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Differential regulation of amounts of the guanine-nucleotide-binding proteins G_i and G_o in neuroblastoma × glioma hybrid cells in response to dibutyryl cyclic AMP

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Incubation of the neuroblastoma × glioma hybrid cell line NG108-15 in tissue culture with dibutyryl cyclic AMP (1 mM) for up to 8 days produced a morphological differentiation of the cells, during which they extended neurite-like processes. Pertussis-toxin-catalysed ADP-ribosylation indicated that amounts of guanine-nucleotide-binding proteins (G-proteins), which are substrates for this toxin, were approximately doubled in membranes from the 'differentiated' cells in comparison with the control cells. Immunoblotting of membranes derived from either untreated or dibutyryl cyclic AMP-treated cells with anti-peptide antisera specific for the α subunits of the pertussis-toxin-sensitive G-proteins G_i and G_o demonstrated that amounts of these G-proteins were reciprocally modulated during the differentiation process. In comparison with the untreated cells, the amount of G_i in the 'differentiated' cells was decreased, whereas the amount of G_o was substantially increased. Stimulation of high-affinity GTPase activity in response to opioid peptides, which in this cell line interact with an opioid receptor of the δ subclass, was much decreased, and inhibition of adenylate cyclase activity was almost entirely attenuated in the 'differentiated'-cell membranes in comparison with membranes of untreated cells. Opioid receptor number was also decreased in membranes of the dibutyryl cyclic AMP-treated cells in comparison with the control cells. These data demonstrate that relatively small changes in the observed pattern of pertussis-toxin-catalysed ADP-ribosylation of membranes can mask more dramatic alterations in amounts of the individual pertussis-toxin-sensitive G-proteins, and further demonstrate the importance of methodologies able to discriminate between the different gene products.

INTRODUCTION

Of a family of guanine-nucleotide-binding proteins (G-proteins) which function in the transduction of information from agonist-stimulated receptors to second-messenger generation systems [1], a subfamily are substrates for mono-ADP-ribosylation catalysed by pertussis toxin [2,3]. Although it was originally assumed that the inhibitory G-protein of the adenylate cyclase system (G_i) was the sole substrate for pertussis toxin [4,5], improved protein-purification protocols [6,7] and analysis of cDNA clones [8–10] have indicated that, besides G_i, both G_o and other 'G_i-like' proteins can be expressed in various tissues [11–13]. All of these pertussis-toxin-sensitive G-proteins are heterotrimers in which unique but highly homologous α subunits interact with similar β/γ subunits. As the α subunit of each of these proteins is of apparent molecular mass between 39 and 41 kDa, it is essentially impossible to resolve adequately and identify individual members of this family by using denaturing gel electrophoresis based solely on pertussis-toxin-catalysed ADP-ribosylation. With this in mind, we have generated a series of selective polyclonal antisera against either purified or partially purified G-proteins [14–16], or more recently against peptide sequences predicted from cDNA clones to be present in different members of this family of proteins [17–19].

On the basis of the high degree of homology between the different G-proteins, it is of particular importance and interest to attempt to define the specificity, or otherwise, of interactions of particular receptors with these G-proteins in systems in which more than one type of pertussis-toxin-sensitive G-protein is expressed. One possible approach to this problem might be to manipulate systems in such a fashion that the concentrations of the different G-proteins are modified. The neuroblastoma × glioma hybrid cell line NG108-15 has previously been shown to express both G_i and G_o [11]. In the present paper we demonstrate that morphological differentiation of these cells caused by exposure to dibutyryl cyclic AMP is coincident with reciprocally altered amounts of the α subunits of G_i and G_o. The magnitude of responsiveness of membranes of these cells to opioid peptides was decreased in the differentiated state in comparison with the control cells. In these conditions, the amounts of the α subunit of G_i in the membrane was decreased to some 30% of that in the control cells, whereas the amount of the α subunit of

Abbreviation used: G-protein, guanine-nucleotide-binding protein.

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G_o was increased to some 430% of the amount of G_o in the control cells. Modulation of the amounts of these proteins occurred throughout the differentiation process.

MATERIALS AND METHODS

Materials

The sources of all materials in this study for cell growth and for the immunoblotting experiments have previously been recorded [11,12,15,18], except that N^6,$O^{2'}$-dibutyryl cyclic AMP was purchased from Boehringer-Mannheim. [^{32}P]NAD$^+$ was purchased from Dupont/New England Nuclear, [γ-^{32}P]GTP was from Amersham International, and pertussis toxin was obtained from Porton Products, Porton Down, Wiltshire, U.K. All other chemicals were purchased from either Sigma or Boehringer-Mannheim and were of the highest quality available.

Antibody production and characterization

Production of antiserum AS7 has previously been detailed [20]. Briefly, the antiserum was raised in a rabbit against a conjugate of a synthetic peptide corresponding to the *C*-terminal decapeptide of the α subunit of rod transducin (KENLKDCGLF), which was coupled to keyhole-limpet haemocyanin with glutaraldehyde. As well as transducin, this antiserum recognizes the various forms of 'G_i' [16–20]. Antiserum IM1 was produced by a similar strategy after coupling the synthetic peptide NLKEDGISAAKDVK (amino acids 22–35 of the α subunit of G_o) to keyhole-limpet haemocyanin [21]. The specificity of this antiserum for G_o was demonstrated by antisera-mixing experiments and immunoblotting against purified G_i and G_o from bovine brain, as first shown by us for other selective antisera [15] (see also Fig. 3 and the text). Antiserum OC1 was produced by the same means against the *C*-terminal decapeptide of the α subunit of G_o (ANNLRGCGLY). This antisera also shows specificity for the α subunit of G_o and displays no cross-reactivity with forms of G_i [22]. Antiserum RV6 was raised by immunization of a rabbit with a mixture of the holomeric forms of pertussis-toxin-sensitive G-proteins from bovine brain. Antibodies in this antiserum recognize the β subunit of G-proteins, but no antibodies against G-protein α subunits were produced [15]. Antiserum HPA was raised in a similar manner to antiserum RV6, but contains populations of antibodies directed against the α subunit of G_o and against the β subunit. In this regard it resembles antiserum RV3, which we have extensively characterized previously [15]. The specificity of antiserum HPA was assessed as described above for all the other antisera used in this study. Antiserum HPA was kindly given by Dr. H. Ploegh and Dr. L. van der Voorn (Division of Cellular Biochemistry, Netherlands Cancer Institute, Amsterdam, The Netherlands).

Tissue culture

Control NG108-15 cells were grown in Dulbecco's Modified Eagle's Medium, which was supplemented to 10% (v/v) with foetal bovine serum (Imperial Laboratories). This medium was further supplemented with hypoxanthine, aminopterin, thymidine and 100 units of both penicillin and streptomycin/ml, as previously described [11,23]. 'Differentiated' cells were produced by splitting a sub-confluent culture of control cells 1:5 into fresh tissue-culture flasks and growing the cells for up to 8 days in medium containing all the above components, except for only 2% foetal bovine serum and with the addition of 1 mM-dibutyryl cyclic AMP. Both sets of cells were harvested identically and membranes were prepared from them as previously described [23]. In several cases cells were also grown in the presence of only 2% foetal bovine serum but in the absence of dibutyryl cyclic AMP.

Gel electrophoresis

SDS/polyacrylamide-gel electrophoresis [10% (w/v) acrylamide] was performed as described in ref. [24]. Pertussis-toxin- and cholera-toxin-catalysed ADP-ribosylation were performed in the presence of GTP as described in ref. [25]. Autoradiograms derived from the dried gels were produced by using Kodak X-O-Mat X-ray film. In each case the autoradiogram so produced was used as a template to locate the radiolabelled polypeptides, and the relevant areas of the gel were excised and counted for radioactivity [25]. Immunoblotting with the various selective antisera was performed as detailed in ref. [18]. Dilutions of the primary antisera were routinely 1:200, except for antiserum HPA, which was used at a 1:1000 dilution. Assessment of the relative amounts of G-protein subunits as indicated by the intensity of immunoblot staining was carried out by analysing scans of a film positive of the blot on a Bio-Rad Gel Scanner linked to an Olivetti M24 personal computer. We have previously described this process in some detail [19,26].

High-affinity GTPase activities of the membranes and the modulation of this in response to opioid peptides were measured in refs. [23,27]. Adenylate cyclase activities of the membranes were measured as in ref. [23]. Saturation binding isotherms using either [^{3}H]DALAMID or [^{3}H]DADLE to label the δ opiate receptor in these cells were carried out with concentrations of the radioligands of 0–20 nM. Non-specific binding was defined in the presence of 10 μM of either DADLE or DALAMID. Conditions for the assay were as reported in ref. [23].

RESULTS

Incubation of NG108-15 cells in tissue culture with 1 mM-dibutyryl cyclic AMP for 6 days produced a morphological 'differentiation' of the cells (Fig. 1). In comparison with the untreated cells, the dibutyryl cyclic AMP-treated cells extended numerous neurite-like processes. Crude membranes isolated from the control and 'differentiated' cells were extremely similar when resolved by SDS/polyacrylamide-gel electrophoresis and stained with Coomassie Blue (results not shown). However, when the membranes from both the control and 'differentiated' cells were treated with [^{32}P]NAD$^+$ and either cholera toxin or pertussis toxin, resolved by SDS/polyacrylamide-gel electrophoresis and the resultant gel was autoradiographed, there was increased incorporation of radioactivity into a 45 kDa band in response to cholera toxin and into a broad band near 40 kDa in response to pertussis toxin (Fig. 2) in the 'differentiated'-cell membranes. On cutting and counting radioactivity of the appropriate bands in the experiment displayed in Fig. 2, the radioactivity specifically incorporated into the 45 kDa band was 368 c.p.m. in the control membranes and 500 c.p.m. in the membranes derived from dibutyryl

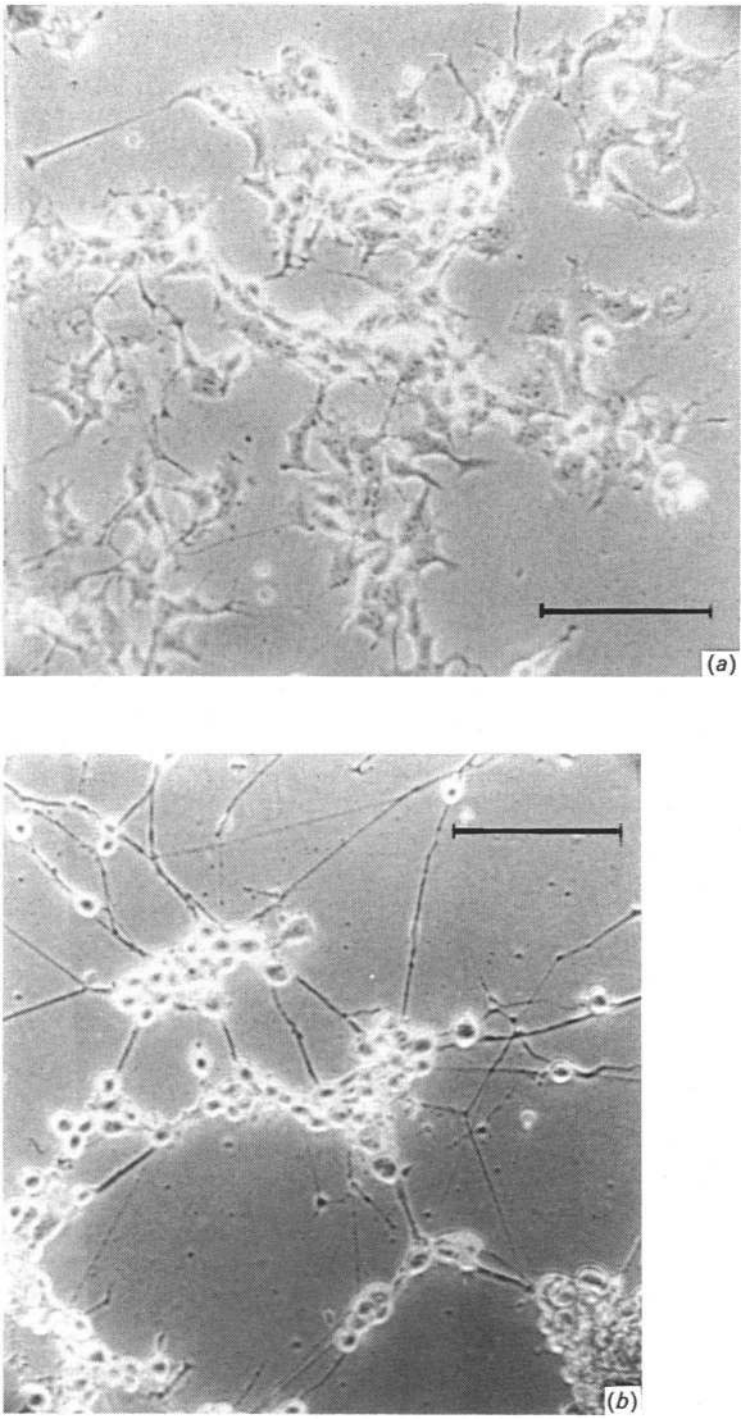

Fig. 1. Morphological appearance of control and dibutyryl cyclic AMP-'differentiated' NG108-15 cells in tissue culture

Control (*a*) and dibutyryl cyclic AMP-'differentiated' (*b*) NG108-15 neuroblastoma × glioma hybrid cells were grown as described in the Materials and methods section. The 'differentiated' cells had been maintained in the presence of dibutyryl cyclic AMP (1 mM) for 6 days when these photographs were taken. The scale bar is equivalent to 10 μm.

cyclic AMP-treated cells, whereas radioactivity specifically incorporated into the 40 kDa band was some 753 c.p.m. in the control membranes and 1233 c.p.m. into membranes of the 'differentiated' cells. Similar data were obtained with each individually harvested batch of cells. In typical experiments in which five replicate assays of a single pair of membrane preparations were ADP-ribosylated with pertussis toxin, the S.E.M. was less than 10% of the mean, and in each case radioactivity incorporated into the 40 kDa band of the 'differentiated'-cell membranes represented a statistically significant increase ($P = 0.001$) over that incorporated into the control membranes.

Although the 45 kDa band labelled by cholera toxin

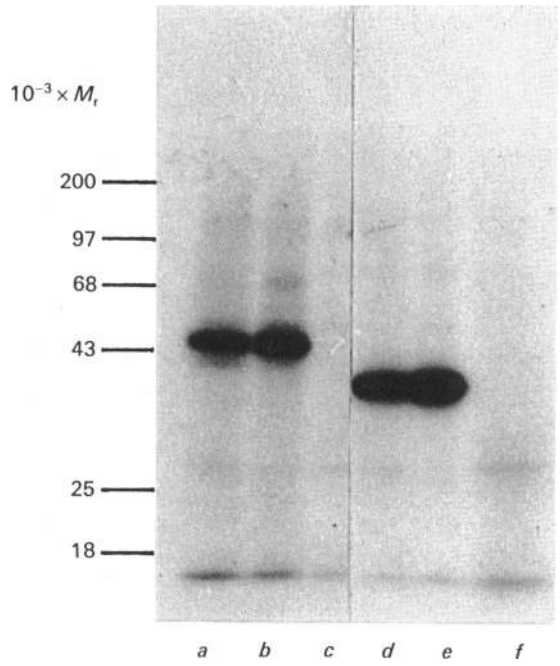

Fig. 2. Cholera- and pertussis-toxin-catalysed ADP-ribosylation in membranes of NG108-15 cells

Membranes (25 μg) of control (*a*, *c*, *d*, *f*) or 6-day dibutyryl cyclic AMP-differentiated (*b*, *e*) NG108-15 cells were treated with [^{32}P]NAD$^+$ and cholera toxin (*a*, *b*), pertussis toxin (*d*, *e*) or without toxin (*c*, *f*) for 1 h as described in the Materials and methods section. Samples were recovered by deoxycholate/trichloroacetic acid precipitation [37] and resolved by SDS/polyacrylamide (10%)-gel electrophoresis. The gel was dried and autoradiographed. The autoradiogram was then used as a template to locate and excise the bands containing radioactivity (see the text).

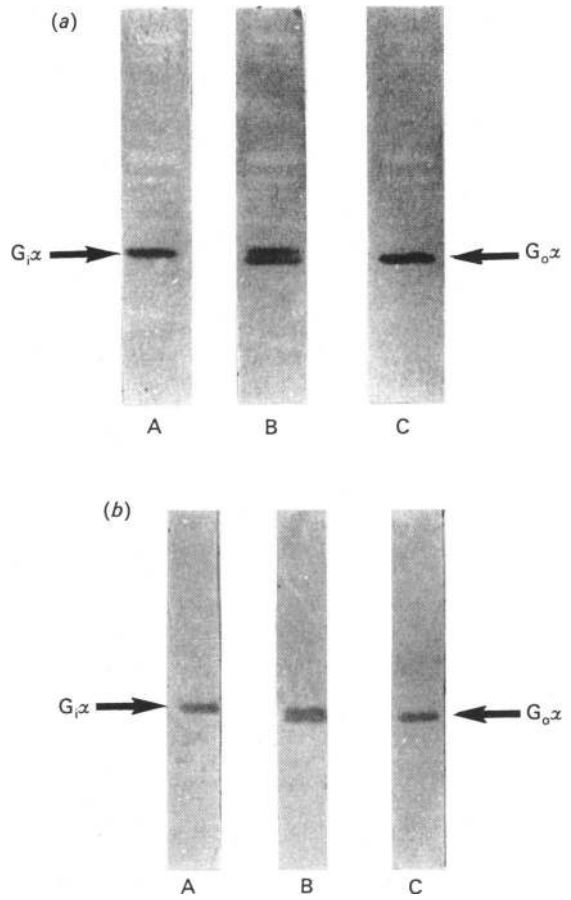

Fig. 3. Antisera AS7 and IM1 identify mutually exclusive polypeptides

(*a*) Antiserum AS7 (A), IM1 (C) or a mixture of these two antisera (B) was immunoblotted against a mixture (330 ng) of G_i and G_o as purified from bovine brain [37] which had been resolved by SDS/polyacrylamide (10%)-gel electrophoresis. The mixture of the antisera identified two polypeptides, of 39 and 40 kDa, whereas each antiserum individually identified only a single species, antiserum AS7 the 40 kDa polypeptides (G_i) and antiserum IM1 the 39 kDa polypeptide (G_o). An identical protocol has previously been used to define the specificity of other anti-G-protein antisera [15]. (*b*) A similar strategy to that of Fig. 3(*a*) was used to demonstrate that antisera AS7 and IM1 identify mutually exclusive polypeptides in bovine brain membranes. Samples (25 μg) of a crude membrane pellet (P2) from bovine brain were alkylated as described in [22] and were resolved by electrophoresis on an SDS/10% polyacrylamide gel and immunoblotted with antiserum AS7 (A), IM1 (C) or a mixture of the two antisera (B).

presumably represents the α subunit of G_s, several different G-proteins can be substrates for pertussis toxin. We thus attempted to assess the relevant contributions of both 'G_i-like' proteins and G_o to the total pattern of pertussis-toxin-catalysed ADP-ribosylations in membranes of the control and 'differentiated' cells, by using specific anti-peptide antisera which selectively recognize the α subunits of each of these G-proteins (Fig. 3). Antiserum AS7, which recognizes an epitope within the *C*-terminal ten amino acids of the α subunit of forms of G_i, identified a 40 kDa polypeptide in Western blots of membranes from both the control and 'differentiated' cells. However, staining of the 40 kDa band was more intense in membranes of the control cells, signalling the presence of higher amounts of a form of G_i in these membranes than in the 'differentiated'-cell membranes (Fig. 4*a*). Densitometric scanning of the immunoblot indicated that the amount of the α subunit of G_i was some 3-fold lower in membranes of the 'differentiated' cells in comparison with the control cells. In a series of different preparations, the amount of G_i was decreased by some 2–3-fold in the 'differentiated'-cell membranes in comparison with the controls. In contrast, antiserum IM1, which was raised against a synthetic peptide corresponding to amino acids 22–35 of the α subunit of G_o, identified a 39 kDa polypeptide (Fig. 4*b*) which was some 4.3-fold more abundant in membranes of the 'differentiated' cells in comparison with the control cells as assessed by densitometric scanning of the immunoblots. Use of a mixture of antiserum AS7 and IM1 in immunoblots of membranes of NG108-15 cells confirmed that these antisera identified mutually exclusive polypeptides in this cell line (results not shown, but see [22]). A second anti-peptide antiserum (OC1) against the α subunit of G_o, which recognizes the *C*-terminal ten amino acids of this G-protein [22], produced similar results to those with IM1 (results not shown). Similar results with each of the selective G-protein antisera were obtained in at least three membrane preparations derived from cells grown as individual cultures. Growth of NG108-15 cells in the

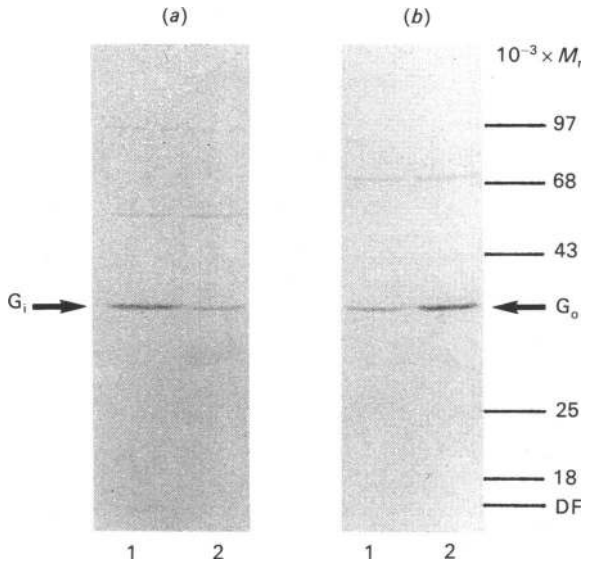

Fig. 4. Immunoblotting of membranes of control and 'differentiated' NG108-15 cells with antisera which discriminate between G_i and G_o

Membranes (150 μg) from (1) control or (2) dibutyryl cyclic AMP (treated 6 days) 'differentiated' NG108-15 cells were resolved by SDS/polyacrylamide (10%)-gel electrophoresis, transferred to nitrocellulose and immunoblotted as described in the Materials and methods section by using either (*a*) antiserum AS7, which recognizes the α subunit of G_i-like proteins, or (*b*) antiserum IM1, which recognizes the α subunit of G_o.

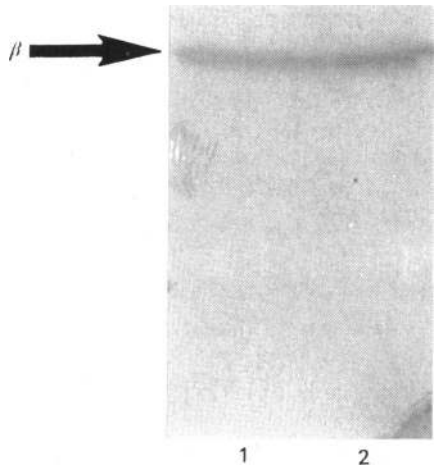

Fig. 5. Detection of the G-protein β subunit

Membranes (150 μg) of either (1) control or (2) 'differentiated' NG108-15 cells as defined in the legend to Fig. 4 were immunoblotted with an antiserum (HPA) which contains antibodies which recognize the β subunit, which appears to be common to all of the G-proteins. The procedure was as described in the Materials and methods section and Fig. 4 legend. This antiserum also contains a subset of antibodies which recognize the α subunit of G_o. In this Figure, only the immunoreactivity against the β subunit is displayed. As with the antipeptide antisera (IM1, OC1) against the α subunit of G_o, antiserum HPA demonstrated elevated amounts of G_o in the 'differentiated'-cell membranes compared with control membranes (results not shown).

presence of only 2% foetal bovine serum but in the absence of dibutyryl cyclic AMP produced neither the morphological differentiation of the cells nor the alterations in G-protein amounts (see Figs. 8 and 9 below), demonstrating that alterations in amounts of the G-proteins were a specific response to the presence of dibutyryl cyclic AMP. Antibodies which recognize the β subunit (antiserum RV6), which appears to be common within the G-protein family, indicated little alteration in the amounts of this polypeptide between membranes of the control and 'differentiated' cells (results not shown). Similar results were obtained for the β subunit in immunoblots using antiserum HPA (Fig. 5).

Opioid peptides activated an opiate receptor of the δ subclass in NG108-15 cells. Stimulation of both control and 'differentiated'-cell membranes with a saturating concentration (1 μM) of the synthetic opioid peptide [D-Ala^2,Leu^5]enkephalin (DADLE) produced an elevation over the basal amounts of membrane high-affinity GTPase activity (Table 1). However, the absolute stimulation was considerably greater in the control membranes than in the 'differentiated'-cell ones, and, although the increase noted in the control cell membranes was statistically significant, that in the 'differentiated'-cell membranes was not. Dose–response curves of GTPase stimulation in response to DADLE indicated a similar EC_{50} (concn. giving 50% of maximal response) for the ligand in the two membrane preparations (Fig. 6).

Forskolin (100 μM)-stimulated adenylate cyclase activity in the membranes of control (274.5 $\pm$ 59.0 pmol/min per mg of protein; mean $\pm$ S.E.M., $n = 4$) and 'differentiated' (306.5 $\pm$ 44.7 pmol/min per mg of protein; mean $\pm$ S.E.M., $n = 5$) cells was very similar. However, this activity was inhibited by DADLE (10 μM) by some 21 $\pm$ 1% in the control cell membranes, whereas opioid-peptide-mediated inhibition of adenylate cyclase in the membranes of the 'differentiated' cells was essentially abolished (1 $\pm$ 1%). Saturation-binding studies using either [^{3}H][D-Ala^2,Met^5]enkephalinamide ([^{3}H]-DALAMID) or [^{3}H]DADLE indicated that only some 55.7 $\pm$ 3.1% (mean $\pm$ S.E.M., $n = 4$) of the number of opioid receptors were present in membranes of the 'differentiated' cells compared with the control cells in the individual sets of membrane preparations (Fig. 7). However, the absolute number of receptors varied over an approx. 2-fold range between different passages of the cells. The receptors present in both control and 'differentiated' membranes displayed equivalent affinities for [^{3}H]DADLE (control, 2.2 $\pm$ 0.3 nM, mean $\pm$ S.E.M., $n = 5$; 'differentiated' 2.8 $\pm$ 0.3 nM, mean $\pm$ S.E.M., $n = 4$). (Fig. 7). No differences were noted in the number of receptors present in individual membrane preparations derived from control cells grown in the presence of either 10% or 2% foetal-calf serum (results not shown).

Immunoblotting with antiserum IM1 of membranes derived from NG108-15 cells which had been treated with dibutyryl cyclic AMP for various times (Fig. 8) showed that amounts of the α subunit of G_o increased throughout the time period used, with particularly marked increases of G_o noted between 3 and 4 days after addition of dibutyryl cyclic AMP. In contrast, however, amounts of G_o were not different in untreated cells which

were harvested at various times after subculture (Fig. 8; cf. lanes *a* and *b*). Similar studies on modulation of the amounts of the α subunit of G_i in response to dibutyryl cyclic AMP with time demonstrated that amounts of immunoreactive G_i fell throughout the period studied (Fig. 9). As for G_o, alteration in concentration of G_i was strictly dependent on the presence of dibutyryl cyclic AMP (Fig. 9).

DISCUSSION

Attempts to assess the fidelity of interaction of receptors with different G-proteins have to a large extent relied on reconstitution studies in which purified or partially purified receptors have been mixed with purified G-proteins and reconstituted into artifical phospholipid vesicles [28,29]. These studies have frequently indicated that a particular receptor type might be able to interact with approximately equal affinity and efficacy with a number of homologous G-proteins, and even to some degree with less closely related G-proteins [30], for which little biochemical or physiological evidence exists in more native systems. One of the major limitations of such reconstitution studies is that, with hindsight, it is unlikely, given the physical similarity of several of the G-proteins, that the experiments have been performed with homogeneous populations of a single G-protein. It may be that suitable reconstitution experiments will require the expression of a single protein derived from a cDNA clone [31,32].

The neuroblastoma × glioma hybrid cell line NG108-15 expresses at least two pertussis-toxin-sensitive G-proteins, a form of G_i and G_o [11]. As this is a homogeneous cell line in tissue culture, we can be sure that both of these highly homologous G-proteins will be at least potentially available to interact with receptors, which have previously been demonstrated to function in a manner which is sensitive to pertussis toxin, in the membrane of these cells [33,34].

Treatment of NG108-15 cells in tissue culture with dibutyryl cyclic AMP caused the cells to adopt a more 'differentiated' morphology [35], in which the cells extended neurite-like processes. This or similar treatments of these cells are frequently used, particularly before electrophysiological experiments. When an attempt to assess amounts of pertussis-toxin-sensitive G-proteins was made by the incorporation of [^{32}P]ADP-ribose from [^{32}P]NAD catalysed by pertussis toxin, incorporation of radioactivity into a broad band of some 40 kDa was increased in the 'differentiated' cells by some 50–100% (see Fig. 2 and the text) in comparison with untreated cells.

As all pertussis-toxin-sensitive G-proteins have α subunits with very similar molecular mass, we attempted to assess the relative contributions of 'G_i' and G_o to the pertussis-toxin-sensitive G-protein pool in both control and 'differentiated' cells by using specific anti-peptide antisera against each of these forms [17–21]. We have shown that these antisera display no cross-reactivity

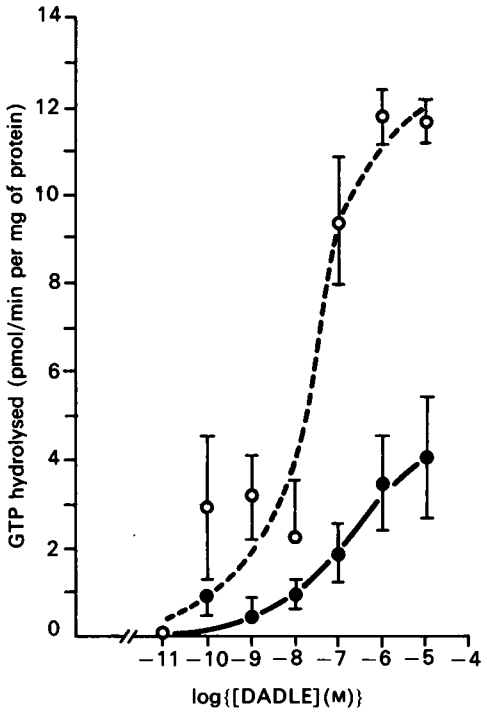

Fig. 6. High-affinity GTPase activity stimulated by opioid peptides in membranes of control and dibutyryl cyclic AMP-'differentiated' NG108-15 cells

The stimulation of high-affinity GTPase activity by various concentrations of DADLE above that noted in the absence of agonist was assessed, as described in the Materials and methods section, in membranes from either control cells (○) or from those which had been treated for 6 days with dibutyryl cyclic AMP (1 mM) (●). The data represent means ± S.E.M. derived from four separate preparations. The basal high-affinity GTPase activity was 26.1 ± 2.9 pmol/min per mg of membrane protein in the control membranes and 24.7 ± 3.5 pmol/min per mg of membrane protein in the membranes from 'differentiated' cells.

Table 1. Maximal stimulation of high-affinity GTPase activity in response to opioid peptides in membranes of control and dibutyryl cyclic AMP-'differentiated' NG108-15 cells

Results represent means ± S.E.M. for six independent experiments performed with membranes derived from separate cultures. The 'differentiated' cultures were treated with 1 mM-dibutyryl cyclic AMP for 6 days before cell harvest. Statistical analysis by use of Student's *t* test for unpaired samples indicated that the DADLE stimulation of GTPase activity was significantly greater in the control membranes than in the differentiated membranes ($P = 0.001$). Similar analysis using Student's *t* test for paired samples indicated that, within each individual experiment, DADLE produced a statistically significant increase in membrane high-affinity GTPase activity in control membranes ($P < 0.05$). However, response to the opioid peptide in the 'differentiated' membranes was not significant ($P > 0.1$).

		High-affinity GTPase activity (pmol/min per mg of protein)	
[DADLE]	Cells...	Control	'Differentiated'
0		26.1 ± 3.2	25.2 ± 3.6
1 μM		35.5 ± 3.8	27.2 ± 3.3

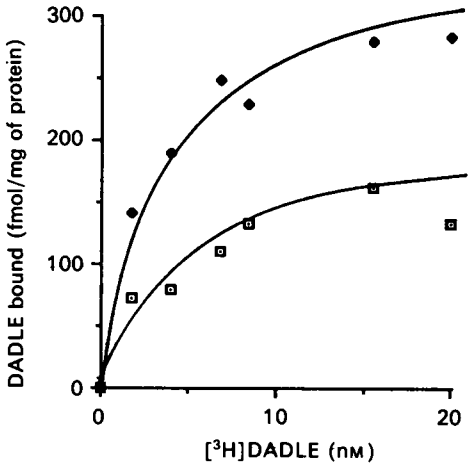

Fig. 7. Binding of [^{3}H]DADLE to membranes of control and 'differentiated' NG108-15 cells

Membranes (150 μg) of control (◆) or 'differentiated' (⊡) NG108-15 cells were incubated with various concentrations of [^{3}H]DADLE in the presence or absence of DALAMID (10 μM) as detailed in [27]. The data in the Figure represent the difference between total and non-specific binding, and hence are indicative of specific binding. Each point is the mean of two replicates which varied by less than 10%.

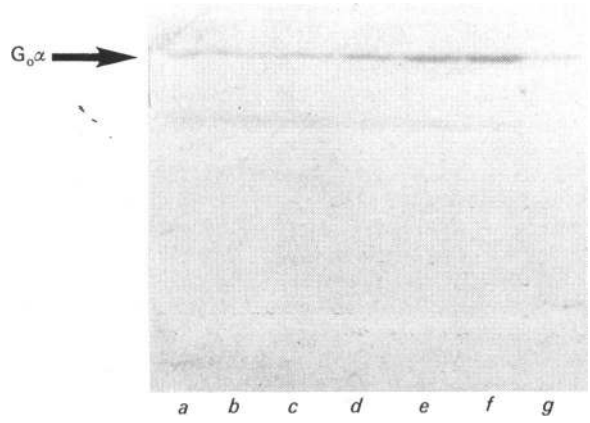

Fig. 8. Time course of dibutyryl cyclic AMP-mediated alterations of amounts of the α subunit of G_o in membranes of NG108-15 cells

Antiserum IM1 was used as first antibody in an immunoblot after resolution of membranes (150 μg) of NG108-15 cells. Lane (*a*), untreated cells grown in the presence of 10% foetal bovine serum, 1 day after subculture; lane (*b*), untreated cells 6 days after subculture; lanes (*c*)–(*f*), dibutyryl cyclic AMP-treated cells after 1 (*c*), 2 (*d*), 4 (*e*) and 6 (*f*) days in the presence of 1 mM-dibutyryl cyclic AMP; lane (*g*), untreated cells grown for 6 days in the presence of 2% foetal bovine serum but without dibutyryl cyclic AMP.

between the two G-proteins (Fig. 3; refs. [20,22]; I. Mullaney & G. Milligan, unpublished work). This analysis showed that, in comparison with the control cells, the 'differentiated' cells expressed lower amounts of G_i and much elevated amounts of G_o (Fig. 4). Interestingly, when several antisera which recognize the

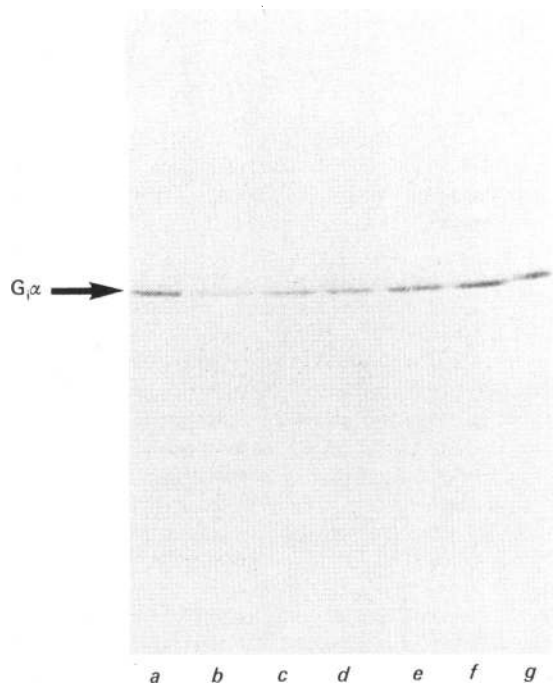

Fig. 9. Time course of dibutyryl cyclic AMP-mediated alterations in the amounts of the α subunit of G_i in membranes of NG108-15 cells

Antiserum AS7 was used as primary antiserum in an immunoblot after resolution of membranes (150 μg) of NG108-15 cells. Lane (*a*), cells grown in the presence of 2% foetal bovine serum but in the absence of dibutyryl cyclic AMP for 6 days; lanes (*b*)–(*e*), cells grown in the presence of 2% foetal bovine serum and 1 mM-dibutyryl cyclic AMP for 6 (*b*), 4 (*c*), 2 (*d*) and 1 (*e*) days; lanes (*f*) and (*g*), cells grown in 10% foetal bovine serum and harvested either 6 days (*f*) or 1 day (*g*) after subculture.

β subunit were used to compare the control and 'differentiated'-cell membranes, little alteration in amount was noted (Fig. 5). This may reflect the fact that expression of the β subunit of G-proteins is not strictly co-ordinated with that of the α subunits or that the reciprocal loss of β subunit associated with G_i balanced the increased amounts associated with G_o. As we have previously noted a concomitant increase in both the α subunit of G_i and the β subunit in adipocytes of rats in response to chemically induced hypothyroidism [18], we tend to favour the second suggestion, but cannot dismiss the first. It is, however, noteworthy that amounts of the α subunits of the two G-proteins assessed in this study were modulated in opposite directions (see also ref. [12]).

We have also examined the responsiveness of both sets of membranes to opioid peptides by measuring the interaction of the receptor with G-protein as assessed by ligand-stimulated GTPase [23,27] and more distally with the adenylate cyclase effector system [23,27]. In both of these cases the maximal effect of the ligand at saturating concentrations was markedly lower, indeed was essentially attenuated, in the 'differentiated'-cell membranes in comparison with those from the control cells. There was no marked difference in the concentrations of the agonist required to produce half-maximal effects. However, as the number of opioid receptors present in the membranes of the differentiated cells was lower than in the control

cells by some 50%, it is not possible to assess with any certainty whether the alteration in receptor numbers or decrease in the amounts of G_i was primarily responsible for these observations.

A recent report by Hescheler and co-workers [36] has used dibutyryl cyclic AMP-'differentiated' NG108-15 cells to conclude that the opiod-receptor effect on Ca^{2+} channels was mediated via G_o and not G_i. These studies relied on the use of purified G_i and G_o, which, on the basis of more recent purification protocols [6,7], were unlikely, as noted above, to have represented homogeneous G-proteins. We have recently utilized anti-peptide antibodies against G_i to prevent, effectively and completely, the interaction of the opioid receptor with its G-protein in these same cells [23], indicating that the δ opioid receptor in these cells interacts with a form of G_i. The apparent dichotomy between these two reports indicates the importance of identification of systems which are likely to allow unambiguous assessment of the specificity or otherwise of the interaction of receptors with individual G-proteins. Whereas the decrease in opioid-receptor number coincident with the alterations of amounts of two individual pertussis-toxin-sensitive G-proteins in these cells in response to dibutyryl cyclic AMP limits the conclusions that can be drawn from these studies, techniques which allow for alteration in amounts of different G-proteins within a single cell type are likely to be of considerable use in the assessment of the specificity of receptor–G-protein interactions.

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