Overexpression of $G_s\alpha$ in NG108-15, neuroblastoma × glioma cells: effects on receptor regulation of the stimulatory adenylyl cyclase cascade

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Abstract **Neuroblastoma × glioma hybrid, NG108-15 cells were stably transfected with an epitope tagged variant of $G_s\alpha$ (HA-$G_s\alpha$). The introduced HA-$G_s\alpha$ was able to interact with the IP prostanoid receptor and was able to stimulate adenylyl cyclase activity as measured by an enhanced capacity of membrane extracts to reconstitute NaF-dependent adenylyl cyclase activity to membranes of S49 lymphoma cyc$^-$ cells. Despite this, neither the maximal stimulation nor the potency of agonist ligands at the IP prostanoid, A_2 adenosine or secretin receptors was altered substantially compared to the parental cells although the basal adenylyl cyclase activity was increased. These data indicate that cellular levels of $G_s\alpha$ do not limit signal transduction capacity in NG108-15 cells, whereas enhanced expression of adenylyl cyclase allows greater maximal cAMP generation following receptor activation (MacEwan, D.J., Kim, G.D. and Milligan, G. (1996) Biochem. J. 318, 1033–1039).**

Key words: Guanine nucleotide binding protein; Prostanoid; Adenylyl cyclase; Neuroblastoma

1. Introduction

Relatively little is known about the absolute levels of expression of the protein components of signal transduction cascades in different cell types and how alterations in levels of these proteins may alter the effectiveness of hormone and neurotransmitter function [1]. In an effort to address these questions we have been using neuroblastoma × glioma, NG108-15, hybrid cells and examining the stimulatory regulation of adenylyl cyclase. These cells express approximately 10^5 copies per cell of the IP prostanoid receptor, 10^6 copies per cell of the α subunit of the G protein G_s and some 2×10^4 copies per cell of adenylyl cyclase [2]. We have previously stably transfected these cells and isolated clones expressing varying levels of the β_2-adrenoceptor and shown that the adenylyl cyclase activity can be maximally stimulated by isoprenaline in cells expressing high levels of the receptor [3,4]. Even in clones expressing relatively low levels of the receptor, maximally effective concentrations of isoprenaline caused the same maximal stimulation of adenylyl cyclase activity but the potency of the agonist was reduced [3,4]. These observations are consistent with the cells expressing high levels of the receptor displaying a large receptor reserve [3,4]. By contrast, increasing total cellular levels of adenylyl cyclase by stable transfection of type II adenylyl cyclase into these cells resulted in a markedly greater maximal activation of adenylyl cyclase without a change in the potency of the agonist ligands [5].

In the present study we overexpress the third component of the stimulatory adenylyl cyclase cascade ($G_s\alpha$) in NG108-15 cells and then assess basal and receptor regulation of adenylyl cyclase activity.

2. Materials and methods

2.1. Materials

All reagents for tissue culture were purchased from Life Technologies, Paisley, Strathclyde, UK. α[^{32}P]ATP and [^{3}H]cAMP were obtained from Amersham International. Iloprost was a kind gift of Schering Health Care, Burgess Hill, Sussex, UK. Other receptor ligands were purchased from the Sigma Chemical Company (Poole, UK). All other chemicals were bought from Sigma or British Drug Houses (BDH) and were of the highest purity available. The cDNA for HA-$G_s\alpha$ in pcDNA1 was a kind gift from Drs M.J. Levis and H.R. Bourne, University of California at San Francisco, CA, USA.

2.2. Methods

2.2.1. Generation and isolation of clones of NG108-15 cell variants expressing HA-$G_s\alpha(L)$. NG108-15 cells were stably co-transfected with plasmid pcDNA1 (10 μg) containing a cDNA (HA-$G_s\alpha$) encoding the long isoform of $G_s\alpha$ in which a section of the haemagglutinin epitope (VPDYA) was constructed between amino acid residues 76–82 [6] and plasmid pBABE hygro (1 μg) which confers resistance to hygromycin B. Clones which were resistant to hygromycin B (200 μg/ml) were selected and expanded. Clone BST15 [7] was selected for analysis in this study (see Fig. 1).

2.2.2. Cell culture. NG108-15 and clone BST15 cells were grown in Dulbecco's modified Eagle's medium (DMEM) which was supplemented to 5% (v/v) with fetal bovine serum. This medium was further supplemented with hypoxanthine, aminopterin, thymidine, 100 U/ml of penicillin and 100 μg/ml streptomycin. Clone BST15 was furthermore maintained in medium containing hygromycin B (200 μg/ml). S49 lymphoma cyc$^-$ cells were grown in DMEM containing 10% heat-inactivated horse serum and maintained between 5×10^5 and 2×10^6 cells/ml.

2.2.3. Membrane preparation. Cells were harvested, washed twice in phosphate buffered saline and pelleted by centrifugation at $500\times g$ for 10 min on a Beckman TJ-6 benchtop centrifuge. Membranes were prepared by homogenizing the cell pellet with 20 strokes of a Teflon/glass homogenizer in 10 mM Tris-HCl, 0.1 mM EDTA pH 7.5 (buffer A). The resultant supernatant was centrifuged at $500\times g$ for 10 min in a Beckman L5-50B centrifuge with a Ti50 rotor, to remove unbroken cells and nuclei. The supernatant was further centrifuged at $48\,000\times g$ for 10 min. The pellet from this centrifugation was resuspended in buffer A and recentrifuged at $48\,000\times g$ for 10 min. The resultant pellet was resuspended in an appropriate volume of buffer A, aliquoted and stored at −80°C until use.

2.2.4. Immunoblotting. Membranes were resolved by SDS-PAGE (10% (w/v) acrylamide) and subsequently immunoblotted with a range of antisera. Antiserum CS, initially characterised by Milligan and Unson [8] was raised in a New Zealand White rabbit after immunization with a glutaraldehyde conjugate of keyhole limpet haemocyanin and a synthetic peptide, RMHLRQYELL, which corresponds to the C-terminal decapeptide common to all isoforms of $G_s\alpha$. This sequence is also preserved in the HA epitope-tagged variant of $G_s\alpha$. Monoclonal antibody 12CA5 which identifies a nonapeptide sequence

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Abbreviations: G protein, guanine nucleotide binding protein; HA, haemagglutinin; DMEM, Dulbecco's modified Eagle's medium; NECA, 5′-*N*-ethylcarboxamidoadenosine

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(YPYDVPDYA) derived from the haemagglutinin protein of human influenza virus was a gift from Dr M.J. Stark, Department of Biochemistry, University of Dundee. Immunoblots were quantitated using a Bio-Rad GS-670 Imaging Densitometer linked to an Apple Macintosh Quadra 800 microcomputer.

2.2.5. Immunoprecipitation of G-proteins. To the 200 μl of SDS-denatured cell suspension was added 800 μl of solubilization buffer (1% (w/v) Triton X-100, 10 mM EDTA, 100 mM NaH_2PO_4, 10 mM NaF, 100 μM Na_3VO_4, 50 mM HEPES pH 7.2), and 100 μl of Pansorbin (Calbiochem). Samples were incubated at 4°C with continuous rotation for 1–2 h for non-specific preclearing. Following centrifugation of the samples (16000×*g*, 2 min, 4°C) the supernatant was collected and subjected to immunoprecipitation by addition of 100 μl of protein A-agarose along with 10 μl of antiserum CS or 1 μl of antibody 12CA5 and incubated at 4°C for 4 h. Immune complexes were then recovered by centrifugation (16000×*g*, 30 s at 4°C) and washed by resuspension-centrifugation three times each with 1 ml of wash buffer (1% (w/v) Triton X-100, 100 mM NaCl, 100 mM NaF, 50 mM NaH_2PO_4, 50 mM HEPES, pH 7.2, 0.5% (w/v) SDS). The final protein A-agarose pellet was resuspended in Laemmli sample buffer, heated at 100°C for 5 min and electrophoresed by SDS/PAGE (10% (w/v) acrylamide). Following resolution of the proteins, the gel was stained with Coomassie blue and then dried. The dried gels were exposed either to photographic film or to phosphor storage screen autoradiography according to the manufacturer's instructions using a Fujix Bio-imaging Analyser linked to an Apple Macintosh Quadra 650 personal computer.

2.2.6. Reverse transcriptase/polymerase chain reaction. The reverse transcriptase/PCR procedure was essentially as described by Steel and Buckley [9]. Total RNA was extracted using RNAzol B (Biogenesis). Purity and quantification of RNA were assessed by spectrophotometric A260/A280 ratios. Samples of 5–10 μg RNA (20 μl) were denatured by incubation at 65°C for 10 min followed by chilling on ice and reverse transcribed in 33 μl of reaction mixture using first strand cDNA synthesis kit (Pharmacia LKB Biotechnology) as detailed by the manufacturer. Incubation was carried out at 37°C for 1 h. The reactions were terminated by heating samples at 95°C for 5 min followed by transfer to ice.

PCR reactions were carried out using the following primers:

$G_s\alpha$-sense, 5′-CCACCTGAATTCTATGAGCATGCC-3′
HA-$G_s\alpha$-sense, 5′-GAGGACGTGCCGGATTACGCG-3′
$G_s\alpha$-antisense, 5′-GCGTGGGTCCTCTCCGGGCTCGGG-3′

Amplifications were performed in 50 μl of buffer containing 25 pmol of primers and 2.5 U of Taq polymerase (Promega) using a HYBAID Omnigene temperature cycler. Amplifications were carried out as follows: 95°C/5 min, 60°C/30 s, 72°C/1 min (1 cycle); 95°C/30 s, 60°C/30 s, 72°C/1 min (30 cycles) 95°C/30 s, 60°C/30 s, 72°C/5 min (1 cycle). Reaction products were then separated by 1.5–1.75% agarose gel electrophoresis. In each case the size of the generated product was that anticipated from the selected primers.

2.2.7. Adenylyl cyclase assays. Adenylyl cyclase assays on membranes of NG108-15 cells and of clone BST15 were performed as described by Milligan et al. [10]. Reconstitution of NaF-stimulated adenylyl cyclase activity to membranes of S49 lymphoma cyc$^-$ cells, which do not express $G_s\alpha$, with sodium cholate extracts of NG108-15 and BST15 membranes was performed as described by McKenzie and Milligan [11].

3. Results

NG108-15 cells were transfected stably with a combination of plasmid pcDNA1 containing a cDNA (HA-$G_s\alpha$) encoding the long isoform of $G_s\alpha$ in which a section of the primary sequence was modified to encode part of the haemagglutinin epitope and plasmid pBABE hygro which confers resistance to hygromycin B [7]. Clones displaying resistance to hygromycin (200 μg/ml) were expanded. Membranes derived from these clones were resolved by SDS-PAGE and immunoblotted with antiserum CS, which identifies the C-terminal decapeptide conserved between all splice variants of $G_s\alpha$ [8]. All of the clones tested endogenously expressed good levels of the long (45 kDa) splice variant of $G_s\alpha$ and a number of them also expressed a second CS immunoreactive polypeptide which migrated somewhat more slowly in SDS-PAGE (Fig. 1A). Clone BST15 was used in this study as it expressed this construct to the highest level and in the passages of cells used for this study membrane levels of HA-$G_s\alpha$ were some 60% of the levels of the endogenously expressed wild type long isoform of $G_s\alpha$ (Fig. 1). Confirmation of the identity of this polypeptide as HA-$G_s\alpha$ was obtained by a number of distinct strategies. RNA isolated from both parental NG108-15 cells and clone BST15 was reversed transcribed and polymerase chain reaction then performed using primer pairs designed to amplify a fragment from authentic $G_s\alpha$ (Fig. 1B, panel A) or from HA-$G_s\alpha$ (Fig. 1B, panel B). A product corresponding to wild type $G_s\alpha$ was obtained from both parental NG108-15 cells and from clone BST15 whereas a product derived from HA-$G_s\alpha$ was obtained only from clone BST15. Immunoprecipitation of membranes of either NG108-15 cells or of clone BST15 cells with antibody 12CA5 (which identifies the HA-epitope) resulted in recovery of a CS immunoreactive polypeptide of the expected molecular mass only from the BST15 cell membranes (Fig. 1C) (although the efficiency of immunoprecipitation of HA-$G_s\alpha$ with antibody 12CA5 was poor) and cholera toxin-catalysed [^{32}P]ADP-ribosylation of membranes of NG108-15 and BST15 cells allowed immunoprecipitation of a single 45 kDa polypeptide (as well as a considerably lower amount of a 42 kDa polypeptide which is the short slice variant of $G_s\alpha$) from NG108-15 cell membranes with antiserum CS (Fig. 1D, lanes 3–4), but a doublet of 45 kDa polypeptides (as well as the 42 kDa polypeptide) from clone BST15 cell membranes (Fig. 1D, lanes 9–10). Immunoprecipitation of the membranes with antibody 12CA5 following cholera toxin-catalysed [^{32}P]ADP-ribosylation failed to immunoprecipitate a radiolabelled polypeptide from membranes of NG108-15 cells (Fig. 1D, lanes 5–6) but isolated a single 45 kDa polypeptide from BST15 cell membranes (Fig. 1D, lanes 11–12) which corresponded to the more slowly migrating polypeptide immunoprecipitated with antiserum CS.

We have previously concluded that the IP prostanoid receptor can interact with both HA-$G_s\alpha$ and $G_s\alpha$ in clone

Table 1
Reconstitution of adenylyl cyclase activity to membranes of S49 cyc$^-$ cells by cholate extracts of NG108-15 and BST15 cells: requirement for NaF

Addition	Cholate extract	
	NG108-15	BST15
	Adenylyl cyclase activity	
	(pmol/min/mg cyc$^-$ membrane)	
None	7.6 ± 0.4	8.0 ± 0.7
NaCl (10 mM)	10.3 ± 0.4	9.7 ± 0.7
NaF (10 mM)	45.2 ± 1.8	73.4 ± 3.5

The adenylyl cyclase activity of 10 μg of S49 cyc$^-$ cell membranes was measured for 1 h at 30°C in the presence of sodium cholate (1% (w/v), 1 h, 4°C) extract derived from 10 μg of membranes of either NG108-15 or BST15 cells. The assays were further supplemented with either NaCl or NaF (10 mM). Data represent means ± S.E.M. of triplicate assays from an experiment which was representative of three performed.

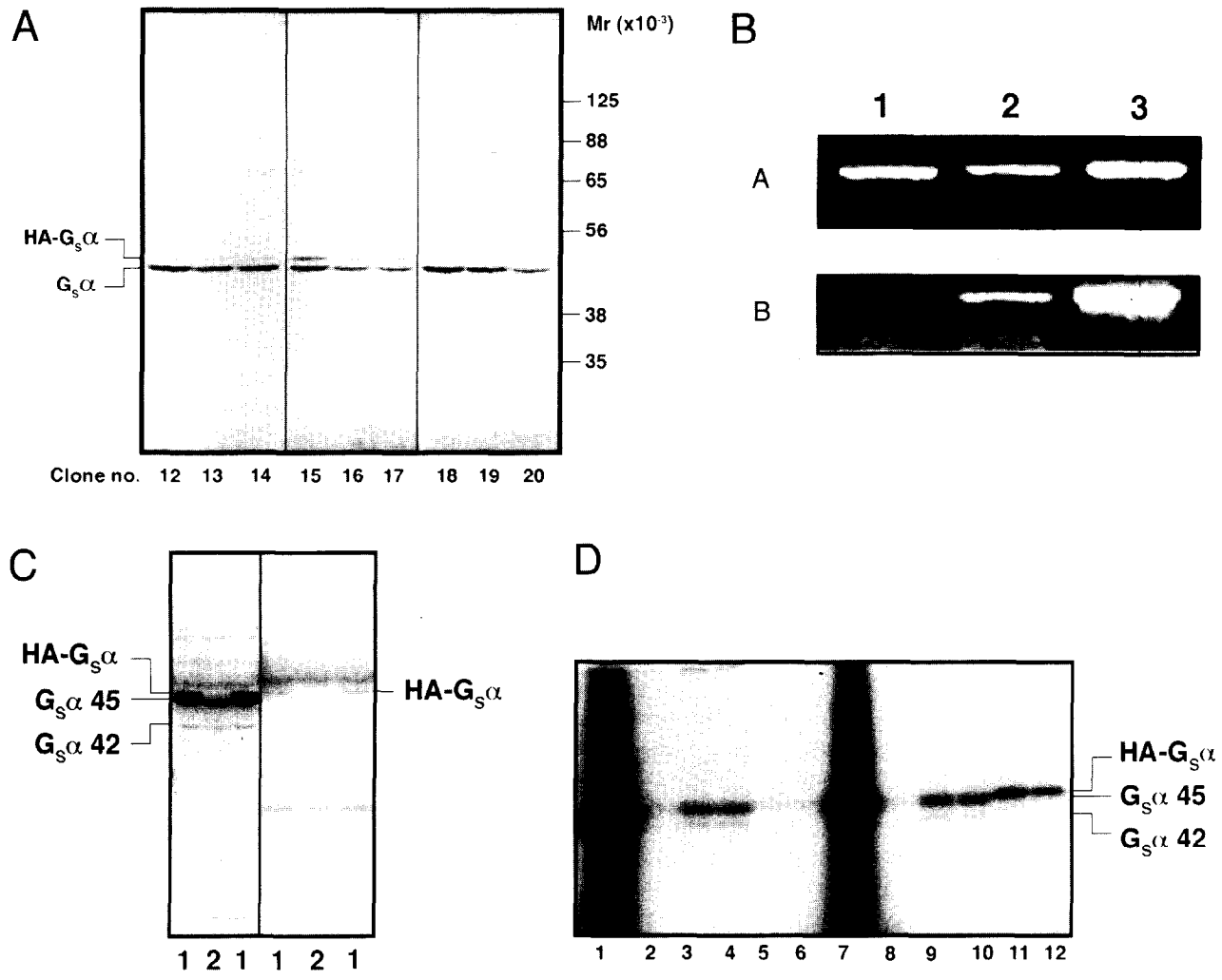

Fig. 1. Overexpression of $G_s\alpha$ in NG108-15 derived clones following transfection with an HA-epitope tagged variant of $G_s\alpha$. A: Immunoblotting for $G_s\alpha$ detects an additional form following stable transfection of NG108-15 cells with HA-$G_s\alpha$. Membranes (25 μg) derived from a series of clones displaying resistance to hygromycin B, derived following co-transfection of NG108-15 cells with plasmids encoding each of HA-$G_s\alpha$ and resistance to hygromycin B were resolved by SDS-PAGE (10% (w/v) acrylamide), transferred to nitrocellulose and immunoblotted using antiserum CS (1:500 dilution) as primary reagent. Clone (BST)15 displayed the highest levels of expression of what is putatively HA-$G_s\alpha$. B: Clone BST15 but not NG108-15 cells expresses mRNA corresponding to HA-$G_s\alpha$. Reverse transcriptase-PCR was performed on RNA isolated from either NG108-15 (1) or clone BST15 (2) cells using primers (see Section 2.2) designed to amplify wild type $G_s\alpha$ (A) or HA-$G_s\alpha$ (B). Positive controls for the PCR reactions using relevant cDNA species are also shown (3). C: Immunoprecipitation of HA-$G_s\alpha$ from membranes of clone BST15 but not NG108-15 cells with an HA-epitope directed antiserum. Membranes (25 μg, left hand panel, 200 μg, right hand panel) from BST15 (1) and NG108-15 (2) cells were immunoblotted with antiserum CS (left hand panel) or immunoprecipitated with antibody 12CA5 prior to immunoblotting with antiserum CS (right hand panel). D: Both $G_s\alpha$ and HA-$G_s\alpha$ act as substrates for cholera toxin-catalysed [^{32}P]ADP-ribosylation. Membranes (100 μg) from NG108-15 (1–6) and BST15 (7–12) cells were subjected to cholera toxin-catalysed [^{32}P]ADP-ribosylation. These were subsequently not treated (1, 7) or immunoprecipitated with normal rabbit serum (2, 8), antiserum CS (3, 4, 9, 10) or antibody 12CA5 (5, 6, 11, 12). The samples were then resolved on SDS-PAGE and autoradiographed.

BST15 cells as assessed by the ability of sustained treatment of the cells with the IP prostanoid receptor agonist iloprost to cause equivalent downregulation of both forms of the G protein [7]. We investigated whether membranes of clone BST 15 expressed higher levels of functionally active $G_s\alpha$ than membranes of NG108-15 cells. As anticipated, membranes of S49 lymphoma cyc$^-$ cells did not express an immunodetectable level of $G_s\alpha$ (data not shown) and addition of either NaF or NaCl (10 mM) failed to cause stimulation of adenylyl cyclase activity (Table 1). Extraction of membranes of NG108-15 cells with sodium cholate (1% (w/v), 1 h, 4°C) followed by reconstitution of the soluble extract with S49 lymphoma cyc$^-$ membranes allowed a marked NaF-stimulation of adenylyl cyclase activity. This was specific for NaF as NaCl was without significant effect (Table 1). Equivalent solubilisation and reconstitution of $G_s\alpha$ from membranes from clone BST15 resulted in an approximately 70% greater NaF stimulation of S49 lymphoma cyc$^-$ membrane adenylyl cyclase activity than achieved with the extract of NG108-15 cells (Table 1). Reconstitution of cyc$^-$ membranes with varying amounts of NG108-15 and BST15 cell membrane sodium cholate extracts resulted in a linear increase in NaF-induced adenylyl cyclase activity over the range of amounts examined (Fig. 2) with greater activity observed for the BST15 cell membrane extracts at all levels used.

The adenylyl cyclase activity of membranes of NG108-15

cells and clone BST15 and its regulation by agonist ligands was compared. There was a significant increase in basal adenylyl cyclase activity in membranes of clone BST15 compared to NG108-15 cells (1.33 ± 0.07 fold, mean ± S.E.M., $n = 8$). Iloprost caused a concentration-dependent stimulation of adenylyl cyclase activity in membranes of both cell lines. The EC_{50} for iloprost ($4.8 \pm 0.6 \times 10^{-8}$ M in NG108-15 cells and $5.2 \pm 2.0 \times 10^{-8}$ M in BST15 cells (means ± S.E.M., $n = 3$)) (Fig. 3) was not different between the two clones and the maximal level of adenylyl cyclase activity which could be achieved was similar (Fig. 3). Agonists at the A_2 adenosine (5′-*N*-ethylcarboxamidoadenosine (NECA), NG108-15 $EC_{50} = 9.1 \pm 2.0 \times 10^{-7}$ M, BST15 $EC_{50} = 6.0 \pm 2.2 \times 10^{-7}$ M) and secretin (secretin, NG108-15 $EC_{50} = 5.7 \pm 2.6 \times 10^{-8}$ M, BST15 $EC_{50} = 8.4 \pm 3.8 \times 10^{-8}$ M, means ± S.E.M., $n = 3$ in each case) receptors also displayed no differences in their concentration-dependence for stimulation of adenylyl cyclase activity (Fig. 4). However, neither of these ligands was capable of producing as large a maximal stimulation of adenylyl cyclase activity as iloprost in either cell line (NECA = 36.6 ± 3.3%, mean ± S.E.M., $n = 3$; secretin = 26.2 ± 6.4%, mean ± S.E.M., $n = 4$).

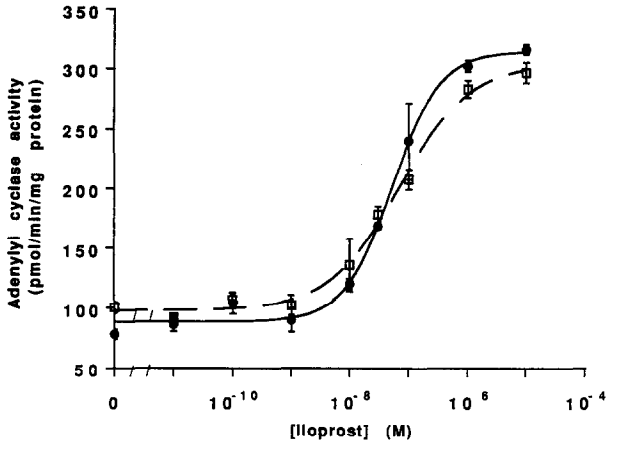

Fig. 3. Iloprost stimulates adenylyl cyclase activity equally in membranes of NG108-15 and BST15 cells. Basal adenylyl cyclase activity and its regulation by varying concentrations of the IP prostanoid receptor agonist iloprost was measured in membranes (10 μg) of NG108-15 (filled symbols) or BST15 cells (open symbols). Data are presented as means ± S.E.M. from quadruplicate assays from a representative experiment of three performed.

4. Discussion

Transgenic alterations in the protein components of cell signalling cascades can now be achieved in mice. Indeed, overexpression of both the β_1 [12] and β_2 [13]-adrenoceptors in a cardiac-specific manner has been reported with the aim of examining the relative role of these two catecholamine receptors in cardiac function and to allow analysis of whether the cardiac insufficiency associated with the failing heart [14] might be alleviated by gene therapy approaches based on alteration in adrenergic stimulation of adenylyl cyclase. Although these studies have been both technically challenging and provided new insights into cardiac regulation, a more systematic approach to monitoring the levels of expression of the different protein components of signalling cascades and to understanding how modification of the levels of each may alter both the potency of agonists ligands and the maximal potential output from the cascade need to be performed in parallel to provide insights into rational strategies to alter cell signalling efficiency. We are involved in this using neuroblastoma × glioma hybrid, NG108-15, cells as a model test system [2–5]. We have previously characterised the steady-state levels of expression of each of the IP prostanoid receptor, $G_s\alpha$ and adenylyl cyclases, which comprise the primary response components of the stimulatory adenylyl cyclase cascade, in these cells [2] and demonstrated that increasing expression of a $G_s\alpha$-linked receptor (the β_2-adrenoceptor) results in increasing potency [3,4] and intrinsic activity [4] of adrenergic ligands without altering the maximal possible output of the cascade (except at very low receptor densities [4]). By contrast, clones stably transfected to express higher levels of adenylyl cyclase responded with a greater maximal output following receptor occupancy without altering either the potency or efficacy of agonist ligands [5]. In the current study we have used a clone of these cells transfected to stably overexpress $G_s\alpha$ and examined the functional effects of this alteration.

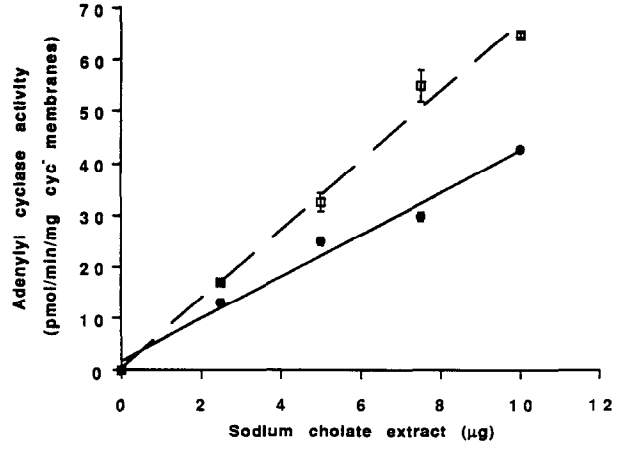

Fig. 2. Membranes of BST15 cells contain greater levels of S49 lymphoma cyc⁻ reconstitution activity than NG108-15 cells. Adenylyl cyclase assays were performed on S49 lymphoma cyc⁻ membranes (10 μg) in the presence of 10 mM NaF as in Table 1 except that sodium cholate extracts corresponding to varying amounts of membrane protein derived from NG108-15 (filled symbols) and BST15 (open symbols) cells were reconstituted with the cyc⁻ membranes. Adenylyl cyclase activity measured in the absence of addition of the sodium cholate extracts was not stimulated by the presence of NaF (see Table 1) and was subtracted from all the other values. Two further experiments produced similar results.

The strategy adopted was to transfect the cells with an HA epitope-tagged variant of $G_s\alpha$ to allow independent detection of expression of this polypeptide from the endogenously expressed form of this G protein. Serendipitously, however, we observed that the HA-tagged form of $G_s\alpha$ migrated more slowly in SDS-PAGE than the wild type protein and as such we could conveniently use an antiserum directed against a common region to probe the relative levels of expression of the two forms of $G_s\alpha$ (Fig. 1). Although the degree of overexpression (as fold) of $G_s\alpha$ in clone BST15 compared to NG108-15 cells may not seem particularly great, as noted above, this corresponds to the expression of approximately 6×10^5 extra copies per cell of $G_s\alpha$ in clone BST15 compared

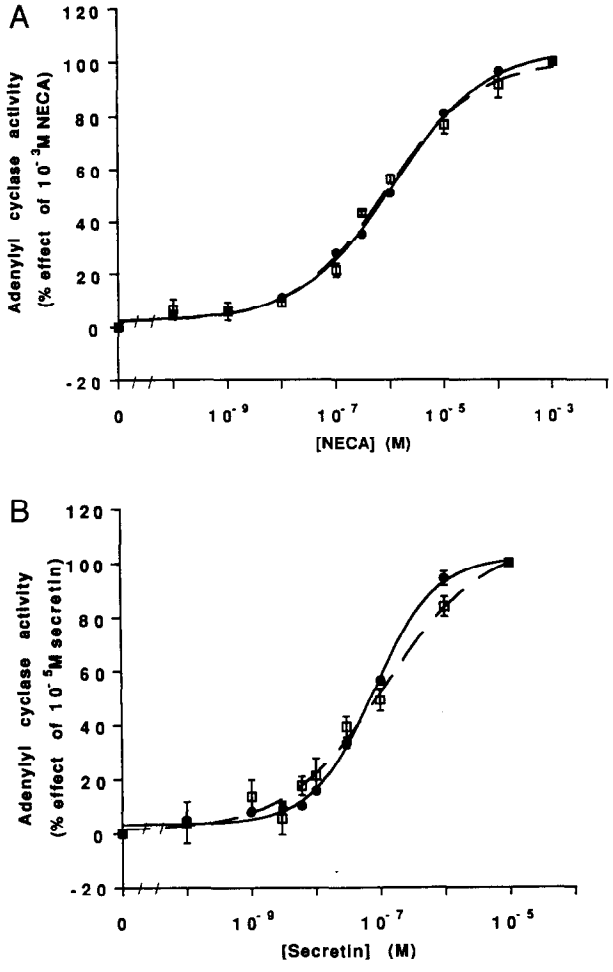

Fig. 4. The regulation of adenylyl cyclase activity by the A_2 adenosine and secretin receptors in similar in NG108-15 and BST15 cell membranes. Adenylyl cyclase activity and its regulation by varying concentrations of NECA (A) or secretin (B) was measured in membranes of NG108-15 (filled symbols) or BST15 (open symbols) cells. Data is presented as% over basal activity compared to that achieved by maximally effective concentrations of NECA (10^{-3} M) or secretin (10^{-5} M). Points represent means ± S.E.M. of quadruplicate assays from representative experiments of three performed with each agonist.

to NG108-15 cells. Despite this, the maximal capacity of the IP prostanoid receptor to cause stimulation of adenylyl cyclase was not increased nor was the potency of the prostanoid agonist ilopost increased to any significant extent (Fig. 3). Very similar results to those of the IP prostanoid agonist were obtained with agonists at both the A_2 adenosine and secretin receptors (Fig. 4) even though we have previously shown that the levels of expression of these two receptors is insufficient to allow maximal activation of the adenylyl cyclase cascade in NG108-15 cells [4,5]. Indeed, the only obvious alteration in adenylyl cyclase regulation in clone BST15 compared to NG108-15 cells was an increase in the basal activity of this cascade. It was thus vital to validate that the relatively small effects observed was not a reflection that HA-$G_s\alpha$ was incapable of causing stimulation of adenylyl cyclase activity. This was achieved by demonstrating that sodium cholate extracts of BST15 cell membranes had approximately 70% greater capacity to allow reconstitution of NaF-stimulated adenylyl cyclase activity to membranes of S49 lymphoma cyc^- cells than equivalent extracts of NG108-15 cells (Fig. 2 and Table 1).

As such, transgenic strategies to increase levels of expression of $G_s\alpha$ do not seem a promising means to regulate signal transduction effectiveness. This may not be surprising in NG108-15 cells as we have noted previously that steady-state levels of $G_s\alpha$ in these cells are substantially higher than levels of either the IP prostanoid receptor (some 10-fold) or adenylyl cyclase (some 70-fold) [2]. As such, further expression of G_s might be anticipated to have a rather limited capacity to modify signal transduction efficiency. It will be of interest, in time, to try to reduce the cellular expression of $G_s\alpha$ and then examine if this can reduce the effectiveness of adenylyl cyclase regulation. Virtually nothing is known of the levels of expression of signal transducing components in the majority of cells and tissues but it is interesting to note that $G_s\alpha$ also appears to be expressed to considerable molar excess over adenylyl cyclase in cardiac myocytes [15] and thus it should not be surprising that overexpression of $G_s\alpha$ in the heart of mice also resulted in little functional modulation of the adenylyl cyclase cascade [16].

A further potential concern in studies such as this is to be able to demonstrate that the excess immunodetectable expressed protein actually has a cellular distribution appropriate to its function and that it is actually accessed by agonist-occupied receptors. We have previously demonstrated this for HA-$G_s\alpha$ in clone BST15 by demonstrating that levels of both this form of the protein and the wild type, endogenous $G_s\alpha$ are regulated equivalently by activation of the IP prostanoid receptor [7]. High level expression of G proteins in transient transfection studies can often be seen to result in greater maximal effectiveness of co-transfected receptors. However, such studies tend to define potentials for receptor-G protein interactions and rarely attempt to provide quantitative details on levels of expression of the proteins of interest. The current study indicates that transgenic increases in levels of expression of G_s are unlikely to provide large functional benefits unless the cell system under study can be shown to express endogenously only low levels of this G protein.

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