Adipocyte G-proteins and adenylate cyclase

Effects of adrenalectomy

Manuel ROS,* John K. NORTHUP† and Craig C. MALBON*‡

*Department of Pharmacological Sciences, Diabetes and Metabolic Diseases Research Program, School of Medicine, Health Sciences Center, State University of New York at Stony Brook, Stony Brook, NY 11794-8651, U.S.A.,

and †Department of Pharmacology, Yale University School of Medicine, New Haven, CT 06510, U.S.A.

Steroid hormones modulate the ability of cells to respond to hormones that act via cyclic AMP. In adipocytes of adrenalectomized rats, cyclic AMP accumulation and lipolysis in response to adrenaline are attenuated. However, the mechanism(s) of these effects are poorly understood. The effects of altered glucocorticoid status *in vivo* on the steady-state amounts of components of the hormone-sensitive adenylate cyclase were analysed in rat adipocytes. β -Adrenergic receptors were analysed by using radioligand binding and immunoblotting with an anti-receptor antiserum. Neither the amount of radioligand binding nor the amount of β -adrenergic-receptor peptide (M_r 67 000) was altered by adrenalectomy, whereas treatment of adrenalectomized rats with dexamethasone was found to increase both parameters by more than 25 % with respect to the control. Forskolin-stimulated adenylate cyclase activity was unchanged in membranes isolated from adipocytes of adrenalectomized rats, but was decreased (50 %) in those from dexamethasone-treated rats. The α -subunit of G_s was probed by using cholera-toxin-catalysed ADP-ribosylation. Immunoblotting was used to analyse the steady-state amounts of G-protein β -subunits (β - $G_{35/36}$). Adrenalectomy was associated with decreases in the steady-state amounts of α - G_s (30 %) and β - $G_{35/36}$ (50 %). Dexamethasone treatment of adrenalectomized animals partially restored the lipolytic response of adipocytes to adrenaline and the amounts of α - G_s , increased the amounts of β - $G_{35/36}$ subunits from 50 % to 150 % of control values, increased β -adrenergic receptors by more than 25 % and decreased adenylate cyclase activity (50 %). These results suggest that the steady-state amounts of components of hormone-sensitive adenylate cyclase are differentially regulated by glucocorticoids.

INTRODUCTION

Steroid hormones modulate the ability of cells and tissues to respond to hormones that act via the second messenger cyclic AMP. In adipose tissue, in particular, adrenalectomy has been shown to attenuate catecholamine-stimulated lipolysis (Allen & Beck, 1972; Lamberts *et al.*, 1975; Exton *et al.*, 1972). In liver, glucocorticoids also have been shown to exert permissive effects on the catecholamine-stimulated ureogenesis and glycogenolysis (Garcia-Sainz *et al.*, 1988; Exton *et al.*, 1972). However, the biochemical mechanism(s) underlying these effects remain to be elucidated. Since glucocorticoids can modulate the action of hormones that act via cyclic AMP, a fundamental question that arises is, can glucocorticoids regulate the steady-state amounts of individual components of the hormone-sensitive adenylate cyclase system? Thyroid hormones, like glucocorticoids, also exert permissive effects on the actions of hormones that act via cyclic AMP (Malbon *et al.*, 1988). It has been shown that thyroid hormones can differentially regulate the steady-state amounts of several G-protein subunits such as α - G_i (α - G_{41}), α - G_o (α - G_{39}) and the β - $G_{35/36}$ in adipocytes (Malbon *et al.*, 1985; Saggerson, 1986; Milligan *et al.*, 1987; Ros *et al.*, 1988).

In liver, thyroid status and adrenalectomy also have been shown to modulate the steady-state amounts of G-proteins (Stiles *et al.*, 1981; Garcia-Sainz *et al.*, 1988) and β -adrenergic receptors (Wolfe *et al.*, 1976; Rajerison *et al.*, 1974). In an effort to gain a greater insight into the biochemical basis of glucocorticoid effects on the adenylate cyclase system, the effects of adrenalectomy and dexamethasone treatment *in vivo* on the steady-state expression of several components of the hormone-sensitive adenylate cyclase system were investigated.

EXPERIMENTAL

Animals

Female Sprague–Dawley rats weighing 150–175 g were used in all experiments. Adrenalectomy was performed under ether anaesthesia. Adrenalectomized animals received 0.9 % NaCl in their drinking water and were fed *ad libitum* until killed, 10 days after surgery. Dexamethasone (0.4 mg/day) was administered subcutaneously to adrenalectomized animals, commencing 6 days after surgery and 4 days before use. Control animals, littermates of adrenalectomized rats, received the saline vehicle subcutaneously.

Abbreviations used: α - G_s , the α -subunit of the regulatory component of hormone-sensitive adenylate cyclase that mediates stimulation; α - G_i , the M_r 41 000 α -subunit that mediates inhibition of the hormone-sensitive adenylate cyclase; α - G_o , the M_r 39 000 α -subunit that is found in high abundance in bovine brain; β - $G_{35/36}$, the M_r 35 000–36 000 β -subunits of G-proteins; PIA, N^6 -phenylisopropyladenosine; ICYP, (–)-[¹²⁵I]iodonopindolol; BSA, bovine serum albumin.

‡ To whom correspondence should be addressed.

Isolation of cells and membranes

Adipocytes were obtained by collagenase digestion of parametrial adipose tissue by the method of Rodbell (1964), as described previously (Malbon *et al.*, 1978). Highly purified plasma membranes were prepared by the method of McKeel & Jarret (1970). The isolated cells were washed twice with and homogenized in buffers containing the following proteinase inhibitors: leupeptin (5 µg/ml), aprotinin (5 µg/ml) and freshly prepared phenylmethanesulphonyl fluoride (30 µg/ml). In all cases, membranes were freshly prepared from control, adrenalectomized, and adrenalectomized dexamethasone-treated animals, concurrently.

Cyclic AMP assay

Cyclic AMP accumulation by adipocytes was measured as described previously (Czech *et al.*, 1980).

Adenylate cyclase assay

Freshly prepared membranes (30 µg) were incubated at 30 °C in a final volume of 0.1 ml containing 50 mM-Hepes (pH 8.0), 25 mM-Tris/HCl, 11 mM-MgCl₂, 10 mM-phosphocreatine, 2 mM-mercaptoethanol, 1 mM-cyclic AMP, 0.3 mM-ATP containing 1–2 µCi of [α -³²P]ATP, creatine kinase (2 mg/ml), bovine serum albumin (1 mg/ml) and 0.4 mM-EGTA, in the absence or the presence of 10 µM-forskolin. The assay was initiated by addition of the membranes and was terminated after 10 min by addition of a solution containing 2% SDS, 1.4 mM-cyclic AMP and 40 mM-ATP. The cyclic AMP generated was isolated and quantified by the method of Salomon *et al.* (1974). Assays were routinely performed in triplicate.

Bacterial toxin-catalysed labelling

Membranes (0.1 mg of membrane protein) were incubated in the presence of [α -³²P]NAD⁺ and cholera toxin as described by Malbon *et al.* (1984). SDS/polyacrylamide-gel electrophoresis and autoradiography were performed as described by Malbon *et al.* (1984). The amount of radiolabel incorporated into G-protein α -subunits was quantified by the method of Lai *et al.* (1981).

Protein determination

Protein was assayed by the method of Lowry *et al.* (1951), with bovine serum albumin as the standard.

Immunoblotting

Immunoblotting was performed as described by Ros *et al.* (1988). Briefly, membranes (0.04–0.3 mg of membrane protein) or purified G-protein subunits were subjected to SDS/polyacrylamide-gel electrophoresis on 1.5 mm-thick 10%-polyacrylamide slab gels. The separated proteins were then transferred from the gel to nitrocellulose paper (Schleicher and Schuell) by the use of an electroblotting apparatus (Bio-Rad) operated at a constant current of 0.15 mA for 6 h. The blots of resolved proteins were probed with rabbit polyclonal antisera as indicated and then with ¹²⁵I-labelled goat anti-rabbit IgG. Regions of the blots that corresponded to the bands identified by autoradiography were excised, and the amount of radioactivity in each band was measured by γ -radiation spectrometry.

Protein purification and antibodies

β -G_{35/36} subunits were purified as described by Evans *et al.* (1986). Polyclonal antisera against G-protein subunits were raised in rabbits (Evans *et al.*, 1986). β -Adrenergic receptors of guinea-pig lung membranes were purified to apparent homogeneity (specific activity > 9 nmol/mg of protein) by using affinity chromatography and size-exclusion h.p.l.c. as described by Cubero & Malbon (1984). The anti-(β -adrenergic-receptor) antiserum raised in rabbits against purified guinea-pig lung receptors has been characterized elsewhere (Moxham *et al.*, 1986, 1988; George *et al.*, 1988).

Radioligand-binding assay

[¹²⁵I]Iodocyanopindolol (ICYP) binding to the membranes was performed at 22 °C for 1 h (Malbon, 1980). All receptor-binding parameters were analysed by computer by using a non-linear least-squares curve-fitting procedure and the modified LIGAND program (Biosoft–Elsevier, Cambridge, U.K.).

Glycerol release

Neutralized extracts from incubations were assayed for glycerol as described by Malbon *et al.* (1978).

Materials

All radiochemicals were purchased from Du Pont–New England Nuclear. Bacterial toxins were purchased from List Biological Laboratories. All other reagents were purchased from standard commercial sources.

Presentation of data

The results were obtained from a minimum of three independent experiments, each performed on separate occasions with different groups of animals. The data are presented either as the mean values $\pm$ S.E.M. of separate experiments or as representative of a group.

RESULTS AND DISCUSSION

Lipolysis and adenylate cyclase

Adrenalectomy has been shown to impair catecholamine stimulation of lipolysis in adipose tissue (Allen & Beck, 1972; Exton *et al.*, 1972; Lamberts *et al.*, 1975). This is illustrated in Figs. 1 and 2, where, in agreement with the above-mentioned reports, our results show that adrenalectomy markedly impairs lipolysis, measured as glycerol release (Fig. 1), and cyclic AMP accumulation (Fig. 2) in response to adrenaline. Dexamethasone treatment of adrenalectomized animals partially reverses the attenuation of the lipolytic response to adrenaline (Fig. 1).

When lipolysis was stimulated with the plant diterpene forskolin, results similar to the adrenaline response were obtained, i.e. a decrease with adrenalectomy and a partial recovery with dexamethasone treatment. Interestingly, forskolin-stimulated cyclic AMP accumulation (Fig. 2b) was found to be unchanged by adrenalectomy and was decreased to 50% of the control value after dexamethasone treatment. These data suggest that adrenalectomy does not alter the catalytic activity of the adenylate cyclase, whereas dexamethasone treatment decreases it. To address this question more fully, adenylate cyclase activity of membranes isolated from adipocytes was measured in each case. Fig. 3 shows that

Fig. 1. Adrenaline- and forskolin-stimulated lipolysis in rat adipocytes: effects of adrenalectomy

Adipocytes from adrenalectomized (○; □, ADX), control (●; ■, CTRL) and adrenalectomized dexamethasone-treated (△; ▨, ADX+DEX) rats were isolated and incubated in Krebs–Ringer phosphate buffer containing 3% BSA in the presence of adrenaline or forskolin at the indicated concentrations for 1 h. Samples (50 μl) from each tube were collected in triplicate and the concentration of glycerol was assayed as described in the Experimental section. These data are from a single experiment representative of three experiments performed on as many separate membrane preparations. The s.e.m. bars for each data point are smaller than the symbols; *significantly different from control ($P < 0.05$).

Fig. 2. Adrenaline- and forskolin-stimulated cyclic AMP accumulation in rat adipocytes: effects of adrenalectomy

Adipocytes from adrenalectomized (○; □, ADX), control (●; ■, CTRL) and adrenalectomized dexamethasone-treated (△; ▨, ADX+DEX) rats were isolated and incubated in Krebs–Ringer phosphate buffer containing 3% BSA in the presence of adrenaline or forskolin at the concentrations indicated. After 2 min incubation the cyclic AMP content of the cells was assayed as described in the Experimental section. The data are from a single experiment representative of three such experiments, each performed on as many separate cell preparations. The s.e.m. bars for each data point are smaller than symbols; *significantly different from control ($P < 0.05$).

adrenalectomy does not affect the forskolin-stimulated adenylate cyclase activity, whereas dexamethasone treatment of adrenalectomized rats decreases it by 50%. Adrenal status has been reported to modulate the activity of cyclic AMP phosphodiesterase (Mangianello & Vaughan, 1973). Therefore we also measured the activity of adenylate cyclase in the presence of RO 20-1724, a

potent inhibitor of phosphodiesterase activity. As expected, RO 20-1724 increased cyclic AMP in all cases (Fig. 3b), but the pattern among all three conditions did not change. These results are consistent with the proposal that adrenalectomy does not affect the catalytic activity of adenylate cyclase. Dexamethasone treatment, in contrast, results in a decrease in adenylate cyclase activity.

Fig. 3. Effects of adrenalectomy on the adenylate cyclase activity of rat adipocyte membranes

Freshly-prepared membranes of adipocytes isolated from adrenalectomized (□), control (▤) and adrenalectomized dexamethasone-treated (▨) rats were incubated in the absence (B) or in the presence (Forsk) of 10 μ M-forskolin and in the absence (a) or in the presence (b) of 0.1 mM-RO 20-1724. Adenylate cyclase activity was assayed as described in the Experimental section. Assays were routinely performed in triplicate. The results shown are mean values $\pm$ S.E.M. of three experiments, performed on as many separate cell membrane preparations: *significantly different from control ($P < 0.05$).

Fig. 4. Scatchard plots of specific binding of ICYP to membranes prepared from adipocytes from control, adrenalectomized and adrenalectomized dexamethasone-treated rats

Freshly prepared membranes from adrenalectomized (○; ADX), control (●) or adrenalectomized dexamethasone-treated (△; ADX + DEX) rats were incubated at 22 °C for 60 min with increasing concentrations of radioligand. Specific binding was assayed as described in the Experimental section. The data in the plot are means of triplicate determinations of a single experiment representative (variance $< 10\%$) of six separate experiments performed on as many individual fat-cell membrane preparations. Mean values ($\pm$ S.E.M.) of K_d and B_{max} , evaluated from six separate experiments as described in the Experimental section, are listed below: *significantly different from control ($P < 0.05$).

	ADX	Control	ADX + DEX
B_{max} (fmol/mg)	290 ± 30	295 ± 15	$370 \pm 25^*$
K_d (nM)	0.16 ± 0.04	0.24 ± 0.02	0.20 ± 0.04

This decrease provides an explanation for the lowered cyclic AMP response (Fig. 2) and only partial recovery of adrenaline stimulation of lipolysis (Fig. 1) in adipocytes from dexamethasone-treated rats.

An alteration in the inhibitory pathway might contribute to the alteration in the stimulatory response to hormones. To address this possibility, the ability of the adenosine agonist *N*⁶-phenylisopropyladenosine (PIA) to inhibit forskolin-stimulated lipolysis and cyclic AMP accumulation was investigated. The ability of PIA (1–100 nM) to inhibit lipolysis and cyclic AMP accumulation was found to be unaltered by adrenalectomy or glucocorticoid treatment (results not shown).

Status of β -adrenergic receptors

β -Adrenergic receptors represent the first element in the transmembrane signalling system which controls stimulation of the adenylate cyclase and ultimately the activation of lipolysis. β -Adrenergic receptors have been shown to be regulated by glucocorticoids in several tissues (Wolfe *et al.*, 1976; Guellaen *et al.*, 1978; Collins *et al.*, 1988; Hadcock & Malbon, 1988). The status of β -adrenergic receptors was probed in membranes freshly prepared from adipocytes of control rats, rats that had been adrenalectomized, and rats that had been adrenalectomized and treated with dexamethasone *in vivo* for 4 days. Specific binding of the high-affinity β -adrenergic antagonist ligand ICYP to adipocyte membranes at equilibrium was measured at radioligand concentrations of 10–1000 pM. The data were transformed by the method of Scatchard (1949). As shown in Fig. 4, in membranes prepared from adrenalectomized, control, and adrenalectomized/dexamethasone-treated animals, the Scatchard plots of specific ICYP binding were linear under the conditions employed. The maximal

Fig. 5. Effects of adrenalectomy on the steady-state amounts of β -adrenergic receptors in rat adipocytes: analysis by immunoblotting

Membranes (0.3 mg of protein) isolated from adipocytes from adrenalectomized (A), control (C) and adrenalectomized dexamethasone-treated rats (D) were reduced with 20 mM-dithiothreitol and then subjected to SDS/polyacrylamide-gel electrophoresis. Immunoblotting of the resolved proteins was performed with an anti- β -adrenergic-receptor antiserum, 6EN-1 (Moxham *et al.*, 1986), and a second ^{125}I -labelled goat anti-rabbit IgG in tandem as described in the Experimental section. The results are expressed as the percentage of the value obtained with fat-cell membranes from control rats and are means of three separate experiments. The right panel shows the autoradiogram of a representative immunoblot.

binding capacity (B_{max}) of β -adrenergic receptors in the membranes of adrenalectomized rats was unaltered, whereas in the case of adrenalectomy followed by glucocorticoid treatment the B_{max} increased significantly (Fig. 4 legend). The affinity of the receptors for ICYP was unaffected by each of these endocrine manipulations *in vivo* (Fig. 4 legend). Immunoblotting of the membrane proteins separated on SDS/polyacrylamide-gel electrophoresis was performed to quantify the amount of receptor in the membrane by a technique that was independent of ligand binding. Blots were probed with the anti-receptor antiserum 6EN-1 (Moxham *et al.*, 1986). The immunoblots revealed no change in adrenalectomized animals, and an increase in the dexamethasone-treated animals (Fig. 5). These data, in agreement with those obtained by radioligand binding, show that adrenalectomy does not change the steady-state amount of β -adrenergic receptors. However, adipocyte membranes isolated from dexamethasone-treated adrenalectomized rats did display a 30% increase in the steady-state amount of β -adrenergic receptors. These data agree with those of Lacasa *et al.* (1988), who also have reported that incubation of rat adipose-tissue fragments with dexamethasone increases the number of β -adrenergic receptors. It has also been shown that β -adrenergic receptors are regulated at the level of mRNA by glucocorticoids (Collins *et al.*, 1988; Hadcock & Malbon, 1988; Malbon & Hadcock, 1988).

Status of G-protein subunits

Since transmembrane signalling between catecholamine receptors and adenylate cyclase is transduced by

Fig. 6. Polyacrylamide-gel analysis of radioactive products resulting from cholera-toxin-catalysed ADP-ribosylation of membranes of adipocytes from adrenalectomized (A), control (C) and adrenalectomized dexamethasone-treated rats (D)

Equal amounts of membrane protein (0.1 mg) were incubated with $[\alpha\text{-}^{32}\text{P}]\text{NAD}^+$ (1 μM) and GTP (0.1 mM) in the presence of pre-activated cholera toxin (100 $\mu\text{g}/\text{ml}$) and then subjected to SDS/polyacrylamide-gel electrophoresis and autoradiography as described in the Experimental section. The results shown in the left panel are expressed as the percentage of the values obtained in controls, and are means $\pm$ S.E.M. for three to five separate experiments: $\boxtimes$, α -G_s 42 (i.e. M_r 42000); $\square$, α -G_s 46; *significantly different from control ($P < 0.05$). The right panel is an autoradiogram of a gel from a representative experiment.

Fig. 7. Effects of adrenalectomy on the steady-state amount of β -G_{35/36} subunits in rat adipocyte membranes

Membranes (0.04 mg of membrane protein) isolated from fat-cells from adrenalectomized (A), control (C) and adrenalectomized dexamethasone-treated (D) rats were subjected to SDS/polyacrylamide-gel electrophoresis and then to immunoblotting with antiserum G β -7 and a second ¹²⁵I-labelled goat anti-rabbit IgG, in tandem. The right panel shows an autoradiogram of the blot from a representative experiment. Regions of the blot corresponding to β -G_{35/36} subunits were excised, and the amount of radioactivity in the bands was measured as described in the Experimental section. The results are expressed as the percentage of the values obtained with control fat-cell membranes, and are means $\pm$ S.E.M. for five separate experiments (left panel); *significantly different from control ($P < 0.05$).

G_s, we investigated if glucocorticoids exerted an effect on the steady-state amounts of α -G_s. The relative amounts of α -G_s of adipocyte membranes were measured by using cholera-toxin-catalysed ADP-ribosylation at the expense of [³²P]NAD⁺. This method has been shown to be useful for establishing the relative amounts of α -G_s in cell membranes (Malbon & Gill, 1979; Stiles *et al.*, 1981; Watkins *et al.*, 1987). Cholera toxin catalysed the ADP-ribosylation of two major peptides, of M_r 42000 and 46000, in adipocyte membranes from adrenalectomized, control, and dexamethasone-treated adrenalectomized rats alike (Fig. 6). The amount of incorporation of label into these peptides was quantified and compared. Adrenalectomy was associated with a 30% decrease in the amounts of ADP-ribose incorporated into both of the cholera-toxin substrates. In the membranes of dexamethasone-treated/adrenalectomized rats, a small variable increase was observed in the amounts of the M_r 42000 and 46000 radiolabelled peptides, suggesting a reversal by glucocorticoid administration *in vivo*. The patterns of membrane proteins made visible by staining with Coomassie Blue, in contrast, were not altered by these endocrine manipulations *in vivo* (results not shown).

Glucocorticoids regulate several targets in the cell, and their final actions probably cannot be explained by a single change. Adrenal steroids have been reported to modulate the activity of cyclic AMP phosphodiesterase (Mangianello & Vaughan, 1973). Lamberts *et al.* (1975) have shown that glucocorticoids also induce cyclic AMP-dependent kinase activity. The decrease in α -G_s reported herein, together with the above-mentioned

alterations in the phosphodiesterase and/or in the cyclic AMP-dependent kinase, could explain the impaired response to adrenaline in adipocytes from adrenalectomized rats when cyclic AMP or lipolysis was measured (Figs. 1 and 2). However, the fact that the changes in lipolysis are more dramatic than those produced in cyclic AMP in response to forskolin in adipocytes from adrenalectomized rats suggest that other steps beyond cyclic AMP generation (i.e. lipases) are also regulated by glucocorticoids. That adipocytes from adrenalectomized dexamethasone-treated rats display partial recovery of the lipolytic response, despite their low extent of cyclic AMP accumulation in response to adrenaline, highlights this point.

In view of the effects of adrenal steroids on the α -subunit of G_s, it was critical to assess the influence of adrenalectomy on the β -subunits, apparently common to several G-proteins. The status of the β -subunits were investigated by immunoblotting with antiserum G β -7, which recognizes both the 35000- and 36000- M_r forms of β -subunits (Evans *et al.*, 1987). Unlike the α -subunit discussed above, the β -subunits are not known to be substrates for bacterial-toxin-catalysed ADP-ribosylation. Adipocyte membranes from adrenalectomized, control, and adrenalectomized/dexamethasone-treated rats were separated on SDS/polyacrylamide gels, transferred to nitrocellulose and probed with antisera that specifically recognize these G β -subunits. Peptides of M_r 35000 and 36000 were prominently stained in immunoblots of membranes from all three sources (Fig. 7). Quantitative immunoblotting techniques were applied to the analysis of the steady-state amounts of these

peptides, as previously described (Watkins *et al.*, 1987; Ros *et al.*, 1988). Adrenalectomy was found to result in a 50% decrease in the steady-state amounts of the β -subunits of G-proteins. Treatment with dexamethasone not only reversed the decline in the steady-state amounts of β -subunits, but also elevated the steady-state amounts of these proteins in adipocyte membranes of adrenalectomized rats to 150% of control values. Thus adrenal steroids *in vivo* appear to modulate the steady-state amounts of β -subunits of G-proteins.

An inhibitory role for β -subunits of G-proteins in the hormonal control of adenylate cyclase has been proposed (Katada *et al.*, 1986; Gilman, 1984). Taking these considerations into account, enhanced cyclic AMP accumulation in response to adrenaline might be expected in adipocytes of adrenalectomized rats. However, the simultaneous decrease in β -subunits may keep the ratio of α/β -subunits of G-proteins in the same range as in control animals. Alterations in the steady-state amounts of α -subunits may be compensated by similar changes in β -subunits, i.e. an adaptive mechanism of the tissue to the adrenalectomy. Dexamethasone treatment of adrenalectomized animals results in an increase in β -subunits and α -G_s contents. This, together with the decline in the catalytic activity of adenylate cyclase, may explain the diminished lipolytic and cyclic AMP responses of adipocytes from adrenalectomized dexamethasone-treated rats. β -G_{35/36} are apparently common to several G-proteins, and any alteration in their steady-state amounts may influence by mass action the equilibrium between β -subunits and α -subunits of other G-proteins in addition to α -G_s. Two different genes encoding the β -G₃₆ (β -1) and β -G₃₅ (β -2) subunits have been isolated (Codina *et al.*, 1986; Fong *et al.*, 1987). However, the specific function unique to each of these proteins, if any, still remain unclear.

Concluding remarks

Modulation of the steady-state amounts of G-protein subunits and G-protein-linked receptors appears to be a likely mechanism by which glucocorticoids can modulate transmembrane signalling. Hormones, other than glucocorticoids, have been shown to exert a modulatory effect at the level of G-proteins, catalytic activity of the adenylate cyclase or G-protein-linked receptors such as β -adrenergic receptors. Hypothyroidism has been shown to increase the steady-state amounts of α -G_i, α -G_o and β -G_{35/36} in adipocytes (Malbon *et al.*, 1985; Saggerson, 1986; Milligan *et al.*, 1987; Ros *et al.*, 1988). Hyperthyroidism, on the other hand, has been shown to decrease the activity of adenylate cyclase in the same system (Rapiejko & Malbon, 1987). Permissive effects of thyroid and steroid hormones have also been described in other tissues. In liver, hypothyroidism is associated with an increased responsiveness to catecholamine regulation of glycogenolysis (Malbon *et al.*, 1978). The number of β -adrenergic receptors was shown to increase several-fold in the hypothyroid state (Malbon, 1980). The steady-state amounts of α -G_s have also been shown to be modulated by thyroid status in rat reticulocytes (Stiles *et al.*, 1981). In liver, adrenalectomy decreases the Ca²⁺-mediated α -adrenergic stimulation of glycogenolysis (Exton *et al.*, 1972; Studer & Borle, 1984). This effect has been associated with a decrease in the rise in Ca²⁺ in response to α -adrenergic stimulation (Freudenrich & Borle, 1988). In parallel with these

physiological effects, changes in the steady-state amounts of several G-protein subunits and β -adrenergic receptors also have been described in liver. Adrenalectomy has been shown to decrease the steady-state amount of α -G_s and at the same time increase the steady-state amount of α -G_i (Garcia-Sainz *et al.*, 1988) and the number of β -adrenergic receptors (Rajerison *et al.*, 1974; Wolfe *et al.*, 1976; Guellaen *et al.*, 1978).

In the present report, we show that adrenalectomy brings about a decrease in the steady-state amounts of α -G_s and β -G_{35/36} without affecting β -adrenergic-receptor number or the catalytic activity of the adenylate cyclase. Dexamethasone treatment, in contrast, reversed the effects of adrenalectomy on G-protein subunits, increased the number of β -adrenergic receptors and decreased the activity of adenylate cyclase. Glucocorticoids have been shown to increase adenylate cyclase and the expression of the α -subunit of G_s in GH₃ cells (Chang & Bourne, 1987). Thus glucocorticoids, as thyroid hormones, appear to regulate differentially the steady-state amounts of the components of the hormone-sensitive adenylate cyclase. Alteration of the steady-state amounts of β -G subunits as well as other G-protein subunits may alter the equilibrium that exist between α -subunits and their β/γ complexes. Changes in the $\alpha:\beta/\gamma$ ratio may then alter the responsiveness of the cell to a given hormone. This appears to represent at least one biochemical mechanism by which glucocorticoids can exert their effects.

Glucocorticoids regulate many different targets in the cell. The decrease in the steady-state amount of α -G_s described herein, together with the reported changes in the phosphodiesterase activity (Mangianello & Vaughan, 1973), can explain, at least in part, the impaired cyclic AMP accumulation and lipolysis by adipocytes from adrenalectomized rats. However, the poor correlation between both forskolin-stimulated cyclic AMP accumulation and lipolysis found in adipocytes from adrenalectomized rats suggest that other step(s) distal to cyclic AMP accumulation (i.e. lipases) are regulated by glucocorticoids.

This work was supported by U.S. Public Health Service grants DK30111 and DK25410 from the National Institutes of Health (to C.C.M.). M.R. is the Recipient of a Consejo Superior de Investigaciones Científicas (Spain) postdoctoral fellowship. C.C.M. is the recipient of Research Career Development Award KO4-AM00786 from the National Institutes of Health.

REFERENCES

- Allen, D. O. & Beck, R. R. (1972) *Endocrinology* (Baltimore) **91**, 504–510
- Chang, F.-H. & Bourne, H. R. (1987) *Endocrinology* (Baltimore) **121**, 1711–1715
- Codina, J., Stengel, D., Woo, S. L. C. & Birnbaumer, L. (1986) *FEBS Lett.* **207**, 187–192
- Collins, S., Caron, M. & Lefkowitz, R. J. (1988) *J. Biol. Chem.* **263**, 9067–9070
- Cubero, A. & Malbon, C. C. (1984) *J. Biol. Chem.* **259**, 1344–1350
- Czech, M. P., Malbon, C. C., Kermam, R., Gitomer, W. & Pilch, P. F. (1980) *J. Clin. Invest.* **66**, 574–582
- Evans, T., Brown, M. L., Fraser, E. D. & Northup, J. R. (1986) *J. Biol. Chem.* **261**, 7052–7059

- Evans, T., Fawzi, A., Fraser, E. D., Brown, M. L. & Northup, J. R. (1987) *J. Biol. Chem.* **262**, 176–181
- Exton, J. H., Friedman, N., Hee-Aik, E. W., Brineaux, P., Corbin, J. D. & Park, C. R. (1972) *J. Biol. Chem.* **247**, 3579–3588
- Fong, H. K. W., Amaturda, T. T., Birren, B. W. & Simon, M. (1987) *Proc. Natl. Acad. Sci. U.S.A.* **84**, 3792–3796
- Freudenrich, C. C. & Borle, A. B. (1988) *J. Biol. Chem.* **263**, 8604–8610
- Garcia-Sainz, J. A., Huerta-Baena, M. E. & Malbon, C. C. (1988) *Am. J. Physiol.*, in the press
- George, S. T., Berrios, M., Hadcock, J., Wang, H.-y. & Malbon, C. C. (1988) *Biochem. Biophys. Res. Commun.* **150**, 665–672
- Gilman, A. G. (1984) *Cell* **36**, 577–579
- Guellaen, G., Yates-Aggerbeck, M., Vauquelin, G., Strosberg, D. & Hanoune, J. (1978) *J. Biol. Chem.* **253**, 1114–1120
- Hadcock, J. R. & Malbon, C. C. (1988) *Proc. Natl. Acad. Sci. U.S.A.*, in the press
- Katada, T., Oinuma, M. & Ui, M. (1986) *J. Biol. Chem.* **261**, 5215–5221
- Lacasa, D., Agli, B. & Giudicelli, Y. (1988) *Biochem. Biophys. Res. Commun.* **153**, 489–497
- Lai, E., Rosen, O. M. & Rubin, C. S. (1981) *J. Biol. Chem.* **256**, 12866–12874
- Lamberts, S. W., Timmenmans, H. A., Krammer-Blankestijn, M. & Birkenhager, J. C. (1975) *Metab. Clin. Exp.* **24**, 681–689
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Malbon, C. C. (1980) *Mol. Pharmacol.* **18**, 193–198
- Malbon, C. C. & Gill, D. M. (1979) *Biochim. Biophys. Acta* **586**, 518–527
- Malbon, C. C. & Hadcock, J. R. (1988) *Biochem. Biophys. Res. Commun.* **154**, 676–681
- Malbon, C. C., Moreno, F. J., Cabelli, R. J. & Fain, J. N. (1978) *J. Biol. Chem.* **253**, 8820–8825
- Malbon, C. C., Graziano, M. P. & Johnson, G. L. (1984) *J. Biol. Chem.* **259**, 3254–3260
- Malbon, C. C., Rapiejko, P. J. & Mangano, T. J. (1985) *J. Biol. Chem.* **260**, 2558–2564
- Malbon, C. C., Rapiejko, P. J. & Watkins, D. C. (1988) *Trends Pharmacol. Sci.* **9**, 33–36
- Mangianello, V. & Vaughan, M. (1973) *J. Biol. Chem.* **248**, 7164–7170
- McKeel, D. W. & Jarret, L. (1970) *J. Cell Biol.* **44**, 417–432
- Milligan, G., Spiegel, A. M., Unson, C. G. & Saggerson, E. D. (1987) *Biochem. J.* **247**, 223–227
- Moxham, C. P., George, S. T., Graziano, M. P., Brandwein, H. J. & Malbon, C. C. (1986) *J. Biol. Chem.* **261**, 14562–14570
- Moxham, C. P., Ross, E. M., George, S. T. & Malbon, C. C. (1988) *Mol. Pharmacol.* **33**, 486–492
- Rajerison, R., Marchetti, J., Roy, C., Bockaert, J. & Jard, S. (1974) *J. Biol. Chem.* **249**, 6390–6400
- Rapiejko, P. J. & Malbon, C. C. (1987) *Biochem. J.* **241**, 765–771
- Rodbell, M. (1964) *J. Biol. Chem.* **239**, 375–380
- Ros, M., Northup, J. R. & Malbon, C. C. (1988) *J. Biol. Chem.* **263**, 4362–4368
- Saggerson, E. D. (1986) *Biochem. J.* **238**, 387–394
- Salomon, Y., Londos, C. & Rodbell, M. (1974) *Anal. Biochem.* **58**, 541–548
- Scatchard, G. (1949) *Ann. N.Y. Acad. Sci.* **51**, 660–672
- Stiles, G. L., Stadel, J. M., DeLean, A. & Lefkowitz, R. J. (1981) *J. Clin. Invest.* **68**, 1450–1455
- Studer, R. K. & Borle, A. B. (1984) *Biochim. Biophys. Acta* **804**, 377–385
- Watkins, D. C., Northup, J. R. & Malbon, C. C. (1987) *J. Biol. Chem.* **263**, 10651–10657
- Wolfe, B. B., Harden, T. K. & Molinoff, P. B. (1976) *Proc. Natl. Acad. Sci. U.S.A.* **73**, 1343–1347

Received 11 July 1988/19 September 1988; accepted 27 September 1988