Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 4929–4933, July 1988
Neurobiology

Antibody specific to the α subunit of the guanine nucleotide-binding regulatory protein G_o: Developmental appearance and immunocytochemical localization in brain

(membrane transduction/neurotransmitters/hippocampus/synaptic transmission)

KWEN-JEN CHANG*, WILLIAM PUGH*, STEVEN G. BLANCHARD*, JOHN MCDERMED†, AND JAMES P. TAM‡

Departments of *Molecular Biology and †Organic Chemistry, Burroughs Wellcome Co., Research Triangle Park, NC 27709; and ‡Rockefeller University, 1230 York Avenue, New York, NY 10021

Communicated by George H. Hitchings, March 18, 1988

ABSTRACT **A polyclonal rabbit antibody (9120) against the α subunit of the "other" guanine nucleotide-binding protein G_o (α_o) was raised against a synthetic α_o peptide fragment (Asp-Gly-Ile-Ser-Ala-Ala-Lys-Asp-Val) attached to a branched core system. Antiserum 9120, at a final dilution of 1:400, can detect α_o in as little as 0.2 μg of G_o on immunoblots, and, at a final dilution of 1:20,000, can detect α_o in 1 μg of G_o on immunoblots. Antiserum and affinity-purified antibody are specific to α_o. No cross-reactivity was detected to the α subunits of the stimulatory or inhibitory guanine nucleotide-binding regulatory proteins or of transducin (α_s, α_i, or α_T) or to the β or γ subunits. Immunoblots revealed a high density of α_o in rat brain and lung membrane preparations, but other tissues (such as adipose tissue, heart, erythrocytes, and liver) have no detectable α_o. Developmental studies showed that α_o in rat brain was low before birth, increased after birth, and reached the full adult level at 4 weeks of age. In contrast, ADP-ribosylation of 40-kDa proteins increased for up to 1 week and then decreased. Immunocytochemistry revealed that α_o was localized to somatic and synaptic membranes in rat brain, whereas little or no α_o was detected in the cytoplasm of neuronal cell bodies. Our observations suggest that G_o in brain might have a role in membrane signal transduction at synaptic and extrasynaptic sites.**

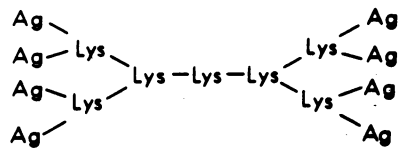
A family of guanine nucleotide-binding regulatory proteins (G proteins) transduces signals from cell-surface receptors to intracellular effectors (1). Members of this family include the structurally homologous stimulatory and inhibitory G proteins of the ligand-sensitive adenylate cyclase system (G_s and G_i, respectively) (2–11) and the transducin (T_r) protein, which couples the light-induced activation of rhodopsin to cGMP phosphodiesterase activity (12). The G_s, G_i, and T_r proteins have been purified as oligomeric proteins composed of α, β, and γ subunits (2–11). The α subunits of G_s, G_i, and T_r are distinct but homologous proteins with molecular masses of 45, 41, and 40 kDa, respectively. The β subunit has a molecular mass of 35 kDa and appears to be identical in all forms of G protein. The γ subunit of G_s, G_i, and T_r is reported to have a molecular mass of $\approx$8 kDa. The α subunits of G_s (α_s) and G_i (α_i) are substrates for cholera and pertussis toxin-catalyzed ADP-ribosylation, respectively. The α subunit of T_r (α_T) can be ADP-ribosylated by cholera and pertussis toxins at distinct sites (13, 14). Another G protein, termed the "other" G protein (G_o), with a similar structure was isolated from bovine brain but has an undetermined function (15, 16). The α subunit of the G_o protein (α_o) has a molecular mass of 39 kDa and is a substrate for pertussis-toxin catalyzed ADP-ribosylation (15, 16).

The complete amino acid sequences of α subunits of G_s, G_i, and T_r have been deduced from the nucleotide sequences of their cDNAs (17–23). A partial amino sequence of α_o has been obtained directly from the purified protein (22). Polyclonal antibodies against α and β subunits have been raised against either the purified proteins (24–27) or the synthetic peptide fragments (24). Since these α subunits are structurally homologous proteins of different functions, a highly specific antibody against each α subunit is desirable for anatomic localization and functional studies. Antibodies reported so far have cross-reacted with multiple α subunits. In this study, we report the properties of a rabbit polyclonal antibody against a synthetic peptide fragment unique to α_o, which is covalently coupled to a heptalysine branched oligomer. This antibody interacts specifically with α_o of rat brain and lung. It does not cross-react with the α subunit from G_s (α_s), with α_i, with α_T, or with α_o from rat heart or adipose tissue, if α_o is present in these tissues.

METHODS AND MATERIALS

Antibody Preparation. A nanopeptide fragment (Asp-Gly-Ile-Ser-Ala-Ala-Lys-Asp-Val) unique to α_o was selected as the immunogen. A heptalysine oligomer carrying eight copies of this peptide fragment ("octopus-immunogen") was synthesized by a solid-phase method (28).

Rabbits were immunized with 100 μg of this octavalent



Octopus-immunogen

peptide in complete Freund's adjuvant and 3 weeks later with a 50-μg booster injection in incomplete Freund's adjuvant. Rabbits received booster injections four times at 3- to 4-week intervals and were bled 2 weeks after the final booster injection. To affinity purify antibody, α_o was separated from other proteins by NaDodSO$_4$/PAGE and electroblotted onto a nitrocellulose sheet. The α-subunit bands were identified, cut out, and used as an affinity matrix. Affinity-purified α_o antibodies were isolated by incubating α_o antiserum with the α_o affinity matrix and eluting specific α_o antibodies with 0.2

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: G protein, guanine nucleotide-binding regulatory protein; G_i, G_o, G_s, the inhibitory, other, and stimulatory G proteins, respectively; T_r, transducin; α_o, α_i, and α_T, α subunit of G_o, G_i, and T_r, respectively.

M glycine (pH 2.8). The eluent was immediately neutralized with Tris base and stored at −80°C.

Preparation of G Proteins. G_o and G_i proteins were purified from rat brain membrane preparations as described by Sternweis and Rabinshow (15). The identities of the G_o and G_i proteins were confirmed by $NaDodSO_4$/PAGE and by ADP-ribosylation catalyzed by pertussis toxin (15, 16). G_s and G_i proteins of human erythrocytes (11) were generous gifts of Codina and Birnbaumer (Baylor College of Medicine).

Immunoblots. To block nonspecific sites, electroblotted nitrocellulose sheets were incubated with 3% (wt/vol) bovine serum albumin for 3 hr at 24°C in a buffer containing 50 mM Tris·HCl, 0.2 M NaCl, and polyethyleneglycol (1 mg/ml; molecular mass, 20 kDa). The blots were incubated with α_o antibody for 3 hr at 24°C, washed with buffer several times over 30 min, and then incubated with goat anti-rabbit IgG-coupled alkaline phosphatase for 1 hr at 24°C. The immunoreactive bands were then visualized by the color reaction of nitro blue tetrazolium chloride and 5-bromo-4-chloro-3-indolyl phosphate catalyzed by alkaline phosphatase at pH 9.5 (29). The color reaction was stopped by washing with 5 mM EDTA solution.

Membrane Preparation. To minimize the proteolytic degradation, all membranes were prepared in the presence of the following proteinase inhibitors: soybean trypsin inhibitor (50 μg/ml), 10^{-5} M phenylmethylsulfonyl fluoride, and leupeptin (20 μg/ml). Rat brain membranes were prepared as described (30). Rat cardiac sarcolemma membranes were prepared by the method of Slaughter *et al.* (31). Membrane particulates from rat liver and lung were prepared by differential sucrose density centrifugation. The post-mitochondria membrane fractions were used. The plasma and microsomal membranes from adipose tissue were prepared by centrifugation of post-mitochondria supernatant at 40,000 × *g* for 30 min and 140,000 × *g* for 60 min, respectively. The membrane ghosts of human and rat erythrocytes were prepared by hypotonic lysis and were extensively washed.

ADP-Ribosylation. Pertussis toxin-induced ADP-ribosylation of various substrates was determined by measuring the incorporation of [^{32}P]ADP-ribose from [*adenylate*-^{32}P]NAD after a 30-min incubation at 30°C in a buffer containing 100 mM Tris·HCl (pH 8.0), 10 mM thymidine, 1 mM ATP, 1 mM EDTA, 2.5 mM $MgCl_2$, 20 μM [^{32}P]NAD (specific activity, ≈2000 cpm/pmol), and pertussis toxin (1 μg/ml). When the substrate was purified G protein, 0.5 mM L-α-dimyristoyl phosphatidylcholine was added to the reaction mixture. The ADP-ribosylation reaction was stopped by adding 20% (wt/vol) trichloroacetic acid. The precipitated proteins were collected by centrifugation and dissolved in $NaDodSO_4$/PAGE sample buffer and neutralized. Proteins were separated by $NaDodSO_4$/PAGE with exponential 7%–15% [2:1 (vol/vol)] crosslinked gels, and gels were stained with Coomassie brilliant blue and dried. Radioactive bands were visualized by autoradiography with Kodak XAR-5 film.

Immunocytochemistry. Adult male Sprague–Dawley rats were deeply anesthetized and sequentially perfused through the ascending aorta with 100 ml of heparinized saline and with 500 ml of 4% (vol/vol) formaldehyde in 0.05 M Tris-buffered saline (pH 7.7). The brain was removed, cut into blocks, and frozen in liquid nitrogen. Cryostat sections (10 μm thick) were collected on gelatin-coated slides and dried overnight at room temperature. Sections were preincubated in serum buffer containing 0.05 M Tris·HCl (pH 7.7), 0.1 M NaCl, 0.05% Triton X-100, bovine serum albumin (2.5 mg/ml), and 2% (vol/vol) normal goat serum for 2 hr at room temperature. Primary antibody (9120) was diluted 1:2000 in serum buffer, and the sections were incubated in this solution with agitation overnight at 4°C. All subsequent reactions were carried out at room temperature. The sections were rinsed in serum buffer and incubated for 2 hr in serum buffer containing a 1:200 dilution of biotinylated goat anti-rabbit IgG for 2 hr. Sections were rinsed in 0.1 M sodium phosphate buffer (pH 7.6) and incubated for 2 hr in 0.1 M sodium phosphate buffer (pH 7.6) containing a 1:200 dilution of avidin–biotin-peroxidase complex (Vector Laboratories, Burlingame, CA) for 1 hr. Sections were washed in 0.1 M sodium phosphate (pH 7.6), incubated first in 0.8 mM diaminobenzidine for 20 min and then in the same solution plus 0.025% H_2O_2 for 20 min, and then dried.

Adjacent sections were processed with normal rabbit serum, preimmune serum (all at a 1:2000 dilution), or excess (0.2 mM) antigen to assess immunohistochemical specificity. Additional sections were stained with thionin. All sections were examined by bright- and dark-field light microscopy to identify and localize immunoreactive products and to identify the cytoarchitecture of the section.

RESULTS

Characterization of Antibody Against α_o. Antibodies against α_o were identified in sera from three of the four

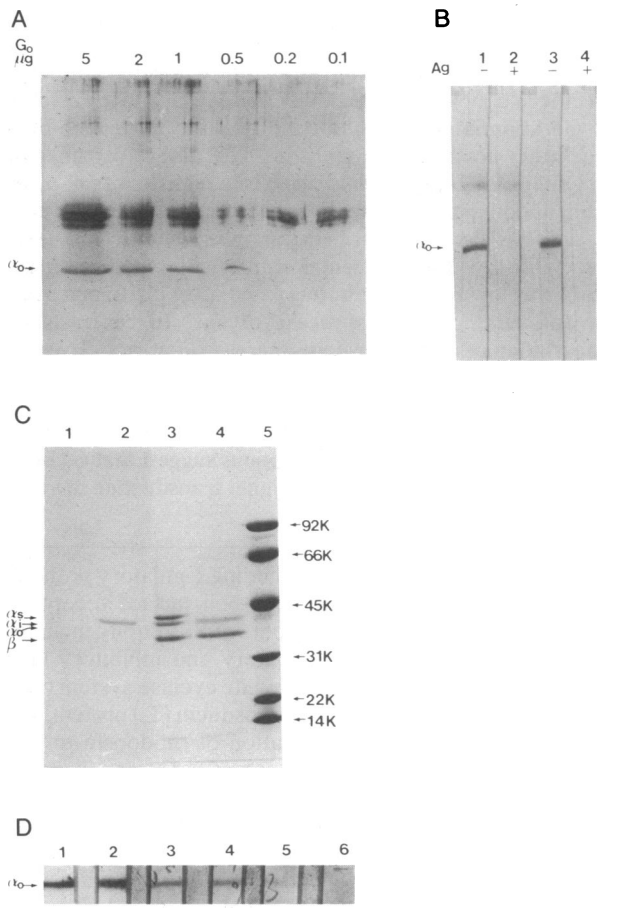


FIG. 1. Characteristics of the α_o antiserum 9120 in immunoblotting. Purified G proteins from $NaDodSO_4$/polyacrylamide gels were electroblotted onto nitrocellulose sheets. (*A*) Sensitivity of antiserum at 1:400 dilution to various concentrations of G_o proteins as indicated. (*B*) Specificity of antiserum 9120 and affinity-purified antibody. Lanes: 1, antiserum alone; 2, antiserum plus antigen peptide (10^{-4} M); 3, affinity-purified antibody; 4, affinity-purified antibody plus antigen peptide. (*C*) Specificity of antibody to G proteins. Lanes: 1, immunostaining of G_s and G_i from human erythrocytes; 2, immunostaining of G_o and G_i from rat brain; 3, Coomassie blue staining of G_s and G_i; 4, Coomassie blue staining of G_o and G_i; 5, molecular size marker proteins. (*D*) Dilution of antiserum against 1 μg of purified G_o protein. The dilution factors are 1:200 (lane 1), 1:600 (lane 2), 1:2000 (lane 3), 1:6000 (lane 4), 1:20,000 (lane 5), and no antiserum (lane 6).

rabbits after four booster injections. One antiserum, designated 9120, was used for the remaining studies because of its high titer. At a final dilution of 1:400, antiserum 9120 could detect α_o on an immunoblot containing 0.2 μg of G_o protein (Fig. 1*A*). This same antiserum contained antibody against several unidentified 50- to 60-kDa proteins that often contaminated the gel. The affinity of antibodies for these contaminating proteins appeared to be low, and the proteins were not related to G_o protein, since immunostaining was not inhibited by the antigen peptide fragment (Fig. 1*B*). Immunostaining of 50- to 60-kDa proteins was greatly decreased by extensive washing of the blot before incubation with alkaline phosphate-conjugated goat anti-IgG (Fig. 1*B*). Immunostaining of α_o could be detected by this antiserum at a final dilution of 1:20,000. It appeared to be saturated at a 1:600 dilution (Fig. 1*D*). We routinely used a 1:400 dilution for most experiments.

Monospecific α_o antibody was affinity-purified by absorption onto pieces of a nitrocellulose blot containing only α_o and subsequent elution with glycine buffer (pH 2.8). This affinity-purified antibody interacted exclusively with α_o (Fig. 1*B*), even on blots of crude membrane preparations (data not shown).

Immunostaining by antiserum 9120 and by affinity-purified antibody was specific to α_o, since it was inhibited by adding an antigen peptide fragment or G_o (Fig. 1*B*). The specificity of this α_o antibody was further confirmed by its lack of immunoreactivity with as much as 6 μg of G_s or G_i proteins purified from human erythrocytes protein (Fig. 1*C*), whereas it detected α_o protein in only 0.2 μg of purified preparations of G_o protein or 7 μg of total brain membrane proteins. This α_o antibody also did not detect α_o in membrane protein preparations from bovine rod outer segment (data not shown), rat liver, human, or rat erythrocytes (Fig. 2), which is consistent with the α_o specificity of this antibody and the absence of G_o from retina, liver, and erythrocytes.

Comparison of α_o in Various Tissues. Two bands of proteins in adipose tissues with molecular masses of ≈40 kDa have been shown to be ADP-ribosylated by pertussis toxin, suggesting the existence of G_o and G_i in this tissue (31, 32). Our studies also showed that there was a broad band of ADP-ribosylated proteins at ≈40 kDa in the adipose tissue membrane preparations (Fig. 3). However, no immunoreactive bands were detected with antibody 9120 even when a large quantity of membrane proteins were used (Fig. 2). These data suggest that either G_o is absent from adipose tissue or does not cross-react with our antibody.

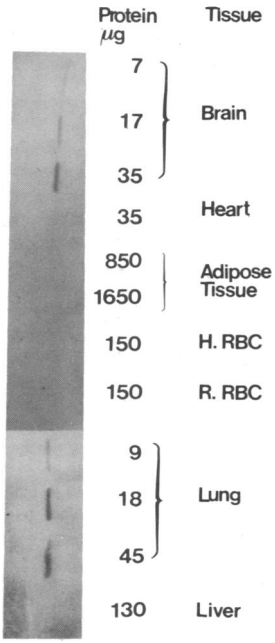


FIG. 2. Immunoblots of membranes of various tissues with 1:400 dilution of α_o antiserum 9120. Amounts of membrane proteins applied and tissues are indicated.

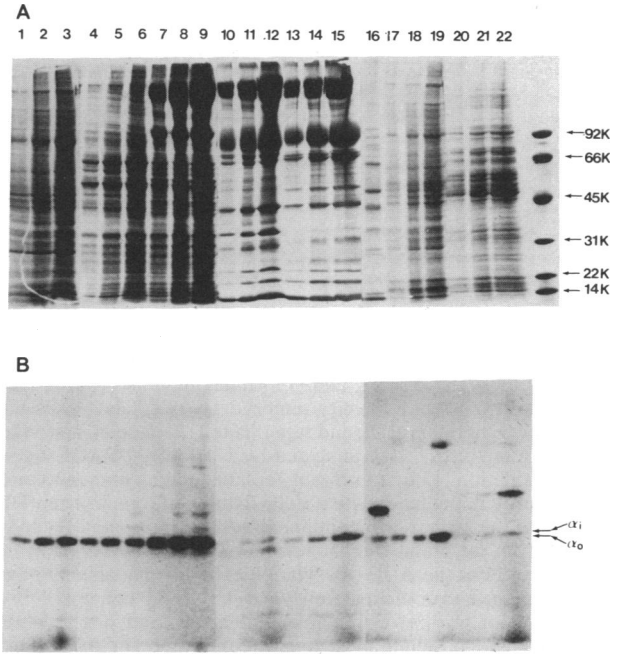


FIG. 3. Pertussis toxin-stimulated ADP-ribosylation of membrane proteins from various tissues. (*A*) The Coomassie blue staining of membrane proteins. (*B*) Autoradiography of [^{32}P]ADP-ribosylated proteins in the presence of pertussis toxin (1 μg/ml). Lanes containing brain membranes: 1, 7 μg; 2, 17 μg; and 3, 35 μg. Lanes containing adipose tissue plasma membranes: 4, 17 μg; 5, 43 μg; and 6, 85 μg. Lanes containing adipose tissue microsomal membranes: 7, 63 μg; 8, 165 μg; and 9, 320 μg. Lanes containing human erythrocyte membranes: 10, 27 μg; 11, 54 μg; and 12, 130 μg. Lanes containing rat erythrocyte membranes: 13, 27 μg; 14, 54 μg; and 15, 130 μg. Lane containing rat heart membranes: 16, 17 μg. Lanes containing rat lung membranes: 17, 2 μg; 18, 5 μg; and 19, 10 μg. Lanes containing rat liver membranes: 20, 4 μg; 21, 9 μg; and 22, 18 μg. Lane to the right in *A* contains molecular size markers identified in kDa (K).

Similarly, G_o was reported (22) in bovine cardiac sarcolemma membranes by pertussis toxin-induced ADP-ribosylation and immunoblot with an antibody against an α_o fragment. With our antibody, we were unable to detect any immunoreactive band for rat or porcine cardiac sarcolemma membrane preparations (Fig. 2); however, these membrane preparations did contain 40-kDa proteins that were ADP-ribosylated by pertussis toxin (Fig. 3).

Membranes from lung tissue contained a 40-kDa protein that was immunoreactive and ADP-ribosylated (Figs. 2 and 3). The density of this protein in lung appears to be as high as in brain membranes.

ADP-ribosylation was observed for a 95-kDa lung protein, a 55-kDa heart protein, and an 80-kDa liver protein, although ADP-ribosylation of all proteins was not pertussis toxin-dependent (Fig. 3*B*).

Developmental Study of α_o in Rat Brain. Developmental studies of G_o were carried out by immunoblotting and by pertussis toxin-stimulated ADP-ribosylation of membranes prepared from brains of rat embryos 1 week before birth, of newborn rats, and of 1-, 2-, and 4-week-old rats. No α_o was detected in immunoblots from embryonic brain tissue, but α_o was detected at birth and increased continuously up to 4 weeks of age (Fig. 4*A*). Surprisingly, the pattern of ADP-

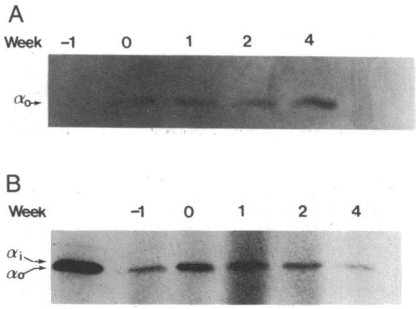


FIG. 4. Neonatal developmental appearance of G_o in rat brain. (*A*) Immunoblot. (*B*) Autoradiography of pertussis toxin-dependent [^{32}P]ADP-ribosylation of G_o and G_i. Equal amounts of brain membrane from rats at various ages [embryo 1 week (lane −1) before birth, a newborn rat (lane 0), and rats 1 (lane 1), 2 (lane 2), and 4 (lane 4) weeks after birth] were analyzed by NaDodSO$_4$/PAGE for the ADP-ribosylation (15 μg of protein per lane) and by immunoblotting (30 μg of protein per lane) with a 1:400 dilution of α_o antiserum 9120. The lane to the far left in *B* contains the ADP-ribosylated G_o and G_i purified from rat brain. Similar results have been obtained in four experiments. For the ADP-ribosylation experiments, an aliquot was analyzed at the end of the reaction for the [^{32}P]NAD content by TLC. About 90% of [^{32}P]NAD remained intact and did not show any difference among membranes; thus the difference in ADP-ribosylation is not due to the degree of the availability of substrate.

ribosylation was quite different. ADP-ribosylated protein was detected in embryonic brain, reached a peak level 1 week after birth, and declined after 2 weeks (Fig. 3*B*). The 4-week-old rat showed a minimal level of ADP-ribosylation that was even lower than that of embryonic brain membranes (Fig. 4*B*), despite its having the highest level of immunoreactive α_o (Fig. 4*A*).

Immunocytochemical Localization of α_o in Rat Brain. We have observed a heterogeneous distribution of α_o in rat brain similar to that described by Worley *et al.* (27). Control preparations included omitting primary antibody or adding normal rabbit serum, preimmune serum, or excess antigen to the primary antibody reaction mixture; all yielded negative immunostaining, suggesting a high antibody specificity for α_o. The hippocampus displayed strong immunoreactivity and had a laminated cytoarchitecture. Fig. 5 *A*, *C*, and *E* shows a Nissl-stained pyramidal cell layer in the CA1 region of the dorsal hippocampus. Under low magnification, immunoreactive staining was widely distributed, abundant in the neuropil and low in cell bodies (Fig. 5*B*). Fig. 5*D* shows weak immunostaining in the pyramidal cell layer but very strong immunoreactivity in the suprajacent stratum oriens and the subjacent stratum radiatum. However, upon close examination at higher magnification (Fig. 5*F*), immunoreactivity can be seen at the somal membrane of neuronal cell bodies but not in the cytoplasm. Similar observations were also found in other regions of brain, especially the cortex. We interpret the above observation to be the result of membrane-to-cytosol ratios. In the hippocampus, the apical and basilar dendritic fields (strata oriens and radiatum) are dense in membrane but low in cytosol, which results in a dense immunostaining. Conversely, in the pyramidal cell layer the ratio is opposite—a low membrane-to-cytosol ratio—which results in a low density of immunostaining.

DISCUSSION

Our α_o antibody was raised by injecting a simple heptalysine oligomer carrying eight copies of the immunogenic α_o peptide fragment (octopus-immunogen). This is in contrast with the traditional method, in which a small peptide must be coupled to a large protein carrier (i.e., bovine serum albumin or thyroglobulin) to be an effective immunogen. The carrier we used is a simple and defined structure, and the whole octopus-immunogen can be synthesized by the solid-phase method.

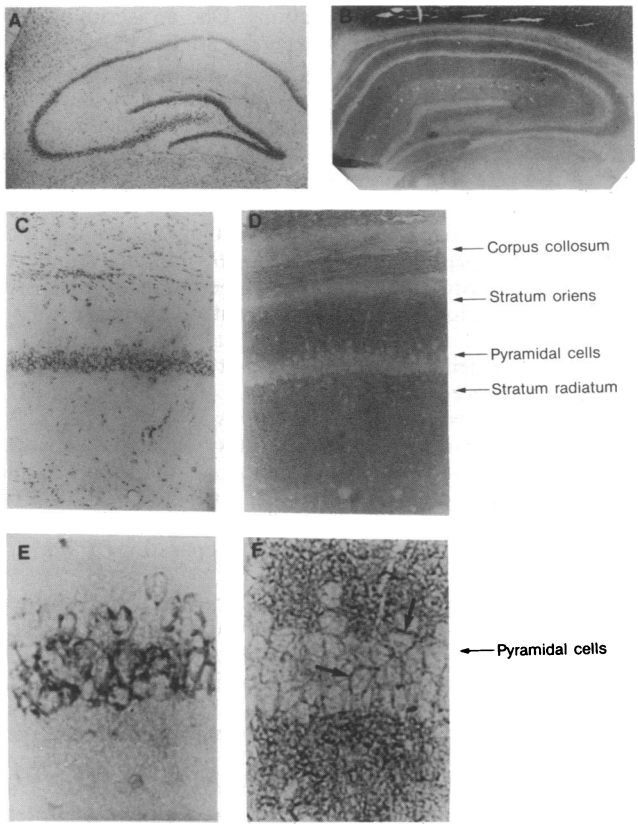


FIG. 5. Photomicrographs of the hippocampus. (*A*, *C*, and *E*) Thionin stained. (*B*, *D*, and *F*) Immunostained for α_o. (*A* and *B*) Immunoreactivity in the pyramidal cells of the CA1 and CA3 region as well as granule cells of the dentate gyrus appears very low. (*C* and *D*) Strong immunoreactivity is seen in the strata oriens and radiatum, representing the apical and basilar pyramidal cell dendritic fields of the CA1 dorsal hippocampus. (*E* and *F*) Upon close examination at high magnification, immunoreactivity (arrows) is associated with the somal membranes but not the cytoplasm of pyramidal cells. (For *A* and *B*, ×20; for *C* and *D*, ×100; for *E* and *F*, ×400.)

Although several α_o antibodies have been reported, all lacked high specificity to α_o. Ten percent cross-reactivity to α_i was observed for the antibody reported by Worley *et al.* (27). By using α_o fragment Asn-**Leu-Lys-Glu-Asp**-Gly-Ile-Ser-**Ala**-Ala-Lys-Asp-**Val-Lys**, Mumby *et al.* (24) produced an α_o antibody with some cross-reactivity to α_T. This, perhaps, is not unexpected, since this peptide is partially (50%) homologous to α_T fragment Lys-**Leu-Lys-Glu-Asp**-Ala-Glu-Lys-Asp-**Ala**-Arg-Thr-**Val-Lys**. By using purified G_o, Gierschik *et al.* (26) produced a polyclonal antibody that can recognize α_o and the common β subunit. In our study, we selected the peptide fragment unique to α_o (Asp-Gly-Ile-Ser-Ala-Ala-Lys-Asp-Val) for the immunogen. The results clearly show high specificity to α_o. Our studies also show it is applicable to immunoblotting studies and immunohistochemical localization of G_o proteins.

The function of G_o remains unknown. The presence of G_o protein in adipose tissue and bovine heart muscle has been suggested (24, 33). In the adipose tissues the evidence is obtained from the pertussis toxin-stimulated doublet [^{32}P]-ADP-ribosylation of a 40-kDa protein. These substrates ADP-ribosylated by pertussis toxin can also be ADP-ribosylated by cholera toxin, suggesting they are different from G_i or G_o proteins (34). In bovine cardiac tissue, the possible reactivity of the α_o antibody to α_T has not been completely excluded. Our data suggest that neither tissue contains α_o

protein, although the difference between our study and that reported by Mumby *et al.* (24) could be a species difference. Alternatively, the immunoreactive band in cardiac tissue detected by Mumby *et al.* (24) might have resulted from cross-reaction with another similar protein (i.e., α_T). Despite the presence of ≈40-kDa proteins in adipose tissue, liver, and erythrocytes that can be ADP-ribosylated by pertussis toxin, our antibody does not detect any α_o, even when large amounts of membranes were applied to the gel, suggesting that G_i but not G_o is present in those tissues. In contrast, lung tissue appears to contain a large quantity of α_o. The cellular localization of α_o in lung tissue may be useful in probing the function of G_o.

Developmental appearance of G_o proteins in rat brain was examined by immunoblotting and by pertussis toxin-mediated ADP-ribosylation of 40-kDa proteins. Immunoblots revealed that the amount of α_o increased as the animal developed. In contrast, ADP-ribosylation reached its maximum at ≈1 week of age and decreased thereafter. This study raises the issue of whether or not ADP-ribosylation accurately reflects the absolute quantity of G proteins present, since ADP-ribosylation is dependent upon the quantity and the functional state of G proteins. For instance, it is known that the ADP-ribosylation of G_i, and perhaps G_o, is reduced when activated by GTP (35). It was also shown (36) that thrombin activation of platelets greatly reduces the pertussis toxin-induced ADP-ribosylation of α_i. Alternatively, ADP-ribosylation in this report could represent mainly α_i ADP-ribosylation, but this is unlikely since large quantities of α_o are detected by immunoblot and since G_o is ≈5-fold more abundant than G_i in brain (15, 16). Thus, our results may suggest that most of the G_o protein in 3- and 4-week-old rats is in a functionally activated state that prevents the ADP-ribosylation of G_o.

It has been inferred that G proteins have a role in the regulation of phosphotidylinositol cycle, since GTP stimulated phospholipase C activation in membranes and in permeabilized cells (37, 38). Pertussis toxin, which ADP-ribosylated G_o and G_i, inhibited ligand-induced phospholipase activation and the production of two second messengers, diacylglycerol and inositol 1,4,5-trisphosphate, in certain cells (39–41). A G protein that is a pertussis toxin substrate was also implicated in coupling receptors directly to ion channels (42, 43). The similar localization of G_o and protein kinase C in neuronal synaptic zones has led Worley *et al.* (27) to further suggest that G_o plays a role in the brain that is related to regulation of the phosphotidylinositol cycle in synapses. Our studies have revealed a more extensive localization G_o in the plasma membrane of neuronal cell bodies in addition to synaptic zones, suggesting a broader membrane transduction function for G_o in brain than just synaptic transmission.

Note. After this paper was submitted for publication two papers appeared. One paper reported similar postnatal developmental appearance of α_o in rat brain (44). The other paper reported that there are three types of α_i. The α_i peptides corresponding to the α_o peptide used in our antibody production are Asp-Gly-Glu-Lys-Ala-Ala-Arg-Glu-Val for α_{i1} and α_{i2} and Asp-Gly-Glu-Lys-Ala-Ala-Lys-Glu-Val for α_{i3} (45). Both peptides differ from our α_o antigen peptide, and, since we could not detect immunoreactivity to α_i purified from human erythrocytes and to tissues that were reported to contain α_i, it is unlikely that antiserum 9120 cross-reacts with α_i.

We thank Mark A. Collins for his skillful technical assistance.

1. Gilman, A. G. (1984) *Cell* **36**, 577–579.
2. Northup, J. K., Sternweis, P. C., Smigel, M. D., Schleifer, L. S., Ross, E. M. & Gilman, A. G. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 6516–6520.
3. Sternweis, P. C., Northup, J. K., Smigel, M. D. & Gilman, A. G. (1981) *J. Biol. Chem.* **256**, 11517–11526.
4. Sternweis, P. C. & Gilman, A. G. (1979) *J. Biol. Chem.* **254**, 3333–3340.
5. Schleifer, L. S., Kahn, R. A., Hanski, E., Northup, J. K., Sternweis, P. C. & Gilman, A. G. (1982) *J. Biol. Chem.* **257**, 20–23.
6. Northup, J. K., Smigel, M. D. & Gilman, A. G. (1982) *J. Biol. Chem.* **257**, 11416–11423.
7. Codina, J., Hildebrandt, J. D., Birnbaumer, L. & Sekura, R. D. (1984) *J. Biol. Chem.* **259**, 11408–11418.
8. Bokoch, G. M., Katada, T., Northup, J. K., Hewlett, E. L. & Gilman, A. G. (1983) *J. Biol. Chem.* **258**, 2072–2075.
9. Bokoch, G. M., Katada, T., Northup, J. K., Ui, M. & Gilman, A. G. (1984) *J. Biol. Chem.* **259**, 3560–3567.
10. Katada, T., Bokoch, G. M., Northup, J. K., Ui, M. & Gilman, A. G. (1984) *J. Biol. Chem.* **259**, 3568–3577.
11. Codina, J., Hildebrandt, J., Iyengar, R., Birnbaumer, L., Sekura, R. D. & Manclark, C. R. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 4276–4280.
12. Stryer, L., Hurley, J. B. & Fung, B. K.-K. (1981) *Curr. Top. Membr. Transp.* **15**, 93–108.
13. Abood, M. E., Hurley, J. B., Pappone, M.-C., Bourne, H. R. & Stryer, L. (1982) *J. Biol. Chem.* **257**, 10540–10543.
14. Watkins, P. A., Moss, J., Burns, D. L., Hewlett, E. L. & Vaughan, M. (1984) *J. Biol. Chem.* **259**, 1378–1381.
15. Sternweis, P. C. & Robishaw, J. D. (1984) *J. Biol. Chem.* **259**, 13806–13813.
16. Neer, E. J., Lok, J. M. & Wolf, L. G. (1984) *J. Biol. Chem.* **259**, 14222–14229.
17. Hurley, J. B., Fong, H. K. W., Teplow, D. B., Dreyer, W. J. & Simon, M. I. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 6948–6952.
18. Tanabe, T., Nukada, T., Nishikawa, Y., Sugimoto, K., Suzuki, H., Takahashi, H., Noda, M., Haga, T., Ichiyama, A., Kangawa, K., Minamino, N., Matsuo, H. & Numa, S. (1985) *Nature (London)* **315**, 242–245.
19. Harris, B. A., Robishaw, J. D., Mumby, S. M. & Gilman, A. G. (1985) *Science* **229**, 1274–1277.
20. Lochrie, M. A., Hurley, J. B. & Simon, M. I. (1985) *Science* **228**, 96–99.
21. Toshihide, N., Tanabe, T., Takahashi, H., Noda, M., Haga, K., Ichiyama, A., Kangawa, K., Hiranaga, M., Matsuo, H. & Numa, S. (1986) *FEBS Lett.* **197**, 305–310.
22. Hurley, J. B., Simon, M. I., Teplow, D. B., Robishaw, J. D. & Gilman, A. G. (1984) *Science* **226**, 860–862.
23. Sullivan, K. A., Liao, Y.-C., Alborzi, A., Beiderman, B., Chang, F.-H., Masters, S. B., Levinson, A. D. & Bourne, H. R. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 6687–6691.
24. Mumby, S. M., Kahn, R. A., Manning, D. R. & Gilman, A. G. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 265–269.
25. Huff, R. M., Axton, J. M. & Neer, E. J. (1985) *J. Biol. Chem.* **260**, 10864–10871.
26. Gierschik, P., Milligan, G., Pines, M., Goldsmith, P., Codina, J., Klee, W. & Spiegel, A. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 2258–2262.
27. Worley, P. F., Baraban, J. M., Van Dop, C., Neer, E. J. & Snyder, S. H. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 4561–4565.
28. Merrifield, R. B. (1983) *J. Am. Chem. Soc.* **85**, 2149–2154.
29. Leary, J. J., Brigati, D. J. & Ward, D. C. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 4045–4049.
30. Chang, K.-J. & Cuatrecasas, P. (1979) *J. Biol. Chem.* **254**, 2610–2618.
31. Slaughter, R. S., Sutko, J. L. & Reeves, J. P. (1983) *J. Biol. Chem.* **258**, 3183–3190.
32. Malbon, C. C., Rapiejko, P. J. & Garcia-Sainz, J. A. (1984) *FEBS Lett.* **176**, 301–306.
33. Gierschik, P., Morrow, B., Milligan, G., Rubin, C. & Speigel, A. (1986) *FEBS Lett.* **199**, 103–106.
34. Owens, J. R., Frame, L. T., Ui, M. & Cooper, D. M. F. (1985) *J. Biol. Chem.* **260**, 15946–15952.
35. Ui, M. (1984) *Trends. Pharmacol. Sci.* **5**, 277–279.
36. Lapetina, E. G., Reep, B. & Chang, K.-J. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 5880–5883.
37. Litosch, I., Wallis, C. & Fain, J. N. (1985) *J. Biol. Chem.* **260**, 5464–5471.
38. Cockcroft, S. & Gomperts, B. D. (1985) *Nature (London)* **314**, 534–536.
39. Brandt, S. J., Dougherty, R. W., Lapetina, E. G. & Niedel, J. E. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 3277–3280.
40. Nakamura, T. & Ui, M. (1985) *J. Biol. Chem.* **260**, 3584–3593.
41. Smith, C. D., Lane, B. C., Kusaka, I., Verghese, M. W. & Snyderman, R. (1985) *J. Biol. Chem.* **260**, 5875–5878.
42. Pfaffinger, P. J., Martin, J. M., Hunter, D. D., Nathanson, N. M. & Hille, B. (1985) *Nature (London)* **317**, 536–538.
43. Breitwieser, G. E. & Szabo, G. (1985) *Nature (London)* **317**, 538–540.
44. Milligan, G., Streaty, R. A., Gierschik, P., Spiegel, A. M. & Klee, W. A. (1987) *J. Biol. Chem.* **262**, 8626–8630.
45. Beals, C. R., Wilson, C. B. & Perlmutter, R. M. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 7886–7890.