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# Alterations in G-protein expression and the hormonal regulation of adenylate cyclase in the adipocytes of obese (*fa/fa*) Zucker rats

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Attenuated maximal activations by forskolin, $Mn^{2+}$, NaF or guanosine 5'-[γ-thio]triphosphate (GTP[S]) were noted for adenylate cyclase activity in adipocytes from obese (*fa/fa*) Zucker rats compared with their lean (*Fa/Fa*) littermates. GTP[S] achieved half-maximal activation of adenylate cyclase at some 10-fold lower concentrations in membranes from lean animals compared with those from obese. Levels of the 42 and 45 kDa forms of $G_s$ were some 40–50% lower in membranes from obese animals, and levels of $G_i$-1 and $G_i$-3 were some 62–65% lower. No differences in levels of $G_i$-2 α-subunits or G-protein β-subunits were observed. $G_i$ function, as assessed by inhibiting forskolin-stimulated adenylate cyclase activity, was detected in membranes from lean, but not obese, animals. Receptor-mediated inhibition of adenylate cyclase, achieved by prostaglandin $E_1$, nicotinate and phenylisopropyladenosine, was similar in membranes from both lean and obese animals. Levels of β-adrenoceptors were some 50% lower in membranes from obese animals. It is suggested that the attenuated activation of adenylate cyclase by stimulatory ligands in membranes from obese animals may be caused by decreases in both $G_s$ and receptors, and that this may contribute to the attenuated lipolytic response seen in adipocytes from such animals.

## INTRODUCTION

Adenylate cyclase performs a key regulatory role in controlling cellular function. It transduces the action of many hormones in a variety of target cells, where its functioning can be controlled by receptors exerting either stimulatory or inhibitory effects through the guanine-nucleotide-binding regulatory proteins (G-proteins) $G_s$ and $G_i$ respectively. These G-proteins are heterotrimeric entities consisting of distinct α-subunits, together with β- and γ-components. The α-subunits both bind and hydrolyse GTP as well as serving to couple to both an appropriate receptor and the catalytic unit of adenylate cyclase itself [1–3]. The stimulatory protein $G_s$ also affords an amplification of receptor-stimulated adenylate cyclase activity at low receptor occupancy: this it achieves by effecting a Collision Coupling Mechanism [4,5]. The inhibitory protein $G_i$ can attenuate the functioning of stimulatory hormones by either direct action of its α-subunit (α-$G_i$) or by release of β-subunits [1–3]. The functioning of $G_i$, however, can be eliminated by the action of pertussis toxin, which causes the ADP-ribosylation and inactivation of α-$G_i$. The activity of adenylate cyclase and its ability to respond to hormonal activation are thus intimately related to regulation by both $G_s$ and $G_i$. This is clear from analysis of its activity in lymphoma cells [1–3], in diabetic states [6–8] and in pituitary tumours [9], where changes in G-protein functioning, elicited by either mutation or covalent modification, lead to dramatic alterations in adenylate cyclase activity.

Elevations of cyclic AMP concentrations stimulate lipolysis in adipocytes, and thus this process is intimately connected with changes in adenylate cyclase activity [10,11]. However, in a number of model systems of obesity, alterations in adipocyte catecholamine-stimulated lipolysis have been noted [12–16]. One such system is the obese Zucker rat, where lesions in glucagon's ability to regulate adenylate cyclase in the hepatocytes of such obese animals, compared with lean controls, have been noted [17]. The Zucker obese rat (*fa/fa*) suffers from genetic post-weaning obesity [15], mild hyperglycaemia [18], insulin-resistance [19] and hypothyroidism [20]. Here we have investigated both the regulation of adenylate cyclase by catecholamines and other hormones, as well as the expression of G-protein components in the adipocytes from adult normal (*Fa/Fa*) and obese (*fa/fa*) Zucker rats.

## MATERIALS AND METHODS

### Materials

ATP, BSA, thyroid-stimulating hormone (TSH), glucagon, secretin, (−)-isoprenaline (+)-bitartrate, $N^6$-(L-2-phenylisopropyl)adenosine (PIA), prostaglandin $E_1$ ($PGE_1$), nicotinic acid and NaF were all from Sigma. Phosphocreatine, creatine kinase and GTP were all from Boehringer Mannheim. Radiochemicals were from Amersham International. All other chemicals were of A.R. grade from BDH Chemicals.

### Animals

Male genetically obese (*fa/fa*) and lean (*Fa/Fa*) Zucker rats were obtained from Olac, Shaws Farm, Bicester, Oxon., U.K., and killed by cervical dislocation at 20–25 weeks of age for the immediate preparation of white adipocytes. All animals were allowed *ad libitum* access to standard rat chow.

### Preparation of membranes

Rats were killed by cervical dislocation, and white epididymal fat-pads were removed. Adipocytes were prepared by the method of Rodbell [21], and isolated adipocytes were suspended in 4 times their packed cell volume of buffered sucrose (0.25 M-sucrose/50 mM-Tris/HCl/1 mM-EDTA/3 mM-ATP, pH 7.4) at 20–25 °C and homogenized by hand in a Potter–Elvehjem homogenizer (10 strokes). The resulting homogenate was then

Abbreviations used: G-protein, guanine-nucleotide-binding protein; $G_i$, inhibitory G-protein; $G_s$, stimulatory G-protein; PIA, $N^6$-(phenylisopropyl)adenosine; $PGE_1$, prostaglandin $E_1$; TSH, thyroid-stimulating hormone; DDA, 2',5'-dideoxyadenosine; GTP[S], guanosine 5'-[γ-thio]triphosphate; p[NH]ppG, guanylyl 5'-imidodiphosphate.

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centrifuged at 1500 *g* for 10 min at 4 °C, the resulting fat cake was removed, and the supernatant was centrifuged at 15000 *g* for 15 min at 4 °C. The pellet was resuspended in buffered sucrose at 4 °C by 5 strokes of a hand-operated Potter–Elvehjem homogenizer and centrifuged at 15000 *g* for 15 min at 4 °C. The resulting pellet was resuspended in 1 mM-EDTA/5 mM-Tris/HCl, pH 7.4, and centrifuged at 30000 *g* for 10 min at 4 °C, and the resulting pellet was resuspended in the same buffer to a concentration of 0.2–0.8 mg/ml and rapidly frozen to −80 °C. Membranes stored under these conditions retained full activity for at least 8 weeks.

### Assay of adenylate cyclase activity

This was done either radiochemically by the method of Salomon *et al.* [22] or by a cyclic AMP-binding method [23]. Briefly, reactions were done in triethanolamine/HCl buffer, final pH 7.4, containing 5 mM-$MgSO_4$, 0.1 mM-Ro 20-1724, 1 mM-EDTA, 22 mM-phosphocreatine (disodium), 1 mM-ATP, 1 mM-dithiothreitol and 1 unit of adenosine deaminase/ml. Incubations were carried out over periods of up to 30 min in a final volume of 0.1 ml with 3–7 $\mu$g of membrane protein at 30 °C. During this time, reactions were linear and initial rates were measured only.

### Immunoblotting of membranes

The specific antisera capable of recognizing the gene products of the three $G_i$ gene products so far identified ($G_i$-1, $G_i$-2 and $G_i$-3) and procedures employed to analyse adipocyte membranes by immunoblotting were performed as detailed previously by us [6–8,24]. The antiserum AS7 was used to detect the $\alpha$-subunits of $G_i$-1 and $G_i$-2, and the antiserum I3B was used to detect the $\alpha$-subunit of $G_i$-3. Antiserum CS1, which was generated against the *C*-terminal decapeptide of $\alpha$-$G_s$, was used to detect the 42 kDa and 45 kDa forms of $\alpha$-$G_s$. The antiserum BN1, formed from a peptide specific for G-protein $\beta$-subunits, was used to detect these in adipocyte membranes [24]. A $^{125}$I-labelled second antibody was used to detect the bands as previously [6–8].

### Binding of $^{125}$I-cyanopindolol to adipocyte membranes

This was done as described in detail by others [25]. Briefly, assays (250 $\mu$l) were done in 20 mM-Hepes, final pH 8.0, con-

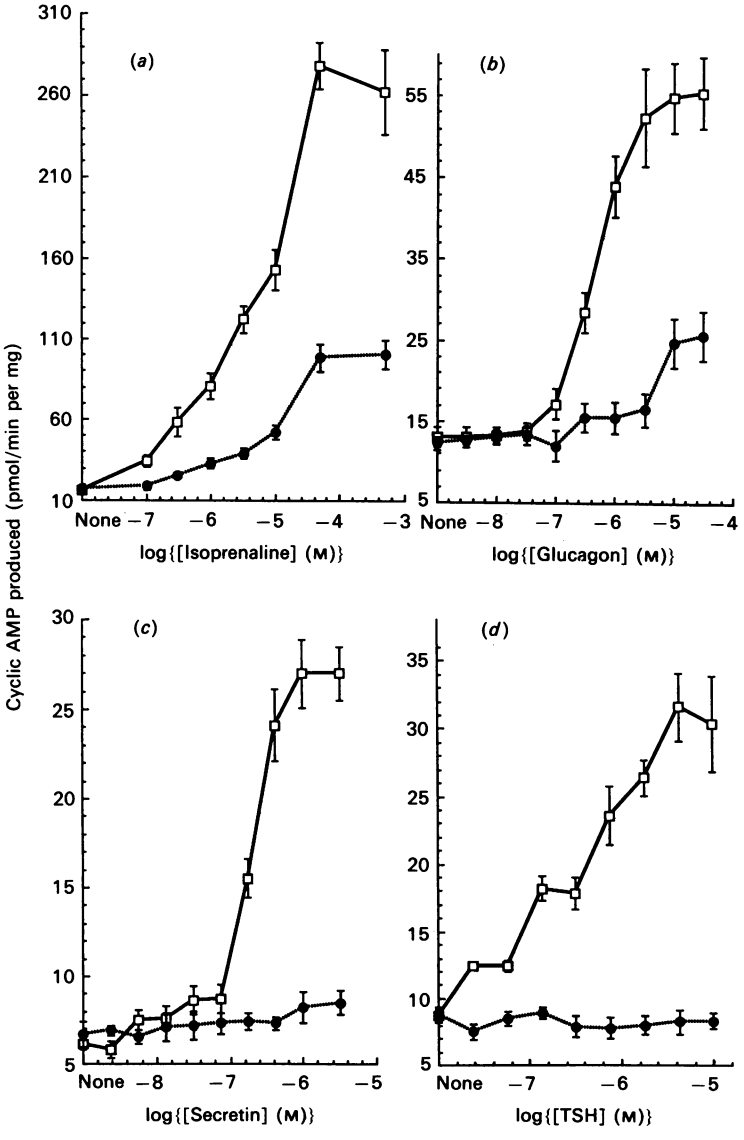


**Fig. 1. Hormonal stimulation of adenylate cyclase**

A dose–effect analysis for (*a*) isoprenaline, (*b*) glucagon, (*c*) secretion and (*d*) TSH. All assays were done in the presence of 100 $\mu$M-GTP with adipocyte membranes from either lean (□) or obese (●) animals. Results are means ± S.D. for six different experiments with different animals.

taining 5 mM-$MgCl_2$ and 1 mM-EDTA. Binding with membranes and $^{125}$I-cyanopindolol was conducted over 45 min at 30 °C, by which time equilibrium had been achieved. Bound $^{125}$I-cyanopindolol was separated from unbound by vacuum filtration on Whatman GF-C filters, which were then washed with 4 × 4 ml of ice-cold buffer before radioactivity counting. A saturation isotherm was determined over the range 0–200 pM-$^{125}$I-cyanopindolol. Non-specific binding was determined by the inclusion of 10 $\mu$M-isoprenaline. This was subtracted from total binding to obtain the amount of material bound specifically.

## RESULTS

Isoprenaline was able to exert a potent stimulation of adenylate cyclase activity in adipocyte membranes from lean Zucker rats. However, this action was markedly decreased when membranes from obese animals were used (Fig. 1; Table 1). The attenuated ability of adenylate cyclase in adipocytes from obese animals to be stimulated by hormones was also seen when any one of the hormones glucagon, TSH and secretin were employed. Indeed, we failed to observe any stimulatory effect of either TSH or secretin on the adenylate cyclase activity of adipocyte membranes from obese animals (Fig. 1). The concentrations of isoprenaline and glucagon required to exert half-maximal activation of adenylate cyclase were slightly higher with membranes from obese animals (Table 1).

In contrast with these differences, the specific activities of adipocyte membrane adenylate cyclase from lean and obese animals, in the presence of GTP, were very similar (Table 1). This was also true for assessment of membrane adenylate cyclase activity when other stimulatory ligands, which exert actions through $G_s$, were employed, such as 20 mM-NaF (47 ± 3; 45 ± 5) and 0.1 mM-guanosine 5′-[γ-thio]triphosphate (GTP[S]) (77 ± 3; 70 ± 5); values (in pmol/min per mg of membrane protein) are means ± S.D. for $n = 4$ separate experiments with membranes from different lean and obese animals respectively. Similarly, for ligands acting directly on the catalytic unit of adenylate cyclase, such as forskolin (170 ± 15; 160 ± 12) and 5 mM-$MnCl_2$ (220 ± 12; 210 ± 13), no apparent differences were observed; values (pmol/min per mg) are means ± S.D. for $n = 6$ separate membrane preparations.

Analysis of the ability of GTP[S] to stimulate adenylate cyclase activity showed that, although similar maximum activities were obtained, half-maximal activation was achieved at much lower concentrations of GTP[S] with membranes from lean animals (Fig. 2*a*; Table 1). If such dose–effect curves were done in the presence of isoprenaline, then, similarly, a large (10-fold) difference in the $EC_{50}$ for activation was achieved, with similar maximal stimulations being achieved (Fig. 2*b*; Table 1). Interestingly, when GTP[S] was substituted for GTP while determining dose–effect curves for isoprenaline, this caused a major change in the plots obtained with membranes from lean animals, but had little observable effect if membranes from obese animals were employed (Table 1). Thus, for lean animals, the maximum specific activity attained was decreased markedly, and a dramatic

**Table 1. Regulation of adenylate cyclase in adipocyte membranes from lean (*Fa/Fa*) and obese (*fa/fa*) Zucker rats**

Results are from three separate experiments using different membrane preparations. Values in parentheses show fold activation achieved with the test ligand compared with the activity in its absence. Key: n.d., not detectable; n.p., not performed; $EC_{50}$, concentration at which half-maximal effects were achieved. Activity is given in pmol of cyclic AMP produced/min per mg of membrane protein.

| | Lean | | Obese | |
|---|---|---|---|---|
| | Max. activity | $EC_{50}$ | Max. activity | $EC_{50}$ |
| GTP (alone) | 16 ± 2 | n.p. | 17 ± 2 | n.p. |
| GTP (+5 μM-isoprenaline) | 84 ± 4 | 42 ± nM | 53 ± 8 | 380 ± 20 nM |
| Isoprenaline (+100 μM-GTP) | 262 ± 26 (16) | 6.3 ± 0.8 μM | 100 ± 9 (6) | 8.9 ± 1.1 μM |
| Glucagon (+100 μM-GTP) | 55 ± 4 (5) | 0.5 ± 0.1 μM | 26 ± 3 (2) | 1.3 ± 0.5 μM |
| TSH (+100 μM-GTP) | 30 ± 4 (4) | 0.27 ± 0.05 μM | n.d. | n.p. |
| Secretin (+100 μM-GTP) | 27 ± 2 (4) | 0.14 ± 0.02 μM | n.d. | n.p. |
| GTP[S] (alone) | 77 ± 3 | 8.5 ± 1.2 nM | 70 ± 5 | 28 ± 3 nM |
| GTP[S] (+50 μM-isoprenaline) | 112 ± 10 | 0.4 ± 0.1 nM | 118 ± 15 | 4 ± 1 nM |
| Isoprenaline (+100 μM-GTP[S]) | 136 ± 10 | 0.8 ± 0.2 μM | 119 ± 11 | 8 ± 2 μM |

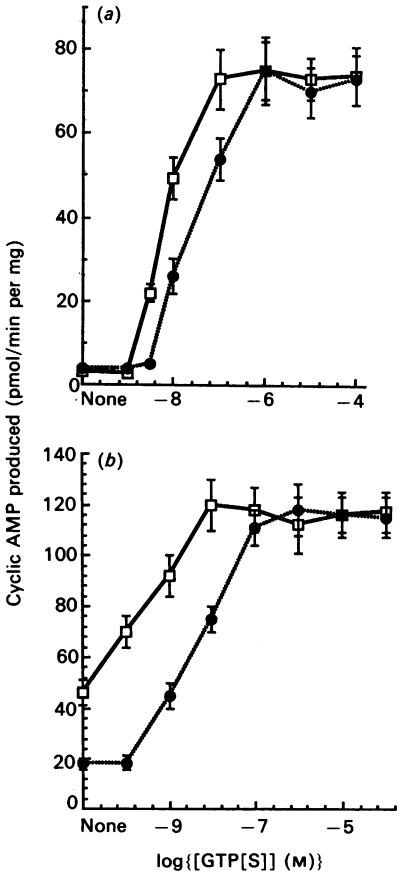


**Fig. 2. Stimulation of adenylate cyclase by GTP[S]**

A dose–effect analysis was done in the absence (*a*) or the presence (*b*) of 50 μM-isoprenaline: analysis of isoprenaline stimulation performed in the presence of 100 μM-GTP[S]. Experiments were done with membranes from either lean (□) or obese (●) animals. Results are means ± S.D. for three different experiments using different animals.

decrease occurred in the concentration of isoprenaline needed to achieve half-maximal activation (Table 1) of adenylate cyclase. Analysis of the ability of GTP to activate adenylate cyclase in the presence of isoprenaline demonstrated a nearly 10-fold lower $EC_{50}$ for activation of the enzyme activity in membranes from lean animals compared with those of the obese (Table 1).

Low concentrations of p[NH]ppG have been employed to detect functional $G_i$ activity by inhibiting basal adenylate cyclase activity that has been amplified by forskolin [6–8,17,26,27]. Using such an approach, we were able to detect functional $G_i$ in membranes from lean, but not obese, animals (Fig. 3). In contrast with using this non-hydrolysable analogue to activate $G_i$ directly, we also used ligands capable of interacting with receptors coupled to $G_i$ in order to inhibit adenylate cyclase. In such experiments we were able to detect receptor-coupled $G_i$ activity in membranes from both lean and obese animals (Table 2). This activity was detectable whether adenylate cyclase was stimulated either directly by forskolin or through the $\beta$-adrenoceptor (Table 2). Direct inhibition of the catalytic unit by occupancy of the adenosine P-site with the agonist 2′,5′-dideoxyadenosine (DDA) showed it to be similarly effective in membranes from lean and obese animals.

Using anti-peptide antisera, we ascertained the relative levels of various G-protein components in membranes from lean- and obese-rat adipocytes (Fig. 4). The antiserum AS7 clearly identified a large decrease in $\alpha$-$G_i$-1 (65±6% decrease; $n = 4$; $P < 0.001$) in membranes from obese animals, with only a small, and not significant, decrease in that of $\alpha$-$G_i$-2 (16±9%; $n = 4$), and levels of $\alpha$-$G_i$-3 (62±7% decrease; $n = 4$; $P < 0.001$) were decreased markedly, as detected by the antiserum I3B. The 42 kDa (50±6% decrease; $n = 4$; $P < 0.001$) and the 45 kDa (42±7% decrease; $n = 4$; $P < 0.001$) forms of the stimulatory $G_s$ were also decreased substantially, as detected with the anti-peptide antibody CS1. In contrast, G-protein $\beta$-subunits detected with the antiserum BN1 were little different in the membranes from the obese animals (5±8% decrease; $n = 4$; not significant).

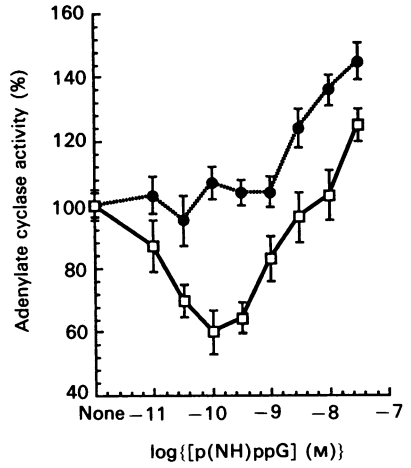


**Fig. 3. Inhibition of adenylate cyclase with low concentrations of p[NH]ppG**

Adenylate cyclase was assayed in the presence of 100 $\mu$M-forskolin and increasing concentrations of p[NH]ppG. Membranes from lean (□) and obese (●) animals were used. Results are means±S.D. of three experiments with membranes from different animals.

**Table 2. Receptor-mediated inhibition of adenylate cyclase in adipocyte membranes from lean and obese Zucker rats**

Assays were all done in the presence of 100 $\mu$M-GTP and saturating concentrations of inhibitory ligands (maximally effective). Experiments show means±S.D. for four experiments done with different membrane preparations. Specific activities of the forskolin-stimulated activity were 180±12 and 185±15 pmol/min per mg of membrane protein for lean and obese animals respectively. Those for the isoprenaline-stimulated activities were 280±18 and 102±12 pmol/min per mg of membrane protein.

| | Inhibition of denoted stimulated activity (%) | | | |
|---|---|---|---|---|
| | Lean | | Obese | |
| Inhibitory ligand | 100 $\mu$M-forskolin | 100 $\mu$M-isoprenaline | 100 $\mu$M-forskolin | 100 $\mu$M-isoprenaline |
| PIA (1 $\mu$M) | 48±4 | 65±8 | 49±3 | 63±8 |
| Nicotinate (10 $\mu$M) | 45±3 | 54±7 | 41±4 | 58±5 |
| DDA (100 $\mu$M) | 83±5 | 85±9 | 87±7 | 85±6 |
| $PGE_1$ (1 $\mu$M) | 47±5 | 55±12 | 46±4 | 58±9 |

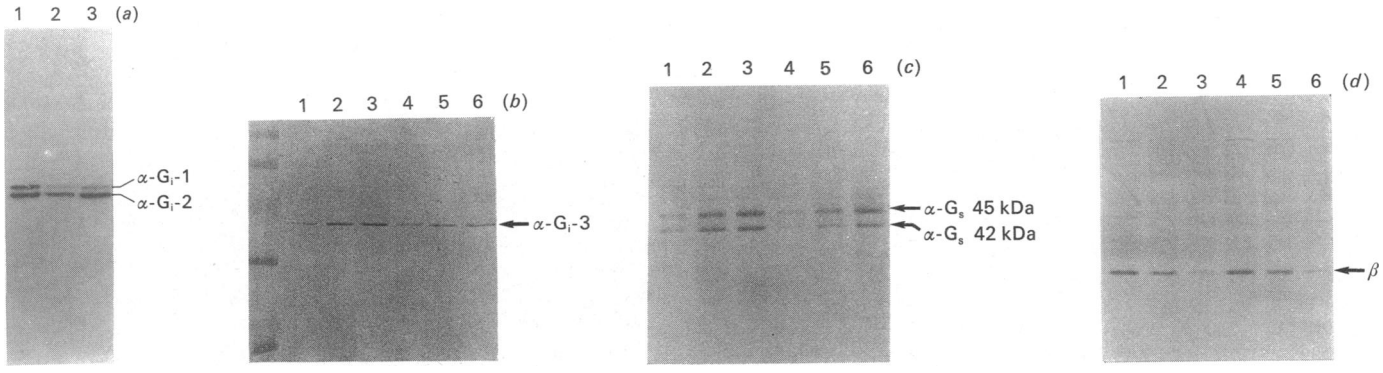


**Fig. 4. Immunoblotting of G-protein subunits in adipocytes from lean and obese animals**

(*a*) Detection of the $\alpha$-subunits of $G_i$-1 and $G_i$-2 with antiserum AS7; track 1, lean (80 $\mu$g); track 2, obese (80 $\mu$g); track 3, obese (160 $\mu$g). (*b*) Detection of the $\alpha$-subunit of $G_i$-3 with antiserum I3B for membranes of lean (tracks 1, 2, 3) and obese (tracks 4, 5, 6) animals, with 50 $\mu$g (tracks 1, 4), 100 $\mu$g (tracks 2, 5) and 150 $\mu$g (tracks 3, 6) of membranes. (*c*) Detection of the 42 kDa and 45 kDa forms of $G_s$ with antiserum CS1 with membranes from lean (tracks 1, 2, 3) and obese (tracks 4, 5, 6) animals at 20 $\mu$g (tracks (1, 4), 40 $\mu$g (tracks 2, 5) and 60 $\mu$g (tracks 3, 6) of membranes. (*d*) Detection of G-protein $\beta$-subunit with antibody BN1 with membranes of lean (tracks 4, 5, 6) and obese (tracks 1, 2, 3) animals at 40 $\mu$g (tracks 3, 6), 80 $\mu$g (tracks 2, 5) and 120 $\mu$g (tracks 1, 4) of membranes. Plasma membranes were subjected to SDS/PAGE and immunoblotted with anti-peptide antisera as described in the Materials and methods section. Data shown are typical experiments of those done three times using membranes from different lean and obese animals.

The high-affinity $\beta$-adrenoceptor antagonist cyanopindolol was used in its $^{125}$I-labelled form to probe $\beta$-adrenoceptors. The binding of this agonist, on Scatchard analysis, showed half-maximal binding in adipocyte membranes at $35 \pm 5$ pM for lean and $35 \pm 7$ pM for obese animals. In contrast, maximal binding was $85 \pm 7$ fmol/mg and $42 \pm 5$ fmol/mg for adipocyte membranes from lean and obese animals respectively ($n = 6$ different membrane preparations). We were unsuccessful in attempting to perform binding analyses using $^{125}$I-glucagon as a specific ligand for glucagon receptors. This methodology has been applied to hepatocytes, but such cells present not only a far richer source of glucagon receptors but also exhibit $EC_{50}$ values for binding and activation of adenylate cyclase at approx. 4 nM [28], rather than micromolar levels observed here. As glucagon binds efficaciously to hydrophobic surfaces, these factors, coupled with any extraneous lipidic material from the adipocytes, may conspire against any successful binding study.

## DISCUSSION

In the Zucker rat, obesity occurs as a result of a single gene defect, although the nature of this remains to be determined [29,30]. Nevertheless, this defect leads to a marked decrease in hormone-stimulated lipolysis in the obese rats [14], a process controlled by the cyclic AMP-dependent activation and phosphorylation of hormone-sensitive triacylglycerol lipase [10]. Here we show that the ability of lipolytic hormones to stimulate adenylate cyclase is dramatically attenuated in adipocyte membranes from adult obese animals compared with their lean controls (Fig. 1). However, there did not appear to be any defect in the catalytic unit of adenylate cyclase itself. This was because, with membranes from either lean or obese animals, similar activities of adenylate cyclase were noted under circumstances when this enzyme was stimulated directly by either the diterpene forskolin [2] or $Mn^{2+}$ ions, which serve to uncouple regulatory G-proteins from adenylate cyclase as well as activating the catalytic unit directly [2,31–33]. Coupling between the stimulatory G-protein $G_s$ and adenylate cyclase was determined by using GTP or its non-hydrolysable analogue GTP[S], as well as NaF, which activates GDP-bound $G_s$. Again, similar responses were obtained with both membrane preparations at maximally effective concentrations of this ligand. However, dose–effect analyses of the ability of GTP[S] to activate adenylate cyclase showed a marked difference in the $EC_{50}$ values for the two membrane preparations. It was clear that, as in adipocytes from *ob/ob* mice [11], proportionally lower concentrations of GTP[S] were required to activate adenylate cyclase in membranes from lean animals even when isoprenaline, a ligand for the stimulatory $\beta$-adrenoceptor acting on adenylate cyclase, was present in the assays. This could be due to either a modification of $G_s$ or a decrease in its amount in membranes from obese animals. Analysis of adipocyte membranes from obese animals by using specific antisera to $G_s$ showed that, relative to lean animals, the levels of both forms of $G_s$ were decreased by almost one-half. That membranes from lean animals have more $G_s$ in them may thus provide an explanation for the lower $EC_{50}$ values seen for GTP[S] activation of adenylate cyclase in these membranes. It is also possible that this situation could be exacerbated by the fact that, despite lower levels of $G_i$-1, $G_i$-2 and $G_s$ in membranes of obese animals, there was little change in G-protein $\beta$-subunits. If this results in the presence of free $\beta$-subunits, then such a situation may inhibit $G_s$ dissociation, through Mass Action, hence resulting in a shift in the dose–effect curves for GTP[S]. Interestingly, however, similar levels of maximal activation of adenylate cyclase were achieved in membranes from both lean and obese animals. This may be because this non-hydrolysable analogue binds and constitutively activates a pool of $G_s$ which is of sufficient size to activate adenylate cyclase fully in both sets of membranes.

In marked contrast, the ability of stimulatory hormones to activate adenylate cyclase was dramatically lower in adipocyte membranes from obese animals compared with lean (Fig. 1; Table 1). The maximal effects of isoprenaline and glucagon were decreased by over 50%, and the functioning of TSH and secretin in membranes from obese animals was almost abolished. This may well contribute to the decreased hormone-stimulated lipolysis seen in the adipocytes of obese animals [14]. Analysis of $\beta$-receptor number indicated that levels of this receptor were approx. 50% lower in membranes from obese animals compared with controls. Although this value is strikingly similar to the decrease in isoprenaline-stimulated adenylate cyclase observed, it is possible that this may be fortuitous. Our reason for suggesting this is that stimulatory receptors coupled to adenylate cyclase, such as $\beta$-adrenoceptors and the glucagon receptor in the presence of GTP, have been shown to activate adenylate cyclase through a Collision Coupling Mechanism [4,5]. This mechanism does not yield a linear relationship between receptor occupancy and subsequent adenylate cyclase activity. Rather, one obtains proportionately higher activities at lower occupancy, thus effecting an amplification which can buffer the system against receptor loss [4]. We suggest that the attenuation of isoprenaline-stimulated activity may thus be due not only to a decrease in the numbers of both receptors but also to the observed decrease in the amount of $G_s$.

Analysis of $G_i$ functioning was done here either by assessing receptor-mediated $G_i$ action or by activating $G_i$ directly with low concentrations of the non-hydrolysable analogue p[NH]ppG (Fig. 3; Table 2). Such experiments showed that the magnitude of receptor-mediated $G_i$ inhibition, elicited through a number of distinct receptors, was no different in membrane preparations from lean compared with obese animals. Evaluation of levels of $G_i$ $\alpha$-subunits, however, showed that levels of $G_i$-1 and $G_i$-3 were decreased by over 60% in membranes of obese compared with lean animals, whereas levels of $G_i$-2 were only slightly decreased. That no difference was seen in receptor-mediated functioning would be consistent with various studies [34–37] which have suggested that it is $G_i$-2, rather than either $G_i$-1 or $G_i$-3, which is the true $G_i$, capable of inhibiting adenylate cyclase. In contrast, only in membranes from lean animals was guanine-nucleotide-effected $G_i$ function evident. Such observations parallel those made by us [8] comparing $G_i$ function in the adipocytes of streptozotocin-diabetic animals. We have shown that in the hepatocytes of streptozotocin-induced diabetic rats, as well as those from obese Zucker rats, guanine-nucleotide-induced $G_i$ function is abolished [7,34,38–40]. This effect has been attributed to the phosphorylation of $G_i$-2 caused by the action of protein kinase C [7,34,38–40]. However, it seems that receptor-linked $G_i$ function is not abolished by such a modification [8,38], and this has been suggested [8,38] to be because coupling of the inhibitory receptor to this G-protein causes an overpowering conformational change in $G_i$, allowing inhibition to ensue. Thus phosphorylation is rather different from the ADP-ribosylation of $G_i$, catalysed by pertussis toxin, which blocks receptor-coupled $G_i$ function by modifying a cysteine residue in the receptor-binding domain on $G_i$ and, depending on the system studied, either inactivates or attenuates GTP-dependent $G_i$ function [1–3]. There are thus similarities in the alterations of $G_i$ function found both in obese Zucker rats and in streptozotocin-induced diabetes, although differences, as regards G-protein expression, are observed. In this regard, streptozotocin-induced diabetes causes adipocyte levels of $G_i$-3 to increase while leaving those of $G_i$-2, $G_i$-1 and $\beta$-subunits unchanged [8].

Other studies on obese Zucker rats have shown that

isoprenaline- and glucagon-stimulated adenylate cyclase activity is decreased in cardiac tissue [39] and hepatocytes [17], although not to the extent noted here in adipocytes. Similarly, loss of guanine-nucleotide-mediated inhibition of adenylate cyclase was also identified in hepatocytes of obese animals [17], along with an increase in the phosphorylation of $G_i$-2 [40].

It thus seems that defects in the adenylate cyclase signalling system characterize obese Zucker rats. This may account for defects in the control of fat [14,15] and carbohydrate [18,19] metabolism as well as contributing to the impaired cardiac inotropic response found in obese animals [41]. Certainly, many model systems of obesity show impaired functioning of lipolytic hormones on adipocytes [11–13,16,42], indicating that this factor may contribute to the hyperadiposity seen. Such a correlation holds with aging and the development of obesity in the Zucker rat [39]. Indeed, a detailed analysis of cardiac tissue showed that the responsiveness to isoprenaline decreased with age [39]. Furthermore, whereas in the present study it was clear that adult obese animals had a dramatically decreased response to adrenaline compared with their lean littermates, only slight differences were evident in studies done on immature (5–7 week old) animals [43]. The consequence of the large decreases in adipocyte $G_i$-1 and $G_i$-3 of obese animals, however, remains to be ascertained. It is also noteworthy that, although obese Zucker rats are hypothyroid [20], such differences in G-protein expression between the lean and obese littermates is in marked contrast with the effect of chemically induced hypothyroidism in rats [44], where levels of $G_i$ (1+2) were found to be approximately doubled. This lead to the enhanced functioning of inhibitory receptors controlling adenylate cyclase [43]. It must be that in the obese Zucker rat other mechanisms provide a dominant control on G-protein expression. Indeed, it has been shown that adipocytes from obese humans exhibit lowered levels of both $G_i$ (1+2) and $G_s$ [45], which is supportive of the proposal [29] that the obese Zucker rat is a useful model of human obesity.

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