The turkey erythrocyte β-adrenergic receptor couples to both adenylate cyclase and phospholipase C via distinct G-protein α subunits

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By contrast with mammalian β-adrenergic receptors, the avian isoform elicits two distinct effector responses, activation of adenylate cyclase and polyphosphoinositide-specific phospholipase C (PLC) leading to the accumulation of both cyclic adenosine monophosphate (cyclic AMP) and inositol phosphates. We have investigated the mechanisms of β-adrenergic receptor signalling in turkey erythrocytes. Stimulation of adenylate cyclase by the β-adrenergic-receptor agonist isoprenaline exhibits a 30-fold lower EC_{50} than that for PLC activation, which may indicate a marked receptor reserve for the former effector. Similar K_i values were obtained for the inhibition of both responses by four β-adrenergic antagonists, arguing that a single receptor population is responsible for both effects. Antibodies raised against G-protein peptide sequences were used to show that the identity of the G-protein mediating the PLC response was an avian homologue of G_{11}, the level of expression of which was very similar to that of the stimulatory G-protein of adenylate cyclase, G_s. Thus a single population of β-adrenergic receptors apparently interacts with distinct G-proteins to activate different effectors. The stoichiometries of the receptor–G-protein–effector interactions are therefore similar for both second-messenger responses and the data are discussed in terms of the different efficacies observed for each response.

INTRODUCTION

Activation of the family of phosphoinositide-specific phospholipases C (PLCs) results in the formation of two intracellular second messengers involved in diverse cellular events such as activation, proliferation and secretion [1]. Inositol 1,4,5-trisphosphate increases cytosolic free calcium concentrations by inducing release from intracellular stores and possibly influx from the extracellular medium [1]. Diacylglycerol is involved in the activation of phospholipid- and calcium-dependent isoforms of protein kinase C [2].

Turkey erythrocytes have been used as a cell-free model to investigate various aspects of signal transduction via the hydrolysis of inositol phospholipids. These cells express P_{2y} purinergic receptors and β-adrenergic receptors [3–5], both of which are coupled to a β-isoform of PLC in a guanine nucleotide-dependent manner. Furthermore, while > 90% of the endogenous PLCβ is cytosolic [6], the receptor- and G-protein-induced activation of the phosphoinositide signalling cascade can be achieved in ghost membrane preparations washed free of the cytosolic enzyme. In such preparations, the endogenous receptor- and G-protein-responsive PLC has been shown to be associated with the actin cytoskeleton [7].

The G_q family of G-proteins was recently shown to be responsible for mediating activation of β-isoforms of PLC [8,9], which can be mimicked using purified components in reconstitution assays. The gene encoding a turkey PLC-activating G-protein α subunit was recently cloned from a turkey brain cDNA library and the encoded protein was found to be most similar to mammalian G_{11} of the G_q family, based upon sequence homology [10]. In addition, the βγ subunits of heterotrimeric G-proteins have been reported to activate three of the four known β isoforms of PLC [11,12], an effect which may account for pertussis toxin-sensitive stimulation of PLC, and adds further complexity to receptor stimulation of the phosphoinositide signal-transduction pathway. The βγ-mediated stimulation of PLCβ contrasts with that for adenylate cyclase where βγ stimulation of adenylate cyclases II and IV is absolutely dependent on the presence of an active α subunit of G_s[13] and therefore serves to potentiate the effect of a cyclase agonist. βγ-Mediated PLC stimulation can be demonstrated in the absence of G-protein α subunits.

The mechanism of activation of PLCβ by the β-adrenergic receptor in turkey erythrocytes has not been confirmed. Three modes of activation of PLCβ which have been previously outlined in ref. [14] are considered in this paper. First, the turkey erythrocyte may express multiple subtypes of the β-adrenergic receptor which couple to distinct G-proteins. A second possibility is that the turkey erythrocyte expresses a single population of β-adrenergic receptors which stimulates adenylate cyclase via $G\alpha_s$ and PLCβ activation is secondary to the formation of, and mediated by, free βγ subunits from heterotrimeric G_s. Thirdly, a single population of receptors may couple to multiple G-proteins and thereby regulate separate effectors. We have attempted to distinguish between these possibilities using pharmacological and immunological tools to dissect the participating β-adrenergic receptors and the G-protein α subunits with which they interact. The results suggest that a pharmacologically homogeneous population of β-adrenergic receptors can activate two distinct G-proteins, G_s and G_{11} respectively, with the former mediating the activation of adenylate cyclase and the latter regulating PLC.

MATERIALS AND METHODS

Materials

Isoprenaline was from Sigma and adenosine 5′-[β-thio]diphosphate (ADP[S]) was from Boehringer Mannheim.

Abbreviations used: AlF_4^-, fluoroaluminate; βγ, βγ dimer from heterotrimeric G-protein; G-protein, heterotrimeric GTP-binding protein; PLC, polyphosphoinositide-specific phospholipase C; ADP[S], adenosine 5′-[β-thio]diphosphate; GTP[S], guanosine 5′-[γ-thio]triphosphate.

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Antibody CQ1 was raised against a C-terminal decapeptide of G_q and purified as described [15]. The anti-G_s antibody, RM1 [16], was a kind gift from Dr. Allen Spiegel (NIH, Bethesda, MD, U.S.A.). Anti-G_q-specific serum (IQB) was raised against a peptide representing amino acids 119–134 of the G_q primary sequence [17], which is not well-conserved in G_{11} isoforms. The purified α subunit of turkey erythrocyte G_{11} was provided by Dr. T. K. Harden, University of North Carolina. Purified G_q and G_{11} from bovine liver were kind gifts from Dr. J. Exton, Vanderbilt University. [^{3}H]Inositol, [^{14}C]cyclic AMP and [^{3}H]ATP were purchased from Amersham International.

Tissue preparation

Rat frontal cortex (0.5 g weight) was suspended in 5 ml of 10 mM Tris/HCl, pH 7.5, 0.1 mM EDTA (buffer A) and disruption of the tissue achieved with 25 strokes of a hand-held Teflon-on-glass homogenizer. The resulting homogenate was centrifuged at 500 *g* for 10 min in a Beckman L5-50B centrifuge with a Ti 50 rotor to remove unbroken cells and nuclei. The supernatant fraction from this treatment was then centrifuged at 48000 *g* for 10 min and the pellet was washed and resuspended in 10 ml of buffer A. After a second centrifugation at 48000 *g* for 10 min, the membrane pellet was resuspended in buffer A to a final protein concentration between 1 and 3 mg/ml and stored at −80 °C until required.

Erythrocyte membrane preparation

Turkey erythrocytes were collected, washed and lysed as previously described [5]. Membranes were prepared from intact turkey erythrocytes or from ghosts by addition of 0.25 vol. of 1.5 mM Hepes/150 mM NaCl, pH 7.4, followed by disruption with glass beads (0.25 mm) by vortex-mixing four times for 30 s using a bench-top 'vortex' mixer, interspersed by periods on ice. The glass beads were allowed to settle and the supernatant was centrifuged for 15 min at 500 *g* at 4 °C to remove nuclei. The membrane-containing supernatant was either used immediately for SDS/PAGE or washed further with 20 mM Tris/HCl, pH 7.5, 1 mM EDTA and centrifuged in a bench-top microfuge at $\approx$ 12000 *g*.

SDS/PAGE analysis

Separation of proteins by SDS/PAGE and blotting were performed on 13% (w/v) polyacrylamide gels, exactly as described by Blank et al. [18]. After proteins were transferred to 'ProBlott' poly(vinylidene difluoride) membranes (Applied Biosystems), proteins were probed with anti-peptide sera specific for the C-termini of forms of $G_s\alpha$, $G_q\alpha$ or $G_q\alpha/G_{11}\alpha$. Visualization of the protein–antibody complexes was achieved using a horseradish peroxidase-linked secondary antibody and the ECL 'Vectastain' system (Vecta Laboratories).

To quantify the level of expression of G-proteins, turkey erythrocyte membranes, prepared as described in the Materials and methods section, along with various amounts of *Escherichia coli*-produced recombinant $G_q\alpha$ or the long splice variant of $G_s\alpha$ were resolved by SDS/PAGE, transferred to nitrocellulose and probed with anti-peptide sera specific for the C-termini of $G_s\alpha$ and $G_{11/q}\alpha$. Densitometric scanning of protein bands using a Shimadzu CS-9000 dual-wavelength flying-spot laser densitometer [17] allowed construction of a standard curve for each G-protein (see [19]). As the anti-G_q/G_{11} serum identifies each of these proteins equally because the epitope recognized is identical between mammalian $G_q\alpha$ and $G_{11}\alpha$ and turkey $G_{11}\alpha$, then mammalian $G_q\alpha$ provides an appropriate standard curve for turkey $G_{11}\alpha$.

PLC responses

Turkey erythrocyte PLC responses were performed as previously described [5,7]. Briefly, ghosts were labelled with [^{3}H]inositol, incubated with 10 mM MgATP, thoroughly washed and stimulated with the indicated concentrations of either purinergic (ADP[S]) or β-adrenergic (isoprenaline) agonists in the presence or absence of guanosine 5′-[γ-thio]triphosphate (GTP[S]). Reactions were performed at 37 °C and terminated after 4 min by addition of ice-cold 6.25% (w/v) perchloric acid. After neutralization with a 1:1 mixture of trioctylamine/trichlorotrifluoroethane, inositol phosphates were resolved using mini-columns of Bio-Rad AG-1 (200–400 mesh in the formate form).

Adenylate cyclase assays

Turkey erythrocyte ghosts were stimulated as above in the presence of [^{3}H]ATP and in the presence or absence of the indicated β-adrenergic antagonists. After termination with 5% (v/v) perchloric acid, cell extracts were spiked with a known quantity of [^{14}C]cyclic AMP and applied to Bio-Rad AG-50W X8 cation-exchange resin columns as above. Free ATP was eluted with water and cyclic AMP was eluted with a second volume of water. The eluate was allowed to run into columns of alumina and cyclic AMP was eluted from this with 50 mM Tris, pH 8. Production of cyclic AMP was quantified by scintillation spectrometry and losses corrected for by reference to the ^{14}C standard.

Pretreatment with antibodies

In experiments designed to study the role of different G-proteins, ghosts were labelled with [^{3}H]inositol, incubated with 10 mM MgATP for 15 min, washed free of unincorporated label and incubated (1 mg of ghost protein per ml), with the appropriate anti-G-protein antibody dilutions on ice to minimize metabolism of ^{3}H-labelled lipids. After 1 h, ghosts were washed once with 10 mM Hepes, pH 7.4, 5 mM $MgCl_2$, diluted in the same buffer and used immediately.

RESULTS

Isoprenaline-stimulated PLC responses

It has been reported previously that stimulation of turkey erythrocytes via the β-adrenergic receptor activates phosphoinositide hydrolysis independently of cyclic AMP production [4,5]. To determine the relative efficacy of the β-adrenergic agonist, isoprenaline, for stimulation of PLC, versus adenylate cyclase, turkey erythrocyte ghosts were stimulated as described in the Materials and methods section with increasing concentrations of isoprenaline in the presence of 10 μM GTP[S] (Figure 1). When assayed under identical conditions, PLC responses to isoprenaline lay significantly to the right of adenylate cyclase responses (Figure 1 and [5]), and determination of the EC_{50} values for each response demonstrated that the PLC response was approx. 30-fold less sensitive than the adenylate cyclase response (4.4 nM versus 126 nM).

The above data would be consistent either with the presence of two populations of β-receptors with different sensitivities to isoprenaline or with a requirement for greater receptor occupancies for the activation of PLC compared with adenylate cyclase. To distinguish between these two possibilities, adenylate

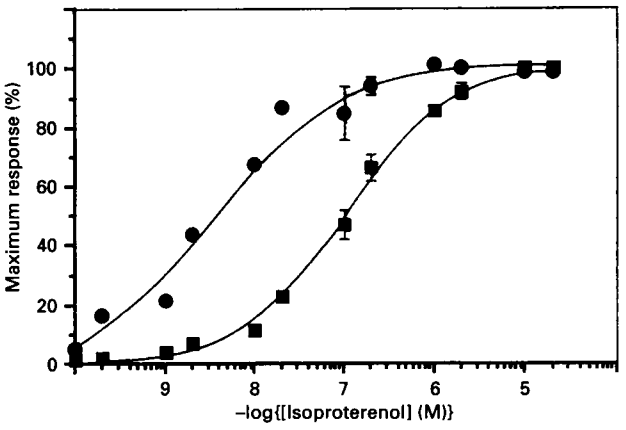

Figure 1 Dose–response relationships for isoprenaline-stimulated second messenger responses

[^{3}H]Inositol-labelled turkey erythrocyte ghosts were assayed for PLC activity (■) in the presence of 10 μM GTP[S] and increasing concentrations of isoprenaline. Assays were performed for 4 min at 37 °C and were stopped and assessed for inositol phosphate production as described in the Materials and methods section. Data are expressed as percentages of the maximum response observed. These data are taken from previously published data from this laboratory [5]. Adenylate cyclase activation (●) was assayed under identical conditions and data are similarly expressed as percentages of the maximum response. Each point is the mean of duplicates ± range and data are representative of three experiments.

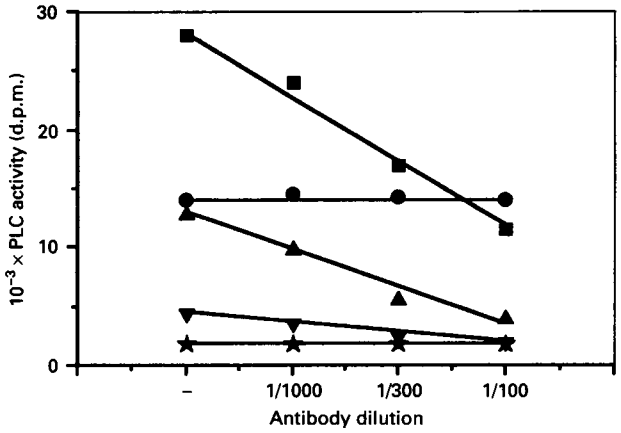

Figure 2 The effect of pretreatment of turkey erythrocyte ghosts with increasing concentrations of anti-G_q antibodies on agonist and guanine nucleotide-stimulated PLC responses

[^{3}H]Inositol-labelled turkey erythrocyte ghosts were incubated with increasing dilutions of anti-G_q antibodies on ice for 1 h. Ghosts were then assayed for PLC responses as described in the Materials and methods section, using 1 μM GTP[S] (▼), 10 μM ADP[S] plus 1 μM GTP[S] (■), isoprenaline plus 1 μM GTP[S] (▲) or 10 μM AlF_4^- (●). Control experiments without addition (★) were also performed.

Table 1 β-receptor antagonists block isoprenaline-stimulated adenylate cyclase and PLC with equal potencies

Adenylate cyclase and PLC assays were performed as described in the Materials and methods section, in the presence of 10 μM isoprenaline and increasing concentrations of the antagonists. K_i values were calculated using EC_{50} values of 4.4 nM and 126 nM for stimulation of adenylate cyclase and PLC respectively. Values are from three separate experiments and are means ± S.E.M.

Antagonist	K_i against PLC (nM)	K_i against adenylate cyclase (nM)
Propranolol	1.12 ± 0.18	2.06 ± 0.31
ICI 118551	37 ± 6	64 ± 9
CGP 20172A	74 ± 12	78 ± 18
Betaxolol	139 ± 31	170 ± 25

cyclase and PLC assays were performed in the presence of a single concentration of isoprenaline and increasing concentrations of various β-receptor antagonists. K_i values were calculated for each antagonist using the Cheng–Prusoff equation: $K_i = IC_{50}/[1+(A/EC_{50})]$, where A is the single concentration of isoprenaline used. Using this form of the equation compensates for the intrinsic activity of the agonist to elicit an effector response [20] and allows comparison of each antagonist to block both PLC and adenylate cyclase responses. EC_{50} values of 4.4 nM and 126 nM for adenylate cyclase and PLC respectively were derived from Figure 1. The results are shown in Table 1. K_i values were not significantly different for either effector for all antagonists. These data suggest that a homogeneous population of β-adrenergic receptors mediates activation of adenylate cyclase and PLC with distinct sensitivities, suggesting a requirement for a higher degree of receptor occupancy to elicit the maximum PLC response.

Table 2 CQ1 anti-$G_{q/11}$ serum has no effect on turkey erythrocyte ghost adenylate cyclase responses

Turkey erythrocyte ghosts were pretreated with 1/100 dilution of CQ1 antiserum or with vehicle as described in the Materials and methods section and assayed for receptor and G-protein-regulated adenylate cyclase activity in the presence of the indicated agonists. Assays were stopped and cyclic AMP production assessed as described in the Materials and methods section. Values are means of duplicate determinations and are representative of three experiments which gave essentially identical results.

Treatment	Adenylate cyclase activity (pmol of cyclic AMP per 4 min assay)	
	Control	CQ1 pretreated
Buffer	2.5	3.0
1 μM GTP[S]	6.0	7.0
10 μM Isoprenaline + 1 μM GTP[S]	48.0	51.0
10 μM Adenosine + 1 μM GTP[S]	21.0	21.0
AlF_4^-	103.0	101.0

Identification of the G-protein mediating PLC activation

To determine whether the PLC response was due to direct activation of a G-protein of the G_q family, turkey erythrocyte ghosts were labelled with inositol and pre-incubated on ice for 1 h with various dilutions of the anti-G_q/G_{11} antibody, CQ1. Subsequent PLC responses were increasingly inhibited, as demonstrated by the smaller production of inositol phosphates (Figure 2). Responses to GTP[S], ADP[S] and isoprenaline in the presence of GTP[S] were all inhibited over the same range of antibody titres. CQ1 was equally potent for isoprenaline- and ADP[S]-stimulated responses. Interestingly, PLC responses to

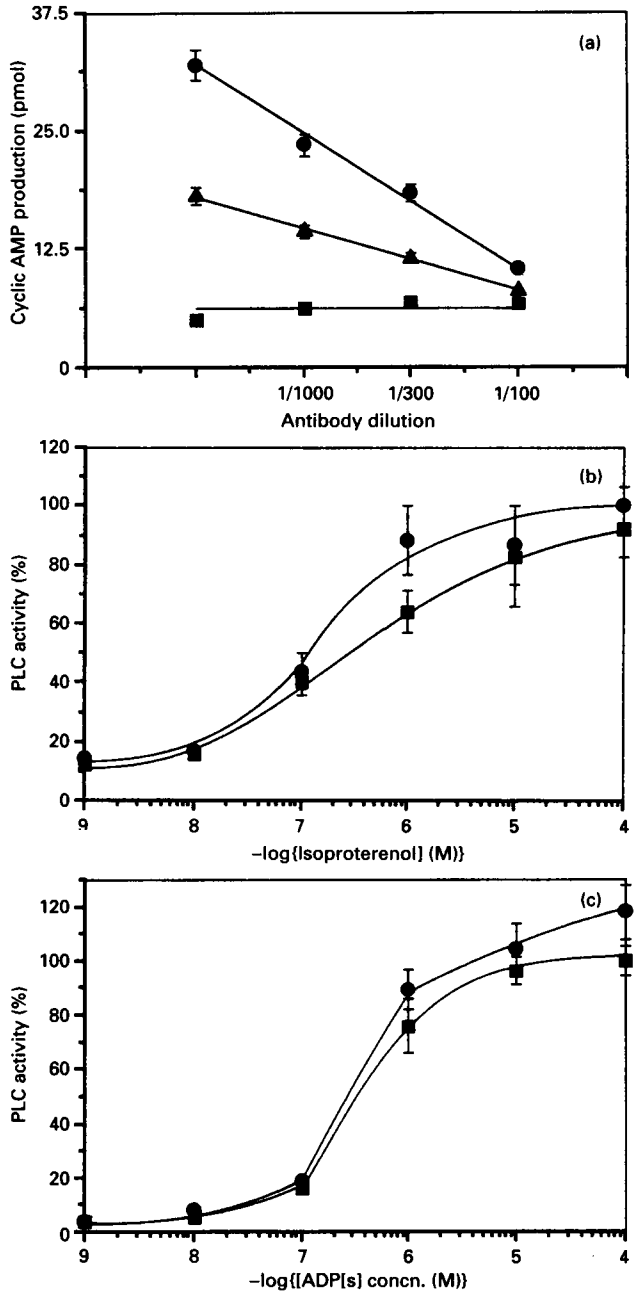

Figure 3 The effect of pretreatment of turkey erythrocyte ghosts with anti-G_q antibodies on agonist and guanine nucleotide-stimulated responses

Turkey erythrocyte ghosts were treated as described in the legend to Figure 2 and assayed for (**a**) adenylate cyclase responses to 100 μM isoprenaline plus 1 μM GTP[S] (●), GTP[S] alone (■) and AlF_4^- alone (▲); or (**b**) PLC responses to isoprenaline and (**c**) ADP[S] in the presence of 1 μM GTP[S]. In (**b**) and (**c**) ■ represents controls and ● represents RM1-pretreated samples. Assays were performed for 4 min at 37 °C.

AlF_4^- were not affected by the presence of CQ1 antibodies. A similar distinction between GTP[S]- and AlF_4^--evoked responses in various cell types was previously reported for antibody X384 raised to the same epitope of G_q [21], and has been observed with antibodies raised to the C-terminus of G_s [16,22].

Time-course experiments, wherein the period of stimulation was increased to as much as 1 h, showed that the CQ1-induced inhibition was not simply a time lag effect, as PLC responses

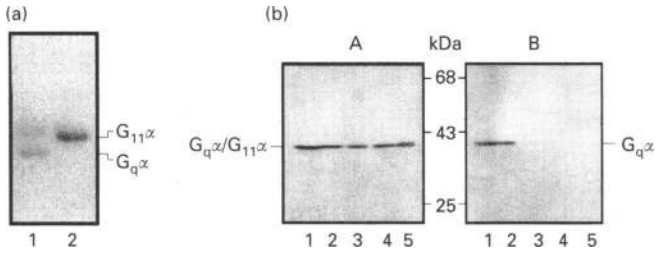

Figure 4 The turkey erythrocyte PLC-stimulatory G-protein is a single species equivalent to mammalian G_{11} rather than G_q

(**a**) 10 ng of either purified mammalian G_q and G_{11} (lane 1) or purified turkey erythrocyte PLC-stimulatory protein (lane 2) were resolved by SDS/PAGE as described in [17]. These samples were immunoblotted using antiserum CQ1 and protein bands visualized as described in the Materials and methods section. (**b**) Membranes from rat frontal cortex (50μg, lane 1) or turkey erythrocytes (50 μg, lane 3 and 100 μg, lane 4) were resolved in SDS/PAGE [10% (w/v) acrylamide] alongside a purified mixture of rabbit liver G_q/G_{11} (20 ng, lane 2) and purified turkey G_{11} (20 ng, lane 5). Samples were transferred to nitrocellulose and immunoblotted with either (panel A) antiserum CQ1 (which identifies $G_q\alpha$ and $G_{11}\alpha$ equally) or (panel B) with antiserum IQB (which identifies only $G_q\alpha$). Protein bands were visualized as for (**a**).

remained significantly smaller in CQ1-treated ghosts over all periods of stimulation (S. R. James and C. P. Downes, unpublished work). Thus pretreatment with anti-G_q/G_{11} antibodies appeared to disable GDP-bound G-proteins in the ghost preparations, inhibiting both purinergic- and β-adrenergic-induced responses. This indicates that PLC activation by isoprenaline is due to activation of a G-protein of the G_q family and not solely by a $\beta\gamma$-stimulated mechanism. The effect of CQ1 was specific for PLC responses and cyclic AMP production was not affected (Table 2), indicating that the G-protein responsible for activating adenylate cyclase was not affected by this antibody. By comparison, pretreatment of ghosts with anti-G_s antibody blocked adenylate cyclase activity (Figure 3a) but had no effect on the PLC response to guanine nucleotide and isoprenaline (Figure 3b) or ADP[S] (Figure 3c). Stimulation with either isoprenaline or ADP[S] in the presence of GTP[S] was not significantly altered in anti-G_s-treated ghosts from controls and agonist dose–response curves were virtually superimposable.

The above results indicate that a member of the G_q family of G-proteins mediates isoprenaline-stimulated PLC responses. To clarify the identity of the particular G-protein with which the β-adrenergic receptor interacted, proteins from turkey erythrocyte membranes were resolved by SDS/PAGE and compared with a mixture of mammalian liver G_q and G_{11} (Figure 4). After transferring to nitrocellulose, blots were probed with CQ1 antibody as described in the Materials and methods section. Figure 4(a) shows that the mammalian mixture of G_q and G_{11} was clearly resolved into two polypeptides ($G_q\alpha$ migrates more slowly in such conditions; [22a]) while the avian PLC-stimulatory G-protein migrated as a single band in a position similar to mammalian G_{11}. Cloning and sequencing of the turkey protein indicates it to be closely related to mammalian G_{11} [10]. In a second experiment, a mirror-image Western blot was probed with CQ1 (left-hand side) and with IQB (right-hand side) specific for α_q (Figure 4b). Turkey erythrocyte membrane proteins (25 or 50 μg) were compared with protein derived from rat frontal cortex, a purified mixture of rabbit liver G_q and G_{11} and purified α_{11} from turkey erythrocytes. Blots probed with CQ1 yielded a protein band in all lanes at approx. 43 kDa consistent with this antibody recognizing proteins of the G_q family of G-proteins. By contrast, the G_q-specific antibody IQB, displayed positive

immunoreactivity with both mammalian samples but indicated a lack of expression of $G_q\alpha$ in turkey erythrocyte membranes. These results therefore indicate that β-adrenergic stimulation of PLCβ in turkey erythrocytes is mediated by an avian homologue of mammalian G_{11}.

Relative concentrations of G_s and G_{11} in turkey erythrocytes

From the data shown in Figure 1, the β-adrenergic agonist isoprenaline appears to be a more effective activator of adenylate cyclase than PLC in the turkey erythrocyte. To determine whether differences in G-protein expression mediate the greater efficacy of isoprenaline as an activator of adenylate cyclase, the concentrations of both G_s and G_{11} were determined in turkey erythrocyte membranes by Western blotting of proteins resolved by SDS/PAGE, followed by densitometric scanning of the protein bands as described in the Materials and methods section. Results showed that the concentrations of G_s and G_{11} were 0.48 ± 0.05 μg/mg and 0.62 ± 0.11 μg/mg respectively (mean ± S.D., three experiments). Thus levels of expression of each protein were similar and it appears that the greater sensitivity of adenylate cyclase activation via the β-adrenergic receptor is not simply due to a higher level of expression of G_s.

DISCUSSION

The turkey erythrocyte ghost represents a useful cell-free system for the study of various aspects of signal transduction via the hydrolysis of polyphosphoinositides. Extensive characterization has revealed that both purinergic and β-adrenergic receptors are expressed which couple to a cytoskeleton-associated PLCβ in a G-protein-dependent manner. In this study, we have extended the characterization of this system to show that stimulation of both PLC and adenylate cyclase by the β-adrenergic agonist, isoprenaline, is mediated via a single receptor population which shows greater affinity for G_s and adenylate cyclase. In addition, the results indicate that PLC activation is not due solely to release of βγ subunits from G_s, but includes interaction of the β-adrenergic receptor with a member of the G_q family of G-proteins and confirm that the G-protein expressed in turkey erythrocytes is G_{11}.

While it is clear that stimulation of PLCβ isoforms by βγ subunits can occur independently of activated α subunits [11,12], it is formally possible that the isoprenaline-induced effects observed here are due to synergy between GTP[S]-liganded α_{11} and free βγ subunits released from G_s, leading to an amplified PLC response. This interpretation of our data can be discounted for several reasons. First, the anti-G_s antibody RM1 had no effect on isoprenaline- and ADP[S]-induced PLC responses. This antibody and another similar to it are believed to bind to GDP-coupled G_s holomers, preventing guanine nucleotide exchange and maintaining the heterotrimeric form of the G-protein [16,22]. Although there has been some debate concerning the order of events leading to G_s activation [23], we have no evidence to indicate that RM1 does not behave in this manner in turkey erythrocytes. Therefore, if synergy between G_s-derived βγ subunits and GTP[S]-coupled α_{11} were the cause of the isoprenaline-stimulated PLC responses, antiserum RM1 would be expected to inhibit this in a dose-dependent fashion. This was not observed. Secondly, addition of purified βγ subunits to turkey erythrocyte ghosts had no effect on GTP[S]-stimulated PLC responses [24], indicating that elevations in endogenous free βγ subunit concentrations would not amplify PLC responses to GTP[S]. Thirdly, pretreatment of turkey erythrocytes with cholera toxin would be predicted to cause dissociation of G_s heterotrimers, thus elevating free βγ concentrations. When this experiment was done, subsequent agonist- and GTP[S]-evoked responses were not affected [4]. These data argue that PLC responses observed after β-adrenergic receptor stimulation in turkey erythrocytes are not due to synergy between GTP[S]-liganded α_{11} and free βγ subunits and are more likely due to β-receptor activation of α_{11} directly, supporting our hypothesis.

The avian β-adrenergic receptor is one of a growing number of examples displaying promiscuous signalling behaviour where a receptor is able to activate multiple effectors (reviewed in [14]). Thus, stimulation of the β-adrenergic receptor causes the accumulation of both cyclic AMP and inositol phosphates [4,5]. We have established that the activation of multiple effectors by the turkey erythrocyte β-adrenergic receptor is probably mediated by a homogeneous population of receptors which interacts with both G_s and G_{11}. Mammalian homologues of the β-adrenergic receptor, while being prototypic activators of adenylate cyclase, do not appear to interact with multiple effectors. The basis for the difference in behaviour of the avian β-adrenergic receptor compared with the mammalian receptor is not known. However, the receptors show greatest sequence homology and similarity in the transmembrane portions of the receptor (72% identity; [25]) and exhibit marked differences in the less hydrophobic regions, particularly the portion of primary sequence forming the third intracellular loop. Since this region of receptors is thought to be highly influential in determining interactions with G-proteins [26], it seems reasonable to suggest that sequence differences here may underlie the avian receptor's ability to interact with multiple G-proteins. However, it cannot be ruled out formally that the turkey erythrocyte expresses distinct subtypes of the β-adrenergic receptor, despite only a single receptor clone being obtained [25] and the inability to distinguish them pharmacologically (Table 1).

The agonist sensitivities of the adenylate cyclase and PLC responses were markedly different in the turkey erythrocyte, with the β-adrenergic agonist isoprenaline showing greater efficacy as an activator of adenylate cyclase. This was not due to marked differences in the levels of expression of $G_s\alpha$ and $G_{11}\alpha$ in turkey erythrocytes. Previous measurements of the expression of the β-adrenergic receptor in turkey erythrocyte membranes have shown that the concentration is approx. 460 fmol per mg of protein [27]. Thus, both G_s and G_{11} are expressed at approx. 30-fold excess over the β-adrenergic receptor. Secondly, PLCβ has been shown to act as a GTPase-activating protein to G-proteins of the G_q family [28] and the sensitivity of the PLC response to β-adrenergic agonists could be reduced relative to that of adenylate cyclase by the ability of the effector to enhance the rate of inactivation of G_{11}. However, the experiments reported here were performed using the non-hydrolysable analogue of GTP, GTP[S], thereby eliminating the GTP hydrolysis step in these assays. Thus it seems unlikely that enhanced guanine nucleotide hydrolysis by G_{11} induced by PLC caused the lower sensitivity of the PLC response.

The relative coupling efficiencies between the avian β-adrenergic receptor and adenylate cyclase versus PLC might alternatively reflect differences in the level of expression of the effector enzymes themselves. Not only have the expression levels of PLCβ and adenylate cyclase in this system not been quantified, but it is clear that only a small fraction of the PLC itself is required to couple efficiently to fully activated G_{11} [7]. Optimum stoichiometries for these interactions are probably more easily defined in reconstituted systems than in intact membranes.

Using antibodies raised to various peptide sequences within G_s and G_{11}, which represent relatively simple biochemical tools, we have confirmed that avian erythrocytes do not contain a G_q G-protein, but rather that receptor-induced activation of PLC is

mediated by a G-protein with characteristics most similar to the mammalian G_{11} G-protein. This G-protein appears to be present in apparently similar quantities to G_s, yet coupling of it to the β-adrenergic receptor is apparently less efficient than that exhibited by G_s. Further studies are therefore required to determine the cause of the disparity between the ability of this receptor to couple to distinct G-proteins and whether this is a feature common to other promiscuous receptors in other systems.

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