百日咳毒素修飾部位を持たない2種の新しいG蛋白質αサブユニットの同定

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Identification of Two Novel GTP-binding Protein α-Subunits that Lack Apparent ADP-ribosylation Sites for Pertussis Toxin

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SUMMARY

Two novel G-protein α-subunits, which are referred to as $G_L1\alpha$ and $G_L2\alpha$, have been identified by isolating bovine liver cDNA clones that cross-hybridized at reduced stringency with bovine G_i1 α-subunit cDNA. The deduced amino acid sequences of $G_L1\alpha$ and $G_L2\alpha$ share 83% identity with each other. $G_L1\alpha$ and $G_L2\alpha$ show 82-98% amino acid identity with $G_Q\alpha$ and Gα11, which have recently been cloned from a mouse brain cDNA library and shown to activate phospholipase C. The high value of amino acid identity (98%) between $G_L2\alpha$ and Gα11 suggests that $G_L2\alpha$ corresponds to the bovine version of Gα11. $G_L1\alpha$ and $G_L2\alpha$ show 45-59% amino acid identity with other known G-protein α-subunits. $G_L1\alpha$ and $G_L2\alpha$, as well as $G_Q\alpha$, lack a consensus site for ADP-ribosylation by pertussis toxin. These G-protein α-subunits appear to form a new subfamily of G-protein that is involved in pertussis toxin-insensitive signal transduction systems.

Messenger RNA corresponding to $G_L2\alpha$ was detected in bovine cerebral cortex, liver, atrium, lung, and kidney but $G_L1\alpha$ mRNA was detected only in liver, lung, and kidney. Antiserum prepared against a synthetic pentadecapeptide corresponding to the deduced carboxyl terminus of $G_L2\alpha$ specifically reacted with a 40-kDa protein in mouse liver, brain, lung, heart, kidney, and spleen. The amount of the 40-kDa protein was highest in brain and lung.

INTRODUCTION

A family of heterotrimeric guanine nucleotide-binding regulatory proteins (G-proteins) is essential for mediating signal transduction between cell surface receptors and intracellular effectors such as adenylate cyclase, phospholipase C (PLC), phospholipase A2, cyclic GMP phosphodiesterase, and ion channels (for review Gilman, 1987; Neer and Clapham, 1988; Ross, 1989; Birnbaumer et al., 1990; Kaziro et al., 1991; Simon et al., 1991).

G-proteins are composed of three subunits termed α, β, and γ. The α-subunit has a guanine nucleotide binding site and intrinsic GTPase activity. β and γ subunits associate with each other under biological conditions. Fig. 1 shows the basic mechanism of the G-protein mediated receptor-effector coupling. In the inactive state, the GDP-bound G-protein α-subunit forms a heterotrimer with the $\beta\gamma$-subunits. Agonist-occupied receptor interacts with the $\alpha\beta\gamma$ complex. This interaction stimulates the release of GDP from the α-subunit and subsequent binding of GTP. The binding induces the dissociation of the α-subunit from the $\beta\gamma$ subunits. The dissociated subunits then interact with effectors. The bound GTP is hydrolyzed to GDP by the intrinsic GTPase activity of the α-subunit. This hydrolysis leads to the association of GDP-bound α-subunit with $\beta\gamma$-subunits, a return of the G-protein to an inactivate state.

Prior to my studies on two cDNA clones encoding $G_L1\alpha$ and $G_L2\alpha$ (Nakamura et al., 1991), nine mammalian α-subunit cDNA clones had been reported: $G_S\alpha$ (Nukada et al., 1986a), $G_{olf}\alpha$ (Jones and Reed, 1989), $G_i1\alpha$ (Nukada et al., 1986b), $G_i2\alpha$

(Itoh et al., 1986), $G_i3\alpha$ (Didsbury et al., 1987), $G_o\alpha$ (Van Meurs et al., 1987), $G_{trod}\alpha$ (Tanabe et al., 1985), $G_{tcone}\alpha$ (Lochrie et al., 1985), and $G_z\alpha$ [Fong et al., 1988; also referred to as $G_x\alpha$ (Matsuoka et al., 1988)]. Four different kinds of $G_s\alpha$ and two kinds of $G_o\alpha$ proteins are generated from a single gene by alternative splicing (Kozasa et al., 1988; Hsu et al., 1990). Distinct β-subunits, called β_1, β_2, and β_3, also exist (Sugimoto et al., 1985; Fong et al., 1987; Levine et al., 1990). At least three cDNAs encoding distinct γ-subunits have also been identified (Hurley et al., 1984; Gautam et al., 1989; Gautam et al., 1990).

Functions of some G-proteins are affected by the ADP-ribosylation catalyzed by cholera and pertussis toxins. The α-subunits of G_s and G_{olf}, both of which stimulate adenylyl cyclase, are modified by cholera toxin and constitutively activate adenylyl cyclase (Cassel and Selinger, 1977; Jones et al., 1990a). Adenylyl cyclase-inhibiting G-protein, G_i, is modified and uncoupled from cell surface receptors by pertussis toxin (Bokoch et al., 1983). Three subtypes of G_i α-subunits, $G_i1\alpha$, $G_i2\alpha$, and $G_i3\alpha$, have been identified by cDNA cloning and each has been demonstrated to be ADP-ribosylated by pertussis toxin (Linder et al., 1990). However, the precise function of each $G_i\alpha$ subtype has not yet been determined. Two variants of $G_o\alpha$, which are also modified by pertussis toxin (Sternweis and Robishaw, 1984; Hsu et al., 1990), are involved in the inhibition of L-type Ca^{2+} channel in GH3 cell lines (Kleuss et al., 1991). The α-subunit of transducin (G_{trod}), which activates cyclic GMP phosphodiesterase, is modified by both toxins (Van Dop et al.,

1984; West et al., 1985) and its GTPase activity is inhibited (Watkins et al., 1984). By contrast, the G_z α-subunit, which lacks a consensus site for ADP-ribosylation by pertussis toxin, has been shown not to be a substrate for ADP-ribosylation catalyzed by either pertussis toxin or cholera toxin (Casey et al., 1990). The function of G_z has not yet been determined.

The sensitivity toward pertussis toxin of G-protein involved in PLC activation differs among tissues and cell lines. In neutrophils and HL-60 cell lines, pertussis toxin completely blocks the activation of PLC by N-formyl-methionyl-leucyl-phenylalanine (Gierschik et al., 1989). Likewise, brain G_o, which is sensitive to pertussis toxin, has been shown to activate PLC in *Xenopus* oocytes. (Moriarty et al., 1990). On the other hand, vasopressin receptors in liver (Uhing et al., 1986) and muscarinic acetylcholine receptors in brain (Chiu et al., 1988) are thought to stimulate PLC via pertussis toxin-insensitive G-proteins.

Since activation of PLC in liver has been shown to be mediated by G-protein in pertussis toxin-insensitive manner, a bovine liver cDNA library was constructed and two novel clones encoding G-protein α-subunits that lack the ADP-ribosylation sites for the toxin were isolated (Nakamura et al., 1991). Here I will discuss the details of their properties and functions.

Independent of this investigation, two additional cDNA clones encoding $G_q\alpha$ and $G\alpha 11$ have recently been reported (Strathmann and Simon, 1990). These $G\alpha$ proteins are thought to be identical to the liver (Taylor et al, 1990) and brain

(Pang and Sternweis, 1990) 42-kDa α-subunits that activate PLC (Smrcka et al., 1991; Taylor et al., 1991). In addition, three cDNA clones encoding Gα12, Gα13 (Strathmann and Simon, 1991) and Gα16 (Amatruda et al., 1991) have also recently been reported.

MATERIALS AND METHODS

Materials- The cDNA Synthesis System and cDNA Cloning System λgt10 were obtained from Bethesda Research Laboratories (BRL) and Amersham, respectively. Sequenase® DNA Sequencing Kits were purchased from United States Biochemical Corporation (USBC), Kilo-Sequence Deletion Kits from Takara, Oligolabelling Kits from Pharmacia and the 0.24-9.5 Kb RNA Ladder from BRL.

cDNA Cloning- The cDNA library was constructed using the cDNA Synthesis System and the cDNA Cloning System λgt10. The first cDNA strand was synthesized by random-primed reverse transcription of poly(A)$^+$-enriched bovine liver RNA. The synthesis of the first and second strands, ligation of *Eco*RI linker to the cDNA and cloning of the cDNA into the unique *Eco*RI site of λgt10 was performed according to the procedures described by the vendors. The cDNA libraries were screened by the plaque-hybridization method (Benton and Davis, 1977). Hybridization was performed at 37°C overnight in solutions described by Jones and Reed (Jones and Reed, 1987) except that 50% (vol/vol) or 30% formamide was included under a high- or low-stringency condition, respectively. The *Eco*RI(-71)/*Ava*II(1181) fragment excised from clone pGα28 (Nukada et al., 1986) encoding $G_i1\alpha$ subunit and the ~560-base-pair (bp) *Eco*RI/*Eco*RI fragment from clone pGL28 (see below) were labelled using the Oligolabelling Kit with [α-^{32}P]dCTP as probes (specific activity, 1 x 10^8 cpm/μg DNA). Filters were rinsed at room temperature in 6 x SSC (1 x SSC = 0.15M NaCl,

15mM sodium citrate), 0.1% sodium dodecyl sulfate (SDS) and washed at 37°C in 2 x SSC, 0.1% SDS or 0.3 x SSC, 0.1% SDS.

cDNA Sequencing- The *Eco*RI/*Eco*RI inserts of clones pGL28, pGL2, pGL3, pGL4, pGL5, and pGL7 were subcloned in pUC18. The *Eco*RI(-581)/*Hin*dIII(919) and *Hin*dIII(919)/ *Sma*I(1091) fragments of clone pGL1 were subcloned in the *Eco*RI/*Sma*I site of pBLUESCRIPT SK(+) (STRATAGENE), since clone pGL1 contained an *Eco*RI site in the deduced amino acid coding region of $G_L1\alpha$ (Fig. 1A). Restriction fragments were subcloned in pUC119 and sequenced using the Sequenase® DNA Sequencing Kit. Alternatively, deleted fragments of the DNA insert by Exonuclease III and Mung Bean nuclease using the Kilo-Sequence Deletion Kit were sequenced.

Isolation of RNA and RNA Blot Hybridization Analysis- Total RNA was extracted from bovine cerebral cortex, liver, atrium, lung, and kidney as in (Chirgwin et al., 1979), and liver poly(A)$^+$ RNA for cDNA library construction was isolated as described in (Aviv and Leder, 1972). RNA blot hybridization analysis was performed by the procedures described in (Thomas, 1980; Nukada et al., 1987); the amount of total RNA used was 20µg. The hybridization probes (specific activities, 1 x 10^9 cpm/µg DNA) were the *Sma*I(-137)/ *Sma*I(1091) fragment excised from clone pGL1 (Fig. 1A) and the *Pvu*II(66)/*Pst*I(1031) fragment from clone pGL7 (Fig. 1B), and labelled by the Oligolabelling Kit with [α-^{32}P]dCTP. The size markers used were the 0.24-9.5 Kb RNA Ladder.

Immunoblot Analysis- The pentadecapeptide (Lys-Asp-Thr-Ile-Leu-Gln-Leu-Asn-Leu-Lys-Glu-Tyr-Asn-Leu-Val) corresponding to a deduced amino acid sequence found in $G_L2\alpha$ (amino acid residues 339-353 in Fig. 4) was synthesized using an Applied Biosystems 430A automated peptide synthesizer. The pentadecapeptide (5 mg) was conjugated to 5 mg of keyhole limpet hemocyanin using 0.1% (vol/vol) glutaraldehyde according to the procedures described in (Goldsmith et al., 1987). Immunization of rabbits with the coupled peptide and generation of antiserum AGL2 was performed as reported (Goldsmith et al., 1987). Immunoblot analysis was carried out as described in (Goldsmith et al., 1987; Harris et al., 1985), except that homogenates of mouse brain, liver, heart, lung, kidney, and spleen were used and the antiserum AGL2 incubated for 2 h at room temperature in the presence or absence of 0.2 mg/ml of the pentadecapeptide.

RESULTS

Isolation of cDNA Clones - Initially a bovine liver cDNA library (~4 x 10^5 primary recombinants) was screened at low stringency with a cDNA probe excised from clone pGα28 (Nukada et al., 1986b) encoding G_i1 α-subunit ($G_i1\alpha$). About 30 positive clones were detected. Partial nucleotide sequence analysis revealed that clone pGL28 isolated from these clones encoded a novel G-protein α-subunit (which is designated as $G_L1\alpha$: the L in $G_L\alpha$ refers to liver). Clone pGL28 cDNA was then hybridized at high stringency to about 4 x 10^5 plaques from the same cDNA library, and 7 positive clones were detected. Subsequent analysis indicated that two (clones pGL1 and pGL4) of these positive clones encoded $G_L1\alpha$ and four (clones pGL2, pGL3, pGL5, and pGL7) encoded another novel G-protein α-subunit (which is referred to as $G_L2\alpha$). Two clones (clones pGL1 and pGL7) containing the entire coding regions were analyzed further by nucleotide sequencing of both strands of the cDNA inserts (Fig. 2).

Deduced Amino Acid Sequences of $G_L1\alpha$ and $G_L2\alpha$ - Fig. 3 shows the 1671-nucleotide sequence of the cDNA insert of clone pGL1. Translation of the $G_L1\alpha$ cDNA sequence in one open reading frame predicted a 355-residue protein sequence (M_r = 41496) that showed 54% amino acid sequence identity with $G_i1\alpha$, $G_i2\alpha$, and $G_i3\alpha$ (Nukada et al., 1986b; Itoh et al., 1986; Didsbury et al., 1987); 54-53% identity with $G_o\alpha$ (Van Meurs et al., 1987) and $G_o2\alpha$ (Hue et al., 1990); 54-52% identity with $G_{trod}\alpha$ (Tanabe et al., 1985) and $G_{tcone}\alpha$ (Lochrie et al., 1985); 52% identity with $G_z\alpha$ (Fong et al., 1988); 51-45% identity with $G_s\alpha$ (Nukada et al., 1986a) and $G_{olf}\alpha$ (Jones and

Reed, 1989); 82% identity with $G_q\alpha$ and Gα11 (Strathmann and Simon, 1990); 46-49% identity with Gα12 and Gα13 (Strathmann and Simon, 1991); and 57% identity with Gα16 (Amatruda et al., 1991). By analogy with the homologous Gα proteins, the initiation codon was assumed to be the ATG at nucleotide position 1-3. This assignment is supported by the fact that the nucleotide sequence surrounding this ATG triplet agrees with the favoured sequence that flanks functional initiation codons in eukaryotic mRNAs (Kozak, 1984; Kozak, 1987). The DNA sequence 5' to the coding region includes no nonsense codons nor ATG triplets in frame, however. Thus, the possibility that the initiating methionine is located upstream of the 5'-end of the cDNA insert of clone pGL1 cannot be excluded.

Fig. 4 shows the 1391-nucleotide sequence of the cDNA insert of clone pGL7. Translation of the cDNA sequence in one open reading frame gave a protein sequence (referred to as $G_L2\alpha$) which is homologous to Gα proteins including $G_L1\alpha$. There were three possible initiation sites (ATG triplets at nucleotide positions -18 to -16, -3 to -1, and 1 to 3) in the downstream of a nonsense codon (TAG at position -225 to -223) found in frame. The initiation site was tentatively assigned to the third methionine by homology to other Gα proteins and the most favorable nucleotide sequence with Kozak's eukaryotic initiation translational consensus sequence (Kozak, 1984; Kozak, 1987).

The $G_L2\alpha$, thus, consists of 353 amino acid residues (M_r = 41375) and shows 83% amino acid sequence identity with $G_L1\alpha$; 55-54% identity with $G_i1\alpha$, $G_i2\alpha$, and $G_i3\alpha$; 51-52% identity

with $G_o\alpha$ and $G_o2\alpha$; 53% identity with $G_{trod}\alpha$ and $G_{tcone}\alpha$; 49% identity with $G_z\alpha$; 48-46% sequence identity with $G_s\alpha$ and $G_{olf}\alpha$; 89% identity with identity $G_q\alpha$; 98% with $G\alpha11$; 46-49% identity with $G\alpha12$ and $G\alpha13$; and 59% identity with $G\alpha16$.

Protein Homologies - A comparison of the amino acid sequences of bovine $G_L1\alpha$ and $G_L2\alpha$ with those of other $G\alpha$ proteins (mouse $G\alpha11$, $G_q\alpha$, human $G\alpha16$, bovine $G_s\alpha$, rat $G_{olf}\alpha$, bovine $G_i1\alpha$, rat $G_i2\alpha$, rat $G_i3\alpha$, bovine $G_o\alpha$, hamster $G_o2\alpha$, bovine $G_{trod}\alpha$, $G_{tcone}\alpha$, rat $G_z\alpha$, mouse $G\alpha12$, and $G\alpha13$) is shown in Fig. 5. Three highly conserved regions (segments I, II and III) exhibit sequence homology with elongation factor-Tu and *ras* p21 proteins and correspond to functional regions for guanine nucleotide binding (la Cour et al., 1985; Jurnak, 1985; McCormick et al., 1985; de Vos et al., 1988; Pai et al., 1989; Milburn et al., 1990; Schlichting et al., 1990). Another highly conserved region (segment IV) of G-protein α-subunits shows no sequence homology with elongation factor-Tu nor *ras* p21 proteins. This region may be involved in unique functions of $G\alpha$ proteins, such as interactions with cell surface receptors, G-protein $\beta\gamma$-subunits and different intracellular effector systems. Amino acid sequence differences that are unique to $G_L1\alpha$ and $G_L2\alpha$ are scattered throughout the sequence, and some of these changes are also found within stretches of highly conserved amino acid sequence of the other $G\alpha$. The dispersed distribution of the amino acid sequence differences observed in $G_L1\alpha$, $G_L2\alpha$, and the other $G\alpha$ suggest that $G_L1\alpha$ and $G_L2\alpha$ are not derived by differential splicing from a known $G\alpha$ gene.

Fig. 6 shows the phylogenetic trees of mammalian G-protein α-subunits calculated by progressive sequence alignment method (Feng and Doolittle, 1987). The α-subunits can be grouped into five subfamilies: i) G_s subfamily, G_s and G_{olf}; ii) G_i subfamily, G_i1-3, G_o (G_o1), G_o2, G_{trod}, G_{tcone}, and G_z; iii) GL subfamily, $G_L1\alpha$, $G_L2\alpha$, $G_q\alpha$, and $G\alpha11$; iv) $G\alpha12$ and $G\alpha13$; and v) $G\alpha16$.

RNA Blot Hybridization Analysis - In order to analyze the tissue distribution of $G_L1\alpha$ and $G_L2\alpha$ mRNAs, total RNA from bovine cerebral cortex, liver, atrium, lung, and kidney were examined for the species hybridizing with bovine $G_L1\alpha$ and $G_L2\alpha$ cDNA probes (Fig. 7). A RNA species of ~3.7-kb nucleotides that hybridized with the $G_L2\alpha$ cDNA probe was observed in all tissues examined (Fig 7B). On the other hand, low levels of $G_L1\alpha$ mRNA were detected in liver, lung, and kidney (Fig. 7A). In each case, two mRNA species of ~3.9-kb and ~2.4-kb were observed. The highest levels of $G_L1\alpha$ mRNAs were detected in kidney.

Immunoblot Analysis- A pentadecapeptide, Lys-Asp-Thr-Ile-Leu-Gln-Leu-Asn-Leu-Lys-Glu-Tyr-Asn-Leu-Val, corresponding to the deduced carboxyl terminal amino acid sequence of $G_L2\alpha$, was synthesized (Fig. 4). Antiserum, AGL2, was then raised against the $G_L2\alpha$-pentadecapeptide coupled to keyhole limpet hemocyanin. Immunoblotting analysis with homogenates of mouse spleen, kidney, lung, liver, brain, and heart was performed to determine the tissue distribution of the $G_L2\alpha$ proteins. The antiserum, AGL2, reacted with several polypeptides in most tissues tested (Fig. 8A). The reactivity of AGL2 with a 40-kDa polypeptide was inhibited by the co-incubation of AGL2

with the $G_L2\alpha$-pentadecapeptide in all tissues examined, however (Fig. 8B). This result indicates that an immunoreactive polypeptide of 40-kDa was recognized specifically by the antiserum against the synthetic peptide. The 40-kDa polypeptide was detected in all tissues examined, but was most predominant in brain and lung. The antiserum AGL2 did not cross-react specifically with $G_i1\alpha$ and $G_o\alpha$ proteins purified from porcine brain (data not shown). The 40-kDa polypeptide was observed in all tissues where the $G_L2\alpha$ mRNA was expressed.

DISCUSSION

Two novel Gα proteins, $G_L1\alpha$ and $G_L2\alpha$ have been identified on the basis of their unique primary structures, the sizes and patterns of their expressed mRNAs and proteins. $G_L1\alpha$ and $G_L2\alpha$ show 45-59% amino acid identity with other known Gα proteins except $G_Q\alpha$ and Gα11. Amino acid identity among $G_L1\alpha$, $G_L2\alpha$, $G_q\alpha$, and Gα11 is 82-98%, suggesting that these Gα proteins form a separate subfamily of G-protein α-subunits. Comparable values of the identity are observed among the G_i-like α-subunits (Nukada et al., 1986b; Itoh et al., 1986; Didsbury et al., 1987), and between $G_{trod}\alpha$ and $G_{tcone}\alpha$ (Tanabe et al., 1985; Lochrie et al., 1985). The 98% identity between $G_L2\alpha$ and Gα11 suggests that $G_L2\alpha$ corresponds to the bovine version of Gα11, since more than 98% identity of amino acid sequences is observed among different mammalian species of $G_i2\alpha$, $G_i3\alpha$, $G_o\alpha$, and $G_z\alpha$ (Kaziro et al., 1991).

The cysteine residue in the fourth position from the carboxyl terminus has been identified as the ADP-ribosylation site by pertussis toxin in $G_{trod}\alpha$ (West et al., 1985) and is a feature common to all G-protein α-subunits susceptible to modification by the toxin. $G_L1\alpha$ and $G_L2\alpha$ lack this cysteine residue as do $G_s\alpha$, $G_z\alpha$, and $G_Q\alpha$, which are known not to be ADP-ribosylated by the toxin (Bokoch et al., 1983; Casey et al., 1990; Pang and Sternweis, 1990). This suggests that these two Gα proteins can not be ADP-ribosylated by pertussis toxin.

It is not known whether $G_L1\alpha$ and $G_L2\alpha$ are susceptible to ADP-ribosylation by cholera toxin. Although the arginine residue that has been identified as the site of modification

by cholera toxin in $G_{trod}\alpha$ (Van Dop et al., 1984) is conserved in $G_L1\alpha$ and $G_L2\alpha$, the ability of these proteins to be ADP-ribosylated by this toxin may be compromised due to differences in the amino acid sequences adjacent to analogous arginine (Arg^{179} and Arg^{177}, respectively) in $G_L1\alpha$ and $G_L2\alpha$.

Recently, $G_i\alpha$, $G_o\alpha$, and $G_z\alpha$ have been shown to be modified by amide-linked myristoylation (Schultz et al, 1987; Buss et al., 1987; Jones et al., 1990b; Mumby et al., 1990). Published analyses of the location of amide-linked myristate in proteins have thus far revealed acylation of the amino terminal glycine of each protein (Schultz et al., 1988). Replacement of the second glycine from amino terminus with alanine by site-directed mutagenesis in $G_i1\alpha$ and $G_o\alpha$ abolishes the myristoylation and changes the localization of these proteins from the cell membrane to the cytosol (Jones et al., 1990b; Mumby et al., 1990). $G_L2\alpha$ lacks the amino terminal glycine and $G_L1\alpha$ contains glycine not at position 2 but at position 3 (Fig. 5). This suggests that amino terminal myristoylation does not occur in $G_L2\alpha$ and may not occur in $G_L1\alpha$.

RNA blot hybridization analysis indicated that the ~3.9-Kb and ~2.4-Kb $G_L1\alpha$ mRNAs are expressed in low level in liver, lung and kidney. These two transcripts may result from alternative splicing or the selection of alternative polyadenylation or transcriptional initiation sites. The possibility that the transcripts are derived from distinct but highly conserved genes can not be excluded, however. On the other hand, $G_L2\alpha$ gene is expressed in high level in a wide range of tissues.

Antiserum raised against a synthetic pentadecapeptide corresponding to the carboxyl terminus of $G_L2\alpha$ specifically cross-reacted only with a 40-kDa protein in mouse homogenates of spleen, kidney, lung, liver, brain, and heart (Fig. 8). The molecular size observed by immunoblot analysis, 40-kDa, also correlates with the size predicted from the cDNA sequence of $G_L2\alpha$. However, the amino acid sequence of the synthesized peptide is identical to the carboxyl terminal amino acid sequence of $G_L1\alpha$ at 13 of the 15 residues and to that of $G_q\alpha$ at 15 of the 15 residues (Fig. 5). Thus, this antiserum reacts with both $G_L2\alpha$ and $G_q\alpha$, and may also cross-react with $G_L1\alpha$.

Recently, a 42-kDa Gα protein purified from rat brain (Pang and Sternweis, 1990) has been demonstrated to activate bovine brain PLC (Smrcka et al, 1991). Peptide sequence analysis has revealed that the 42-kDa protein is a mixture of G$q\alpha$ and $G_L2\alpha$ (Gα11) as major and minor components, respectively. A G-protein α-subunit, GPA-42, purified from bovine liver (Taylor et al., 1990), from which $G_L1\alpha$ and $G_L2\alpha$ cDNAs were cloned, also activates brain PLC β1 isozyme (Taylor et al., 1991). Antisera against two synthetic peptides corresponding to the partial sequences of $G_q\alpha$ (*A* and *B* in Fig. 5) have been demonstrated to cross-react with GPA-42 (Taylor et al., 1991). The antiserum against *B* fragment has the possibility to cross-react with $G_L2\alpha$, since the sequence of *B* fragment is identical to the corresponding region of $G_L2\alpha$ at 15 of the 18 residues. These data suggest that GPA-42 may contain $G_L2\alpha$ as well as G$q\alpha$. Neither GPA-42 nor the 42-kDa Gα protein have been shown to be ADP-ribosylated by pertussis

toxin. Furthermore, antiserum against a synthetic peptide corresponding to the carboxyl termini of $G_q\alpha$ and $G_L2\alpha$ attenuates the PLC activation stimulated by vasopressin receptor in rat liver membranes (Gutowski et al., 1991).

Thus, it is possible that $G_L2\alpha$ as well as $G_q\alpha$ mediates the receptor-induced activation of PLC in pertussis toxin-insensitive manner. Since $G_L1\alpha$ lacks the pertussis toxin ADP-ribosylation site, it might be also involved in pertussis toxin-insensitive signal transduction systems. It remains to be determined if there are functional differences among $G_q\alpha$, $G_L1\alpha$, and $G_L2\alpha$ with regards to the specificity for receptors and effectors.

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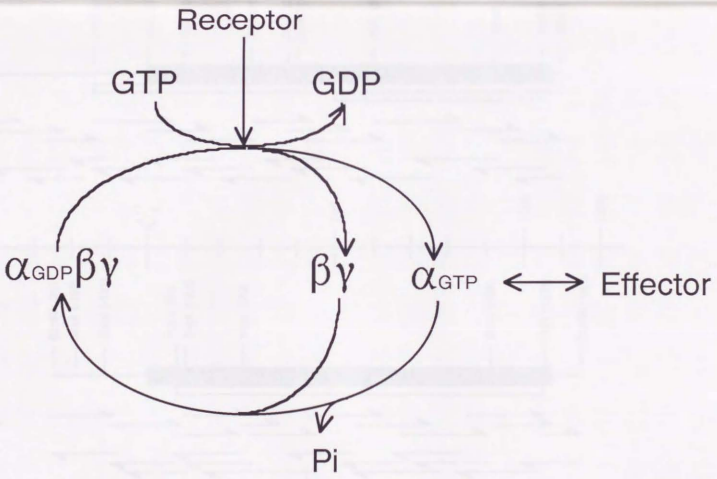

Fig.1 The functional role of G-proteins in signal transduction. $\alpha_{GDP}\beta\gamma$ represents the heterotrimer complex containing the α-subunit with bound GDP and the $\beta\gamma$-subunits. α_{GTP} is the α-subunit containing bound GTP. See text in details.

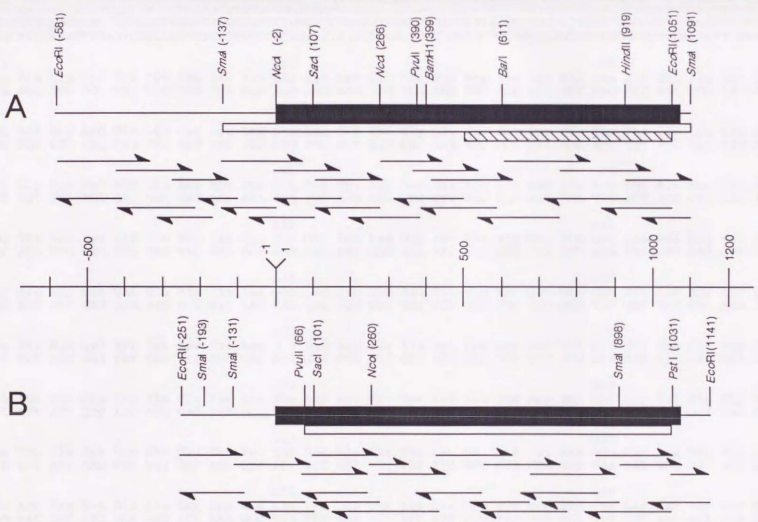

Fig. 2. Strategy for sequencing cloned cDNAs encoding GL1 and GL2 α-subunits. The restriction maps show only the relevant restriction sites, identified by numbers indicating the 5'-terminal nucleotide generated by cleavage (for the nucleotide numbers, see Fig. 3 and Fig. 4). The protein-coding regions for GL1 (A) and GL2 (B) α-subunits are indicated by closed boxes, the sequences used as hybridization probes for RNA blot hybridization analysis by open boxes and the corresponding sequence (excised from pGL28) used as a hybridization probe for selecting clones by a hatched box. The direction and extent of sequence determinations are shown by horizontal arrows under each clone.

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5'--- [illegible]                                                                                                                  -481
CCAGTCTGTCTTAGGCTGCGGTCACTGCTGAGGTTTTCAGAACTTTGCAAGAGAGGGCCAGCTAGGACCTCTGCCTCCAACCCACTCCTTGCCTGCCGCCTGCCTGGGACATCCCCGAGT  -361
CCAGGAGATCTCGCTCCCCGACGCCCCAACTTTTGGCGAGCAATCTGCGCTCCCAGACTGTCGAGCTCCTGTGCCAAGGGGCGTGCTGGTTTTCGGGGGCCGGCAACACTCTCCCTCGGC  -241
TCGCCTTCCACCTACCCCGGCGTGGGGCTGGGTGAGTTGGGATCTGCGGTGCACTTGAGCGTTCCCAGAGCTCTGGATTCGCCCTTCCAGGGGGGATTGAACCCGGGCGGTCCCGGCCTC  -121
TTACTCCGAAACTCGAGTCGGCCCCGGTCTTTTCCCTCCGAACTCCGGTGCCGTCGCGGGTCGGCCGGCGGAGCGTCCGGGCGCTCGAGACTTCGTGGCCTGTCGGGGGCTGTGCGTACC    -1

  1                                   10                                      20                                      30
Met Ala Gly Cys Cys Cys Leu Ser Ala Glu Glu Lys Glu Ser Gln Arg Ile Ser Ala Glu Ile Glu Arg Gln Leu Arg Arg Asp Lys Lys
ATG GCC GGC TGC TGC TGC CTG TCA GCG GAG GAG AAG GAG TCG CAG CGC ATC AGC GCC GAG ATC GAG CGG CAG CTT CGC CGG GAC AAG AAG    90

                                      40                                      50                                      60
Asp Ala Arg Arg Glu Leu Lys Leu Leu Leu Leu Gly Thr Gly Glu Ser Gly Lys Ser Thr Phe Ile Lys Gln Met Arg Ile Ile His Gly
GAC GCG CGC CGC GAG CTC AAG TTG CTG CTG CTG GGA ACT GGT GAA AGT GGC AAA AGC ACC TTT ATC AAG CAG ATG AGA ATC ATC CAC GGG   180

                                      70                                      80                                      90
Ser Gly Tyr Ser Asp Glu Asp Arg Lys Gly Phe Thr Lys Leu Val Tyr Gln Asn Ile Phe Thr Ala Met Gln Ala Met Ile Arg Ala Met
TCT GGG TAC AGC GAC GAA GAC AGA AAG GGG TTC ACG AAG CTG GTT TAC CAA AAC ATA TTC ACT GCC ATG CAA GCC ATG ATC AGA GCC ATG   270

                                     100                                     110                                     120
Asp Thr Leu Lys Ile Gln Tyr Val Cys Glu Gln Asn Lys Glu Asn Ala Gln Leu Ile Arg Glu Val Glu Val Asp Lys Val Ser Thr Leu
GAC ACC CTG AAG ATA CAG TAC GTG TGT GAG CAG AAT AAG GAA AAT GCC CAG CTA ATC AGA GAA GTG GAA GTA GAC AAG GTG TCC ACA CTC   360

                                     130                                     140                                     150
Ser Arg Asp Gln Val Glu Ala Ile Lys Gln Leu Trp Gln Asp Pro Gly Ile Gln Glu Cys Tyr Asp Arg Arg Arg Glu Tyr Gln Leu Ser
TCC AGG GAC CAG GTG GAG GCC ATC AAG CAG CTG TGG CAG GAT CCT GGA ATC CAG GAG TGC TAC GAT CGG AGG CGG GAG TAC CAA CTG TCA   450

                                     160                                     170                                     180
Asp Ser Ala Lys Tyr Tyr Leu Thr Asp Ile Asp Arg Ile Ala Met Pro Ala Phe Val Pro Thr Gln Gln Asp Val Leu Arg Val Arg Val
GAC TCT GCC AAA TAT TAC CTG ACG GAC ATT GAC CGG ATC GCC ATG CCA GCG TTC GTG CCC ACA CAG CAG GAT GTG CTC CGT GTC CGA GTG   540

                                     190                                     200                                     210
Pro Thr Thr Gly Ile Ile Glu Tyr Pro Phe Asp Leu Glu Asn Ile Ile Phe Arg Met Val Asp Val Gly Gly Gln Arg Ser Glu Arg Arg
CCC ACC ACT GGC ATC ATT GAG TAT CCG TTT GAC CTG GAA AAC ATC ATC TTT CGG ATG GTG GAT GTT GGT GGC CAG CGA TCT GAA AGA CGG   630

                                     220                                     230                                     240
Lys Trp Ile His Cys Phe Glu Ser Val Thr Ser Ile Ile Phe Leu Val Ala Leu Ser Glu Tyr Asp Gln Val Leu Ala Glu Cys Asp Asn
AAA TGG ATT CAC TGC TTT GAG AGT GTC ACC TCC ATT ATT TTT TTG GTT GCT CTG AGT GAA TAT GAC CAG GTC CTG GCT GAG TGT GAC AAT   720

                                     250                                     260                                     270
Glu Asn Arg Met Glu Glu Ser Lys Ala Leu Phe Lys Thr Ile Ile Thr Tyr Pro Trp Phe Leu Asn Ser Ser Val Ile Leu Phe Leu Asn
GAG AAC CGC ATG GAA GAG AGT AAA GCC TTA TTT AAA ACT ATC ATC ACC TAC CCC TGG TTT CTG AAC TCA TCA GTG ATT CTG TTC TTA AAC   810

                                     280                                     290                                     300
Lys Lys Asp Leu Leu Glu Glu Lys Ile Met Tyr Ser His Leu Ile Ser Tyr Phe Pro Glu Tyr Thr Gly Pro Lys Gln Asp Val Lys Ala
AAG AAG GAT CTT TTG GAA GAG AAA ATC ATG TAC TCT CAT CTA ATT AGC TAT TTT CCA GAA TAC ACT GGA CCA AAG CAA GAC GTC AAA GCT   900

                                     310                                     320                                     330
Ala Arg Asp Phe Ile Leu Lys Leu Tyr Gln Asp Gln Asn Pro Asp Lys Glu Lys Val Ile Tyr Ser His Phe Thr Cys Ala Thr Asp Thr
GCC AGA GAT TTC ATC CTG AAG CTT TAT CAA GAT CAG AAT CCT GAC AAA GAG AAG GTC ATC TAC TCC CAC TTC ACA TGT GCT ACA GAC ACA   990

                                     340                                     350                 355
Glu Asn Ile Arg Phe Val Phe Ala Ala Val Lys Asp Thr Ile Leu Gln Leu Asn Leu Arg Glu Phe Asn Leu Val
GAG AAT ATC CGC TTT GTG TTT GCT GCT GTG AAA GAC ACA ATC CTA CAA CTG AAC CTG AGG GAA TTC AAC CTG GTT TAA AAGCTGCTGTGCACC  1083

CCTCACCC ---3'
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Fig. 3. Nucleotide sequence of the cDNA encoding the G_L1 α-subunit.

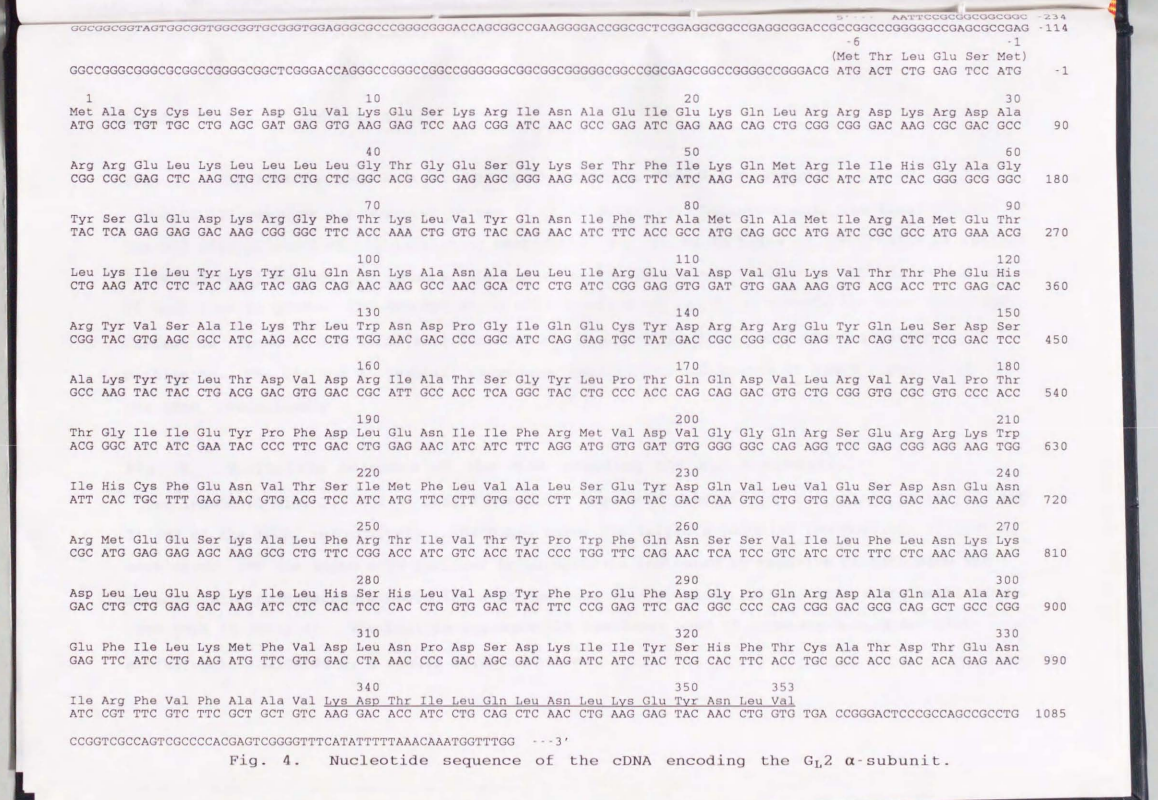

Fig. 4. Nucleotide sequence of the cDNA encoding the G_L2 α-subunit.

Legends to Fig. 3. and Fig. 4.

Fig. 3. Nucleotide sequence of the cDNA encoding the G_L1 α-subunit.

Nucleotide residues are numbered in the 5′ to 3′ direction, beginning with the first residue of the ATG triplet encoding the initiating methionine, and the nucleotides on the 5′-side of residue 1 are indicated by negative numbers; the number of the nucleotide residue at the right-hand end of each line is given. The deduced amino acid sequence of the G_L1 α-subunit is shown above the nucleotide sequence, and amino acid residues are numbered beginning with the initiation methionine. The 5′- and 3′-terminal sequences presented do not extend to the 5′- and 3′-end of the mRNA, respectively.

Fig. 4. Nucleotide sequence of the cDNA encoding the G_L2 α-subunit.

See legend to Fig. 3. The 5′- and 3′-terminal sequences presented do not extend to the 5′- and 3′-end of the mRNA, respectively. There are three ATG triplets encoding the possible initiating methionine, and the amino acid residues in parentheses indicated by negative numbers show the deduced amino acid sequence beginning with two less-likely, but possible, initiating methionines (see text in details). The peptide sequence (15 residues) used to generate a $G_L2\alpha$-specific antiserum is indicated by a single solid underline.

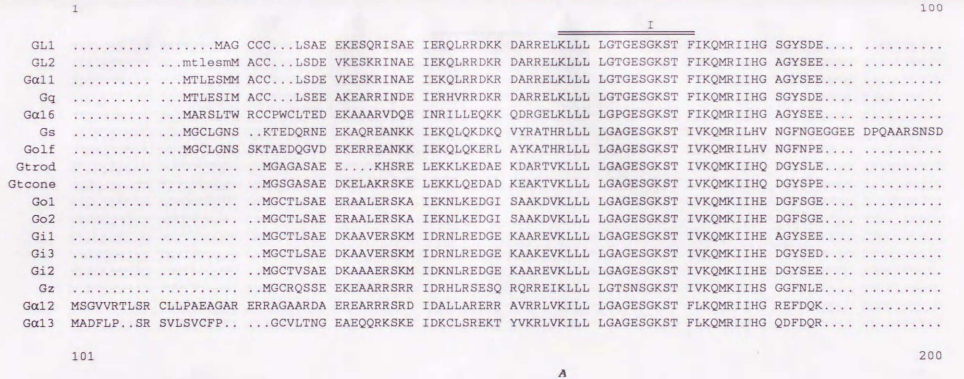
1
100
I
GL1 ....MAG CCC...LSAE EKESQRISAE IERQLRRDKK DARRELKLLL LGTGESGKST FIKQMRIIHG SGYSDE.... .....
GL2 ....mtlesmM ACC...LSDE VKESKRINAE IEKQLRRDKR DARRELKLLL LGTGESGKST FIKQMRIIHG AGYSEE.... .....
Gα11 ....MTLESMM ACC...LSDE VKESKRINAE IEKQLRRDKR DARRELKLLL LGTGESGKST FIKQMRIIHG AGYSEE.... .....
Gq ....MTLESIM ACC...LSEE AKEARRINDE IERHVRRDKR DARRELKLLL LGTGESGKST FIKQMRIIHG SGYSDE.... .....
Gα16 ....MARSLTW RCCPWCLTED EKAAARVDQE INRILLEQKK QDRGELKLLL LGPGESGKST FIKQMRIIHG AGYSEE.... .....
Gs ....MGCLGNS ..KTEDQRNE EKAQREANKK IEKQLQKDKQ VYRATHRLLL LGAGESGKST IVKQMRILHV NGFNGEGGEE DPQAARSNSD
Golf ....MGCLGNS SKTAEDQGVD EKERREANKK IEKQLQKERL AYKATHRLLL LGAGESGKST IVKQMRILHV NGFNPE.... .....
Gtrod ....MGAGASAE E....KHSRE LEKKLKEDAE KDARTVKLLL LGAGESGKST IVKQMKIIHQ DGYSLE.... .....
Gtcone ....MGSGASAE DKELAKRSKE LEKKLQEDAD KEAKTVKLLL LGAGESGKST IVKQMKIIHQ DGYSPE.... .....
Go1 ....MGCTLSAE ERAALERSKA IEKNLKEDGI SAAKDVKLLL LGAGESGKST IVKQMKIIHE DGFSGE.... .....
Go2 ....MGCTLSAE ERAALERSKA IEKNLKEDGI SAAKDVKLLL LGAGESGKST IVKQMKIIHE DGFSGE.... .....
Gi1 ....MGCTLSAE DKAAVERSKM IDRNLREDGE KAAREVKLLL LGAGESGKST IVKQMKIIHE AGYSEE.... .....
Gi3 ....MGCTLSAE DKAAVERSKM IDRNLREDGE KAAKEVKLLL LGAGESGKST IVKQMKIIHE DGYSED.... .....
Gi2 ....MGCTVSAE DKAAAERSKM IDKNLREDGE KAAREVKLLL LGAGESGKST IVKQMKIIHE DGYSEE.... .....
Gz ....MGCRQSSE EKEAARRSRR IDRHLRSESQ RQRREIKLLL LGTSNSGKST IVKQMKIIHS GGFNLE.... .....
Gα12 MSGVVRTLSR CLLPAEAGAR ERRAGAARDA EREARRRSRD IDALLARERR AVRRLVKILL LGAGESGKST FLKQMRIIHG REFDQK.... .....
Gα13 MADFLP..SR SVLSVCFP.. ...GCVLTNG EAEQQRKSKE IDKCLSREKT YVKRLVKILL LGAGESGKST FLKQMRIIHG QDFDQR.... .....
101
200
A
GL1 .DRKGFTKLV YQNIFTAMQA MIRAMDTL.. KIQYVCEQNK ENAQLIREV. .EVDKVSTLS RD......QV EAIKQLWQDP GIQECYDRRR EYQLSDSAKY
GL2 .DKRGFTKLV YQNIFTAMQA MIRAMETL.. KILYKYEQNK ANALLIREV. .DVEKVTTFE HR......YV SAIKTLWNDP GIQECYDRRR EYQLSDSAKY
Gα11 .DKRGFTKLV YQNIFTAMQA MVRAMETL.. KILYKYEQNK ANALLIREV. .DVEKVTTFE HQ......YV NAIKTLWSDP GVQECYDRRR EFQLSDSAKY
Gq .DKRGFTKLV YQNIFTAMQA MIRAMDTL.. KIPYKYEHNK AHAQLVREV. .DVEKVSAFE NP......YV DAIKSLWNDP GIQECYDRRR EYQLSDSTKY
Gα16 .ERKGFRPLV YQNIFVSMRA MIEAMERL.. QIPFSRPESK HHASLVMSQ. .DPYKVTTFE KR......YA AAMQWLWRDA GIRACYERRR EFHLLDSAVY
Gs GEKATKVQDI KNNLKEAIET IVAAMSNLVP PVELANPENQ FRVDYILSVM N........V PDFDFPPEFY EHAKALWEDE GVRACYERSN EYQLIDCAQY
Golf .EKKQKILDI RKNVKDALVT IISAMSTIIP PVPLANPENQ FRSDYIKSIA P........I TDFEYSQEFF DHVKKLWDDE GVKACFERSN EYQLIDCAQY
Gtrod .ECLEFIAII YGNTLQSILA IVRAMTTL.. NIQYGDSARQ DDARKLMHMA DTIEE.GTMP KE......MS DIIQRLWKDS GIQACFDRAS EYQLNDSAGY
Gtcone .ECLEYKAII YGNVLQSILA IIRAMPTL.. GIDYAEVSCV DNGRQLNNLA DSIEE.GTMP PE......LV EVIRKLWKDG GVQACFDRAA EYQLNDSASY
Go1 .DVKQYKPVV YSNTIQSLAA IVRAMDTL.. GVEYGDKERK ADSKMVCDVV SRMEDTEPFS AE......LL SAMMRLWGDS GIQECFNRSR EYQLNDSAKY
Go2 .DVKQYKPVV YSNTIQSLAA IVRAMDTL.. GVEYGDKERK ADSKMVCDVV SRMEDTEPFS AE......LL SAMMRLWGDS GIQECFNRSR EYQLNDSAKY
Gi1 .ECKQYKAVV YSNTIQSIIA IIRAMGRL.. KIDFGDSARA DDARQLFVLA GAAEE.GFMT AE......LA GVIKRLWKDS GVQACFNRSR EYQLNDSAAY
Gi3 .ECKQYKVVV YSNTIQSIIA IIRAMGRL.. KIDFGEAARA DDARQLFVLA GSAEE.GVMT SE......LA GVIKRLWRDG GVQACFSRSR EYQLNDSASY
Gi2 .ECRQYRAVV YSNTIQSIMA IVKAMGNL.. QIDFADPQRA DDARQLFALS CAAEEQGMLP ED......LS GVIRRLWADH GVQACFGRSR EYQLNDSAAY
Gz .ACKEYKPLI IYNAIDSLTR IIRALAAL.. KIDFHNPDRA YDAVQLFALT GPAESKGEIT PE......LL GVMRRLWADP GAQACFGRSS EYHLEDNAAY
Gα12 .ALLEFRDTI FDNILKGSRV LVDARDKL.. GIPWQHSENE KHGMFLMAFE NKAGLP.... VEPATFQLYV PALSALWRDS GIREAFSRRS EFQLGESVKY
Gα13 .AREEFRPTI YSNVIKGMRV LVDAREKL.. HIPWGDNKNQ LHGDKLMAFD TRAPMAAQGM VETRVFLQYL PAIRALWEDS GIQNAYDRRR EFQLGESVKY

Fig. 5.

```
        201                                                                                                          300
                                ▼                                             II
GL1     YLTDIDRIAM PAFVPTQQDV LRVRVPTTGI IEYPFDLENI IFRMVDVGGQ RSERRKWIHC FESVTSIIFL VALSEYDQVL AECDNENRME ESKALFKTII
GL2     YLTDVDRIAT SGYLPTQQDV LRVRVPTTGI IEYPFDLENI IFRMVDVGGQ RSERRKWIHC FENVTSIMFL VALSEYDQVL VESDNENRME ESKALFRTIV
Gα11    YLTDVDRIAT VGYLPTQQDV LRVRVPTTGI IEYPFDLENI IFRMVDVGGQ RSERRKWIHC FENVTSIMFL VALSEYDQVL VESDNENRME ESKALFRTII
Gq      YLNDLDRVAD PSYLPTQQDV LRVRVPTTGI IEYPFDLQSV IFRMVDVGGQ RSERRKWIHC FENVTSIMFL VALSEYDQVL VESDNENRME ESKALFRTII
Gα16    YLSHLERITE EGYVPTAQDV LRSRMPTTGI NEYCFSVQKT NLRIVDVGGQ KSERKKWIHC FENVIALIYL ASLSEYDQCL EENNQENRMK ESLALFGTIL
Gs      FLDKIDVIKQ DDYVPSDQDL LRCRVLTSGI FETKFQVDKV NFHMFDVGGQ RDERRKWIQC FNDVTAIIFV VASSSYNMVI REDNQTNRLQ EALNLFKSIW
Golf    FLERIDSVSL VDYTPTDQDL LRCRVLTSGI FETRFQVDKV NFHMFDVGGQ RDERRKWIQC FNDVTAIIYV AACSSYNMVI REDNNTNRLR ESLDLFESIW
Gtrod   YLSDLERLVT PGYVPTEQDV LRSRVKTTGI IETQFSFKDL NFRMFDVGGQ RSERKKWIHC FEGVTCIIFI AALSAYDMVL VEDDEVNRMH ESLHLFNSIC
Gtcone  YLNQLDRITA PDYLPNEQDV LRSRVKTTGI IETKFSVKDL NFRMFDVGGQ RSERKKWIHC FEGVTCIIFC AALSAYDMVL VEDDEVNRMH ESLHLFNSIC
Go1     YLDSLDRIGA ADYQPTEQDI LRTRVKTTGI VETHFTFKNL HFRLFDVGGQ RSERKKWIHC FEDVTAIIFC VALSGYDQVL HEDETTNRMH ESLMLFDSIC
Go2     YLDSLDRIGA ADYQPTEQDI LRTRVKTTGI VETHFTFKNL HFRLFDVGGQ RSERKKWIHC FEDVTAIIFC VALSGYDQVL HEDETTNRMH ESLKLFDSIC
Gi1     YLNDLDRIAQ PNYIPTQQDV LRTRVKTTGI VETHFTFKDL HFKMFDVGGQ RSERKKWIHC FEGVTAIIFC VALSDYDLVL AEDEEMNRMH ESMKLFDSIC
Gi3     YLNDLDRISQ TNYIPTQQDV LRTRVKTTGI VETHFTFKEL YFKMFDVGGQ RSERKKWIHC FEGVTAIIFC VALSDYDLVL AEDEEMNRMH ESMKLFDSIC
Gi2     YLNDLERIAQ SDYIPTQQDV LRTRVKTTGI VETHFTFKDL HFKMFDVGGQ RSERKKWIHC FEGVTAIIFC VALSAYDLVL AEDEEMNRMH ESMKLFDSIC
Gz      YLNDLERIAA PDYIPTVEDI LRSRDMTTGI VENKFTFKEL TFKMVDVGGQ RSERKKWIHC FEGVTAIIFC VELSGYDLKL YEDNQTSRMA ESLRLFDSIC
Gα12    FLDNLDRIGQ LNYFPSKQDI LLARKATKGI VEHDFVIKKI PFKMVDVGGQ RSQRQKWFQC FDGITSILFM VSSSEYDQVL MEDRRTNRLV ESMNIFETIV
Gα13    FLDNLDKLGV PDYIPSQQDI LLARRPTKGI HEYDFEIKNV PFKMVDVGGQ RSERKRWFEC FDSVTSILFL VSSSEFDQVL MEDRQTNRLT ESLNIFETIV

        301                                                                                                          400
                 III                                          B                                                       IV
GL1     TYPWFLNSSV ILFLNKKDLL EEKIM..YSH LISYFPEYT. .......... .GPKQDVKAA RDFILKLYQD QNPDK..... .......EKV IYSHFTCATD
GL2     TYPWFQNSSV ILFLNKKDLL EDKIL..HSH LVDYFPEFD. .......... .GPQRDAQAA REFILKMFVD LNPDS..... .......DKI IYSHFTCATD
Gα11    TYPWFQNSSV ILFLNKKDLL EDKIL..HSH LVDYFPEFD. .......... .GPQRDAQAA REFILKMFVD LNPDS..... .......DKI IYSHFTCATD
Gq      TYPWFQNSSV ILFLNKKDLL EEKIM..YSH LVDYFPEYD. .......... .GPQRDAQAA REFILKMFVD LNPDS..... .......DKI IYSHFTCATD
Gα16    ELPWFKSTSV ILFLNKTDIL EEKIP..TSH LATYFPSFQ. .......... .GPKQDAEAA KRFILDMYTR MYTGCVDGPE GSKKGARSRR LFSHYTCATD
Gs      NNRWLRTISV ILFLNKQDLL AEKVLAGKSK IEDYFPEFAR YTTPEDATPE PGEDPRVTRA KYFIRDEFLR ISTASGDGRH YC........ .YPHFTCAVD
Golf    NNRWLRTISI ILFLNKQDML AEKVLAGKSK IEDYFPEYAN YTVPEDATPD AGEDPKVTRA KFFIRDLFLR ISTATGDGKH YC........ .YPHFTCAVD
Gtrod   NHRYFATTSI VLFLNKKDVF SEKIK..KAH LSICFPDYN. .......... .GPNTYEDAG .NYIKVQFLE LNMRR..... ......DVKE IYSHMTCATD
Gtcone  NHKFFAATSI VLFLNKKDLF EEKIK..KVH LSICFPEYD. .......... .GNNSYEDAG .NYIKSQFLD LNMRK..... ......DVKE IYSHMTCATD
Go1     NNKFFIDTSI ILFLNKKDLF GEKIK..KSP LTICFPEYP. .......... .GSNTYEDAA .AYIQTQFES KNRSP..... ......N.KE IYCHMTCATD
Go2     NNKWFTDTSI ILFLNKKDIF EEKIT..RSP LTICFPEYT. .......... .GPSAFTEAV .AHIQGQYES KNKSA..... ......H.KE IYTHFTCATD
Gi1     NNKWFTDTSI ILFLNKKDLF EEKIK..KSP LTICYPEYA. .......... .GSNTYEEAA .AYIQCQFED LNKRK..... ......DTKE IYTHFTCATD
Gi3     NNKWFTDTSI ILFLNKKDLF EEKIK..RSP LTICYPEYT. .......... .GSNTYEEAA .AYIQCQFED LNRRK..... ......DTKE VYTHFTCATD
Gi2     NNKWFTDTSI ILFLNKKDLF EEKIT..QSP LTICFPEYT. .......... .GANKYDEAA .SYIQSKFED LNKRK..... ......DTKE IYTHFTCATD
Gz      NNNWFINTSL ILFLNKKDLL SEKIR..RIP LSVCFPEYK. .......... .GQNTYEEAA .VYIQRQFED LNRNK..... ......ETKE IYSHFTCATD
Gα12    NNKLFFNVSI ILFLNKMDLL VEKVK..SVS IKKHFPDFK. .......... .GDPHRLEDV QRYLVQCFDR KRRN...... ......RSKP LFHHFTTAID
Gα13    NNRVFSNVSI ILFLNKTDLL EEKVQ..VVS IKDYFLEFE. .......... .GDPHCLRDV QKFLVECFRG KRRDQ..... ......QQRP LYHHFTTAIN
```

Fig. 5.

```
        401                                   427
        IV                                    ∇
  GL1   TENIRFVFAA VKDTILQLNL REFNLV*
  GL2   TENIRFVFAA VKDTILQLNL KEYNLV*
 Gα11   TENIRFVFAA VKDTILQLNL KEYNLV*
   Gq   TENIRFVFAA VKDTILQLNL KEYNLV*
 Gα16   TQNIRKVFKD VRDSVLARYL DEINLL*
   Gs   TENIRRVFND CRDIIQRMHL RQYELL*
 Golf   TENIRRVFND CRDIIQRMHL KQYELL*
Gtrod   TQNVKFVFDA VTDIIIKENL KDCGLF*
Gtcone  TQNVKFVFDA VTDIIIKENL KDCGLF*
  Go1   TNNIQVVFDA VTDIIIANNL RGCGLY*
  Go2   TNNIQFVFDA VTDVIIAKNL RGCGLY*
  Gi1   TKNVQFVFDA VTDVIIKNNL KDCGLF*
  Gi3   TKNVQFVFDA VTDVIIKNNL KECGLY*
  Gi2   TKNVQFVFDA VTDVIIKNNL KDCGLF*
   Gz   TSNIQFVFDA VTDVIIQNNL KYIGLC*
 Gα12   TENIRFVFHA VKDTILQENL KDIMLQ*
 Gα13   TENIRLVFRD VKDTILHDNL KQLMLQ*
```

Fig. 5. Alignment of the amino acid sequences for the deduced GL1 and GL2 α-subunits and the α-subunits for mouse Gα11, Gq, human Gα16, bovine Gs, rat Golf, bovine Gtrod , bovine Gtcone, bovine Gi1, rat Gi2, rat Gi3, bovine Go (Go1), hamster Go2, rat Gz, mouse Gα12, and Gα13 (from top to bottom). Multiple sequence alignments were generated by the progressive alignment method (Feng and Doolittle, 1987) in UWGCG package (Devereux et al, 1984). The one-letter amino acid notation is used. Dots indicate gaps inserted to optimize homology. The sequence of GL2α indicated by letter characters is the deduced amino acid sequence beginning with two less-likely, but possible, initiating methionines. The highly conserved regions I, II, III, and IV are indicated by double lines above the sequences. Arrowheads point to sites of ADP-ribosylation by pertussis toxin (∇) and cholera toxin (▼) analogous to the sites identified in Gtrodα (Van Dop et al., 1984; West et al., 1985). Two shaded sequences *A* and *B* correspond to the synthesized peptides to generate specific antiserum against Gqα (Pang and Sternweis, 1990).

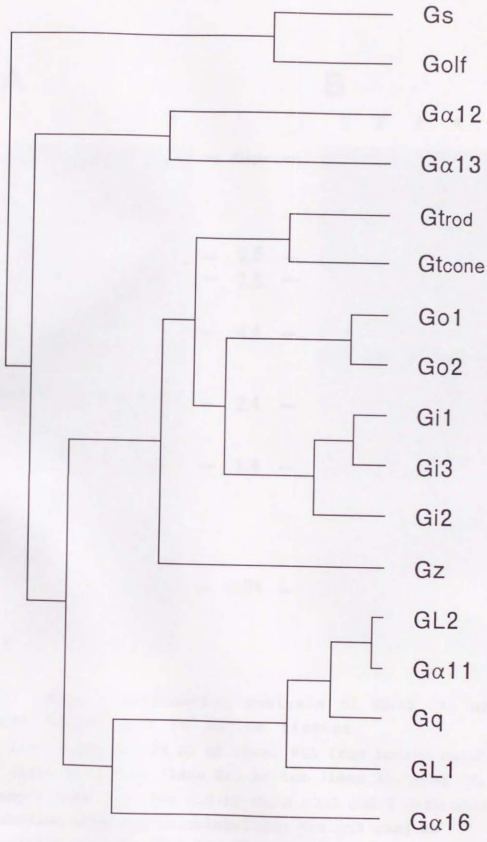

Fig. 6 Phylogenetic trees of mammalian G-protein α-subunits. α-subunits are grouped by a series of progressive pairwise alignments (Feng and Doolittle, 1987).

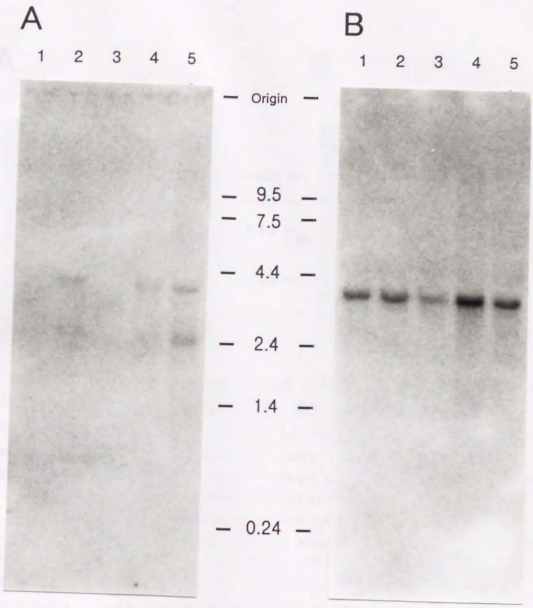

Fig. 7. Blot hybridization analysis of GL1α (A) and GL2α (B) mRNA transcripts in bovine tissues.

Each lane contained 20 μg of total RNA from bovine cerebral cortex (lane 1), liver (lane 2), atrium (lane 3), lung (lane 4) or kidney (lanes 5). The 1.0-kb GL2α cDNA and 1.2-kb GL1α cDNA were labelled with the Oligolabelling Kit and used as hybridization probes. RNA lengths were determined relative to the RNA Ladder size markers. Autoradiography was performed at -70°C with intensifying screen. Exposure time: GL1α, 10 days; GL2α, 3 days.

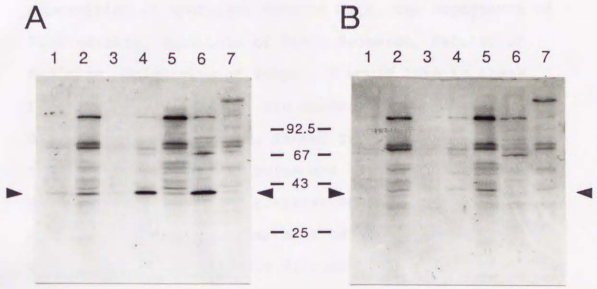

Fig. 8. Immunoblot analysis of mouse tissue homogenates resolved by SDS-polyacrylamide gel electrophoresis.

Homogenates (24 µg except 48 µg in lane 1) from mouse spleen (lanes 1 and 3), kidney (lane 2), lung (lane 4), liver (lane 5), brain (lane 6) and heart (lane 7) were processed by 12% SDS-PAGE, and the resolved proteins were transferred to Immobilon PVDF (Millipore). Immunoblot analysis was performed as described under "Materials and Methods" using antiserum AGL2 (at a 1:3000 dilution): the incubation with the antiserum was for 2 h at room temperature in the absence (A) or presence (B) of 0.2 mg/ml GL2α-peptide. Both the second antibody, biotinylated donkey anti-rabbit Ig and peroxidase-streptavidin were used at a 1:1000 dilution, and respective incubations were for 1 h at room temperature. The Konica Immunostaining Kit (Konica) was used as the substrate for peroxidase. The 40-kDa polypeptides inhibited specifically by co-incubation of the antiserum with the GL2a-peptide are indicated by arrowheads. The size markers used were phosphorylase B (92.5 kDa), bovine serum albumin (67 kDa), actin (43 kDa), and chymotrypsinogen (25 kDa).

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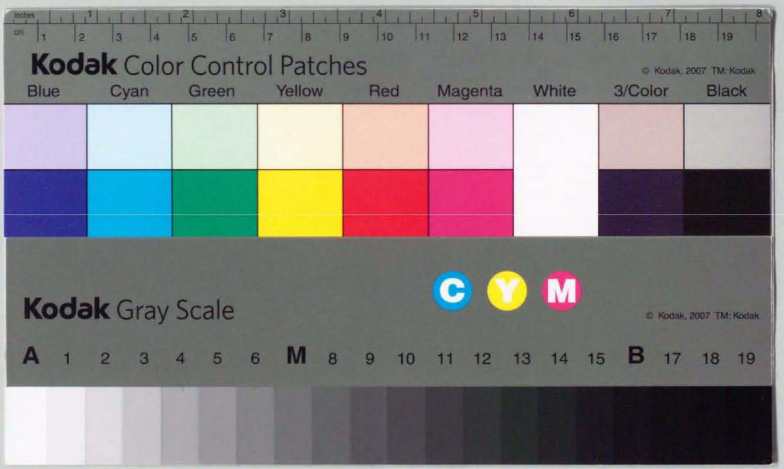
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A 1 2 3 4 5 6 M 8 9 10 11 12 13 14 15 B 17 18 19