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G_{i2} mediates α_2-adrenergic inhibition of adenylyl cyclase in platelet membranes: *In situ* identification with G_α C-terminal antibodies

(signal transduction/guanine nucleotide-binding proteins/receptors)

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ABSTRACT **A panel of antibodies to synthetic decapeptides corresponding to the C termini of guanine nucleotide-binding regulatory protein (G protein) α subunits has been generated in rabbits. The specificity of each antibody was assessed by ELISA for peptide binding and by immunoblotting for binding to defined, recombinant G_α subunits expressed in *Escherichia coli*. Immunoblotting of human platelet membranes with these antibodies identified a variety of endogenous G proteins including G_s (stimulatory), G_{i2} (inhibitory), G_{i3}, and $G_{x(z)}$ (unknown function). Pretreatment of platelet membranes with C-terminal antibodies reactive with G_{i2}, but not with antibodies to G_{i3} or $G_{x(z)}$, blocked α_2-adrenergic inhibition of adenylyl cyclase. This identifies G_{i2} as the dominant mediator of cyclase inhibition in this pathway. This approach may provide a general means of identifying relevant functional interactions of G proteins with receptors and effectors *in situ*.**

Guanine nucleotide binding regulatory proteins (G proteins) comprise a family whose members function as key transducing elements in a variety of transmembrane signaling pathways (1–3). Activation of a heterotrimeric G protein by an agonist-liganded receptor results in exchange of bound GDP for GTP at the binding site present in the α subunit and dissociation of the α-subunit GTP from the $\beta\gamma$ complex. It is the G_α subunits that are thought to confer functional specificity to each G protein, allowing discrimination among multiple receptors and effectors, by virtue of the relative diversity of α-subunit structure. Indeed, nine distinct G_α structures have been identified to date by recombinant DNA cloning: G_{t1}, G_{t2}, G_s, G_{i1}, G_{i2}, G_{i3}, G_o (1, 2), $G_x(G_z)$ (3–5), and G_{olf} (6) (t, transducin; s, stimulatory; i, inhibitory; o, other; x, unknown; olf, olfactory). With the exception of G_t and G_s, the receptor and effector specificities of these various G_αs remain largely uncertain. Reconstitution experiments with purified G proteins have often suggested relative promiscuity of interaction of G proteins with receptors (7, 8) and effectors (9) but must be cautiously interpreted because the experimental stoichiometry may not reflect the relative concentration of signaling elements in the native membrane. Another potential shortcoming of these experiments is unrecognized heterogeneity in "purified" G-protein fractions that can resist resolution even after multiple chromatographic steps (10–12).

To circumvent these difficulties, we explored the use of antibodies to G_α subunits to probe the functional interactions of G proteins with receptors and effectors in native membranes. We developed a panel of antibodies to synthetic decapeptides representing the C termini of G_α subunits because of evidence that this domain governs in part the specific interaction of G proteins with receptors (13). This evidence includes the functional uncoupling of several G proteins from receptor after the covalent modification of the cysteine four residues from the C terminus by pertussis toxin ADP-ribosyltransferase (1–3), homology between this region of transducin-α and a 48-kDa retinal protein that competes for binding to rhodopsin (14) and the Arg^{389} to Pro mutation in the $G_s\alpha$ C terminus that accounts for the loss of receptor coupling in the *unc* variant of S49 murine lymphoma cells (15, 16). The feasibility of using specific G_α C-terminal antibodies as functional probes in native membranes was suggested in recent studies with the $G_s\alpha$ C-terminal antibody RM, which uncoupled G_s-mediated adenylyl cyclase stimulation from β-adrenergic agonists in wild-type S49 cell membranes (17). We now report detailed characterization of the specificity of this panel of G_α C-terminal antibodies and their application in human platelet membranes to identify the endogenous G-protein mediator of α-adrenergic inhibition of adenylyl cyclase.

MATERIALS AND METHODS

Peptide Synthesis, Antisera, ELISA, and Immunoblotting. Decapeptides were synthesized by the Merrifield solid-phase method (18) to yield free C-terminal carboxylic acids. Peptides were conjugated to keyhole limpet hemocyanin (Sigma) with glutaraldehyde (19), and rabbits were immunized as described (20, 21). Direct binding assays for antibody specificities were performed in 96-well polystyrene microtiter plates (Immunoplate I, Nunc) coated overnight at 4°C with 200 μl of synthetic peptide solution per well [1 μg/ml in 0.2 M sodium carbonate buffer (pH 9.6)] by ELISA using affinity-purified anti-peptide antibodies and goat anti-rabbit IgG conjugated with alkaline phosphatase (Kirkegaard and Perry Laboratories, Gaithersburg, MD). SDS/PAGE and immunoblotting were performed as described (21–23).

Expression of Recombinant G_α Subunits in *Escherichia coli*. The method for expression of G_α subunits in *E. coli* K38 cells is described in detail by Mattera *et al.* (24). G_α subunits were expressed as fusion polypeptides with nine amino acids added to their natural amino termini. The cDNAs inserted

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Abbreviations: G protein, guanine nucleotide-binding regulatory protein; G_s, the stimulatory G protein of adenylyl cyclase; G_{i1}, G_{i2}, G_{i3}, the G proteins homologous to G_i, the pertussis toxin substrate mediating inhibition of adenylyl cyclase, numbered in order of their cDNA cloning; G_o, a major pertussis toxin substrate of brain "other" than G_i that may modulate Ca^{2+} and/or K^+ channel function; G_{olf}, a G protein expressed uniquely in sensory neurons of the olfactory mucosa; G_t, transducin, a retinal G protein mediating visual transduction present in rod (G_{t1}) and cone (G_{t2}) photoreceptor cells; $G_{x(z)}$, a G protein of unknown function identified by DNA cloning; G_{41}, α_{41}, β_{36}, etc., numerical subscripts of G proteins or their subunits indicate relative mobility on SDS/PAGE in kilodaltons; PGE$_1$, prostaglandin E$_1$.

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into the pT7 expression vector were the 379-amino acid form of α_s (25), human α_{i1} and α_{i3}, mouse α_{i2}, and rat α_o and α_x (3). Total bacterial lysates were prepared as described (24) and used in immunoblots in amounts specified in the figure legends. Total bacterial lysates were centrifuged, and soluble supernatant fractions were used for some immunoblots. Only the soluble fraction was available for α_{i1}; for α_x, no soluble material was available, since essentially all expressed protein was associated with the particulate fraction.

Preparation of Platelet Membranes and Antibody Pretreatment. Human platelet membranes were prepared essentially as described (26) from outdated human platelets obtained from the Clinical Center, National Institutes of Health. The membranes were suspended in storage buffer (20 mM Tris·HCl, pH 7.5/1 mM EDTA/0.1 mM dithiothreitol) to a protein concentration of 3–6 mg/ml, and stored in aliquots at −70°C. For use in functional studies, 1 ml of frozen platelet membranes was thawed and diluted with 10 ml of ice-cold 100 mM NaCl/20 mM Tris·HCl, pH 7.5 (NT buffer). After centrifugation in the SS34 rotor at 20,000 rpm (≈48,000 × *g*) for 30 min, the membrane pellet was resuspended in 3–4 ml of NT buffer. Aliquots of this membrane suspension (1–2 mg/ml) were preincubated with or without normal rabbit immunoglobulin (Sigma) or affinity-purified G_α C-terminal antibodies at a final antibody concentration of 20–50 μg/ml for 1 hr at 4°C.

Adenylyl Cyclase Assays. Adenylyl cyclase activity in 30-μl aliquots of platelet membranes preincubated as described above was determined in a 100-μl final assay volume that contained in addition 0.1 mM [α-^{32}P]ATP (2–5 × 10^6 cpm; Du Pont/New England Nuclear), 1 mM dithiothreitol, creatine phosphokinase (0.2 mg/ml), creatine phosphate (1.8 mg/ml), 100 μM cAMP, 2 mM $MgCl_2$, 50 mM NaCl, 10 μM GTP, 1 mM isobutylmethylxanthine, bovine serum albumin (0.25 mg/ml), and 50 mM Tris·HCl (pH 7.5). Other components when present included prostaglandin E_1 (Sigma), *l*-norepinephrine (Regis, made up with water from the solid bitartrate monohydrate salt each day), and yohimbine hydrochloride (United States Biochemical) at final concentrations of 10 μM, 50–100 μM, and 500 μM, respectively. Reactions were carried out at 30°C for 20 min and terminated by addition of carrier ATP and [^{3}H]cAMP. Radiolabeled cAMP was isolated by the method of Salomon *et al.* (27) as described (28).

RESULTS

To [illegible]erate antisera capable of recognizing each of the kn[illegible] G_α subunits, we synthesized decapeptides corresp[illegible] to the C termini of G_t (20, 22), G_o (12), G_s, $G_{x(z)}$, and G_i [illegible] we used these to immunize rabbits (Table 1). We then te[illegible] ELISA the ability of each of the antisera to bind to ea[illegible] the immunogenic peptides. Antisera RM and QN bound essentially only to their corresponding immunogenic peptide; the remaining antisera, moreover, showed little if any binding to peptides RM and QN. For peptides KE, EC, and GO, the immunogenic peptide was in each case best recognized by its corresponding antiserum, but there was considerable cross-reactivity. Antiserum EC showed greater cross-reactivity for peptide KE than did antiserum GO, consistent with the greater degree of identity between peptides KE and GO (Table 1). Surprisingly, however, the cross-reactivity of antiserum GO against peptide EC was equivalent to that of antiserum AS, and, for the GO peptide, antiserum EC showed as much reactivity as did GO antiserum, whereas AS antiserum showed only weak reactivity.

Table 1. C-terminal decapeptides and corresponding antisera

Peptide	Sequence*	Antiserum†	G_α subunit‡
RM	RMHLRQYELL	RM	G_s
QN	QNNLKYIGLC	QN	$G_{x(z)}$
GO	ANNLRGCGLY	GO	G_o
EC	KNNLKECGLY	EC	G_{i3}
KE	KENLKDCGLF	AS	G_{t1}, G_{t2}
—	KNNLKDCGLF	—	G_{i1}, G_{i2}

*Single-letter amino acid code.
†Antiserum raised by immunization with the designated peptide.
‡Designated peptide represents C terminus of corresponding G_α subunit (3).

To assess the reactivity and specificity of these antibodies against proteins encompassing the immunogenic peptide sequences, we prepared immunoblots on bacterial lysates containing unique, defined G_α subunits expressed by recombinant DNA techniques. Individual G_α subunits (with the exception of G_s) are visible as Coomassie blue-stained bands in the bacterial lysates (Fig. 1), and their relative migration on SDS/PAGE is equivalent to that observed for the products of *in vitro* translation in the reticulocyte lysate system (22). Note that relative to the other G_α subunits, G_x migrates as a ≈40-kDa protein, and that the "small" form of $G_{s\alpha}$ cDNA (25) used for expression produces a ≈42-kDa product. On immunoblot, RM and QN antisera were absolutely specific for G_s and G_x, respectively. G_s and G_x, moreover, were not reactive with GO, EC, or AS antisera (Fig. 1). AS antiserum reacts strongly and equivalently with G_{i1} and G_{i2}, shows weak reactivity against G_{i3}, and shows no reactivity against G_o (Fig. 1). GO and EC antisera react best with G_o and G_{i3}, respectively, but they display very substantial reciprocal cross-reactivity. Both antisera show weak but definite cross-reactivity against G_{i1} and G_{i2} (Fig. 1).

Within the limits of specificity demarcated by the ELISA and immunoblotting studies presented here, the panel of G_α C-terminal antibodies offered promise in application to functional questions of G-protein interactions in native membranes. Not only did multiple lines of evidence point to a role for the G_α C terminus in receptor interaction (see above), but the known accessibility of pertussis toxin to the C terminus of G_i and G_o in membranes (1, 2) suggested that this region might be available to interact with antibodies *in situ*. A potent inhibition of adenylyl cyclase mediated by α_2-adrenergic receptors in platelets (29, 30) involves a pertussis toxin-sensitive, functionally defined G_i (26), the identity of which is unknown. We chose this system for detailed immunologic and functional analysis with the battery of G_α C-terminal antibodies.

We first defined the G-protein complement in human platelet membranes on immunoblots, in reference to parallel blots of the G proteins present in a cholate extract of bovine brain (Fig. 2). SDS/PAGE was performed on 10% gels with a low percentage of *bis*acrylamide to improve resolution in the G_α region (22). The β-specific antiserum MS (22) readily detected G_β in platelet membranes, although it was much more abundant in brain. A distinct β_{36}/β_{35} doublet was evident in blots of platelet membranes analyzed on standard 10% gels (data not shown). Whereas the antibody RM (17) demonstrated a relative abundance of the "large" forms of G_s in brain, the "small" forms predominated in platelets (25). Antibody QN, which is absolutely specific among recombinant G_α subunits for the 40-kDa protein encoded by G_x DNA on immunoblots, identified no antigen in blots of brain membranes but gave a distinct signal at 40 kDa in platelet membrane blots. This immunoreactivity could be completely abolished by coincubation with the QN peptide (data not shown). Pending further characterization, this protein is tentatively identified as $G_{x(z)}$ (4, 5). As previously reported (12, 20), AS reacts with a doublet of 41 and 40 kDa in bovine brain, corresponding to the G_{i1} and G_{i2}, respectively, identified by cDNA cloning (see references in refs. 1–3) and sequencing of purified proteins (31, 32). The heavy α_{41} and

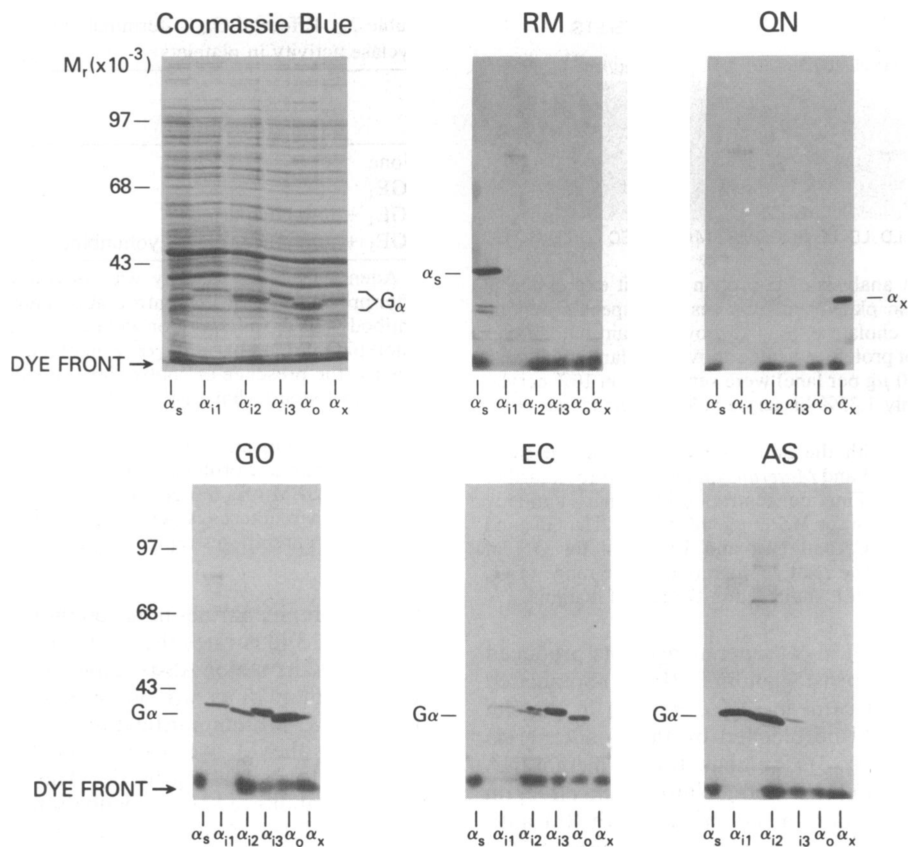

FIG. 1. Analysis of specificity of G-protein antisera by immunoblot of G_α subunits expressed in *E. coli*. Total lysates of *E. coli* expressing α subunits of G_s, G_{i2}, G_{i3}, G_o, and G_x, and the soluble fraction of an *E. coli* lysate expressing α_{i1} were diluted 1:1 in SDS sample preparation buffer and applied to SDS/10% polyacrylamide gels. For protein staining with Coomassie blue, a total of 40 μl per lane was applied for G_s and G_{i2}, 20 μl per lane was applied for G_{i3}, G_o, and G_x, and 100 μl per lane was applied for G_{i1}; half of the respective amounts were applied to gels for transfer to nitrocellulose and subsequent immunoblotting. For the latter, five replica filters were incubated with affinity-purified antibodies (RM and QN, 5 μg/ml; GO, EC, and AS, 1 μg/ml) for 2 hr at room temperature, and immunoreactive bands were detected with peroxidase-conjugated goat anti-rabbit IgG as described (21, 23). The positions of molecular size markers (Bethesda Research Laboratories prestained "high" standards; 97 kDa, phosphorylase B; 68 kDa, bovine serum albumin; 43 kDa, ovalbumin) are indicated, as are the Coomassie blue-stained G_α subunits (*Upper Left*) and the immunoreactive G_α subunits in the five blots. The dye front, representing pyronin-Y on the immunoblots, is also indicated (the fainter staining in the α_{i1} lane of the blots reflects the use of buffer for this sample containing a lower pyronin-Y concentration). The expressed G_α subunits are visible as Coomassie blue-stained bands with the exception of α_s, which is obscured by the abundant, endogenous *E. coli* protein beneath the 43-kDa marker. Immunoreactivity in the α_x lane (*Lower Left*) and in the α_{i2} lane (top band) (*Lower Middle*) represents "spillover" from the adjacent lane.

lighter α_{40} signals in AS immunoblots of brain contrast with results in platelets, in which faint 41-kDa and heavy 40-kDa staining is seen. That these correspond, respectively, to G_{i3} and G_{i2} in platelets is supported by auxiliary immunoblotting with the G_{i3} C-terminal antibody EC and antibodies LD and LE specific for an internal sequence in G_{i1} and G_{i2}, respectively (20, 22). In platelets, EC also labels a doublet of 41 and 40 kDa, which, unlike the pattern with AS, is heavier in the upper band. As blotting with the G_{i1}-specific antibody LD gives no detectable signal at 41 kDa in 150 μg of platelet membranes, but a measurable 41-kDa band in as little as 25 μg of brain extract, we conclude that G_{i1}, if present at all in platelets, must be expressed to a level less than one-sixth that in bovine brain. (A faint band at 40 kDa in platelet membranes seen with LD antibody attests to its weak cross-reactivity with the abundant α_{i2} present, confirmed on blots of recombinant $G_{i2\alpha}$ not shown here.) The EC-reactive 41-kDa entity in platelets is thus tentatively identified as G_{i3}, which would account for a weak AS cross-reactivity. That the 40-kDa species in platelets, which reacts strongly with AS and weakly with the poorly discriminating EC, is G_{i2} is supported by LE staining (Fig. 2). As shown previously (20), LE antibody strongly labels the AS-reactive 40-kDa species in brain corresponding to G_{i2}. The G_o-specific antibodies GC and GO (12) give no detectable signal against 150 μg of platelet membranes, while heavily staining the $G_{o\alpha}$ present at 39 kDa in 25 μg of brain extract. The abundance of G_o accounts for the heavy EC cross-reactivity in this region of brain immunoblots.

The analysis of platelet membrane G proteins by immunoblotting demonstrates the presence of G_s, G_{i2}, G_{i3}, and presumptive $G_{x(z)}$, with no detectable G_{i1} or G_o. These results are consistent with the findings of a recent purification of pertussis toxin substrate G proteins from human platelet membranes (33). The major G protein purified had an α subunit of 40 kDa with a partial amino acid sequence revealing identity with G_{i2} (31, 32). A minor pertussis toxin substrate with G_α of 41 kDa was also found but could not be unequivocally identified as G_{i1} or G_{i3} by available antibodies (33). The anomalous position of cysteine at the extreme C terminus of $G_{x(z)}$ (4, 5), in contrast to its position four amino acid residues from the C terminus in the known pertussis toxin substrates (1, 2), makes it uncertain whether this G protein is a substrate for ADP-ribosylation by this toxin or would have been undetected in this purification (33).

With the identity of the major heterotrimeric G proteins in platelet membranes established by immunoblotting, we questioned whether the G_α C-terminal antibodies could be used in functional studies to elucidate the pathway of α-adrenergic adenylyl cyclase inhibition in this system. As others have

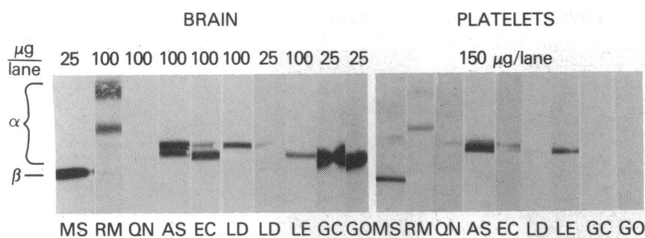

FIG. 2. Immunoblot analysis of G-protein subunit expression in bovine brain and human platelet membranes with specific peptide antisera. Aliquots of a cholate extract of bovine brain membranes containing the amount of protein indicated above each lane and human platelet membranes (150 μg per lane) were separated on 10% acrylamide gel [containing only 1.35% bisacrylamide to enhance G_α resolution (22)] by SDS/PAGE. After transfer to nitrocellulose, immunoblotting was performed with the anti-peptide antiserum indicated below each lane (see Table 1 and *Materials and Methods* for a further description of these sera). Final concentration of primary antibody was 1:250 of crude antiserum for MS; 2 μg/ml for AS, LD, GC, and GO; 5 μg/ml for RM, EC, and LE; and 10 μg/ml for QN of affinity-purified antibody. The relative migration of the range of G_α subunits and of the β subunit is indicated in the lateral margins.

firmly established (26, 29), α_2-adrenergic agonists produced a significant inhibition of prostaglandin E_1 (PGE_1)-stimulated adenylyl cyclase activity (mean, 36%; n = 7) in platelet membranes, which could be blocked by the α_2-adrenergic antagonist yohimbine (Fig. 3). Preincubation of membranes with control immunoglobulin (20–50 μg/ml) had no effect on this inhibition. Preincubation of membranes with RM antibody, specific for the $G_{s\alpha}$ C terminus, produced >75% inhibition of the PGE_1-stimulated cyclase activity, comparable to the effect of RM on β-adrenergic-stimulated adenylyl cyclase activity in S49 cell membranes reported recently (17), without diminishing the inhibitory effects of norepinephrine (Table 2). Antibodies QN and EC, against the C-terminal decapeptides of $\alpha_{x(z)}$ and α_{i3}, respectively, had no effect on α-adrenergic cyclase inhibition (Fig. 3), although, as shown above, they identified G proteins of 40 kDa (putative $\alpha_{x(z)}$; QN antibody) and 41 kDa (α_{i3}; EC antibody) on immunoblots of crude platelet membranes (Fig. 2). In contrast, preincubation with AS antibody significantly diminished the α_2-adrenergic inhibition of cyclase activity (mean, 51% reduction of norepinephrine-induced inhibition;§ range, 28–69%; n = 5). Fig. 3 illustrates the results of a representative experiment. As the major AS-reactive G protein in platelet membrane immunoblots was identified as G_{i2}, consistent with results of G-protein purification (33), and no G_{i1} was detectable (see above), we ascribe the functional effect of AS antibody to its binding to G_{i2}. The effects of AS antibody could be abolished by coincubation with the immunogenic peptide KE (see Table 1; data not shown).

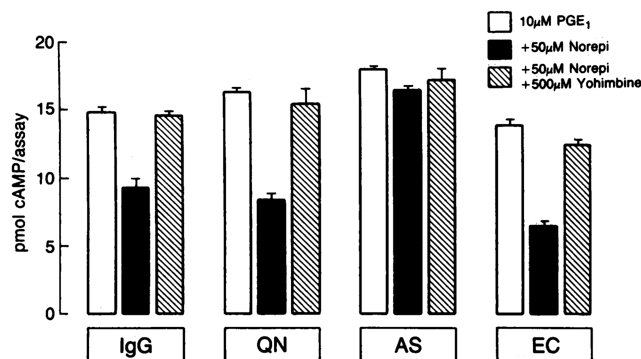

FIG. 3. The effect of G_α C-terminal antibody pretreatment on α-adrenergic inhibition of adenylyl cyclase in human platelet membranes. Aliquots of human platelet membranes were incubated with control rabbit immunoglobulin (IgG) or affinity-purified G_α C-terminal antibodies QN, AS, or EC, as indicated, at a final antibody concentration of 50 μg/ml as described in *Materials and Methods*. At the end of the preincubation, the PGE_1-stimulated adenylyl cyclase activity in 30-μl aliquots was determined without or with l-norepinephrine or yohimbine. Assays contained 54 μg of membrane protein in a 100-μl final reaction volume. Values represent the mean of triplicate determinations, with error bars indicating the SEM.

Table 2. Effect of $G_{s\alpha}$ C-terminal antibody RM on adenylyl cyclase activity in platelets

Addition	Preincubation	
	IgG	RM
None	0	0
PGE_1	16.9	3.68 (78)*
PGE_1 + norepinephrine	8.6 (49)†	1.43 (61)†
PGE_1 + norepinephrine + yohimbine	15.5	2.57

Adenylyl cyclase activity was measured in platelet membranes after preincubation with control rabbit immunoglobulin (IgG) or RM antibody (20 μg/ml), with or without the addition of PGE_1 (10 μM), l-norepinephrine (100 μM), or yohimbine (500 μM). In pilot experiments, the presence of control immunoglobulins at this concentration was found to have no effect on cyclase activity. As indicated, basal cyclase activity above the blank value could not be detected. Shown are the means of triplicate activity determinations in 32 μg of platelet membrane protein, expressed as pmol of cAMP per assay, in which the SEM was 6% or less.

*Value in parentheses is percent inhibition by RM antibody.

†Values in parentheses represent percent inhibition by norepinephrine.

DISCUSSION

All five synthetic C-terminal decapeptides proved to be highly immunogenic, and produced antibodies capable of reacting not only with the immunogenic peptides, but also with endogenous and recombinant G_α subunits. RM and QN peptides produced antisera that are virtually specific for their corresponding peptides and for the α subunits, G_s and $G_{x(z)}$, respectively, that contain these peptide sequences. This degree of specificity reflects the relatively unique sequence of each peptide. The antisera AS, EC, and GO showed no reactivity with G_s and $G_{x(z)}$ and for the remaining G_α subunits displayed the following patterns of relative specificity: for AS, $G_{i1} = G_{i2} >> G_{i3}$, nonreactive with G_o; for EC, $G_{i3} > G_o >> G_{i1} = G_{i2}$; for GO, $G_o > G_{i3} >> G_{i1} = G_{i2}$. The surprising reciprocal cross-reactivity of GO and EC is explained by the presence of a C-terminal tyrosine instead of phenylalanine in their immunogenic peptide sequences (see Table 1), a change with dominant consequences on the pattern of antibody specificity. This was verified by studies of the hybrid peptide KNNLKDCGLY, which reacted with GO/EC but not with AS (cf. Table 1; unpublished data). The careful delineation of antibody specificity established a basis for further immunologic and functional analysis of G proteins in human platelet membranes.

Platelet membranes, which are richly endowed with receptors governing second messenger systems (34), revealed a complement of G_s, G_{i2}, G_{i3}, and $G_{x(z)}$ on immunoblots. The G_{i2} identified with AS and LE antibody corresponds to the major pertussis toxin-sensitive G protein identified by protein purification (33). The minor 41-kDa G_α reported in this purification (33) would represent G_{i3} based on results in the present study. No detectable G_{i1} or G_o was found by immunoblotting. We

§Reduction of α_2-adrenergic inhibition of PGE_1-stimulated adenylyl cyclase activity by antibody is calculated as [1 − (% inhibition in presence of test antibody/% inhibition in presence of nonimmune antibody)] × 100%.

have tentatively identified the QN-reactive 40-kDa species as $G_{x(z)}$ (4, 5) because of the absolute specificity of QN antibodies for the $\alpha_{x(z)}$ C-terminal decapeptide on ELISA and for the 40-kDa recombinant $\alpha_{x(z)}$ on immunoblots. Of course similar results would be obtained if a novel G protein encompassing the $G_{x(z)}$ C-terminal sequence, or a closely related decapeptide, were present in platelets. If $G_{x(z)}$ is indeed pertussis toxin insensitive (4, 5), it is tempting to speculate that it may mediate one of several known signaling pathways sharing this characteristic in platelets (34). Failure to detect any immunoreactivity with QN antibodies does not necessarily indicate that $G_{x(z)}$ is not expressed in brain [indeed the mRNA is found in brain (4, 5)], but rather that its concentration is low and/or its distribution is highly localized.

We identify G_{i2} as the principal mediator of α_2-adrenergic receptor-coupled inhibition of platelet adenylyl cyclase in functional studies with a panel of antibodies with G_α C-terminal specificity. AS antibodies, which uncoupled the α_2-adrenergic inhibitory response in this system, react predominantly with G_{i2} and weakly with G_{i3}. The findings presented here rule out a predominant role for G_{i3} in this inhibitory pathway, however, because the most G_{i3}-selective antibody, EC, had no effect. Our data do not, however, exclude an ancillary role for G_{i3} in cyclase inhibition, nor do they rule out functional coupling of α_2-adrenergic receptors with G_{i3} in other effector pathways. AS antibodies have been used in previous functional studies to block rhodopsin-stimulated GTPase in reconstituted vesicles containing G_t (35) and opioid-stimulated GTPase in NG108-15 cell membranes (36). Since AS antibodies do not distinguish between α_{i1} and α_{i2}, their use to define functional specificity in cells containing both of these G_α subunits is problematic. Furthermore, one cannot generalize from the present results—i.e., identification of G_{i2} as a functional G_i in platelets—to other cells with various proportions of the several pertussis toxin-sensitive G_α subunits.

Reconstitution studies examining the mechanism of cyclase inhibition have consistently found the activity of $\beta\gamma$ subunits more robust than any α_i in this regard (10, 26, 37, 38), consistent with a model in which the deactivation of α_s by the mass action of $\beta\gamma$ binding plays a critical role (1). The present experiments do not resolve which of α_{i2} or $\beta\gamma$ accounts for the inhibitory activity of G_{i2}, but the abundance of this G protein in platelets (33) makes a role for $\beta\gamma$ (1) fully tenable.

The work presented here demonstrates an approach for the *in situ* characterization of G-protein functional interactions by using antibodies to the G_α C terminus. This C-terminal region of G_α appears to be exposed in membranes and accessible to macromolecules including pertussis toxin and immunoglobulins, an availability reflecting, perhaps, its role in receptor interaction (13). Recent functional studies with RM (17) have shown its ability to immunoprecipitate from detergent extract a complex of guanosine 5'-[γ-thio]triphosphate- or fluoride-activated α_s and cyclase. The C-terminal decapeptide region is therefore not essential for effector binding by α_s and may serve as a "handle" to identify G_α–effector complexes under similar conditions of preactivation by using C-terminal antibodies. Such G_α antibodies may prove generally useful to assign functional roles to individual G proteins. A clear definition of the relevant functional interactions of the individual G proteins is a prerequisite to a general knowledge of the structural features that control G-protein discrimination among, and interaction with, receptors and effectors, ensuring fidelity in signal transduction.

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