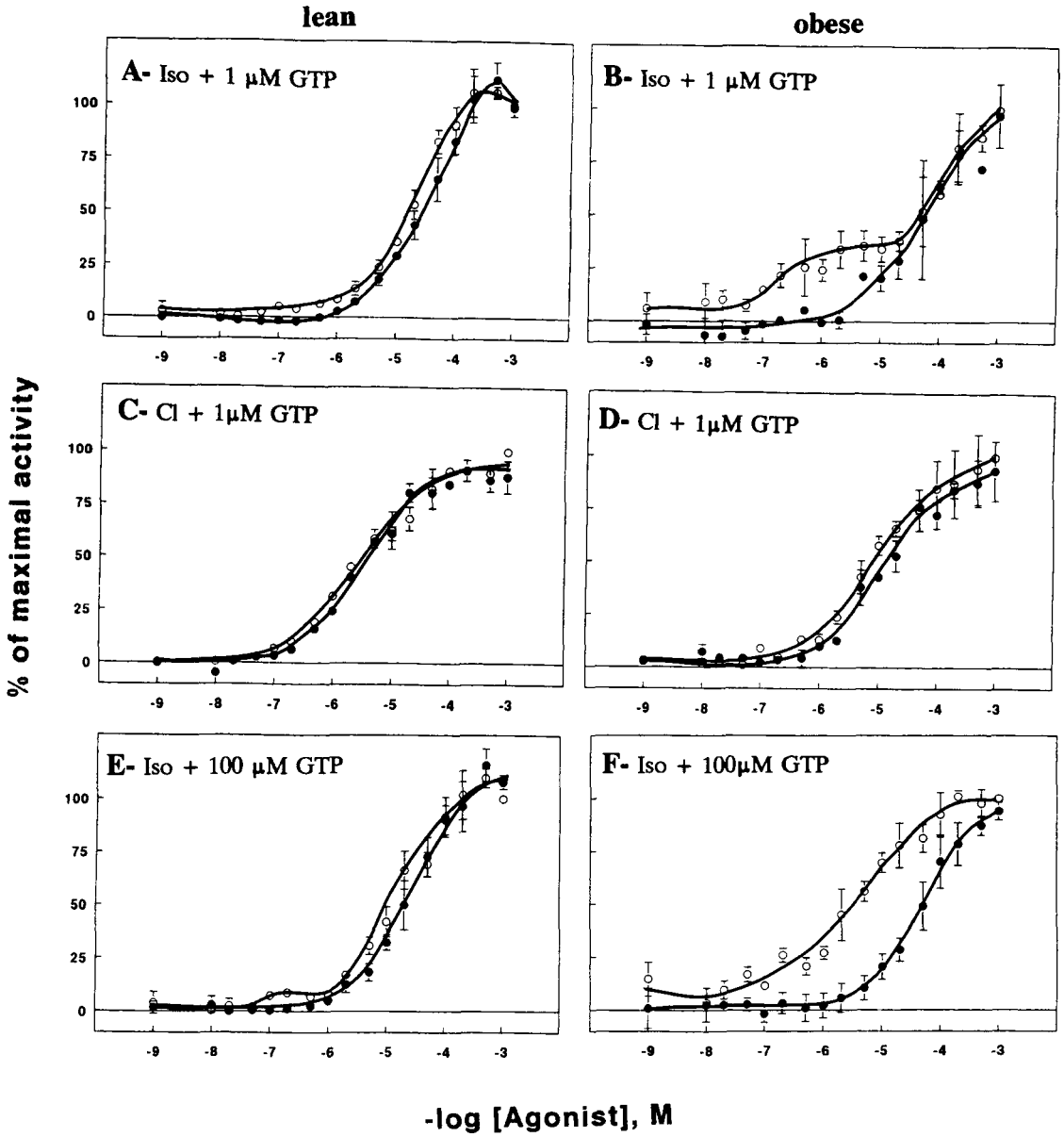

Fig. 1. Isoproterenol- and Cl 316,243-stimulated adenylyl cyclase activity in mouse adipocyte membranes. Membranes from lean (+/+) (**A,C,E**) and obese mice (**B,D,F**) were incubated with the indicated concentrations of isoproterenol (Iso) or Cl 316,243 (Cl) and either 1 or 100 μM GTP, as indicated. Adenylyl cyclase activity was determined from the amount of cAMP produced, as described in the method section. The data are expressed as percentage relative to the activity produced by 100 μM agonist after subtracting the activity produced by GTP alone, the means ±SE of 3–4 independent experiments with different membrane preparations. In each panel, open symbols represent the samples incubated with agonist alone and the closed symbols, samples incubated with agonist +1 μM propranolol.

with a maximal effect at 1–100 μM. With the β_3-agonists CL 316,243 or BRL-37344 (as for all agonists in lean mouse membranes), a biphasic effect of GTP was noted: activation occurred between 1 nM to 1 μM, and the activity diminished with GTP concentrations >1 μM (Fig. 2C).

To confirm whether the bimodal effect of GTP in mouse adipocyte membranes was an effect of activation by β_3-adrenergic receptor, agonists were assayed both without and with 1 μM propranolol at increasing GTP concentrations. This propranolol concentration is expected to inhibit most of the β_1/β_2 activity, but not the activity elicited by agonists acting at the β_3 receptor (cf. Fig. 1). The results shown in Figure 3A–C confirm that the activity elicited by isoproterenol, a nonselective agonist, salbutamol, a β_2-selective

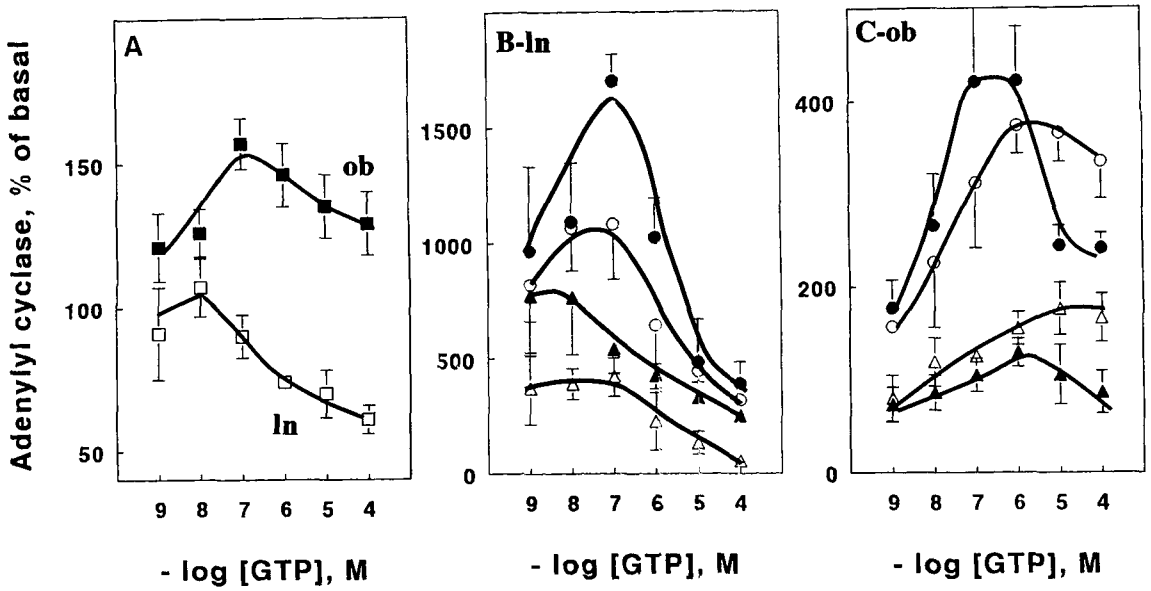

Fig. 2. Activation and inhibition of adenylyl cyclase by β-adrenergic agonists and GTP in mouse adipocyte membranes. Membranes from lean (□) and ob/ob (■) mice were incubated with the indicated concentrations of GTP alone (A) or GTP + 100 μM agonist in (B) lean (+/+) and (C) obese mice (note differences in scale). Adenylyl cyclase activity was determined from the amount of cAMP produced, as described in the method section. The data are expressed as percentage relative to basal activity (cf. Table I), the means ±SE of 3–6 independent experiments with different membrane preparations. ○, isoproterenol; ●, CL 316,243; △, salbutamol; ▲, BRL-37344.

agonist, or CL 316,243, a β_3-selective agonist, was almost indistinguishable in the presence and absence of propranolol in lean mouse membranes. In obese mouse membranes (Fig. 3D–F), propranolol revealed a biphasic effect of GTP with isoproterenol and salbutamol, but it did not modify the biphasic response to CL 316,243.

Since the inhibitory effect of GTP mediated via β-adrenergic agonists has been shown to be attenuated by treatment with pertussis toxin (Murayama and Ui, 1983), the effect of this toxin on CL 316,243-mediated activity was investigated. The results (Fig. 4) show that in both lean- and obese mouse membranes, pertussis toxin treatment attenuated the inhibitory phase of the GTP concentration curve, in the presence of the selective β_3-agonist. This finding is in contrast to the lack of effect of toxin treatment on isoproterenol-mediated activity in obese mouse membranes [Bégin-Heick and Coleman, 1988].

To verify the β-adrenergic receptor subtypes expressed in lean and obese mouse white epididymal adipose tissue, the presence of each of the subtypes was assessed using Northern blot analysis (Fig. 5). The data show that all three types of adrenergic receptors are present in both the lean and the obese mouse tissue. However, whereas β_2 subtypes are present in low, but comparable amounts, the abundance of the β_1 subtype is reduced, and that of the β_3-receptor subtype in the obese-mouse tissue is only a small fraction of that found in the lean mouse.

DISCUSSION

The objectives of this study were to determine what types of β-adrenergic receptor activity are responsible for the activation of adenylyl cyclase in mouse adipocyte membranes and, how β-adrenergic receptor activity is modulated by GTP and how this may be affected by the *ob* gene.

Types of β-Adrenergic Receptors Expressed in Mouse Epididymal Adipocytes

Cloning of a new β-adrenergic receptor subtype from human [Emorine et al., 1989], mouse [Nahmias et al., 1991], and rat [Granneman et al., 1991; Muzzin et al., 1991], and the identification of the gene product in adipose tissues [Fève et al., 1991; Granneman and Lahners, 1992; Muzzin et al., 1991; Nahmias et al., 1991; Revelli et al., 1993; Collins et al., 1994] has firmly established the existence of a third type of β-adrenergic receptor in these tissues. The existence of a third β-adrenergic receptor subtype had long been inferred, based on pharmacological responses of rat adipose tissue to various agonists [Arch et al., 1991]. It is now well accepted

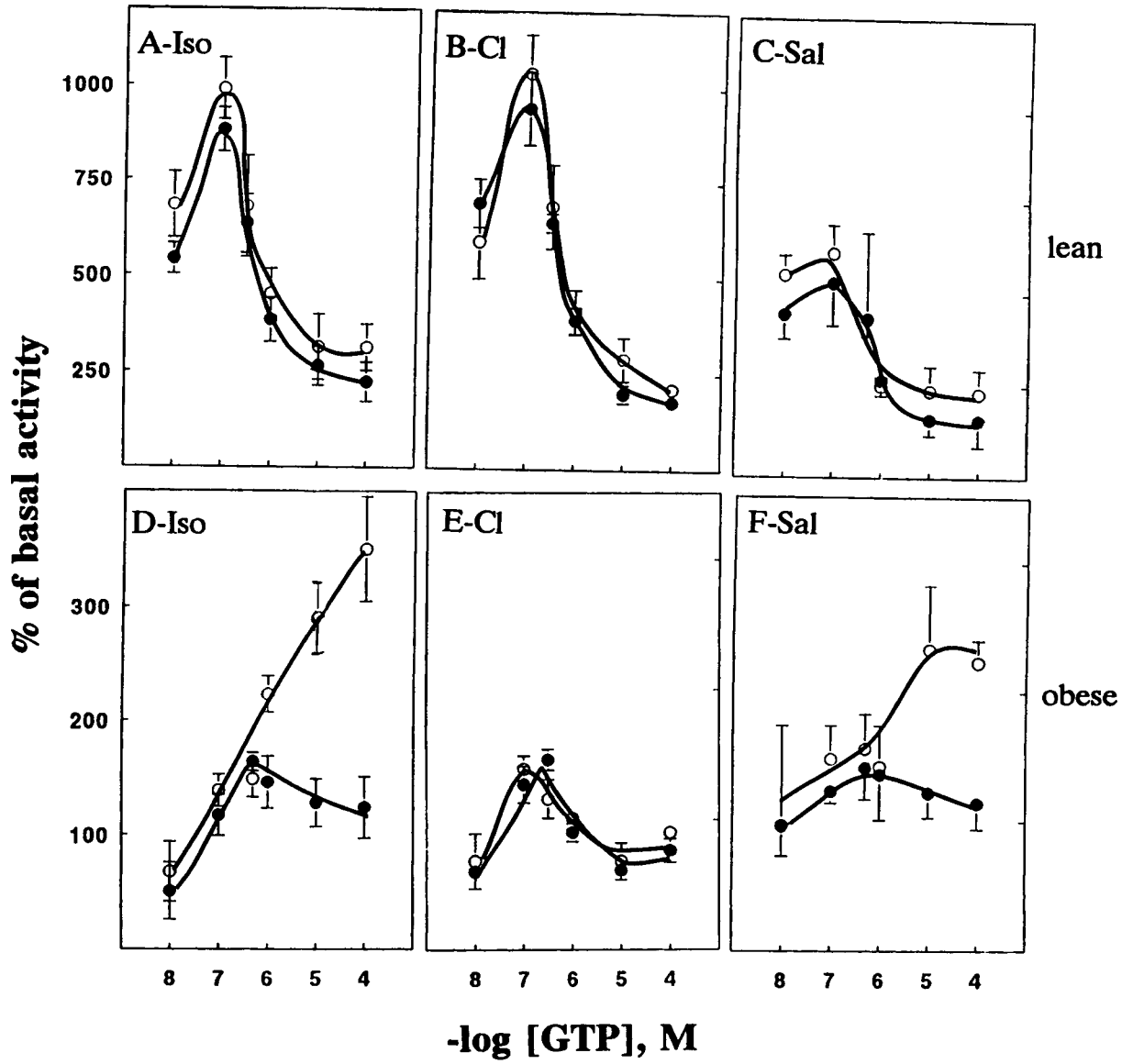

Fig. 3. Effect of propranolol on β-agonist-elicited activity in mouse adipocyte membranes. Membranes from lean (+/+) and obese mice (note difference in scales) were incubated with 10 μM isoproterenol (Iso); 1 μM CL 316,243 (Cl); 100 μM salbutamol (Sal) and the indicated concentrations of GTP in the presence (●) or absence (○) of 1 μM propranolol. Adenylyl cyclase activity was determined from the amount of cAMP produced, as described in the method section. The data are expressed as percentage activity relative to basal activity (cf. Table I), the means ±SE of 4 independent experiments with different membrane preparations.

that three β-adrenergic receptor subtypes (β_1, β_2, and β_3) coexist in the adipose tissue of many species, including rat, hamster, dog, rabbit, and garden dormouse, and mouse [Lafontan and Berlan, 1993; Collins et al., 1994]; β_3-receptor agonists do not elicit activity in guinea pig adipose tissue [Himms-Hagen et al., 1993], and the presence of the β_3-receptor in adult human white adipose tissue is still the object of controversy [Revelli et al., 1993; Thomas et al., 1992].

The results described above show clearly that, in mouse epididymal white adipocyte membranes, adrenergic agents (CL 316,246 [Bloom et al., 1992] and BRL 37344 [Arch et al., 1991]) that interact selectively with the β_3-receptor subtype have a high potency for the activation of adenylyl cyclase. By contrast, agonists that are known activators via β_1- and/or β_2-receptors display a low potency for the activation of adenylyl cyclase.

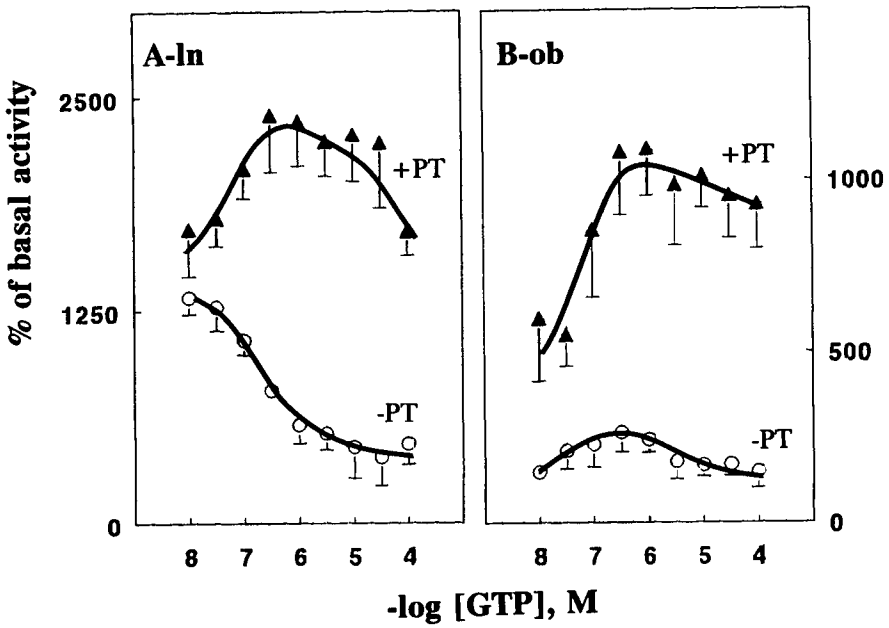

Fig. 4. Effect of pertussis toxin on β_3-adrenergic stimulation. Membranes were isolated adipocytes of control mice (open symbols) or mice treated with pertussis toxin (closed symbols). Adenylyl cyclase activity was assessed in the presence of Cl 316,243 at increasing concentrations of GTP. **A:** Lean mouse membranes. **B:** Obese mouse membranes. (Note differences in scale.) The results are means ±SE of three different experiments, expressed as percentage activity above basal, which were, respectively, for lean control, 51 ± 6; lean PT, 36 ± 2.8; obese control, 15.8 ± 2.1 and obese PT, 12.5 2.5 pmol/min/mg protein.

The results displayed above show that in lean mouse adipocyte, the three β-adrenergic receptor subtypes are expressed at the mRNA level, but that the β_3-adrenergic receptor is largely responsible for the activation of cyclase in membranes.

β-Adrenergic Modulation of Adenylyl Cyclase in Epididymal Adipocytes of the ob/ob Mouse

It is well established that adenylyl cyclase activity in the epididymal adipocyte membranes of the ob/ob mouse responds poorly to adrenergic stimulation [Bégin-Heick, 1985]. By contrast, it has been shown to respond normally to forskolin + Mn^{2+} [Bégin-Heick, 1992]. The kinetics of the receptor, as assessed by [^{125}I]-iodopindolol binding, which measures β_1 and β_2 subtypes, are similar in lean and ob/ob mice [Bégin-Heick, 1992]. This finding suggested that the abundance of the β_1/β_2 components was approximately equal in lean and obese mouse membranes. The lower abundance of G-proteins in the tissue of the ob/ob mouse compared to its lean counterpart has been suggested as a cause for the depressed adrenergic-receptor stimulated cyclase [Bégin-Heick, 1990]. The data presented above show that low β_3-adrenergic receptor activity may be an equally important factor in the poor response of the obese mouse to β-adrenergic agents as the low abundance of G-proteins.

Collins et al. [1994] have reported a study of adrenergic receptor subtypes in the ob/ob mouse. Where our data are comparable, they substantially agree. Thus, I have also found that the mRNA expression of both the β_1 and the β_3 subtypes is reduced in adipose tissue of the ob/ob compared to lean mice, while that of the β_2 subtype is similar.

These data also show that, provided the assays were done at 1 μM GTP or less, significant β_3-adrenergic receptor activity (2–4 times basal activity) could be elicited in the obese mouse membrane with Cl 316,243. While this is significantly less than the activity elicited in lean mouse membranes (more than 10 times basal activity), it contrasts sharply with the extremely low mRNA expression of this receptor subtype in the obese mouse adipocyte, as measured by Northern blot analysis. Such disparity between the expression of receptor subtypes at the mRNA level and functional expression of activity was previously reported for the β-adrenergic receptor subtypes of liver [Arner, 1990], suggesting

that there is not a direct correlation between levels of mRNA expression and abundance of the receptor protein.

Effect of GTP on β_3-Adrenergic Activation

During these studies, it was discovered that the activation of adenylyl cyclase by the β_3-adrenergic receptor is particularly sensitive to the ambient GTP concentration. I have demonstrated previously that adrenergic agonist activation was not accompanied by a biphasic modulation of cyclase by GTP in adipocyte membranes of the ob/ob mouse, as is the case for lean mouse [Bégin-Heick, 1985] and rat [Murayama and Ui, 1883] preparations. The data presented here show that a biphasic response is obtained in obese mouse adipocytes with selective β_3-adrenergic agonists. In addition, provided that β_1/β_2 activation is largely suppressed by the addition of propranolol, nonselective agonists such as isoproterenol also elicit a biphasic effect (see Fig. 3). The inhibitory effect of GTP on β_3-adrenergic receptor-elicited activity in white adipose tissue has also been observed in rat adipose tissue [Chaudhry et al., 1994]. It is similar to the effect seen with other (nonselective) adrenergic agents in rat white-adipose tissue [Murayama and Ui, 1983]. The fact that the inhibitory effects of GTP that accompanies activation by β_3-selective agonists can be attenuated by pertussis toxin suggests that somehow, at high GTP, the β_3-adrenergic-receptor couples via $G_i\alpha$. It is now known that several types of adenylyl cyclase are present in tissues and, more importantly, that their response to the various subunits of the transducing G-protein varies.

The type(s) of cyclase present in white adipose tissue is unknown. However, certain forms are more likely candidates than others due to their properties [DeVino and Iyengar, 1994, and references cited therein]. Adipose tissue AC is inhibited by low concentrations of calcium [Bégin-Heick, 1987], pointing to types V and VI, which are also the most ubiquitous. Except for types II and IV, which are stimulated, and type I, which is inhibited, by $\beta\gamma$ G-protein subunits, the other forms are relatively insensitive to modulation by $\beta\gamma$ subunits, making it unlikely that the release of excess $\beta\gamma$ subunits upon β_3-adrenergic activation directly inhibits AC in adipose tissue.

It is possible, however, that, at high GTP, the β_3-receptor promotes inhibitory effects via an interaction with $G_i\alpha$. Murayama and Ui [1983] have shown that pertussis toxin treatment attenuates the bimodal effect of GTP on isoproterenol-stimulated activity; I have shown that the bimodal effect of isoproterenol [Bégin-Heick, 1985] and of CL 316,243 (Fig. 4) is also attenuated by pertussis toxin in lean mouse adipocyte membranes. This suggests that the β_3-adrenergic agonists can somehow couple to the α-subunits of G_i as well as of G_s. While this appears counterintuitive, there is now evidence showing that the various isoforms of the α_2-adrenergic receptor subtypes can stimulate adenylyl cyclase

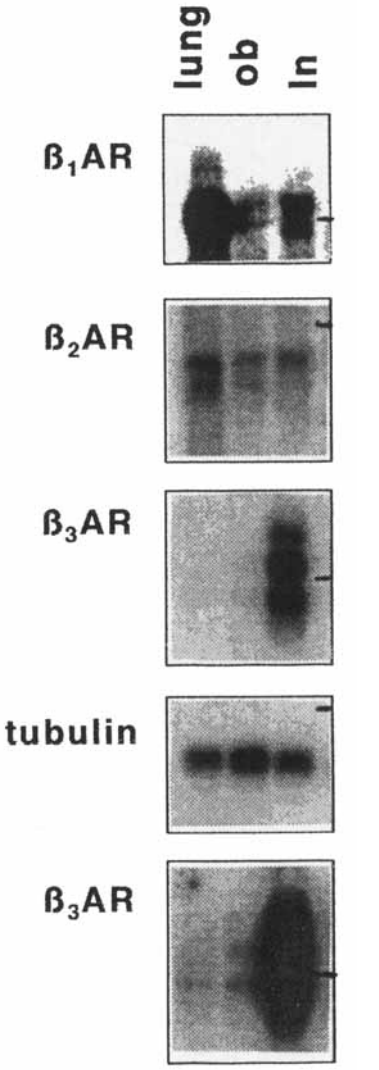

Fig. 5. Northern blot analysis of the β-adrenergic receptor subtypes in white adipocytes of lean and obese mice. 10 μg of polyA$^+$ RNA were blotted as described in the Methods section. Blots were hybridized with labelled cDNA probes corresponding to each of the receptor subtypes and to mouse tubulin. Lung RNA was also used as a positive control for the β_1 and β_2 subtypes and a negative control for the β_3 subtype. The bottom photograph represents overexposure of the β_3 blot to show the levels in obese mouse adipocytes.

activity via interaction with G_s, although this receptor normally couples to G_i [Eason et al., 1992].

GTP on its own can also inhibit AC activity in lean-mouse adipocyte membranes, suggesting that, at high concentrations, the nucleotide can activate $G_i\alpha$ in the absence of agonist. By contrast, GTP alone only stimulates AC in obese mouse membranes. This difference between lean and obese adipocyte membranes may be related to the decreased complement of G-proteins in the membranes from the two types of mice [Bégin-Heick, 1990].

Although GTP has long been known to have a bimodal effect on hormone-stimulated AC in adipose tissue, it is difficult to know the precise physiological significance of this bimodal effect. Total cellular concentration of GTP is believed to be in the high micromolar (μM) range. If similar GTP concentrations are prevalent at the membrane level, at which receptors and G-proteins interact, the net effect of β_3-adrenergic activation might be inhibitory to cAMP production. Confirmation of these possibilities awaits a more detailed analysis of the regulation of the β_3-adrenergic receptor interaction with its transducing G-proteins.

ACKNOWLEDGMENTS

This work was supported by a grant from the Medical Research Council of Canada. The author is indebted to Pascale Reinhardt-Poulin for expert technical assistance, to Dr. H.M.C. Heick for reading the manuscript, and to Dr. M. Chevrette for advice on molecular techniques.

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