Enhanced degradation of the phosphoinositidase C-linked guanine-nucleotide-binding protein $G_q\alpha/G_{11}\alpha$ following activation of the human M1 muscarinic acetylcholine receptor expressed in CHO cells

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Treatment of CHO cells stably expressing the human M1 muscarinic acetylcholine (HM1) receptor with the cholinergic agonist carbachol results in a reduction in cellular levels of $G_q\alpha/G_{11}\alpha$. Half-maximal effects are produced by 3 h, and a new steady state of some 50% of the resting levels of $G_q\alpha/G_{11}\alpha$ is subsequently established [Mullaney, Dodd, Buckley and Milligan, (1993) Biochem. J. **289**, 125–131]. To analyse the mechanism of this effect, we examined the rate of turnover of $G_q\alpha/G_{11}\alpha$ in these HM1-expressing cells in the presence and absence of carbachol (1 mM). In untreated cells the measured removal of ^{35}S-labelled $G_q\alpha/G_{11}\alpha$ was adequately described by a monoexponential curve with a half-time ($t_{0.5}$) of 18.0±2.2 h. When the cells were treated with carbachol a more complex pattern of $G_q\alpha/G_{11}\alpha$ degradation was observed. Upon addition of the agonist, the rate of degradation initially increased markedly ($t_{0.5}$ = 2.9±0.2 h). The maintained presence of the agonist was unable, however, to sustain the enhanced rate of degradation. Beyond 8 h of treatment with carbachol, degradation of $G_q\alpha/G_{11}\alpha$ returned to a rate close to that observed in untreated cells ($t_{0.5}$ = 18.5±1.3 h). Parallel experiments indicated that the effect of carbachol was specific for $G_q\alpha/G_{11}\alpha$, as the $t_{0.5}$ of $G_i2\alpha$ (approx. 30 h) was not affected by the agonist. Analysis of $G_q\alpha/G_{11}\alpha$ mRNA levels by reverse transcriptase/PCR indicated that there was no difference in cells maintained in the absence and presence of carbachol. Such data demonstrate that agonist-induced establishment of a new steady-state level of $G_q\alpha/G_{11}\alpha$ results from an initial receptor-mediated enhancement of protein turnover followed by a desensitization of the receptor response.

INTRODUCTION

A number of recent reports have indicated that agonist activation of G-protein-linked receptors can result in selective 'down-regulation' of the G-protein(s) which that receptor might be anticipated to interact with and hence activate (see [1] for review). The mechanism(s) responsible for these effects has not been addressed, although in some systems the observed additivity in effect of a receptor agonist and the protein synthesis inhibitor cycloheximide in reducing cellular levels of a G-protein has been interpreted to indicate that transcriptional and translational controls are unlikely to contribute significantly to this process [2,3].

The G-proteins G_q and G_{11} have been demonstrated to be involved in receptor-mediated regulation of the phosphoinositidase C-specific phospholipase Cβ1 [4,5] and we have observed that agonist activation of the human M1 muscarinic acetylcholine receptor, stably expressed in CHO cells, results in a decrease of $G_q\alpha/G_{11}\alpha$ by some 40–50%. No significant alterations in other G-protein α subunits were detected in parallel experiments [3]. In this paper we examine the mechanism of agonist-mediated G_q/G_{11} down-regulation in these cells and demonstrate a receptor-mediated enhancement of the degradation of the G-protein(s) which, after a period of some 8 h, subsequently reverts to the basal rate.

MATERIALS AND METHODS

Materials

Trans[^{35}S]-label (1133 Ci/mmol) was supplied by ICN. Protein A–agarose and geneticin were supplied by Sigma. All other reagents for tissue culture were from Gibco/BRL.

Cell generation and culture

Generation and selection of a clone of CHO cells expressing the human muscarinic M1 acetylcholine (HM1) receptor has previously been described in detail [6,7]. Cells were routinely grown in 75 cm^2 tissue culture flasks in Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% newborn calf serum, penicillin (100 units/ml), streptomycin (100 μg/ml) and geneticin sulphate (500 μg/ml) in 5% CO_2 at 37 °C. In preparation for labelling of the cells with Trans[^{35}S]-label, cells were trypsinized from the flasks and seeded in 20 mm-diam. Petri dishes at some 40% confluency and then grown to 90% confluency. The culture medium was then replaced for 2 h with DMEM lacking methionine and cysteine, supplemented with 5% dialysed newborn calf serum. This medium was then aspirated and replaced with 5 ml of DMEM supplemented with 5% dialysed newborn calf serum and containing 5% of the normal methionine concentration plus 30–40 μCi of Trans[^{35}S]-label. After the labelling period (between 20 and 40 h) the labelling medium was removed and cells were subsequently maintained in standard DMEM supplemented with 5% dialysed newborn calf serum in the presence or absence of the cholinergic agonist carbachol (1 mM). Cells were harvested at appropriate times and a crude (P1) membrane fraction was prepared after homogenization of the cells in 10 mM Tris/HCl/0.1 mM EDTA, pH 7.5, using a hand-held Teflon-on-glass homogenizer.

Abbreviations used: HM1 receptor, human M1 muscarinic acetylcholine receptor; DMEM, Dulbecco's modified Eagle's medium; HPRT, hypoxanthine–guanine phosphoribosyltransferase.

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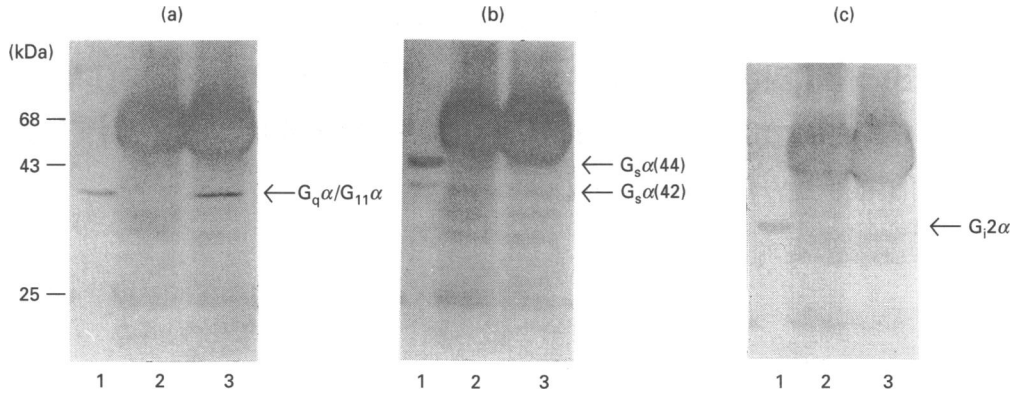

Figure 1 Specific immunoprecipitation of $G_q\alpha/G_{11}\alpha$ from membranes of HM1-expressing CHO cells by antiserum CQ2

Membranes (50 μg) from HM1-expressing CHO cells were either directly resolved by SDS/PAGE [10% (w/v) acrylamide] (lanes 1) or were immunoprecipitated with preimmune serum (lanes 2) or with antiserum CQ2 (lanes 3) prior to addition to the gel. Subsequently, protein was transferred to nitrocellulose and then immunoblotted using (**a**) antiserum CQ2, (**b**) antiserum CS1 or (**c**) antiserum SG1. Only antiserum CQ2 identified a polypeptide(s) in the CQ2 immunoprecipitates (**a**, lane 3), although all of the antisera identified their respective targets in membranes derived from cells (**a**, **b** and **c**, lanes 1).

Immunoprecipitation of ^{35}S-labelled G-proteins

Following measurement of protein amounts by the method of Lowry et al. [8], membranes (30–100 μg of protein) were pelleted in a microcentrifuge and then resuspended with 50 μl of 1% (w/v) SDS. The samples were then heated at 90 °C for 5 min followed by the addition of 950 μl of solubilization buffer (1% 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 at 4 °C [9], containing 1 mM phenylmethanesulphonyl fluoride, 3 mM benzamidine, 0.1 μM soybean trypsin inhibitor, 10 μM leupeptin, 0.2 μM aprotinin and 1.5 μM antipain), and the samples were left on ice for 1 h. Following centrifugation of the samples, the soluble material was removed and precleared by the addition of 50 μl of 4% (w/v) agarose suspension in 0.9% NaCl, which was gently mixed with the sample for 30 min at 4 °C. The agarose was pelleted in a microcentrifuge, and the supernatant was removed and subjected to two further rounds of agarose preclearing. Whole serum (either immune or non-immune), routinely in samples of 10 μl (as this amount of antiserum CQ2 was demonstrated to produce maximally effective immunoprecipitation of $G_q\alpha/G_{11}\alpha$), was then added to the precleared supernatant and the mixture incubated overnight at 4 °C. Protein A–agarose (50 μl/sample) was then added and the samples were mixed gently for 2 h at 4 °C. Immune complexes were then recovered by centrifugation and washed three times with 1 ml of 4% Triton X-100, 100 mM NaCl, 100 mM NaF, 50 mM sodium phosphate, 50 mM Hepes, pH 7.2 [9], and 0.5% SDS. The final Protein A–agarose pellet was resuspended in Laemmli sample buffer, heated to 85–90 °C for 5 min and loaded on to SDS/PAGE [10% (w/v) acrylamide]. Following resolution, the gel was fluorographed by treatment with sodium salicylate (1 M; 2 × 45 min incubations plus 10 min wash with water), dried and autoradiographed using preflashed Fuji X-ray film.

Immunological analysis

The antisera used in these studies have all been characterized previously. Antiserum CQ2 was raised against a conjugate of keyhole-limpet haemocyanin and a synthetic peptide which is predicted to represent the C-terminal decapeptide common to $G_q\alpha$ and $G_{11}\alpha$ [10]. Antisera SG1 [11] and CS1 [12] were generated in similar fashion to identify the C-terminal decapeptides of $G_i1\alpha$ and $G_i2\alpha$ (SG1) and forms of $G_s\alpha$ (CS1).

Preliminary experiments defined the maximally effective amounts of each of these antisera in immunoprecipitation assays in HM1-expressing CHO cells (results not shown). Immunoblotting with each of these antisera was performed as previously described [10].

Data analysis

Autoradiographic films and immunoblots were densitometrically scanned using a Shimadzu CS-9000 dual-wavelength flying spotlaser densitometer and analysed on a Shimadzu FDU-3 central processing unit. Processed data were analysed for goodness of fit to both one- and two-site models using the Kaleidograph curve-fitting programme driven by an Apple Macintosh computer. Statistical comparison of the Chi-square values for one- and two-site curve-fit models was performed by an *F*-test. Only in circumstances in which this statistical test indicated that the two-site model was clearly superior to the one-site model ($P < 0.01$) were the data derived from the two-site model accepted.

Reverse transcriptase/PCR

The overall reverse transcriptase/PCR procedure has been described previously [13].

RNA extractions

Total RNA was extracted according to the acid phenol/guanidinium thiocyanate method of Chomczynski and Sacchi [14]. Purity and quantification were assessed by A_{260}/A_{280} ratios.

Reverse transcription

DNAase digestion of the RNA prior to reverse transcription was omitted since the primers used in this study span several large introns. RNA was precipitated with NaCl/ethanol and redissolved in 5 μl of water. Samples of 1 μg were denatured and reverse-transcribed in 20 μl of buffer composed of 50 mM Tris/HCl, 40 mM KCl, 10 mM $MgCl_2$, 1 mM dithiothreitol, 17 units of RNAguard, 2 μg of oligo$(dT)_{12-18}$ and 13 units of reverse transcriptase (avian myeloblastosis virus; Seikagaku

America Inc). Incubation was carried out at 37 °C for 1 h and stopped by incubation at 80 °C for 10 min. Reaction mixtures were not extracted and were stored at −20 °C prior to amplification.

PCR amplification

PCR reactions were carried out using the following 24-mer primers (synthesized on Applied Biosystems synthesizers): HPRT-231s, CCTGCTGGATTACATTAAAGCACT; HPRT-576a, CCTGAAGTACTCATTATAGTCAAG; $G_q\alpha$-485s, ATGACTTGGACCGTGTAGCCGACC; $G_{11}\alpha$-488s, ACGTGGACCGCATCGCCACAGTAG; $G_{q/11}\alpha$-719a, AGTCAGACAATGAGAACCGCATGG. Amplifications were carried out in 50 μl of buffer containing 10 mM Tris/HCl (pH 9.0), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin, 0.1% Triton X-100, 0.2 mM dNTPs, 20 pmol of primers, template corresponding to 100–200 ng of reverse-transcribed RNA and 1.5 units of Taq polymerase (Promega). Amplification reactions intended for quantification were 'spiked' with 1 μCi of [^{32}P]dATP (NEN; 6000 Ci/mmol). Amplifications were carried out in a Techne PHC-2 thermocycler. Cycles were 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 (25–30 cycles); 95 °C/30 s, 60 °C/30 s, 72 °C/5 min (1 cycle). These conditions ensured a linear relationship between the amount of template mRNA and the amount of amplified product [13].

Reaction products were separated by agarose gel electrophoresis. For quantitative studies, gels were washed in 1 × TAE (0.04 M Tris/acetate, 0.001 M EDTA, pH 8.0), primer bands were excised and the gel was dried under vacuum (80 °C/90 min) prior to exposure to Kodak XAR-5 film. After suitable exposure periods (0.5–18 h), autoradiographs were analysed densitometrically using a Molecular Dynamics densitometer. No signal was observed in samples that were amplified without prior reverse transcription.

RESULTS

The antipeptide antiserum CQ2 was generated against a synthetic peptide predicted to represent the C-terminal decapeptide of $G_q\alpha$ and $G_{11}\alpha$. This sequence is completely conserved between these

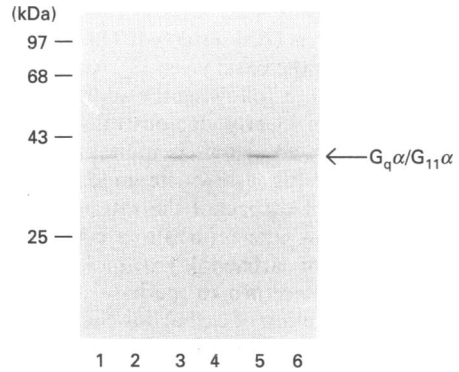

Figure 2 Carbachol treatment down-regulates $G_q\alpha/G_{11}\alpha$ levels in membranes of HM1-expressing CHO cells

Membranes (20 μg, lanes 1 and 2; 30 μg, lanes 3 and 4; 40 μg, lanes 5 and 6) from HM1-expressing CHO cells which were treated without (lanes 1, 3 and 5) or with (lanes 2, 4 and 6) carbachol (1 mM, 16 h) were resolved by SDS/PAGE [10% (w/v) acrylamide] and then immunoblotted using antiserum CQ2 as primary reagent.

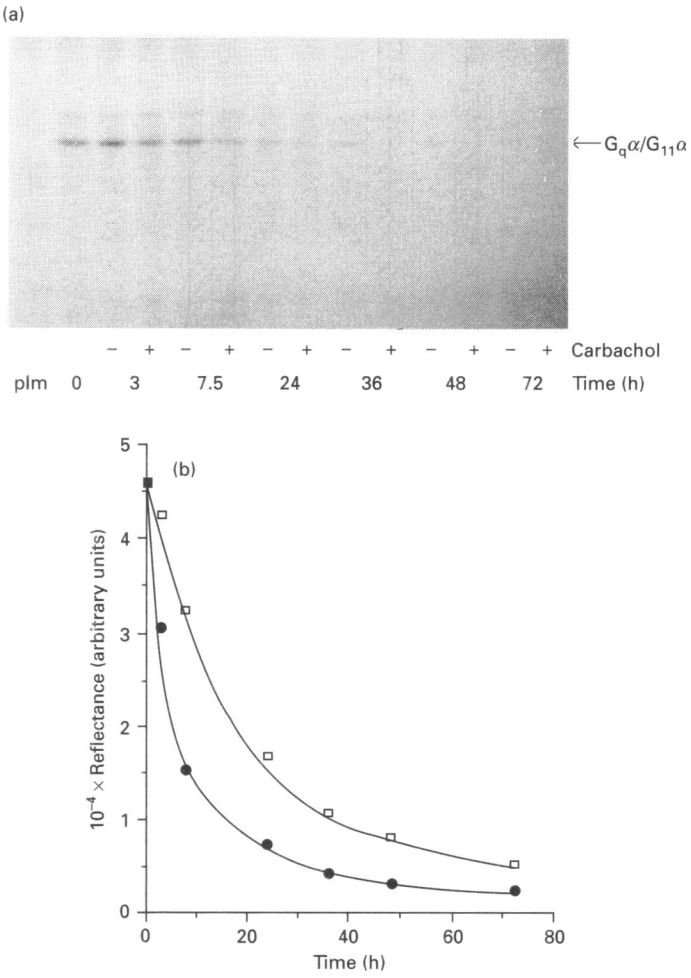

Figure 3 Analysis of the rate of degradation of $G_q\alpha/G_{11}\alpha$ in HM1-expressing CHO cells: effect of receptor activation

HM1-expressing CHO cells were labelled with Trans[^{35}S]-label and then the rate of turnover of $G_q\alpha/G_{11}\alpha$ was measured by immunoprecipitation with antiserum CQ2 as described in the Materials and methods section following various periods in which the cells were maintained in the presence (+) or absence (−) of carbachol (1 mM). (**a**) A fluorogram from a typical experiment is displayed. plm = preimmune serum. Exposure was for 21 days. (**b**) The fluorogram from (**a**) was quantified by densitometric scanning. Signals from $G_q\alpha/G_{11}\alpha$ immunoprecipitated from cells maintained in the absence (□) or presence (●) of carbachol (1 mM) were measured. The data from this figure were analysed for fit consistent with monoexponential decay or for decay with biphasic kinetics. In the absence of carbachol the decay of label in $G_q\alpha/G_{11}\alpha$ was fitted adequately by a monoexponential curve, with $t_{0.5}$ = 16.5 h. The fit was not statistically improved by analysis with more complex models. By contrast, the data from cells maintained in the presence of carbachol were poorly fitted by a one-site model, and the fit was statistically greatly improved when analysed by a two-site model, with a $t_{0.5}$ for the rapid phase of 2.5 h and for the slower phase of 17.5 h.

two G-proteins, and antiserum CQ2 displays high selectivity for G_q/G_{11} in immunoblotting protocols [10]. Immunoprecipitation of membranes of CHO cells stably expressing the HM1 receptor with antiserum CQ2 demonstrated quantitative and specific recovery of $G_q\alpha/G_{11}\alpha$. This was assessed by immunoblotting the immunoprecipitates with a panel of selective anti-Gα antipeptide antisera. The presence of $G_q\alpha/G_{11}\alpha$ in the immunoprecipitates was confirmed using antiserum CQ2 (Figure 1a), but no detectable $G_s\alpha$ (antiserum CS1) (Figure 1b) or $G_i2\alpha$ (antiserum SG1) (Figure 1c) was observed. However, both 44 and 42 kDa splice variants forms of $G_s\alpha$ and the 40 kDa $G_i2\alpha$ were clearly

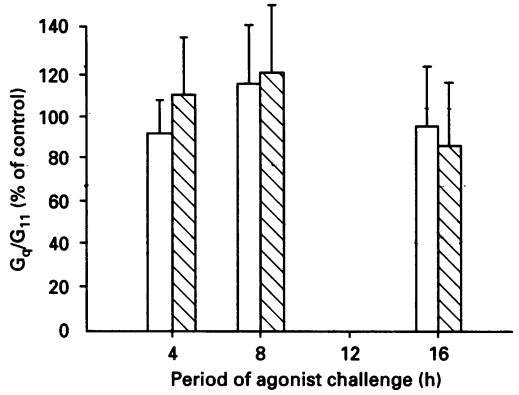

Figure 4 Lack of effect of carbachol on levels of $G_q\alpha/G_{11}\alpha$ mRNA in HM1-expressing CHO cells

Levels of $G_{11}\alpha$ (□) and $G_q\alpha$ (▨) amplified product produced in reverse transcriptase/PCR assays and normalized to the amount of amplified HPRT product (see the Materials and methods section) are shown from HM1-expressing CHO cells exposed to carbachol (1 mM) for various times. Results are expressed as percentages of the values obtained with control treatments. All points represent the means of two independent experiments carried out in duplicate; bars represent S.E.M.

present in HM1-expressing CHO cell membranes which had not been immunoprecipitated (Figure 1, lane 1 of each panel).

Sustained treatment of HM1-expressing CHO cells resulted in a reduction in cellular levels of the α subunit of G_q/G_{11} (Figure 2). To address the mechanism of this phenomenon, HM1-expressing CHO cells were incubated with Trans[^{35}S]-label for 40 h and the decay of radiolabel with time in immunoprecipitated $G_q\alpha/G_{11}\alpha$ was monitored in cells which were either untreated or treated with the cholinergic agonist carbachol (1 mM) (Figure 3a). The specificity of the immunoprecipitation of ^{35}S-labelled material was confirmed by abolition of the presence of radiolabel in the immunoprecipitate when it was performed in the presence of the synthetic peptide used to generate antiserum CQ2 at 1 μg/ml, but not when a peptide corresponding to the equivalent region of forms of $G_s\alpha$ was included (results not shown). Analysis of the rate of decay of ^{35}S-labelled $G_q\alpha/G_{11}\alpha$ (Figure 3b) indicated that in untreated cells this process was monoexponential with an estimated half-time ($t_{0.5}$) of some 18.0±2.2 h (mean±S.E.M.; $n = 7$). By contrast, in the cells maintained in the presence of carbachol the calculated $t_{0.5}$ was clearly more effectively modelled by a two-component fit than by a single exponential. Addition of carbachol was associated with a substantial initial reduction in $t_{0.5}$ for G_q/G_{11} to 2.9±0.2 h ($n = 3$). Interestingly, however, this enhanced rate of G_q/G_{11} degradation was not maintained. At time points beyond 8 h of treatment with carbachol, the $t_{0.5}$ for $G_q\alpha/G_{11}\alpha$ (18.5±1.3 h; $n = 2$) was restored to a value similar to that recorded for untreated HM1-expressing CHO cells.

In contrast to these effects, carbachol (1 mM) treatment of HM1-expressing CHO cells did not stimulate the rate of turnover of $G_i2\alpha$. Immunoprecipitation of membranes, following Trans[^{35}S]-labelling, of untreated cells indicated that the decay of ^{35}S from $G_i2\alpha$ followed a monoexponential curve consistent with a $t_{0.5}$ of 27 h for this polypeptide, while in cells maintained in the presence of carbachol (1 mM) the decay of radiolabel was again represented by a monoexponential function, with a $t_{0.5}$ of 33 h. To assess whether alterations in G_q/G_{11} mRNA levels could also contribute to agonist-mediated regulation of cellular levels of these G-proteins, we performed a comparative reverse transcriptase/PCR assay with RNA isolated from both untreated and carbachol (1 mM)-treated HM1-expressing CHO cells, in order to detect the presence of mRNA encoding $G_q\alpha$ and $G_{11}\alpha$. Levels were normalized to those of hypoxanthine–guanine phosphoribosyltransferase (HPRT). Conditions of amplification were selected [13] to ensure that amplification was in the exponential phase and that a linear relationship existed between initial cDNA concentration and amount of amplified product (results not shown). By relating levels of amplified product corresponding to HPRT to that corresponding to $G_q\alpha/G_{11}\alpha$, it was shown that carbachol treatment of HM1-expressing CHO cells had no significant effect on levels of amplified $G_q\alpha/G_{11}\alpha$ mRNA at any of the time points measured (Figure 4).

DISCUSSION

Carbachol activation of the HM1 receptor results in a down-regulation of cellular $G_q\alpha/G_{11}\alpha$ levels in CHO cells transfected to express this receptor [3]. We have previously noted that the time course of loss of G_q/G_{11} occurs over a period of some 8 h ($t_{0.5}$ = 2–3 h) and that in the continued presence of the agonist a new membrane-bound plateau level of $G_q\alpha/G_{11}\alpha$ is established at some 50–60% of that present in untreated cells [3]. As co-addition of the protein synthesis inhibitor cycloheximide with carbachol provided an additive reduction in cellular $G_q\alpha/G_{11}\alpha$ levels [3], it seemed most likely that enhanced protein degradation was responsible for the agonist-induced reduction in G-protein levels.

To examine this hypothesis, we have used an antiserum which is selectively and quantitatively able to immunoprecipitate $G_q\alpha/G_{11}\alpha$ (Figure 1) to measure the rates of degradation of $G_q\alpha/G_{11}\alpha$ both in untreated cells and in those maintained in the presence of cholinergic agonist carbachol. We have observed that the basal $t_{0.5}$ for $G_q\alpha/G_{11}\alpha$ in these cells is 18.0±2.2 h, and that carbachol stimulation of the cells results in a clear increase in the initial rate of degradation of $G_q\alpha/G_{11}\alpha$ to give a $t_{0.5}$ of approx. 3 h (Figure 3). However, if agonist activation of the receptor was to result in a maintained stimulation of the rate of degradation of the G-protein(s), then in the absence of some further compensatory mechanism, such as an increase in the rate of synthesis of the G-protein(s), the anticipated effect would be to produce a reduction in cellular levels of $G_q\alpha/G_{11}\alpha$ to reach a new steady state which would be a function of the accelerated rate of degradation compared with the basal rate of turnover (i.e. 2.9/18.0 of the initial steady state levels). This, however, is not the phenomenon which has been observed. The new steady state achieved is some 50% of the basal levels [3]. Analysis of the rate of degradation of $G_q\alpha/G_{11}\alpha$ following the addition of carbachol to HM1-expressing cells clearly demonstrates the process is biphasic, while in untreated cells it is monophasic (Figure 3). Analysis of the data using a two-site model indicates that following the addition of carbachol the rate of degradation is very greatly enhanced but later returns to a rate similar to that recorded in the absence of carbachol. Extrapolation of the best-fit data indicates that the return to the basal degradation rate occurs some 8 h after addition of carbachol. Such an effect could be attributed to a 'desensitization' of the receptor response, although it is currently unclear what the molecular mechanism behind this effect might be; alternatively it may reflect the possibility that all of the G-protein which is available to the receptor has been degraded. The effect of carbachol on G-protein degradation is specific. Parallel experiments which examined the turnover of $G_i2\alpha$ indicated that agonist activation of the HM1 receptor did not enhance the rate of turnover of this G-protein.

In an examination of the mechanism of regulation of cellular

levels of G_i2 in DDT_1 MF-2 hamster smooth muscle cells, Hadcock et al. [15] noted that activation of an adenosine A_2 receptor with phenylisopropyladenosine resulted in a decline in the measured half-life of G_i2 from 75 h to 40 h. There was no indication from these studies that the agonist effect on G-protein half-life was transient, but this may reflect the time points chosen for analysis. In related experiments from the same group [16], forskolin, and hence by extension the actions of intracellularly generated cyclic AMP, caused a selective decrease in the half-life of $G_s\alpha$ in S49 mouse lymphoma cells. Again, this appeared to be maintained throughout the period of analysis. Such studies hint at the potential involvement of protein kinase regulation of G-protein half-life, but this clearly must be dependent upon the cell system being studied. For example, in our own studies on receptor-mediated down-regulation of G_2 produced by the activation of an IP prostanoid receptor in neuroblastoma × glioma hybrid NG108-15 cells, the generation of cyclic AMP is not involved in the regulation of cellular G_s levels, as addition of forskolin or membrane-permeant analogues of cyclic AMP were unable to mimic the effect of receptor activation [2]. In this system, indirect evidence indicates that regulation of the rate of degradation of the G-protein will represent the central mechanism for agonist-induced regulation of cellular G-protein levels [2,17]. The absence of any effect of carbachol treatment on steady-state levels of $G_q\alpha$ or $G_{11}\alpha$ mRNA is consistent with the notion that the observed changes in levels of $G_q\alpha/G_{11}\alpha$ protein are not due to a change in the rate of synthesis, although it is still a formal possibility that the rate of translation could alter in the absence of any changes in the steady-state levels of mRNA.

It appears to be clear that G-protein turnover is relatively variable in different cells. As noted above, the basal rate of turnover of G_i2 in DDT_1 MF-2 cells [15] has been reported to be some three times slower than the data we provide herein. Furthermore, Silbert et al. [18] noted very different rates of turnover for $G_o\alpha$ between GH4 pituitary-derived cells and primary cardiocytes, and Brabet et al. [19] have suggested that the rate of degradation of $G_o\alpha$ (and $G_o\alpha$ splice variants) may be a reflection of the state of differentiation of neural cells.

It is likely that only the fraction of the cellular G-protein which is activated by receptor occupancy will act as a target for enhanced proteolytic degradation, and this may explain why dose–response curves of agonist-induced G-protein down-regulation very closely parallel receptor occupation [2,3]. Furthermore, it is clear that agonist-induced down-regulation of G-proteins is not observed as a universal phenomenon, and thus it may be that in systems in which it is not observed the receptor is able to activate only a small proportion of the total membrane amount of the G-protein. As such, enhanced degradation of this small component would not be detectable within the background of a larger amount of the same G-protein being degraded at the basal rate.

Whether receptor activation results in promotion of the rate of degradation of the G-protein by a conformational change which allows greater access to the proteolytic enzyme or results from a covalent modification of the G-protein remains to be examined. It should be noted in this context, however, that cholera toxin treatment of cells, which causes the ADP-ribosylation of $G_s\alpha$, results in a large reduction in cellular levels of $G_s\alpha$ [20–22], but it is unclear whether this is a reflection of the ADP-ribosylation produced by the toxin or because this modification functionally uncouples the G-protein from receptors.

There results demonstrate for the first time that activation of a phosphoinositidase C-linked receptor can increase the rate of turnover of the phosphoinositidase C-linked G-protein(s), and that establishment of a new steady-state level of the G-protein(s) results from a subsequent densitization of the receptor response. Such receptor regulation of $G_q\alpha/G_{11}\alpha$ turnover demonstrates a new mechanism for the control of cellular G-protein levels and indicates that regulation of G-protein levels is a dynamic process in cells which can be regulated by hormones and neurotransmitters.

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