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# Use of specific antibodies to quantitate the guanine nucleotide-binding protein $G_o$ in brain

(pertussis toxin substrate/receptor–effector coupling)

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ABSTRACT **We immunized rabbits with purified guanine nucleotide-binding proteins (G proteins) from bovine brain and obtained an antiserum, RV3, that reacts specifically with the $\alpha$ subunit (39 kDa) of a G protein of unknown function, termed $G_o$, as well as with the $\beta$ subunit (35 kDa) common to all G proteins. RV3 showed no crossreactivity with the $\alpha$ subunits of the stimulatory ($G_s$) or inhibitory ($G_i$) G proteins associated with adenylate cyclase, nor with that of the rod outer segment G protein, transducin. Immunoblots with crude and affinity-purified antiserum showed that RV3 specifically recognizes the $G_o$ $\alpha$ subunit and the $\beta$ subunit in crude brain membranes. Using RV3, we found approximately equal amounts of $G_o$ in brain membranes from frog, chicken, rat, cow, and man. Quantitative immunoblotting gave $G_o$ $\alpha$ subunit/$\beta$ subunit ratios $\approx$ 1 in cerebral cortex, raising the possibility that free $G_o$ $\alpha$ subunit (unassociated with $\beta$ subunit) may exist in brain. The concentration of $G_o$ $\alpha$ subunit in cerebral cortex is about 5 times that of $G_i$ $\alpha$ subunit. The results show that $G_o$ is an immunochemically distinct, highly conserved protein distributed throughout the brain, with particularly high concentrations in forebrain.**

Certain guanine nucleotide-binding proteins (G proteins) are known to function as membrane-bound receptor–effector couplers (1, 2). These include transducin (TD), the G protein of photoreceptor rod outer segments, which couples the photon receptor, rhodopsin, to a specific cGMP phosphodiesterase; and $G_s$ and $G_i$, which couple hormone and neurotransmitter receptors to stimulation ($G_s$) and inhibition ($G_i$) of adenylate cyclase.

TD, $G_s$, and $G_i$ are heterotrimers, each with unique guanine nucleotide-binding $\alpha$ subunits, common 35-kDa $\beta$ subunits, and $\approx$9-kDa $\gamma$ subunits (1, 2). The $\alpha$ subunit of $G_i$, as purified from rabbit liver and human erythrocytes, is a 40-kDa protein that is a specific substrate for pertussis toxin (1). In the purification of $G_i$ from bovine brain, a 39-kDa protein was discovered, in addition to the expected 40-kDa $\alpha$ subunit of $G_i$ (3–5). $\beta$ and $\gamma$ subunits copurified with both the 39- and the 40-kDa proteins. The 39-kDa protein, in common with the 40-kDa $G_i$ $\alpha$ subunit ($G_i$-$\alpha$) is a pertussis toxin substrate and shows high-affinity guanine nucleotide binding as well as GTPase activity. Certain features (e.g., differential susceptibility to tryptic digestion) suggested that the 39- and 40-kDa proteins are distinct entities (4). The protein with the 39-kDa $\alpha$ subunit was termed $G_o$ (for "other"), since its specific function is unknown (4). We previously showed (6) that $G_i$ and $G_o$ from bovine brain are immunochemically distinct. An antiserum against TD-$\alpha$, CW6, crossreacts strongly with $G_i$-$\alpha$ but weakly or not at all (depending on the specific bleeding from which the antiserum was obtained) with $G_o$-$\alpha$. We now report studies with an antiserum, RV3, raised against purified bovine brain G proteins, that specifically recognizes $G_o$. We used these antisera to identify $G_o$ in brains from frog, chicken, and mammals; to measure the amounts of $G_o$ and of $G_i$ in cerebral cortex; and to measure the distribution of $G_o$ in bovine brain.

## MATERIALS AND METHODS

The sources of reagents used for protein purification, NaDodSO$_4$/PAGE, and immunoblotting were as previously cited (3, 6–8).

Purification of brain G proteins (3), human erythrocyte G proteins (8), and TD (7) was performed as described. Fresh bovine brains were delivered to the laboratory on ice from a local slaughterhouse. The brainstem and cerebellum were removed, and membranes were prepared from the remaining brain (4). For measurement of $G_o$ in brain regions, fresh bovine brain was sliced in coronal sections, and tissue from specific regions (see Table 2) was dissected grossly for membrane preparation. Brains from frog (*Xenopus laevis*), rooster (10-week-old White Leghorn), and rat ($\approx$150-g male Sprague–Dawley) were rapidly removed and placed on ice. Human cerebral cortex was obtained postmortem and frozen before membrane preparation. Membranes were prepared (4) from fresh whole frog brain, rooster forebrain (brainstem and cerebellum removed), and rat forebrain and from thawed human cerebral cortex.

Six rabbits were immunized with purified brain G proteins (3), treated with 6 M urea as described (7). Each rabbit was injected with 124 $\mu$g of protein in complete Freund's adjuvant, followed 4 weeks later by 62 $\mu$g in incomplete Freund's adjuvant. Animals were bled 2 weeks after the booster injection and weekly thereafter.

For affinity purification of antisera, 1.5 mg of purified brain G proteins, from early fractions of hydroxylapatite chromatography (3, 6), was coupled to cyanogen bromide-activated Sepharose 4B (Pharmacia) to a final concentration of 1 mg of protein per ml of settled gel. Whole serum, diluted 1:3 with 50 mM glycine/500 mM NaCl at pH 7.5 (buffer A) was incubated overnight at 4°C with the G-protein-linked Sepharose, and after extensive washing with buffer A, specifically bound immunoglobulins were eluted with 100 mM glycine/500 mM NaCl at pH 2.5. The eluate was immediately brought

Abbreviations: G proteins, guanine nucleotide-binding proteins; $G_s$ and $G_i$, the stimulatory and inhibitory G proteins associated with adenylate cyclase; $G_o$, a G protein of unknown function purified from brain; $G_o$-$\alpha$, the $\alpha$ subunit of $G_o$; TD, transducin, the G protein of rod outer segments.

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to pH 7.5, and samples were used directly or stored at −80°C with 0.1% (wt/vol) ovalbumin.

$NaDodSO_4$/PAGE, immunoblotting, and autoradiography were performed as described (6, 7). Quantitative immunoblotting was performed as in ref. 7. In brief, standard amounts of purified $G_i$ plus $G_o$ and unknown samples were loaded onto alternate lanes of 10% polyacrylamide gels, separated by $NaDodSO_4$/PAGE, and transferred to nitrocellulose paper. The nitrocellulose sheets were incubated with appropriate antibodies and then with $^{125}I$-labeled protein A (New England Nuclear, 10 $\mu$Ci/$\mu$g, 150,000 cpm/ml; 1 Ci = 37 GBq). Autoradiography was used to locate the relevant bands, which were then cut out for counting in a gamma spectrometer. Standard curves were constructed based on the radioactivity bound to known amounts of purified $G_i$ and $G_o$ (after subtraction of background). The proportion of each subunit in the purified brain G protein preparation used as standard was determined by densitometry of Coomassie blue-stained gels of the separated, purified proteins. The values for the standard used to obtain the data in Tables 1 and 2 were 37.9% $\beta$, 24.4% $G_o$-$\alpha$, and 18.4% $G_i$-$\alpha$. Linear standard curves were obtained for $G_i$-$\alpha$ between 166 and 500 ng, with antiserum CW6; for $G_o$-$\alpha$ between 220 and 660 ng, with RV3; and for $\beta$ subunit between 340 and 1000 ng, with either antiserum. Unknown (membrane) samples were run that contained between 50 and 150 $\mu$g of protein, for assay with CW6, and between 33 and 100 $\mu$g, for assay with RV3.

## RESULTS

We immunized rabbits with purified bovine brain G proteins, which are a mixture of $G_i$ and $G_o$ (Fig. 1, lane CB). Antisera were tested for reactivity with the purified proteins on immunoblots. Peak reactivity was observed 4 weeks after the booster immunization. All six rabbits (Fig. 1, lanes 1–6) showed reactivity against the $\beta$ subunit, but only one showed strong $\alpha$-subunit reactivity. Antiserum from this animal (RV3) was selected for further characterization.

Comparison of immunoblots probed with RV3 and Coomassie blue-stained gels of $G_i$ plus $G_o$ suggested that RV3 $\alpha$-subunit reactivity was directed against the 39-kDa $G_o$-$\alpha$ rather than the 40-kDa $G_i$-$\alpha$. To prove this, we probed immunoblots of $G_i$ plus $G_o$ with either RV3, or CW6, or mixtures of both antisera. CW6 was previously shown (6) to react with $G_i$-$\alpha$ and not $G_o$-$\alpha$. (Both antisera react with the $\beta$ subunit, with RV3 reacting more strongly than CW6.) When either antiserum was used alone, a single $\alpha$-subunit band was seen (Fig. 2, lanes 1 and 4). Combining the two antisera revealed an $\alpha$ doublet on immunoblot corresponding to the $G_i/G_o$ $\alpha$ doublet on Coomassie blue-stained gels (see Fig. 1). The density of staining of the lower, $G_o$-$\alpha$ band increased with increasing concentration of RV3 on immunoblots; $G_i$-$\alpha$ staining, in contrast, decreased as the concentration of RV3 was increased and, as expected (6), increased as the concentration of CW6 was increased (Fig. 2). Thus, the $\alpha$-subunit reactivity of RV3 is directed against $G_o$-$\alpha$ and not $G_i$-$\alpha$.

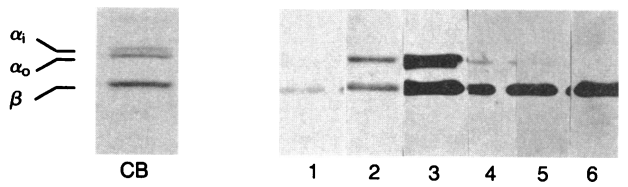


FIG. 1. Immunoblots with sera from rabbits immunized with purified $G_i$ plus $G_o$. Purified brain G proteins were subjected to $NaDodSO_4$/PAGE and either stained with Coomassie blue (lane CB, 2 $\mu$g of protein) or immunoblotted (1 $\mu$g per lane) with a 1:100 dilution of antisera RV1–6 (lanes 1–6, respectively). The protein stain shows the $\alpha$ subunits of $G_i$ and $G_o$ ($\alpha_i$ and $\alpha_o$, 40 and 39 kDa, respectively) and the common 35-kDa $\beta$ subunit.

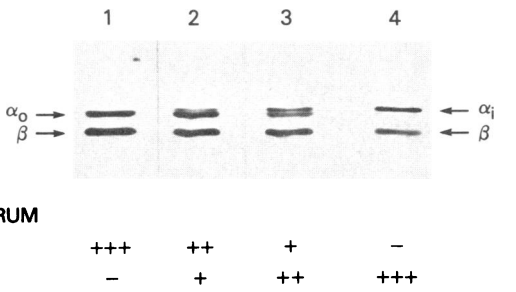


FIG. 2. Immunoblot of purified brain G proteins with antisera RV3 and CW6. $G_i$ plus $G_o$ (2 $\mu$g per lane) were separated by $NaDodSO_4$/PAGE and immunoblotted with a 1:100 dilution of RV3 (lane 1), a 1:200 dilution of RV3 plus a 1:300 dilution of CW6 (lane 2), a 1:300 dilution of RV3 plus a 1:200 dilution of CW6 (lane 3), or a 1:100 dilution of CW6 (lane 4).

We next tested the reactivity of RV3 against other purified G-protein subunits. The antiserum crossreacted with the $\beta$ subunits of purified $G_s$, $G_i$, and TD (Fig. 3).§ The antiserum showed no crossreactivity, however, against the $\alpha$ subunits of bovine TD or of $G_s$ and $G_i$ purified from human erythrocytes (Fig. 3). Lack of reactivity with human erythrocyte $G_i$-$\alpha$ provides further evidence that the $\alpha$-subunit reactivity of RV3 is directed solely against $G_o$.

We next evaluated the reactivity of RV3 on immunoblots of a relatively crude, plasma membrane-containing preparation from bovine brain. RV3 reacted strongly with two bands corresponding in mobility to purified $G_o$-$\alpha$ and $\beta$ but also reacted with several other proteins (Fig. 4, lanes 1 and 2). To assess the specificity of RV3 reactivity, we affinity-purified the antiserum on Sepharose to which purified brain G proteins had been covalently bound, and we tested the affinity-purified antibodies on immunoblots. The results (Fig. 4, lanes 3 and 4) show that, of the bands in brain membranes stained with RV3, only the two comigrating with $G_o$-$\alpha$ and $\beta$ are stained with affinity-purified antibodies. Note that the disappearance of other bands reflects specificity rather than sensitivity of affinity-purified antibodies, since $G_o$-$\alpha$ and $\beta$ are stained as intensely as with crude antiserum. Thus,

§The purified $\beta$ subunit from certain tissues may appear as a doublet on $NaDodSO_4$/PAGE (4). Purified G-protein $\beta$-subunit preparations from brain generally show a predominant lower-mobility form with only minor amounts of a faster migrating component (4, 6). RV3 clearly reacts with the predominant form of $\beta$, but we cannot, at present, assess its reactivity with the minor, faster-migrating component.

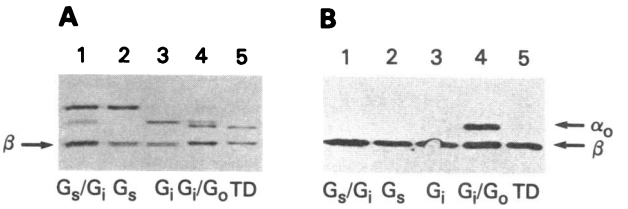


FIG. 3. Crossreactivity of antiserum RV3 with purified G-protein subunits. Proteins were subjected to $NaDodSO_4$/PAGE and stained with Coomassie blue (*A*) or immunoblotted with a 1:100 dilution of RV3 (*B*). Lanes 1: 2 $\mu$g of partially purified G proteins from human erythrocyte, including $G_s$ and $G_i$ $\alpha$ subunits. Lanes 2: 1 $\mu$g of purified $G_s$ from human erythrocyte. Lanes 3: 1 $\mu$g of purified $G_i$ from human erythrocyte. Lanes 4: 2 $\mu$g of purified G protein, including $G_i$ and $G_o$ $\alpha$ subunits, from bovine brain. Lanes 5: 1 $\mu$g of purified bovine transducin.

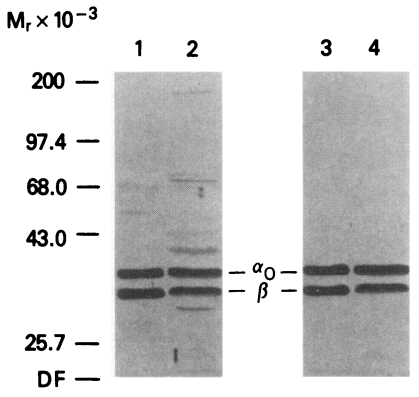


FIG. 4. Immunoblots of brain G proteins with crude and affinity-purified antiserum RV3. Purified $G_i$ plus $G_o$ (1 $\mu$g, lanes 1 and 3) and bovine brain membranes (100 $\mu$g, lanes 2 and 4) were subjected to $NaDodSO_4$/PAGE and immunoblotted with a 1:100 dilution of crude RV3 (lanes 1 and 2) or with affinity-purified RV3 (lanes 3 and 4). The positions of proteins (Bethesda Research Laboratories, prestained high molecular weight) run as standards are indicated at left. $\alpha_o$, $G_o$-$\alpha$; DF, dye front.

antiserum RV3 is capable of specifically recognizing $G_o$-$\alpha$ and $\beta$ on immunoblots of brain membrane proteins.

To evaluate the distribution of $G_o$ in different vertebrates, we used RV3 to probe immunoblots of electrophoretically separated proteins of membrane preparations from frog, chicken, human, rat, and bovine brain. In each species, RV3 reacted strongly, and to a similar degree, with proteins comigrating with purified $G_o$-$\alpha$ and $\beta$ (Fig. 5). The crude antiserum also reacted to a lesser extent with other proteins (the exact pattern was different for each species). As shown above (Fig. 4), these proteins are not stained with affinity-purified antibodies.

To assess the relative amounts of $G_i$ and $G_o$ in bovine cerebral cortical membranes, we performed quantitative immunoblotting with antisera CW6 and RV3. As shown above, CW6 and RV3 specifically recognize $G_i$-$\alpha$ and $G_o$-$\alpha$, respectively. Since both antisera react with the $\beta$ subunit, the latter could be quantitated with both antisera. Table 1 summarizes the results of such measurements. The values for the $\beta$ subunit obtained with the two antisera are in reasonable agreement. The data show that the ratio of $G_o$-$\alpha$ to $G_i$-$\alpha$ in membranes prepared from cerebral cortex is approximately 5:1.

We also measured the concentration of $G_o$ in membranes from different regions of bovine brain by quantitative immunoblotting with RV3. The data for frontal and occipital cortex obtained in this experiment (Table 2) are in reasonable agreement with the value for whole cerebral cortex (Table 1), although frontal cortex appears to contain more $G_o$ than occipital cortex. Because other cortical regions were not studied, we cannot comment on regional differences in $G_o$ content

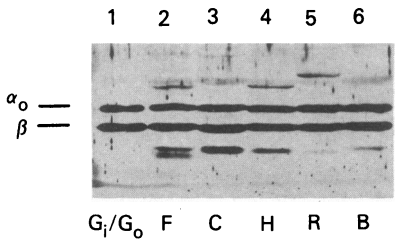


FIG. 5. Immunoblot of brain membranes from several vertebrate species, with a 1:100 dilution of antiserum RV3. Lane 1: purified bovine $G_i$ plus $G_o$ (1 $\mu$g). Lanes 2–6: membranes (150 $\mu$g per lane) from whole frog brain (F), from chicken forebrain (C), and from human (H), rat (R), and bovine (B) cerebral cortex.

Table 1. Quantitation of $G_o$-$\alpha$, $G_i$-$\alpha$, and $\beta$ subunits in immunoblots of bovine cerebral cortical membrane proteins

| Antiserum | Subunit | % total membrane protein |
|---|---|---|
| RV3 | $G_o$-$\alpha$ | 0.74 ± 0.09 (6) |
| | $\beta$ | 0.92 ± 0.07 (6) |
| CW6 | $G_i$-$\alpha$ | 0.16 ± 0.03 (3) |
| | $\beta$ | 1.01 ± 0.24 (6) |

Quantitation was as described in *Materials and Methods*, using antiserum RV3 to measure $G_o$-$\alpha$ and CW6 to measure $G_i$-$\alpha$; common $\beta$ subunits were independently measured with both antisera. Results are shown as mean ± SD, with the number of determinations shown in parentheses.

within cerebral cortex. Two main points emerge from the data in Table 2. (*i*) Brainstem and spinal cord appear to contain substantially lower amounts of $G_o$ than do forebrain structures. (*ii*) The ratio of $G_o$-$\alpha$ to $\beta$ is close to (and for frontal cortex greater than) 1.0 in several regions of bovine brain.

## DISCUSSION

Of six rabbits immunized with purified bovine brain G proteins ($G_i$ plus $G_o$), all six developed antibodies to $\beta$ subunit, and three developed antibodies to $\alpha$ subunit. Antiserum RV3, which showed the strongest $\alpha$-subunit reactivity, was characterized further. We found that the $\alpha$-subunit reactivity of RV3 is directed against bovine brain $G_o$ rather than $G_i$. We previously showed (6, 7) that antiserum CW6 recognizes the 40-kDa $G_i$-$\alpha$ in bovine brain and in human erythrocytes; this provided immunochemical evidence for the similarity, if not identity, of the 40-kDa protein in both tissues. RV3, in contrast, fails to crossreact on immunoblots with the $\alpha$ subunits of $G_s$ and $G_i$ from human erythrocytes or with bovine TD-$\alpha$. This provides immunochemical evidence that $G_o$-$\alpha$ is a distinct protein. Comparison of $G_i$-$\alpha$ and $G_o$-$\alpha$ by peptide mapping leads to a similar conclusion (4, 9).

As shown earlier for TD antisera (6, 7), polyclonal antibodies can distinguish between G-protein $\alpha$ subunits, even though there are regions of amino acid sequence homology [e.g., between TD-$\alpha$ and $G_o$-$\alpha$ (10)]. The specific reactivity of RV3 with $G_o$-$\alpha$ provides another example. RV3 does crossreact with the $\beta$ subunits of all G proteins tested, including TD (see footnote §). This provides further evidence for the close similarity of G-protein $\beta$ subunits (7, 11).

Immunoblots performed with crude and affinity-purified RV3 showed that this antiserum specifically recognizes $G_o$-$\alpha$ and $\beta$ in brain membranes. It is unlikely that any of the other brain-membrane bands that reacted with crude antiserum represent precursors or degradation products of $G_o$-$\alpha$ or $\beta$, since they do not react with affinity-purified RV3. Although $G_o$-$\alpha$ can be radiolabeled by pertussis toxin-catalyzed ADP-

Table 2. Immunoblot quantitation with antiserum RV3 of $G_o$-$\alpha$ and $\beta$ subunits in membranes from various regions of bovine brain

| Region | % total membrane protein | |
|---|---|---|
| | $G_o$-$\alpha$ | $\beta$ |
| Frontal cortex | 1.09 ± 0.18 (6) | 1.08 ± 0.14 (6) |
| Occipital cortex | 0.63 ± 0.16 (5) | 0.78 ± 0.19 (5) |
| Hippocampus | 0.27 ± 0.11 (4) | 0.53 ± 0.13 (5) |
| Striatum | 0.41 ± 0.10 (3) | 0.57 ± 0.15 (3) |
| Thalamus | 0.97 ± 0.13 (6) | 1.11 ± 0.23 (6) |
| Hypothalamus | 0.65 ± 0.16 (6) | 0.90 ± 0.09 (6) |
| Mesencephalon | 0.35 ± 0.09 (5) | 0.54 ± 0.06 (6) |
| Cerebellum | 0.67 ± 0.13 (6) | 0.71 ± 0.08 (6) |
| Pons | 0.22 ± 0.05 (6) | 0.27 ± 0.05 (6) |
| Medulla | 0.22 ± 0.05 (5) | 0.28 ± 0.08 (5) |
| Cervical spinal cord | 0.31 ± 0.07 (6) | 0.48 ± 0.10 (6) |

Results are expressed as in Table 1.

ribosylation (3–5), this reaction is not as specific as immunoblotting with RV3, since $G_i$-$\alpha$ is also a pertussis toxin substrate. Using RV3, we were able to show that proteins with the same mobility on $NaDodSO_4$/PAGE as purified $G_o$-$\alpha$ and $\beta$ are found in approximately equal amounts in brain membranes from frog, chicken, and four orders of mammals [rat, human, and cow (Fig. 5) and guinea pig (data not shown)]. $G_o$-$\alpha$ and $\beta$ are thus highly conserved proteins. Since a common $\beta$ subunit apparently interacts with different G-protein $\alpha$ subunits, structural constraints may limit evolutionary divergence. The function of $G_o$-$\alpha$ is unknown, but the observation that the protein is highly conserved suggests that $G_o$ serves an important function. If $G_o$ acts as a signal transducer, its $\alpha$ subunit presumably interacts with receptors and effectors, in addition to guanine nucleotides. The structural requirements for these interactions again may place constraints on evolutionary divergence in structure.

The initial studies (3–5) on purification of $G_i$ and $G_o$ from bovine brain suggested that these proteins are abundant (about 1–2% of total plasma membrane protein), but they provided no data on the relative amounts of $G_i$ and $G_o$ or on the distribution of $G_o$ in different regions of brain. We addressed these questions with the quantitative immunoblotting method. The method is based on the assumption that proteins behave identically during immunoblotting whether purified or within heterogeneous mixtures (membranes). Also, in comparing concentrations of a protein in different membrane preparations, it is assumed that differential loss of the protein during membrane preparation does not occur. Despite these limitations, the results we obtained with this method for total $G_i$ plus $G_o$ in bovine cerebral cortex (2% of total membrane protein) agree well with the data of Sternweis and Robishaw (4), which were obtained by an entirely different method. Our results for the $\beta$ subunit, obtained independently with different antisera, are also in reasonable agreement, emphasizing the reproducibility of the method.

Our data suggest that $G_o$ is the major G protein in bovine cerebral cortex (concentration about 5-fold higher than $G_i$). The functional significance of such high concentrations of $G_o$ is at present obscure. Although previous studies (3–5) on purification of $G_i$ and $G_o$ from bovine brain did not provide data for the ratio of $G_o$ to $G_i$, analysis of stained gels of purified protein fractions suggests ratios of 2–3:1, about half the value we obtain. This apparent discrepancy could result if free $G_o$-$\alpha$ were lost during purification—a distinct possibility, given the ease with which $G_o$-$\alpha$ dissociates from the $\beta$ subunit. Purifications based on assays such as pertussis toxin ADP-ribosylation, which require the holoprotein (3–5), would be susceptible to such losses.

It is generally assumed (1) that G-protein $\alpha$ subunits are present in membranes as oligomers associated with $\beta$ and $\gamma$ subunits. The *ras* gene products, which may be functionally analogous to G-protein $\alpha$ subunits (1, 10), are not known to associate with $\beta$ subunits and may, therefore, function as free $\alpha$ subunits. Our quantitative immunoblotting data raise the possibility that $G_o$ may be present in membranes and may function not only in a form associated with $\beta$ subunits but also as the free $\alpha$ subunit. The ratio of $G_o$-$\alpha$ to $\beta$ is close to 1 in several regions of bovine brain and is slightly greater than 1 in frontal cortex. Ratios of $G_o$-$\alpha$ to $\beta$ in excess of 1 have also been observed in rat brain (G.M., unpublished observations) and bovine pineal body (P.G., unpublished observations). The ability of free $G_o$-$\alpha$ to bind and exchange guanine nucleotides, its relatively low affinity for the $\beta$ subunit, and its ability to interact with and modulate the agonist affinity of muscarinic cholinergic receptors (12) are all compatible with the idea of free $G_o$-$\alpha$ as a functional entity. Nonetheless, given the limitations of the immunoblot method as a quantitative assay and the possibility (see footnote §) that certain forms of $\beta$ subunit are not detected with our antisera, further work will be needed to assess the possible existence of free $G_o$-$\alpha$ in brain.

We found up to 4-fold differences in the concentration of $G_o$ in different brain regions. In general, forebrain structures contained higher concentrations than did brainstem and spinal cord. No obvious correlation exists between this distribution of $G_o$ and adenylate cyclase activity (13). Since $G_o$ has been shown to interact with muscarinic cholinergic receptors in cerebral cortex (12), it is interesting that our data for $G_o$ distribution in brain roughly parallel the brain distribution of M1, as opposed to M2, muscarinic receptor subtypes (14).

Our data do not answer the key question: what is the specific function of $G_o$? If $G_o$, like $G_s$, $G_i$, and TD, is a membrane-bound signal transducer, elucidation of its specific function will require reconstitution of purified $G_o$ with specific receptors and effectors. G-protein distribution, however, may provide important clues to function. $G_s$ and $G_i$ appear to be universally distributed, reflecting the ubiquity of receptor regulation of adenylate cyclase as a transduction mechanism. TD, in contrast, shows a very limited distribution, reflecting its specific function in visual transduction. The distribution of $G_o$ in tissues other than brain has not been defined, but preliminary reports (4, 9, 15) suggest that the protein is found in some tissues other than brain; e.g., heart. The availability of specific antibodies should prove useful in defining the tissue and cellular distribution of $G_o$. Our preliminary observations (unpublished) indicate that $G_o$ immunoreactivity is present in heart and in cultured 3T3-L1 fibroblasts at concentrations lower than in brain but higher than in liver and kidney. The present data suggest that $G_o$ function is closely associated with events in neuron-rich forebrain regions.

**Note Added in Proof.** After the original submission of this paper, two reports (16, 17) appeared concerning antibodies to $G_o$.

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1. Gilman, A. G. (1984) *Cell* **36**, 577–579.
2. Spiegel, A. M., Gierschik, P., Levine, M. A. & Downs, R. W., Jr. (1985) *N. Engl. J. Med.* **312**, 26–33.
3. Milligan, G. & Klee, W. A. (1985) *J. Biol. Chem.* **260**, 2057–2063.
4. Sternweis, P. C. & Robishaw, J. D. (1984) *J. Biol. Chem.* **259**, 13806–13813.
5. Neer, E. J., Lok, J. M. & Wolf, L. G. (1984) *J. Biol. Chem.* **259**, 14222–14229.
6. Pines, M., Gierschik, P., Milligan, G., Klee, W. & Spiegel, A. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 4095–4099.
7. Gierschik, P., Codina, J., Simons, C., Birnbaumer, L. & Spiegel, A. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 727–731.
8. Codina, J., Hildebrandt, J. D., Sekura, R. D., Birnbaumer, M., Bryan, J., Manclark, C. R., Iyengar, R. & Birnbaumer, L. (1984) *J. Biol. Chem.* **259**, 5871–5886.
9. Malbon, C. C., Rapiejko, P. J. & Garcia-Sainz, J. A. (1984) *FEBS Lett.* **176**, 301–306.
10. Hurley, J. B., Simon, M. I., Teplow, D. B., Robishaw, J. D. & Gilman, A. G. (1984) *Science* **226**, 860–862.
11. Pines, M., Gierschik, P. & Spiegel, A. (1985) *FEBS Lett.* **182**, 355–359.
12. Florio, V. A. & Sternweis, P. C. (1985) *J. Biol. Chem.* **260**, 3477–3483.

13. Daly, J. W., Padgett, W. & Seamon, K. B. (1982) *J. Neurochem.* **38**, 532–544.
14. Flynn, D. D. & Potter, L. T. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 580–583.
15. Malbon, C. C., Mangano, T. J. & Watkins, D. C. (1985) *Biochem. Biophys. Res. Commun.* **128**, 809–815.
16. Huff, R. M., Axton, J. M. & Neer, E. J. (1985) *J. Biol. Chem.* **260**, 10864–10871.
17. Mumby, S. M., Kahn, R. A., Manning, D. R. & Gilman, A. G. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 265–269.