β-Adrenergic signalling in neoplastic lung type 2 cells: glucocorticoid-dependent and -independent defects

KA Droms

Department of Cell Biology and Biochemistry, Texas Tech University Health Sciences Center, 3601 4th St, Lubbock, TX 79430, USA.

Summary Tumorigenic mouse lung-derived type 2 cell lines have large reductions in both β-adrenergic-stimulated cAMP production and ligand binding to β-adrenergic receptors. These tumorigenic cells are also relatively insensitive to glucocorticoids. Because glucocorticoids regulate both β-adrenergic receptor expression and receptor coupling to the stimulatory guanine nucleotide binding protein \(G_s\), interactions between the glucocorticoid and β-adrenergic signalling systems were examined. This study demonstrates that β-adrenergic ligand binding and agonist sensitivity is increased in a tumorigenic cell line stably expressing a normal glucocorticoid receptor transgene. However, although the transfected tumour cells and non-tumorigenic cells have similar amounts and affinities of β-adrenergic agonist and antagonist binding, similar amounts of \(G_s\), subunits and similar forskolin-stimulated adenyl cyclase activities, the former remain much less isoproterenol responsive. Competition binding studies demonstrate that tumour cell β-adrenergic receptors have both high- and low-affinity agonist binding but are functionally uncoupled from \(G_s\). This uncoupling may involve an alteration in \(G_s\) as guanine nucleotides exhibit a reduced ability to stimulate adenyl cyclase. Thus, some aspects of tumorigenic cell dysfunction in β-adrenergic signalling can be ameliorated by interactions with the glucocorticoid pathway, but additional defects are also involved.

Keywords: \(G_s\); β-adrenergic signalling; alveolar type 2 cell; neoplasia; glucocorticoid

β-Adrenergic receptor (βAR) expression and β-adrenergic hormone sensitivity increase during lung development and glucocorticoids accelerate these increases (Cheng et al., 1980; Barnes et al., 1984). In some cell types, glucocorticoids increase βAR expression (Collins et al., 1988) or βAR coupling to \(G_s\) (Davies and Lefkowitz, 1984), the heterotrimERIC guanine nucleotide-binding protein that regulates βAR stimulation of adenyl cyclase (Levitzki, 1988). When compared with two non-tumorigenic mouse lung alveolar type 2-derived cell lines, a number of tumorigenic cell lines of type 2 cell origin exhibit aberrant signal transduction including considerable reductions in sensitivity to both β-adrenergic (Droms et al., 1989; Lange-Carter et al., 1992) and glucocorticoid (Droms et al., 1993) hormones. As both β-adrenergic and glucocorticoid signals are major regulators of type 2 cell function (Ballard, 1986), the defects in these systems may be important aspects of the neoplastic progression of type 2 cells.

The heterotrimeric G protein \(G_s\) consists of α, β and γ subunits. Hormone binding to receptor induces \(G_\alpha\) to exchange bound GDP for GTP, resulting in adenyl cyclase activation. Hydrolysis of the terminal phosphate restores the resting state (Levitzki, 1988). The βAR exhibits two affinity states for agonist binding: one of higher relative affinity (\(k_\alpha\)) and the other of low affinity (\(k_\gamma\)) and glucocorticoids can affect both the relative proportions and affinities of these two sites (Davies and Lefkowitz, 1981). The proposed molecular basis of the high-affinity binding site is that agonist binding to receptor results in formation of a ‘ternary complex’ consisting of hormone, receptor and G protein (De Lean et al., 1980). Thus, the existence of high-affinity agonist binding sites is an indicator of the ‘coupling’ between receptor and G protein. Addition of guanine nucleotides destabilises this ternary complex, resulting in low-affinity hormone binding (Rodbell et al., 1971). The relative ability of guanine nucleotides to induce the low-affinity state is an indicator of functional coupling between receptor and G-protein (Cheung et al., 1989). It is not clear whether glucocorticoid enhancement of βAR–G, coupling involves glucocorticoid effects on the βAR or \(G_s\), or what the mechanisms of these effects might be. However, glucocorticoid treatment of cultured fibroblasts increased GDP-dependent activation of adenyl cyclase in the absence of added hormone (Johnson and Jaworski, 1983), suggesting that some glucocorticoid effects can be exerted distal to hormone receptors.

Tumour-associated defects in β-adrenergic and glucocorticoid adenyl cyclase activity often result from reduced receptor affinity or number (Hunt and Martin, 1980). Indeed, the lack of sensitivity of mouse lung tumour cells to β-adrenergic agonists does involve decreased numbers of βAR (Lange-Carter et al., 1992). In addition, these tumour cells exhibited altered guanine nucleotide analogue binding to a 45 kDa membrane associated protein and enhanced cholera toxin responsiveness (Droms et al., 1989; Lange-Carter et al., 1992), suggesting the hypothesis that G-protein function might be altered in mouse lung tumours. As glucocorticoids enhance both βAR expression and βAR–G, coupling (Davies and Lefkowitz, 1984), both defects in the β-adrenergic signalling pathway could result from the loss of glucocorticoid responsiveness. Glucocorticoids may exert independent effects on the βAR and \(G_s\), or, as even unoccupied βAR can influence G protein activity (Bond et al., 1995), the functional alteration in tumour cell \(G_s\) may result directly from decreased βAR expression. Alternatively, the reduced GDP-dependent coupling of receptors to adenyl cyclase activation observed in hepatomas (Okamura and Terayama, 1976), suggests that independent defects in \(G_s\) may also occur in tumours.

This study addresses the mechanisms of defective β-adrenergic signal transduction in tumorigenic mouse alveolar type 2 cell lines and uncovers both glucocorticoid-dependent and independent mechanisms. Reduced βAR expression can be ameliorated by dexamethasone treatment of a tumorigenic cell line stably expressing a transfected glucocorticoid receptor gene. In response to dexamethasone, this transfected cell line exhibits the same nucleotide and affinities of βAR antagonist and agonist binding sites as non-tumorigenic cells. However, even though these tumour cells exhibit dexamethasone enhancement of isoproterenol-stimulated
intracellular cAMP production, they remain much less isoproterenol responsive than non-tumorigenic cells. This relative insensitivity results from a loss of functional βAR–G₂ coupling that may involve an intrinsic defect in the interaction of G₂ with guanine nucleotides.

Materials and methods

Cell lines

The non-tumorigenic (C10) and tumorigenic (A5) type 2 cell lines were derived and cultured as described previously (Bentel et al., 1989; Droms et al., 1989, 1993). A5 cells were transfected by addition of media conditioned by VDG12P; cells were supplied by Dr. Gary Firestone, University of California, Berkeley, CA, USA). VDG12P; cells release recombinant virus with the rat glucocorticoid receptor gene linked to neomycin resistance (Cook et al., 1988). After selection in 600 μg ml⁻¹ genitin (G418, Gibco, Grand Island, NY, USA), a single resistant colony remained. Cells from this colony were isolated and named A5GR1. A5GR1 cells express the transfected glucocorticoid receptor (Droms, 1995) and are routinely cultured with 200 μg ml⁻¹ genitin to maintain this expression. For all experiments, cells were plated on Corning tissue culture dishes in CMRL 1066 medium with 5% fetal bovine serum (FBS), 100 units ml⁻¹ penicillin, and 100 μg ml⁻¹ streptomycin and allowed to attach overnight. On the following day cells were rinsed twice with 0.9% sodium chloride, and media containing 4% FBS from which endogenous steroids were removed with charcoal was added. At this time, 10 nm dexamethasone was added to the samples indicated in Results. Cells were cultured for an additional 4 days before experimental analyses.

Receptor binding studies

Cells were plated at a density of 1.3 × 10⁵–5 × 10⁶ cells per 100 mm culture dish. Membranes were prepared as described previously (Lange-Carter et al., 1992) and stored for up to 9 days at −80°C before use. For saturation binding studies 5–10 μg of membrane protein per sample was incubated for 90 min at 37°C with (+)⁻¹⁴C-labelled cyanopindolol (¹²⁵IICYP, 2200 Ci mmol⁻¹; DuPont NEN, Boston, MA, USA) ranging in concentration between 10 and 200 pm as described previously (Lange-Carter et al., 1992). Protein concentration was determined by the Lowry method (Lowry et al., 1951). For isoproterenol competition studies, membranes were incubated with 30 pm ¹²⁵IICYP and isoproterenol ranging in concentrations between 1 nm and 1 mM as described previously (Valverius et al., 1987). In some cases, GTP was included at the concentrations indicated. Membranes were harvested on glass fibre filters (Whatman GF/C) and counted in a gamma counter. Non-specific binding ranged between 27% and 32% for all conditions and was determined by adding 1 μM of the unlabelled antagonist propranolol. Non-specific binding has been subtracted from all data presented. Competition binding data were analysed with the Ligand program (Munson and Rodbard, 1983).

cAMP radioimmunoassay

C10 cells were plated at a density of 5 × 10⁴ cells per 60 mm culture dish and A5 and A5GR1 cells at 10⁵ cells per 60 mm dish. Cell lysates were harvested in 10% trichloroacetic acid (TCA) after 1 min treatment with 1 μM isoproterenol in buffer (130 mM sodium chloride, 5 mM potassium chloride, 1 mM calcium chloride, 1 mM magnesium sulphate, 1 mM potassium hydrogen phosphate, 6 mM glucose, 1 mM ascorbic acid, 25 mM Hapes, pH 7.4) at 37°C. After three extractions with diethyl ether, the TCA-soluble fraction was assayed for cAMP using a radioimmunoassay kit (Amersham, Arlington Heights, IL, USA). The TCA-insoluble fraction was solubilised with 0.2 n sodium hydroxide and assayed for protein by the Lowry method (Lowry et al., 1951).

Adenyl cyclase assays

Cell membranes (5–10 μg membrane protein per sample) were incubated for 20 min at 30°C with 0.1 mM unlabelled ATP, 1 μCi per sample of [α-³²P]ATP (30 Ci mmol⁻¹; DuPont NEN, Boston, MA, USA), 1 mM β-mercaptoethanol, 5 mM magnesium acetate, 50 μM cAMP (to competitively saturate phosphodiesterase), 10 mM creatine phosphate and 10 units ml⁻¹ creatine phosphokinase. [³²P]cAMP was separated from [³²P]ATP by ion exchange chromatography as described previously (Salomon, 1979). Some reactions included isoproterenol, guanine nucleotides or forskolin as indicated in Results.

Western blots

Equal amounts of membrane protein (40 μg) from dexamethasone-treated C10 and A5GR1 cells were separated by 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) as described previously (Droms et al., 1989). Resolved proteins were electrophoretically transferred to BA85 nitrocellulose (Schleicher and Schuell, Keene, NH, USA) in buffer containing 190 mM glycine, 20% methanol and 25 mM Tris, pH 8.3. Nitrocellulose membranes were subsequently incubated for 1.5 h in block buffer, consisting of 5% bovine serum albumin and 0.1% NP-40 in PBS (140 mM sodium chloride, 4 mM disodium hydrogen phosphate, 3 mM potassium chloride, 1.5 mM potassium hydrogen phosphate, 0.5 mM calcium chloride, 0.5 mM magnesium chloride, pH 7.4), rinsed 3 × 10 min in PBS and incubated for 12–16 h at 4°C with gentle rocking in block buffer with either a 1:16 000 dilution of an anti-G₂ rabbit polyclonal antibody (UBI, Lake Placid, NY, USA) or with a 1:40 000 dilution of anti-human G₂β common rabbit polyclonal antiserum (UBI). After rinsing 3 × 10 min in PBS, membranes were incubated...
with a 1:4000 dilution of alkaline phosphatase conjugated goat anti-rabbit secondary antibody (Cappel, Westchester, PA, USA). Detection was achieved by incubating the blots in alkaline phosphatase buffer (100 mM sodium chloride, 50 mM magnesium chloride, 100 mM Tris pH 9.5) containing 400 μM 5-bromo-4-chloro-3-indoly-l-phosphate (Sigma, St Louis, MO, USA) and 400 μM nitro blue tetrazolium (Sigma). The resultant blots were scanned with a Mirror 1200 scanner (Mirror Technologies, St Paul, MN, USA) and signals were quantified using the NIH Image program.

Results

The number and affinity of 125I CYP binding sites are similar in dexamethasone-treated C10 and ASGR1 cells

Some tumorigenic mouse lung-derived cell lines have reduced βAR expression compared with non-tumorigenic cells (Lange-Carter et al., 1992). Both the neoplastic A5 cell line and the stable glucocorticoid receptor-transfectant cell line ASGR1 also have very few membrane-associated βAR, as determined by saturation binding of the antagonist 125I CYP (Figure 1). Culturing the A5 cell line with dexamethasone did not increase 125I CYP binding (Figure 1). However, when cultured with 10 nM dexamethasone, the ASGR1 cells exhibited similar numbers of βAR as C10 cells (Figure 1). There were no significant differences in kD or Bmax, between C10 cells cultured without dexamethasone, C10 cells with 10 nM dexamethasone or ASGR1 cells with 10 nM dexamethasone [kD = 22.6 ± 2.6 (s.e.m.), 35.9 ± 6.1 and 30.1 ± 12 pm, P > 0.1; Bmax = 88 ± 10, 91 ± 11, 80 ± 7.7 fmol per mg protein, P > 0.5; n = 6, 4 and 5 respectively]. Statistical analyses were by one-way ANOVA.

Table I Radioimmunoassay of isoproterenol-stimulated cAMP production

| Cell line | 10 nM dex | Basal | 1 μM iso |
|-----------|-----------|-------|---------|
| A5        | –         | 2.3 ± 0.28 | 5.4 ± 0.73 |
|           | +         | 2.0 ± 0.37 | 6.2 ± 0.47 |
| ASGR1     | –         | 4.2 ± 0.11 | 8.0 ± 1.1 |
|           | +         | 2.5 ± 0.35 | 36 ± 7.3 |
| C10       | –         | 11 ± 1.6 | 280 ± 38 |
|           | +         | 12 ± 4.0 | 370 ± 23 |

Intracellular cAMP production after 1 min treatment with 1 μM isoproterenol (iso) was significantly (P < 0.01) enhanced if C10 or ASGR1 cells were cultured for 4 days with 10 nM dexamethasone (dex), whereas A5 cell isoproterenol responsiveness was unaffected. Basal intracellular cAMP was also greater in C10 than in A5 or ASGR1 cells, but was not affected by dexamethasone treatment. Numbers represent the mean ± s.e.m. cAMP produced (pmol cAMP per mg protein). Statistical analyses were by t-tests. n = 6 for each condition.

Table II Basal and isoproterenol-stimulated adenylate cyclase activity

| Cell line | Basal | 100 nM iso | 10 μM iso |
|-----------|-------|------------|-----------|
| ASGR1     | 130 ± 12 | 480 ± 9.8 | 530 ± 6.7 |
| C10       | 260 ± 46 | 2700 ± 110 | 2700 ± 160 |

Membranes from ASGR1 cells cultured with 10 nM dexamethasone have both lower basal and lower isoproterenol (iso)-stimulated adenylate cyclase activities compared with C10 cells cultured with dexamethasone. Numbers represent the mean ± s.e.m. cAMP produced (pmol cAMP per mg protein per 20 min) from two assays (n = 6 for each condition).

Dexamethasone-treated ASGR1 cells are much less isoproterenol responsive than C10 cells

Intracellular cAMP production stimulation by the β-adrenergic agonist isoproterenol did increase approximately 5-fold when ASGR1 cells were cultured with 10 nM dexamethasone, whereas no such increase was observed in A5 cells (Table I). However, although C10 and ASGR1 cells cultured with dexamethasone had similar number of 125I CYP binding sites, ASGR1 cells remained much less responsive to isoproterenol both in whole cells (Table I) and in crude membrane fractions (Table II). The dexamethasone-induced increase in β-adrenergic responsiveness in ASGR1 cells is likely to result from the increase in βAR expression. The approximate 30% increase in isoproterenol responsiveness in C10 cells cultured with dexamethasone compared with control C10 cells was not associated with increased βAR expression and involves enhanced coupling of the βAR to G, (KA Droms, unpublished). C10 cells also exhibited a greater basal level of intracellular cAMP than did A5 or ASGR1 cells (Table I) and an increased basal adenylyl cyclase activity compared with ASGR1 cells (Table II). The increased basal adenylyl cyclase activity in C10 cells may be due to a difference in the activity of Gβ, between cell lines (see below).

C10 and ASGR1 cells have similar forskolin-stimulated adenylyl cyclase activities

As both basal and isoproterenol-stimulated adenylyl cyclase activities are lower in ASGR1 than in C10 cells, a difference between cell lines in the activity of adenylyl cyclase is possible. However, when membranes prepared from C10 and ASGR1 cells cultured with dexamethasone were stimulated with 100 μM forskolin, which activates adenylyl cyclase directly (Downs and Aurbach, 1982), only a slight difference in adenylyl cyclase activity was observed (Figure 2). This difference in forskolin-stimulated adenylyl cyclase activity is much less than the isoproterenol sensitivity difference between cell lines. In fact, as the basal activity is also lower in ASGR1 cells, the fold stimulation by forskolin is greater than in C10 membranes.

Figure 2 Forskolin-stimulated adenylyl cyclase activity. Membranes from C10 and ASGR1 cells cultured with 10 nM dexamethasone do exhibit a slight difference in forskolin-stimulated adenylyl cyclase activity, but this difference is much less than the difference between cell lines in isoproterenol sensitivity. Basal adenylyl cyclase activity is also lower in ASGR1 cells. Data from two assays are combined, n = 6 for each condition. □, Basal activity; ■, + 100 μM forskolin.
C10 and A5GR1 cells have similar levels of Gα and Gβ subunit expression

In some cases, the relative levels of expression of G protein subunits can regulate receptor–G protein coupling (Blumer and Thorner, 1990). Therefore, Gα and Gβ subunit expression were examined on Western immunoblots (Figure 3). For each subunit, three individual blots were quantified as indicated in Materials and methods and the signal from C10 cells was set to 1 (signals from the 45 kDa and 52 kDa forms of Gα were combined). A5GR1 cells did not differ significantly (P > 0.5 for both subunits) from C10 for either Gα (1.1 ± 0.31 s.e.m.) or Gβ (0.91 ± 0.33) expression. Thus, no clear differences between cell lines were observed in the amount of any of the components of the βAR-coupled adenyl cyclase system that might explain their differences in hormone responsiveness.

The βAR and Gα are functionally uncoupled in A5GR1 cells

When A5GR1 and C10 cells are cultured with dexamethasone they express similar amounts of βAR and Gα and have similar forskolin-stimulated adenyl cyclase activities, yet the A5GR1 cells are much less responsive to isoproterenol. Therefore, the interactions between the components of the βAR-coupled adenyl cyclase system were examined. βAR-Gα coupling was analysed by isoproterenol competition for

---

**Figure 3** Gα and Gβ Western blots. C10 (lanes 1) and A5GR1 (lanes 2) cells were cultured with 10 nM dexamethasone and membranes prepared as described in Materials and methods. Representative blots probed with anti-Gα or anti-Gβ are shown. No clear difference between cell lines in the amount of these subunits has been observed. Both the 45 kDa and 52 kDa molecular weight forms of Gα are observed in C10 and A5GR1 cells. For comparison, S49 wt type cells, which express both Gα isoforms, and S49 cyt- cells, which do not express Gα (Harris et al., 1985), are also shown. The blot shown is representative of at least three blots for each subunit.

**Figure 4** Isoproterenol competition for 125IICYP binding. Both C10 (a) and A5GR1 (b) cell membranes exhibited low- and high-affinity isoproterenol binding when incubated without guanine nucleotide. When 8 μM GTP was included, C10 had only low-affinity isoproterenol binding sites. In contrast, even 40 μM GTP only minimally reduced isoproterenol affinity in A5GR1 membranes. Membranes were prepared from cells cultured with 10 nM dexamethasone and incubated with 30 pm 125I-ICYP and the indicated concentrations of unlabelled isoproterenol. Data were analysed using the Ligand program (Munson and Rodbard, 1983). Each point is the mean of duplicate determinations from one assay, whereas each line is the best fit from Ligand analysis of the combined data from at least three independent assays, except for A5GR1-ICYP for which two assays were performed (see Table I). Open symbols, minus GTP; closed symbols, + GTP (8 μM for C10, 40 μM for A5GR1).

---

### Table III Summary of competition binding data

| Cell line | [GTP] | K_H (nM) | K_L (nM) | %R_H | n  |
|-----------|-------|----------|----------|-------|----|
| C10       |       | 14 ± 2.6 | 1500 ± 430 | 68 ± 4.1 | 4  |
| A5GR1     | 8 μM  | 12 ± 2.0 | 230 ± 20  | 60 ± 1.5 | 2  |
|           | 40 μM | 30 ± 16  | 330 ± 70  | 48 ± 10 | 3  |

Membranes from cells cultured with 10 nM dexamethasone were incubated with 125I-ICYP, varying isoproterenol concentrations and the GTP concentrations indicated. The K_H for high (K_H) and low (K_L) affinity isoproterenol binding and the percentage of high-affinity sites (%R_H) are reported. Numbers are the mean ± s.e.m. of parameters estimated from Ligand analysis of individual assays. The number of individual assays for each condition is also reported (n).
125I-CYP binding in the presence or absence of GTP. In such assays, β-adrenergic receptors exhibit two affinity states for agonist binding: one of higher affinity (kH) and the other of lower affinity (kL). As demonstrated in Figure 4 and Table III, both C10 and A5GR1 cells exhibit high- and low-affinity isoproterenol binding in the absence of GTP. The presence of high-affinity sites in both cell lines indicates that there is βAR–G, ‘coupling’ in both cell lines (De Lean et al., 1980). However, inclusion of GTP in the incubations is much more effective at reducing high-affinity isoproterenol binding in C10 than in A5GR1 cells, indicating greater functional βAR–G, coupling in the former (Rodbell et al., 1971; Cheung et al., 1989). It is also interesting to note that kL is much greater in C10 than in A5GR1 cells in the absence of GTP (Table III). This high kL is typically observed when βAR–G, coupling is enhanced by glucocorticoid treatment (Davies and Lefkowitz, 1981), and is consistent with the increased isoproterenol responsiveness that was observed in C10 cells cultured with dexamethasone (Table I). The kL for C10 cells (140±22 nM) that were not cultured with dexamethasone is similar to that for A5GR1 cells (230±20 nM).

Guanine nucleotides stimulate adenylyl cyclase more effectively in C10 than in A5GR1 cells

Neither GTP nor the non-hydrolysable analogue 5-guanosyl-di-phosphate [Gpp(NH)p] activated adenylyl cyclase as effectively in A5GR1 cell membranes as in C10 membranes (Figure 5). In fact, GTP at concentrations as high as 100 μM produced no stimulation of adenylyl cyclase above basal in A5GR1 cells. Although Gpp(NH)p was also much less effective in A5GRI than in C10 cells, 15 μM Gpp(NH)p did stimulate adenylyl cyclase approximately 3-fold above basal activity in the former. As adenylyl cyclase was stimulated by Gpp(NH)p to a similar extent in A5GR1 cells that either were or were not cultured with dexamethasone (Figure 5), a defect in G, that is independent of βAR expression is implicated.

Discussion

Mouse lung tumour cells have considerably reduced sensitivity to β-adrenergic stimulation (Droms et al., 1989; Lange-Carter et al., 1992) and this loss of sensitivity involves reduced βAR expression (Lange-Carter et al., 1992). The present work indicates that glucocorticoid treatment of A5GR1 cells, a tumour cell line stably expressing a glucocorticoid receptor transgene, does allow restoration of βAR expression to a level similar to that observed in the non-tumorigenic C10 cells. The newly expressed βAR is capable of high-affinity ternary complex formation with agonist and G, as indicated by the observation that 60% of isoproterenol binding sites are high affinity (Table III). Reduced tumour cell βAR expression is unlikely to result directly from loss of glucocorticoid stimulation, as C10 cells retain a high level of βAR expression even in the absence of glucocorticoids in the culture medium. Thus, although glucocorticoid treatment does restore βAR expression in A5GR1 cells, the cause of reduced expression in tumour cells is unknown.

Mouse lung tumour cells also exhibited reduced guanine nucleotide analogue binding to a 45 kDa membrane- associated protein and enhanced cholera toxin responsive- ness (Droms et al., 1989; Lange-Carter et al., 1992), suggesting the hypothesis that G protein function is altered in the mouse lung tumours. A functional alteration in tumour cell G, could result from the reduced βAR expression rather than from a direct defect in G, itself, as even unoccupied βAR can influence G protein activity (Bond et al., 1995). However, the present study indicates that functional βAR–G, coupling is considerably reduced in A5GR1 cells even when these cells express as many βAR as C10 cells. This lack of functional βAR–G, coupling in A5GR1 cells is indicated by a relative inability of GTP to destabilise the high-affinity ternary complex and is associated with decreased effectiveness of guanine nucleotides to activate adenylyl cyclase. As A5GR1 cells exhibit a decrease in non-hydrolysable guanine nucleotide activation of adenylyl cyclase that is independent of glucocorticoid treatment and, consequently, βAR expression, an independent defect in tumour cell G, is implicated.

One potential mechanism of the reduced ability of guanine nucleotides to activate G, in tumorigenic mouse lung-derived cell lines is that guanine nucleotide exchange is reduced (Droms et al., 1989). The relative inability of GTP to
destabilize the ternary complex in ASGR1 cells is consistent with this hypothesis. Additionally, the difference between C10 and ASGR1 cells in the effectiveness of GTP at activating adenyl cyclase is even greater than the difference between cell lines when a non-hydrolysable analogue is used. Thus, the GTPase activity of G_i may also be enhanced in the tumour cell system. A reduction in the intrinsic guanine nucleotide exchange rate with an increase in the GTPase activity of G_i in tumour cells would be predicted to produce a decrease in basal adenyl cyclase activity, consistent with the 2- to 3-fold lower basal intracellular cAMP and adenyl cyclase activity observed in A5 and ASGR1 cells compared with C10 cells.

Although a clear role for reduced intracellular cAMP in neoplasia has not been established, agonist-stimulated adenyl cyclase activity is reduced in many tumours. One possibility is that cAMP interferes with mitogenic signal transduction, as observed in Rat1 cells (Cook and McCormick, 1993) and human small-cell lung cancer cells (Viallet et al., 1990). Reduced activity of the cAMP-dependent protein kinase is also a requirement for mitogenesis in some cells (Lamb et al., 1991). In addition, cAMP inhibits growth in soft agar of mouse tumour cell lines (Knupe and Schiller, unpublished observation). These associated reductions in cAMP often result from decreases in the number or affinity of receptors coupled to adenyl cyclase activation (Hunt and Martin, 1980). Alternatively, defects in the G-proteins that couple receptors to adenyl cyclase may also occur. For example, reduced GTP-

dependent coupling of adrenergic receptors to adenyl cyclase had been observed in hepatomas (Okamura and Terayama, 1976). As there are differences between C10 and ASGR1 cells in G_i interactions with both the βAR (guanine nucleotide destabilisation of the ternary complex) and adenyl cyclase (guanine nucleotide activation of cAMP production), the structure of one or more of the subunits of G_i may be altered in mouse lung tumour cells. This possibility is currently being investigated.

References

BALLARD PL. (1986). Hormones and lung maturation. Monographs Endocrinol., 28, 24–193.

BARNES PJ, JACOBS MM AND ROBERTS JM. (1984). Glucocorticoids promote the increase of cellular β-adrenoceptors: autoradiographic evidence. Pediatr. Res., 18, 1191–1194.

BENTEL JM, LYKKE AW AND SMITH GJ. (1989). Cloned murine non-malignant, spontaneously transformed and chemical tumour-derived cell lines related to the type 2 pneumocyte. Cell Biol. Int. Rep., 13, 729–738.

BLUMER KJ AND THORNER J. (1990). β and γ subunits of a yeast guanine nucleotide-binding protein are not essential for membrane association of a subunit but are required for receptor coupling. Proc. Natl Acad. Sci. USA, 87, 4363–4367.

BOND RA, LEFF P, JOHNSON TD, MILANO CA, ROCKMAN HA, McMILLAN TR, APPASUNDARAM S, HYKE MF, KENAKIN TK, ALLEN LF AND LEFKOWITZ RJ. (1995). Physiological effects of inverse agonists in transgenic mice with myocardial overexpression of the β2-adrenoceptor. Nat. Med., 1, 274–277.

CHEUNG JH, GOLDFINEN A, BALLARD PL AND ROBERTS JM. (1992). Glucocorticoids increase pulmonary β-adrenergic receptors in fetal rabbit. Endocrinology, 107, 1646–1648.

CHEUNG AH, SICALIS, DIXON RAF AND STRADER CD. (1989). Agonist-promoted sequestration of the β2-adrenergic receptor requires regions involved in functional coupling with G_s. Mol. Pharmacol., 35, 122–138.

COLLINS S, CARON MG AND LEFKOWITZ RJ. (1988). β2-adrenergic receptors in hamster smooth muscle cells are transcriptionally regulated by glucocorticoids. J. Biol. Chem., 263, 9067–9070.

COOK PW, SWANSON KT, EDWARDS CP AND FIRESTONE GL. (1988). Glucocorticoid receptor-dependent inhibition of cellular proliferation in dexamethasone-resistant and hypersensitive rat hepatoma cell variants. Mol. Cell. Biol., 8, 1449–1459.

COOK SJ AND MCCORMICK F. (1993). Inhibition by cAMP of Ras-dependent activation of Raf. Science, 262, 1069–1072.

DAVIES AO AND LEFKOWITZ RJ. (1981). Agonist-promoted high affinity state of the β2-adrenergic receptor in human neurophilic modulation by corticosteroids. J. Clin. Endocrinol. Metab., 53, 703–708.

DAVIES AO AND LEFKOWITZ RJ. (1984). Regulation of β2-adrenergic receptors by steroid hormones. Annu. Rev. Physiol., 46, 119–130.

DE LEAN A, STADELM JM AND LEFKOWITZ RJ. (1980). A ternary complex model explains the agonist-specific binding properties of the adenyl cyclase-coupled β-adrenergic receptor. J. Biol. Chem., 255, 7108–7117.

DOWNS JR AND AURBACH GD. (1982). The effect of forskolin on adenyl cyclase in S49-wild type and C5(–)–cells. J. Cyclic Nucleotide Res., 8, 235–242.

DROMS KA. (1995). Dexamethasone enhances colonization of soft agar by tumorigenic mouse lung-derived cell lines. Cancer Lett., 95, 99–103.

DROMS KA, HALEY BE, SMITH GJ AND MALKINSON AM. (1989). Decreased NPY-12–13GTP photolabeling of G_s in tumorigenic lung epithelial cell lines: association with decreased hormone responsiveness and loss of contact-inhibited growth. Exp. Cell Res., 182, 330–339.

DROMS KA, HANSON LA, MALKINSON AM AND BEER DG. (1993). Altered dexamethasone responsiveness and loss of growth control in tumorigenic mouse lung cell lines. Int. J. Cancer, 53, 1017–1022.

HARRIS BA, ROBISHAW JD, MUMBY SM AND GILMAN AG. (1985). Molecular cloning of complementary DNA for the alpha subunit of the G protein that stimulates adenyl cyclase. Science, 229, 1274–1277.

HUNT NH AND MARTIN TJ. (1980). Hormone receptors and cyclic nucleotides; significance for growth and function of tumors. Mol. Aspects Med., 3, 59–118.

JOHNSON GS AND JAWSKIRCI CJ. (1983). Glucocorticoids increase GTP-dependent adenyl cyclase activity in cultured fibroblasts. Mol. Pharmacol., 23, 648–652.

LAMB NJ, CAVADORE JC, LABBE JC, MAURER RA AND FERNANDEZ A. (1991). Inhibition of cAMP-dependent protein kinase plays a key role in the induction of mitosis and nuclear envelope breakdown in mammalian cells. EMBO J., 10, 1523–1533.

LANGE-CARTER CA, DROMS KA, VULILLEQUEZ JJ AND MALKINSON AM. (1992). Differential responsiveness to agents which stimulate cAMP production in normal versus neoplastic mouse lung epithelial cells. Cancer Lett., 67, 139–144.

LEVITZKI A. (1988). From epinephrine to cyclic AMP. Science, 241, 800–806.

LOWRY OH, ROSEBROUGH NJ, FARR AL AND RANDALL R. (1951). Protein measurement with the Folin phenol reagent. J. Biol. Chem., 193, 265–275.

MUNSON PJ AND ROCKBARD D. (1983) LIGAND: a versatile computerized approach for characterization of ligand binding systems. Anal. Biochem., 107, 220–239.

OKAMURA N AND TERAYAMA H. (1976). Comparison of the epinephrine-mediated activation of adenyl cyclase in plasma membranes from liver and ascites hepatomas of rats. Biochim. Biophys. Acta, 455, 297–314.

Abbreviations

βAR, β-adrenergic receptor; FBS, fetal bovine serum; G_s, stimulatory heterotrimeric guanine nucleotide binding protein; Gpp(NH)P, 5'-guanylylimidodiphosphate; [35]ICYP, (–)351-cyclopentadecyl-3-endo-255,46,708, inverse agonist-promoted fetal rabbit. Adenylyl cyclase activity is reduced in some cells when McCormick, (Viallet et al., 1990). Reduced activity of the cAMP-dependent protein kinase is also a requirement for mitogenesis in some cells (Lamb et al., 1991). In addition, cAMP inhibits growth in soft agar of mouse tumour cell lines (Knupe and Schiller, unpublished observation). These associated reductions in cAMP often result from decreases in the number or affinity of receptors coupled to adenyl cyclase activation (Hunt and Martin, 1980). Alternatively, defects in the G-proteins that couple receptors to adenyl cyclase may also occur. For example, reduced GTP-dependent coupling of adrenergic receptors to adenyl cyclase had been observed in hepatomas (Okamura and Terayama, 1976). As there are differences between C10 and ASGR1 cells in G_i interactions with both the βAR (guanine nucleotide destabilisation of the ternary complex) and adenyl cyclase (guanine nucleotide activation of cAMP production), the structure of one or more of the subunits of G_i may be altered in mouse lung tumour cells. This possibility is currently being investigated.

Acknowledgements

The author thanks Jerry Allen, Kenneth Chen and Erin Eisen for technical assistance and Dr Laurel M Donahue for helpful comments on the manuscript. This work was supported by grants from the Texas Tech University School of Medicine Seed Research Grant Program and the American Heart Association, Texas Affiliate grant no. 93G-330.
RODBELL M, KRANS MJ, POHL SL AND BIRNBAUMER L. (1971). The glucagon-sensitive adenyl cyclase system in plasma membranes of rat liver. IV. Effects of guanyl nucleotides on binding of 125I-glucagon. J. Biol. Chem., 246, 1872–1876.

SAJMON Y. (1979). adenyl cyclase assay. Adv. Cyclic Nucleotide Res., 10, 35–54.

VALVERUS P, HOFFMAN PL AND TABOKOFF B. (1987). Effect of ethanol on mouse cerebral cortical beta-adrenergic receptors. Mol. Pharmacol., 32, 217–222.

VIALLET J, SHARONI Y, FRUCHT H, JENSEN RT, MINNA JD AND SAUSVILLE EA. (1990). Cholera toxin inhibits signal transduction by several mitogens and the in vitro growth of human small-cell lung cancer. J. Clin. Invest., 86, 1904–1912.