Involvement of $G_i\alpha2$ in sodium butyrate-induced erythroblastic differentiation of K562 cells

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The chronic myelogenous leukaemia cell line K562 can be triggered in culture to differentiate along the erythrocytic pathway in response to a variety of stimulatory agents. In the presence of sodium butyrate, these cells differentiate to erythroblasts and acquire the capability to synthesize haemoglobin. We used this cell system to study alterations in the levels of several G-protein subunits during the cell differentiation programme and to assess the involvement of $G_i\alpha2$ in this process. Western immunoblot analysis revealed the presence of $G_s\alpha1$, $G_s\alpha2$, $G_i\alpha2$, $G_q\alpha$, $G\alpha_{12}$, $G\beta1$ and $G\beta2$ in K562 cells. $G_o\alpha$, $G_z\alpha$, $G\alpha_{13}$ and $G\alpha_{16}$ were not detected. Although the levels of several G-protein subunits were altered after treatment with sodium butyrate, the most striking change was the robust increase in the levels of $G_i\alpha2$, which was accompanied by an increase in the mRNA for $G_i\alpha2$. Inactivation of $G_i\alpha2$ by adding *Bordetella pertussis* toxin to the cultures inhibited erythroblastic differentiation by as much as 62 %, as measured by haemoglobin accumulation. Furthermore, the addition of an oligonucleotide anti-sense to $G_i\alpha2$ inhibited the sodium butyrate-induced robust increase in $G_i\alpha2$ levels, decreasing it to the basal levels seen in control cells; this treatment decreased the erythroblastic differentiation of the cells (as measured by haemoglobin expression) by 50 %. Taken together, these findings imply that increased levels of $G_i\alpha2$ contribute to the sodium butyrate-induced erythroblastic differentiation of K562 cells.

Key words: anti-sense oligonucleotides, G-proteins, haemopoietic cell differentiation, pertussis toxin, Western blotting.

INTRODUCTION

Heterotrimeric guanine nucleotide-binding regulatory proteins (G-proteins) have crucial roles in transmembrane signal transduction by coupling agonist-activated G-protein-coupled receptors to specific effectors in the plasma membrane [1–4]. The levels of G-protein subunits in cells have been shown to vary in response to hormones [5–8], neonatal development [9–11] and cell culture conditions [12,13]. However, the magnitude to which the altered level of a G-protein subunit changes the signalling response elicited by that G-protein has yet to be defined. Although the general roles of G-proteins in transmembrane signalling immediately resulting in the generation of specific second messenger(s) are well established, much less is known about their involvement in the programming of more complex cellular processes such as growth and differentiation.

Haemopoietic cell differentiation, in culture, provides an excellent paradigm for studying these parameters. Originally derived from a patient with chronic myelogenous erythroleukaemia [14], the K562 cell line, when cultured in the presence of sodium butyrate, undergoes differentiation to late erythroblasts [15–17] and acquires the capability to synthesize haemoglobin [15,18]. Here we have explored changes in the cellular levels of selected G-protein subunits during the erythrocytic differentiation of K562 cells. We detected robust increases in the levels of $G_i\alpha2$ as K562 cells differentiated to the erythrocytic phenotype. Suppressing this increase resulted in the attenuation of differentiation. The results are consistent with the interpretation that increased levels of $G_i\alpha2$ contribute to the sodium butyrate-induced erythroblastic differentiation of K562 cells.

MATERIALS AND METHODS

Materials

Sodium butyrate, RPMI 1640 and penicillin/streptomycin solutions were purchased from Sigma (St Louis, MO, U.S.A.). Horseradish-peroxidase-conjugated goat anti-rabbit IgG and other electrophoresis reagents were purchased from Bio-Rad (Hercules, CA, U.S.A.). Fetal bovine serum (FBS) and Trizol reagent were obtained from Gibco (Gaithersburg, MD, U.S.A.). *Bordetella pertussis* toxin (PTX) was purchased from List Biological Laboratories (Campbell, CA, U.S.A.). Anti-sense oligonucleotides were purchased from Operon Technologies (Alameda, CA, U.S.A.). [γ-^{32}P]ATP and enhanced chemiluminescence reagent were purchased from NEN Research Products (Boston, MA, U.S.A.). PVDF membranes (Immobilon-P) were products of Millipore Corp. (Bedford, MA, U.S.A.). Except for antibodies to $G\beta1$ and $G\beta2$ (Santa Cruz Biotechnology, Santa Cruz, CA, U.S.A.), all antibodies used in this study were raised against synthetic peptides and purified by affinity chromatography by using peptide-conjugated Sepharose columns [19]. All other chemicals and enzymes were obtained from commercial sources.

Abbreviations used: FBS, fetal bovine serum; G-proteins, heterotrimeric guanine-nucleotide-binding regulatory proteins; $G\beta1$ and $G\beta2$, isoforms of the β subunit of heterotrimeric G-proteins; $G_i\alpha2$ and $G_i\alpha3$, isoforms of the α subunit of the G-protein that mediates the inhibition of adenylate cyclase; G_o, G-protein involved in K^+ and Ca^{2+} channel regulation; $G_q\alpha$ and $G\alpha_{16}$, G-protein subunits involved in the stimulation of phosphoinositol-specific phospholipase C; $G_s\alpha1$ and $G_s\alpha2$, the isoforms of the α subunit of the G-protein that mediates the activation of adenylate cyclase; $G\alpha_{12}$, $G\alpha_{13}$ and $G_z\alpha$, G-protein subunits of unclear function; PTX, *Bordetella pertussis* toxin.

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Cell culture

The K562 cell line was obtained from the American Type Culture Collection (Manassas, VA, U.S.A.) and maintained at 37 °C in RPMI 1640 medium supplemented with 10 % (v/v) heat-inactivated FBS, 50 i.u. of penicillin and 50 μg of streptomycin/ml of medium, in a humidified air/CO_2 (19:1) atmosphere. Solutions of sodium butyrate were made fresh for each experiment by dissolving sodium butyrate in the culture medium. All additions to the cultures were made 3 days after the initial seeding; at this time, the cells were in exponential phase. When used, anti-sense, sense or random-sequence oligonucleotide was dissolved in PBS and added to serum-free RPMI 1640 medium to attain a final concentration of 30 μM.

Preparation of particulate fractions and immunoblotting analysis

K562 cells were harvested by low-speed (1000 ***g***) centrifugation; the resulting pellet was resuspended and homogenized in homogenization buffer [10 mM Tris/HCl (pH 7.5)/1 mM EDTA/0.1 mM PMSF]. The homogenate was centrifuged at 800 ***g*** for 10 min; the resulting supernatant solution was stored on ice. The pellet was resuspended in the same buffer and the homogenization was repeated. The two supernatant solutions were combined and centrifuged at 60000 ***g*** for 60 min. The pellet was resuspended in the homogenization buffer and re-centrifuged. The resulting pellet, containing particulate fractions, was resuspended in homogenization buffer. The protein content of this preparation was determined by the method of Lowry et al. [20]. All Western blotting analyses were done in conditions under which the signal generated was proportional to the amount of protein sample used; this should permit predictions to be made about the relative abundances of G-proteins in different samples. For each sample, 10 μg of proteins were subjected to SDS/PAGE [with a 3 % (w/v) stacking gel and a 10 % (w/v) separating gel] with a Bio-Rad mini-gel system and transferred for 30 min on Immobilon-P membranes at 40 V in a transfer buffer [25 mM Tris/192 mM glycine (pH 8.3)]. A 5 % (w/v) solution of non-fat dried milk in buffer [20 mM Tris/HCl (pH 7.5)/500 mM NaCl/0.05 % (v/v) Tween 20] was used as a blocking agent. Antibodies against G-protein subunits were diluted in 3 % (w/v) non-fat dried milk in the same buffer. Affinity-purified horseradish-peroxidase-conjugated goat anti-rabbit IgG (dilution 1:3000) was used as the secondary antibody. Detection was enhanced by chemiluminescence in accordance with the manufacturer's protocol (NEN Research Products).

Northern blot analysis

Total cellular RNA was obtained by lysing the cells in a monophasic solution of phenol and guanidine isothiocyanate (TRIzol reagent) by following the manufacturer's instructions (Gibco). To determine the integrity of the preparation, 1–2 μg of the RNA samples was subjected to electrophoresis on 1 % (w/v) agarose gels, stained with ethidium bromide and compared with the 28 S and 18 S ribosomal RNA bands. RNA samples (20 μg) were subsequently separated on a 1 % (w/v) agarose/2.2 M formaldehyde gel and transferred to Nytran membranes (0.2 μm pore size) by downward alkaline transfer for 3 h with the Turboblotter rapid downward transfer system in accordance with the manufacturer's protocol (Schleicher and Schuell). The membranes were dried at 80 °C for 30 min and then prehybridized for 4 h at 42 °C in a solution containing 30 % (v/v) formamide, 10 × Denhardt's reagent (1 × Denhardt's reagent is 0.02 % Ficoll 400/0.02 % polyvinylpyrrolidone/0.002 % BSA), 5 × SSPE [1 × SSPE is 0.15 M NaCl/10 mM sodium phosphate (pH 7.4)/1 mM EDTA], 100 μg/ml salmon testes DNA and 0.5 % SDS. Hybridization was performed in the same solution with approx. 10^6 c.p.m./ml ^{32}P-labelled $G_i\alpha 2$-specific oligonucleotide probe [7] for 18 h at 42 °C. The membranes were washed twice in 2 × SSC/0.1 % SDS [1 × SSC is 0.15 M NaCl/0.015 M sodium citrate (pH 7.0)] and once in 1 × SSC/0.1 % SDS. Membranes were developed and revealed by using a Packard Cyclone phosphorimaging system.

Haemoglobin determination

The haemoglobin content of cells was measured by the method of Mivechi et al. [16]. In brief, cells were lysed in a solution of 0.81 % NaCl/0.12 % Tris/HCl/0.03 % magnesium acetate/0.5 % Nonidet P40. After centrifugation, the lysate was scanned spectrophotometrically at 400–500 nm. The baseline absorbance (at 425 nm) was subtracted from the peak absorbance (at 414 nm) to correct for non-specific absorbance. An A_{414} of 1.0 corresponds to 0.13 mg of haemoglobin per ml of solution [21].

Statistical analysis

Statistical analyses were performed with the RS/1 comprehensive data analysis program (BBN Software Products, Cambridge, MA, U.S.A.).

RESULTS

G-protein subunits in K562 cells and altered levels during differentiation

The K562 cell line, originally developed by Lozzio and Lozzio [14], is a commonly used model system for investigating cellular and molecular events involved in cell proliferation and differentiation [18,22,23]. Because there are no reports detailing the diversity of G-proteins in K562 cells, our initial experiments were directed at establishing the repertoire of G-proteins contained in these cells by Western blotting with antibodies specific for individual G-protein subunits. Of the 12 G-protein subunits surveyed, strong immunological signals were observed for $G_i\alpha 2$, $G_s\alpha 1$, $G_q\alpha$ and $G\beta 2$. $G\alpha_{12}$ and $G\beta 1$ were also detected. $G_i\alpha 3$, $G\alpha_{13}$, $G\alpha_{16}$, $G_o\alpha$ and $G_z\alpha$ were not detected in these cells, although they were detected by using these antibodies in U937 and HL-60 cells (results not shown).

To discern whether the expression of any of these G-proteins was altered during differentiation of the cells, we immunoblotted for several G-protein subunits in cells cultured in the presence of sodium butyrate. It has previously been demonstrated that in the presence of sodium butyrate the K562 cell line differentiates to an erythrocytic phenotype [15–17] capable of expressing haemoglobin [15,18]. Figure 1(A) shows the proliferation pattern of these cells when cultured in the presence of different concentrations of sodium butyrate, up to 10 mM. Although sodium butyrate induces cell differentiation in K562 cells, it is also known to be inhibitory to cell growth [23,24], presumably because fewer cells divide as the population of differentiating cells increases. This inhibition is evident in Figure 1(A). At 10 mM, sodium butyrate was cytotoxic to the cells; this concentration was therefore not used in subsequent experiments. Figure 1(B) confirms that sodium butyrate induced the differentiation of the cells used in this study, as evident from the expression of haemoglobin, a marker for the erythrocytic phenotype. In the absence of sodium butyrate, the cells did not synthesize haemoglobin beyond basal levels.

Treatment with sodium butyrate resulted in differential effects on the expressed levels of the various G-protein subunits

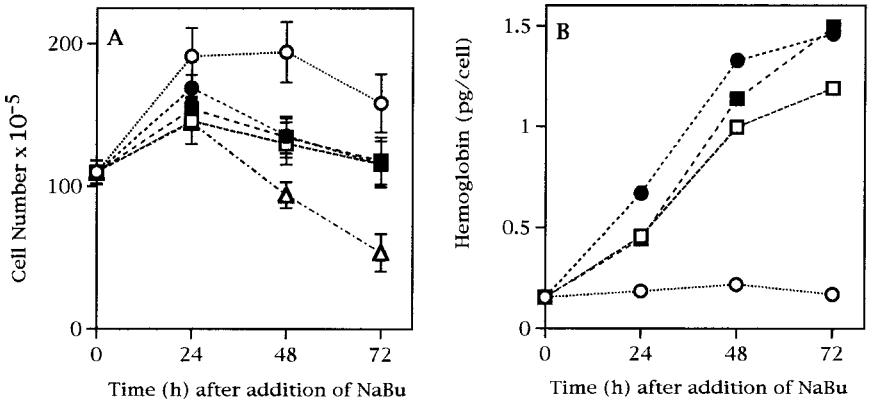


Figure 1 Proliferation patterns and haemoglobin content of K562 cells cultured with various concentrations of sodium butyrate (NaBu)

Approx. 10^6 K562 cells were seeded in flasks containing RPMI 1640 medium supplemented with 10% (v/v) FBS, 50 i.u./ml penicillin and 50 μg/ml streptomycin, and left to proliferate at 37 °C under air/CO_2 (19:1) for 3 days. The medium was then changed to include various concentrations of sodium butyrate. Cell viability was determined by the Trypan Blue dye exclusion assay. (**A**) Number of viable cells in each culture. The results are means ± S.E.M. for three experiments. (**B**) Effect of sodium butyrate on haemoglobin expression. At the time points indicated, cells were removed from the flasks and washed twice in PBS. Haemoglobin accumulation was determined as described in the Materials and methods section. The results shown are means of two experiments. Symbols: ○, control; ●, 1 mM sodium butyrate; □, 2.5 mM sodium butyrate; ■, 5 mM sodium butyrate; △, 10 mM sodium butyrate.

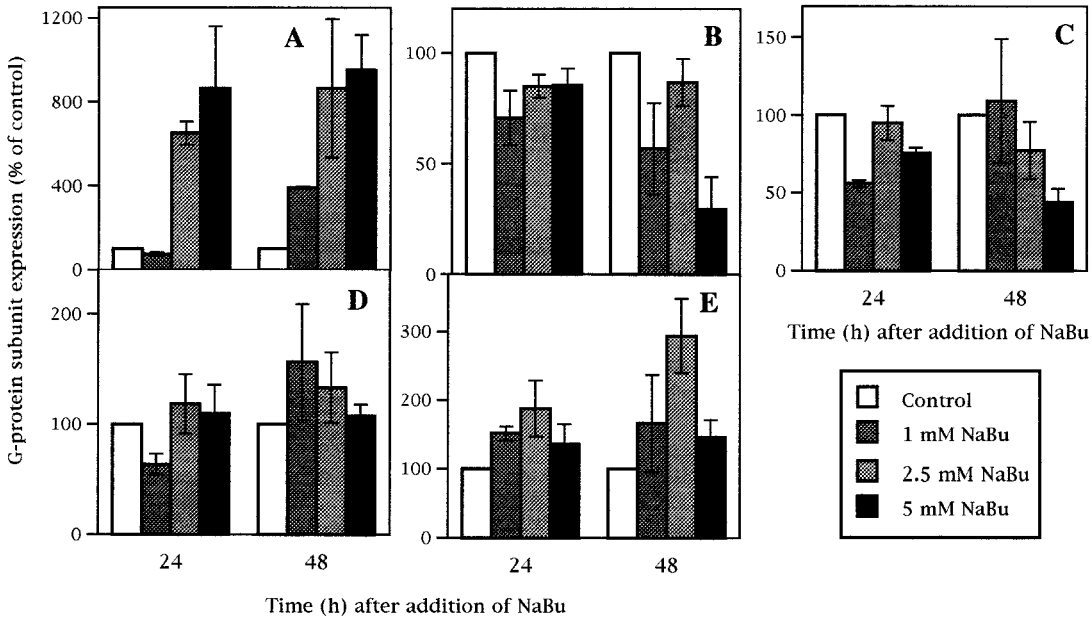


Figure 2 Effect of various concentrations of sodium butyrate (NaBu) on G-protein subunit levels in K562 cells

At the time points indicated, G-protein subunits were immunoblotted from K562 cells grown in the presence or absence of sodium butyrate, with 10 μg of protein per sample. (**A**) $G_i\alpha 2$; (**B**) $G_s\alpha 1$; (**C**) $G_s\alpha 2$; (**D**) $G_q\alpha$; (**E**) $G\beta 2$. The intensities of the immunostained G-protein subunit bands were measured by using an UN-SCAN-IT program available from Silk Scientific (Salt Lake City, UT, U.S.A.). The results are expressed as percentages of G-protein subunit expression in the control cultures (without sodium butyrate). Each assay was done in duplicate. Error bars are means ± S.E.M. for three cell cultures.

measured, the most robust effect being a 9-fold increase in the levels of $G_i\alpha 2$ within 48 h of treatment with 5 mM sodium butyrate (Figure 2A). This increase was sustained beyond 48 h (results not shown). This increase was statistically significant ($P < 0.05$). Lesser increases were observed for $G_q\alpha$ (Figure 2D) and $G\beta 2$ (Figure 2E), although these increases were also statistically significant. In contrast, the levels of $G_s\alpha 1$ and $G_s\alpha 2$ were either unchanged (24 h) or decreased (48 h) in cells treated with 5 mM sodium butyrate. The fact that cell proliferation was inhibited to approximately the same extent with 1–5 mM sodium butyrate (Figure 1A) indicates that the observed alterations in G-protein subunit levels are not a consequence of the inhibited cell proliferation. Because maximal changes in G-protein subunit levels were obtained with 5 mM sodium butyrate, subsequent experiments were performed with this concentration of sodium butyrate. Because of the significantly ($P < 0.05$) robust increase in the levels of $G_i\alpha 2$, a time-course study of this increase was undertaken. As shown in Figure 3, there was little change in $G_i\alpha 2$ expression before 12 h but a marked increase occurred after this time point. Furthermore, with 2.5 and 5 mM sodium butyrate,

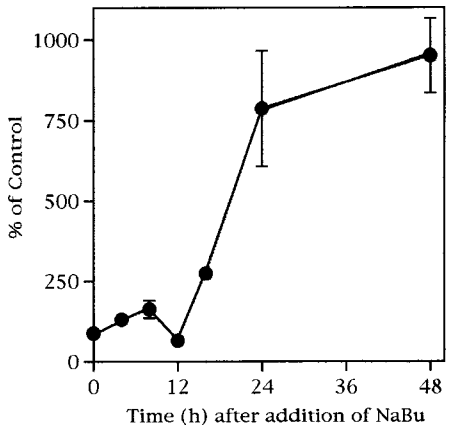
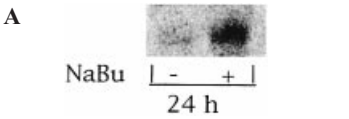


Figure 3 Time course of induction of $G_i\alpha2$ in sodium butyrate (NaBu)-treated K562 cells

K562 cells were grown with or without 5 mM sodium butyrate for the indicated durations. $G_i\alpha2$ levels were determined as described in the legend to Figure 2. Results are expressed as percentages of $G_i\alpha2$ expression in each control sample and are means ± S.E.M. for triplicate determinations of two different cell cultures.



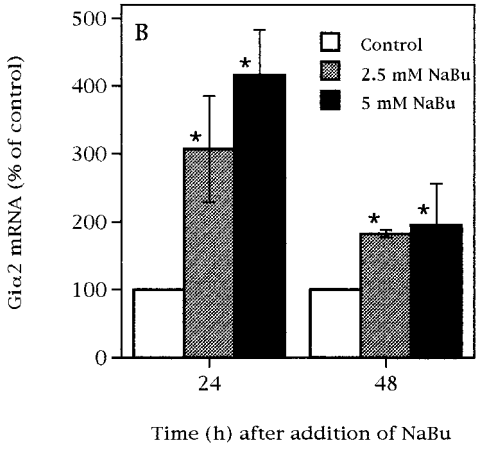


Figure 4 Up-regulation of $G_i\alpha2$ mRNA by sodium butyrate (NaBu) in K562 cells

The cells were grown as described in the legend to Figure 1. Total RNA was isolated from the cells at 24 h intervals after the addition of sodium butyrate. $G_i\alpha2$ mRNA was measured by Northern blotting by using a ^{32}P-labelled oligonucleotide probe specific for $G_i\alpha2$. (**A**) A representative Northern blot from cells grown for 24 h in the presence of 5 mM sodium butyrate. A Packard Cyclone Storage Phosphorimaging System was used for this analysis. (**B**) Quantification of Northern blots by densitometric measurements of autoradiograms. The results shown are means ± S.E.M. for duplicate determinations of two different RNA preparations. *Significantly different ($P < 0.05$) from the control cells.

the sodium butyrate-induced increase in the expression of $G_i\alpha2$ was concurrent with a 3–4-fold increase in mRNA levels for this G-protein subunit at 24 h, as measured by Northern blotting

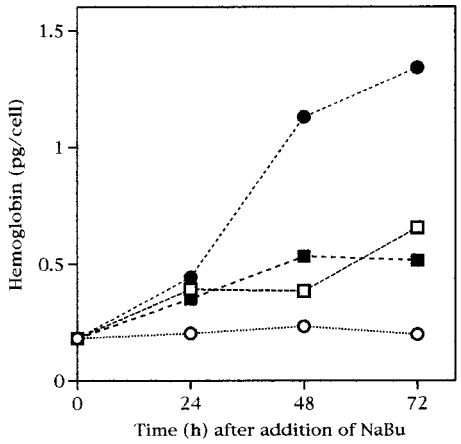


Figure 5 Inhibition of sodium butyrate (NaBu)-induced differentiation in K562 cells by PTX

The cells were seeded as described in the legend to Figure 1. After 3 days the medium was removed and replaced by fresh medium or medium containing 5 mM sodium butyrate with or without PTX (50 ng/ml). Haemoglobin accumulation was measured as described in the legend to Figure 1(B). The results shown are the means of two experiments. Symbols used: ○, Control; ●, 5 mM sodium butyrate; □, 5 mM sodium butyrate plus PTX (PTX was added at 0 h); ■, 5 mM sodium butyrate plus PTX (PTX was added 12 h before sodium butyrate, and again at 0 h).

(Figure 4). This increase in mRNA level was statistically significant ($P < 0.05$).

Relationship of sodium butyrate-induced increase in $G_i\alpha2$ to the differentiation of K562 cells

Because the levels of $G_i\alpha2$ increased as the cells differentiated, it was important to ascertain whether the differentiation event itself required the increase in the expression of this G-protein subunit. We reasoned that if $G_i\alpha2$ were an essential component of the differentiation programme in these cells, then the differentiation response would be inhibited when the expression or the function of $G_i\alpha2$ was interfered with. We therefore tested the effect of PTX on sodium butyrate-induced cell differentiation because PTX is known to ADP-ribosylate, and thus inactivate, the α subunits of the G_i family of G-proteins [25]. Figure 5 shows that treating the cells with PTX drastically inhibited haemoglobin expression, irrespective of when the toxin was added to the culture medium. For example, in cells which received PTX at 0 h and again at 48 h after the addition of sodium butyrate, haemoglobin expression (indicative of erythroid differentiation) was inhibited by 62% at 72 h after treatment with sodium butyrate. These results indicate that a PTX-sensitive substrate is involved in the sodium butyrate-induced differentiation of K562 cells. Of the PTX-sensitive G-protein subunits ($G_i\alpha1$, $G_i\alpha2$, $G_i\alpha3$ and $G_o\alpha$), we did not detect $G_i\alpha3$ or $G_o\alpha$ in K562 cells. $G_i\alpha1$ is reported to be present predominantly in neural tissue [1,26]; it therefore seems that the major PTX-sensitive substrate in the K562 cells is $G_i\alpha2$, thus implicating $G_i\alpha2$ in the erythroblastic differentiation process examined in Figure 5.

To confirm this implication, an anti-sense oligonucleotide (5′-CATCCCGCCGTCCGCCGGCC-3′) was used to directly block the formation of $G_i\alpha2$. This 20-mer is anti-sense to a unique sequence in the human $G_i\alpha2$ gene [27]. The anti-sense oligo was added at a final concentration of 30 μM, 24 h before the addition of sodium butyrate. The sodium butyrate-induced increase in the levels of $G_i\alpha2$ was almost completely abolished in cells treated with the anti-sense oligonucleotide and was decreased to the

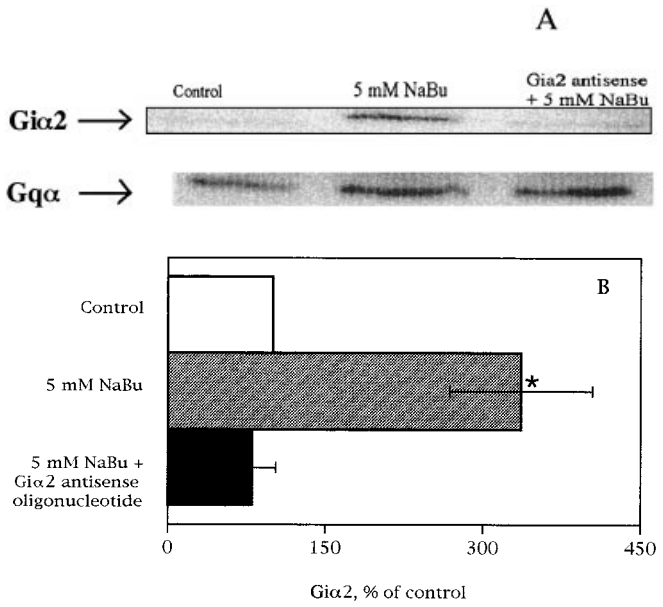


Figure 6 Inhibition of $G_i\alpha2$ expression in K562 cells by $G_i\alpha2$ anti-sense oligonucleotide

Approx. 5×10^5 K562 cells were seeded in three culture flasks and left to proliferate for 72 h in RPMI 1640 medium supplemented with 10% (v/v) FBS and antibiotics. The medium was then removed and the cells were subsequently grown in 1 ml of serum-free medium with or without 30 μM anti-sense oligonucleotide (5′-CATCCCGCCGTCCGCCCGGCC-3′) for 30 min. FBS was then added to 10% (v/v) and the cells were cultured for 24 h, after which 5 mM sodium butyrate (NaBu) was added and the cells were left in culture for a further 24 h. G-protein subunits in cells from each culture were immunoblotted as described in the legend to Figure 2. (**A**) Representative Western immunoblots. (**B**) Densitometric quantification of $G_i\alpha2$ detected on Western blots. The results are means ± S.E.M. for four experiments. *Significantly different ($P < 0.05$) from the untreated control samples.

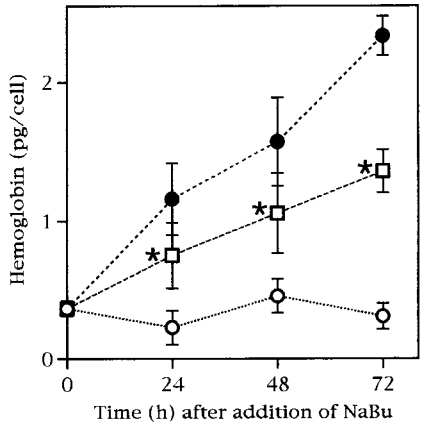


Figure 7 Effect of $G_i\alpha2$ anti-sense oligonucleotide on haemoglobin synthesis in K562 cells grown in the presence of sodium butyrate (NaBu)

Cell culture conditions were the same as those described in the legend to Figure 6. At each time point, haemoglobin was measured as described in the legend to Figure 1(B). Results are means ± S.E.M. for four experiments. Symbols: ○, control; ●, sodium butyrate; □, sodium butyrate plus $G_i\alpha2$ anti-sense oligonucleotide. *Significantly different ($P < 0.05$) from the sodium butyrate-treated samples.

basal levels detected in controls (Figure 6); the expression of other G-proteins, e.g. $G_q\alpha$ (Figure 6A), was not affected, demonstrating that the anti-sense oligonucleotide specifically targeted $G_i\alpha2$. The addition of anti-sense oligonucleotides to the cultures was not detrimental to cell growth, as judged by the lack of effect on cell proliferation in the cultures (results not shown).

The effect of the anti-sense oligonucleotide on cell differentiation was monitored for up to 3 days after the addition of sodium butyrate, by measuring the expression of haemoglobin. Figure 7 shows that the anti-sense treatment decreased haemoglobin expression to approx. 50% of that seen in sodium butyrate-treated control cells; the extent of inhibition at 72 h (51%) was similar to that at 24 h (50%) after the addition of sodium butyrate. Control experiments with either a 20-mer sense oligonucleotide (5′-GTAGGGCGGCAGGCGGCCGG-3′) or a 20-mer random sequence oligonucleotide (5′-GTACTTGCCC-TAAGCCGGCA-3′) had no effect on either $G_i\alpha2$ levels or haemoglobin expression. These results complement those obtained by using PTX to inactivate $G_i\alpha2$. Taken together, the results suggest that the increase in $G_i\alpha2$ is pivotal for the erythroblastic differentiation of K562 cells induced by sodium butyrate; this finding is striking, considering the number of other proteins and factors that must be involved in the complex programme of cell differentiation.

DISCUSSION

The extent to which global alterations in G-protein levels or alterations in the levels of a single G-protein change the magnitude of the associated cellular response has not previously been delineated. The potential association of specific G-protein subunits with cellular differentiation, a more complex biological end-point than the traditionally known G-protein signalling resulting in the formation of specific second messengers, is also poorly defined. Here we have studied the alterations in the levels of several G-protein subunits during the differentiation of K562 cells. Two primary observations were established. First, of the G-protein subunits measured, the levels of $G_i\alpha2$ exhibited the most robust change, increasing up to 900% during the differentiation model used; this increase was associated with an increase in mRNA for $G_i\alpha2$. Secondly, the association of the increased levels of $G_i\alpha2$ with the sodium butyrate-induced cell differentiation was established by inactivating $G_i\alpha2$ with PTX and by using an anti-sense oligonucleotide to block its expression. Both approaches resulted in an inhibition of the sodium butyrate-induced increase in the levels of $G_i\alpha2$ as well as in the sodium butyrate-induced cell differentiation. Taken together, these results indicate that the altered level of $G_i\alpha2$ can influence the magnitude of an associated cellular response; they also suggest that increased levels of $G_i\alpha2$ might have a role in the sodium butyrate-induced differentiation of K562 cells. Although it is unclear to what extent a lack of $G_i\alpha2$ function can be compensated for by other members of the G_i family (i.e. $G_i\alpha1$ and/or $G_i\alpha3$), it is important to note that $G_i\alpha2$ was the only G_i family member detected in our Western immunoblotting assays. This was not surprising, because $G_i\alpha1$ and the isoforms of $G_o\alpha$ are primarily restricted to neuronal tissues [1,26]. The other members of the G_i family (i.e. gustducin and transducin) are expressed specifically in taste and retinal rod cells, which are not haemopoietic [28,29].

The demonstrated involvement of $G_i\alpha2$ in the differentiation of K562 cells raises curiosity about novel role(s) of G-protein subunits in cellular physiology. It has previously been reported that the suppression of $G_i\alpha2$ in fat and liver of transgenic mice expressing a $G_i\alpha2$ anti-sense construct decreases neonatal growth by more than 30%, inhibits glucose tolerance, and induces moderate insulin resistance *in vivo* [30,31]. Murine embryonic $G_i\alpha2$-null cell lines have been reported to maintain the ability to

regulate adenylate cyclase and to differentiate in a manner similar to wild-type embryonic stem cells [32]. Elevated levels of $G_i\alpha2$ suppress the retinoic acid-induced differentiation of F9 teratocarcinoma cells to the primitive endoderm [5]. The inhibitory effect of $G_i\alpha2$ on cellular differentiation in these circumstances sharply contrasts with the findings in this study. For example, the sodium butyrate-induced differentiation in K562 cells occurs in the presence of elevated levels of $G_i\alpha2$; attenuated levels of $G_i\alpha2$ inhibit this differentiation (Figures 6 and 7). This is not true of F9 stem cells, where the levels of $G_i\alpha2$ decrease as the cells are induced to differentiate [5]. This indicates that $G_i\alpha2$ performs different roles related to the growth, development and differentiation of different cell types. Whether similar roles might be performed by other G-proteins of the G_i family requires further study.

The involvement of $G_i\alpha2$ in the sodium butyrate-induced differentiation of K562 cells uncovered in this study evokes comparisons with effects of other G-protein α subunits in other cell systems. For example, $G_s\alpha$ has been shown to suppress the dexamethasone-induced differentiation of 3T3-L1 cells, a suppression that is negated when the cells are treated with oligonucleotides that are anti-sense to $G_s\alpha$ [33]. A comparable role for $G\alpha_{12}$ and $G\alpha_{13}$ in the retinoic acid-mediated differentiation of these cells has also been proposed [34,35]. Strikingly, during the DMSO-induced neutrophilic differentiation of human myeloid HL-60 cells, the expression of the haemopoietic cell-specific G-protein subunit $G\alpha_{16}$ is decreased by 90%, whereas the expression of $G_i\alpha2$ is increased by 160% [36,37]. These divergent findings provide added support to the conclusion that G-protein subunits can influence cell differentiation in different ways, depending on the cell type.

The molecular mechanism or mechanisms by which changes in G-protein subunit levels alter the degree of cell differentiation are at present unknown. Conventional wisdom of $G_i\alpha2$-mediated cell signalling would suggest changes in the intracellular levels of cAMP, in that alterations in the activity of this G-protein subunit can affect the dynamics of cAMP in cells. In the cell differentiation model used in this study, we observed a transient increase (within 1 h) in the levels of cAMP after the addition of sodium butyrate to the culture medium (results not shown); however, the relationship of this transient increase in cAMP levels to the overall differentiation programme is not immediately obvious. In the F9 teratocarcinoma cell system, the inhibitory effect of $G_i\alpha2$ is independent of changes in the amount of cAMP [5]. In a recent paper on the $G_s\alpha$-induced suppression of adipogenesis in 3T3-L1 cells, Liu et al. [38] present excellent data suggesting that changes in cAMP levels might not be involved because the mutagenesis of contact points on $G_s\alpha$ needed for interaction with adenylate cyclase does not impede the $G_s\alpha$-mediated inhibitory effect on cell differentiation. Because alterations in the levels of the α subunit of a G-protein can affect the dynamics of the $\beta\gamma$ component, it might be worthwhile to direct future studies at assessing alterations in downstream components that may be triggered by changes in $\beta\gamma$.

The molecular mechanism(s) by which sodium butyrate induces the late erythroblastic differentiation of K562 cells and the mechanism by which sodium butyrate selectively alters gene expression are currently unknown. However, several reported findings seem pertinent to this question. For example, it is well documented that treatment of cultured cells with millimolar amounts of sodium butyrate stimulates histone hyperacetylation, a phenomenon resulting from the butyrate-induced inhibition of histone deacetylase [39]. Hyperacetylation of histones promotes the relaxation of the chromatin structure and generates negatively supercoiled DNA *in vivo* [40] and *in vitro* [41], making DNA more or less accessible to transcription factors for genes related to cell growth and differentiation [42,43]. In rectal carcinoma cells, early effects of sodium butyrate on cells include a suppression of *p53* and c-*myc* (which is thought to maintain cells in the undifferentiated state) [44]. On the contrary, c-*fos* and c-*jun* gene expressions are elevated in sodium butyrate-treated NIH 3T3 and hepatoma tissue culture cells [45,46]. Velazquez et al. [47] have demonstrated a sodium butyrate-induced increase in the levels of c-Jun, but not c-Fos proteins *in vivo*. However, sodium butyrate does not alter the expression of either c-*fos* or c-*myc* in vascular smooth muscle cells [48]. Other studies have also noted changes (within minutes) in the activation of protein kinase C and other proteins in the tyrosine kinase signalling cascade in sodium butyrate-treated K562 cells [49]. Taken together, these studies suggest that the mechanism by which sodium butyrate alters cell physiology might include modulation of specific gene expression.

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