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Novel Localization of a G Protein, $G_{z\text{-}\alpha}$, in Neurons of Brain and Retina

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Recently, a cDNA coding for a novel G protein α-subunit, $G_{z\text{-}\alpha}$, was isolated from a human retinal cDNA library and shown by Northern blot analysis to be expressed at high levels in neural tissues. We have prepared affinity-purified antibodies specifically directed against synthetic $G_{z\text{-}\alpha}$ peptides and employed immunohistochemical methods to map the localization of $G_{z\text{-}\alpha}$ in human, bovine, and murine retina and brain. By light microscopy, $G_{z\text{-}\alpha}$ was localized to the cytoplasm of neurons, with predominant reactivity in ganglion cells of the retina, Purkinje cells of the cerebellum, and most neurons of the hippocampus and cerebral cortex. Reactivity was confined to perikaryon, dendrites, and a very short segment of proximal axons, except for the retinal ganglion cells, in which the axons in the nerve fiber layer showed intense $G_{z\text{-}\alpha}$ immunoreactivity proximal to the lamina cribrosa. Pre-embedding immunoelectron microscopy demonstrated the presence of focal $G_{z\text{-}\alpha}$ immunoreactivity on the nuclear membranes, endoplasmic reticulum, and plasma membranes of Purkinje cell perikarya and in association with microtubules in their proximal dendrites. Subcellular fractionation studies confirmed the association of $G_{z\text{-}\alpha}$ with plasma and intracellular membranes. The localization of $G_{z\text{-}\alpha}$ and its unique amino acid sequence suggest that it may have a specialized function in neural tissues.

Guanine nucleotide-binding regulatory proteins (G proteins) are generally located on the cytoplasmic face of cell membranes, where they physically couple ligand-bound receptors and initiate a cascade of biochemical processes that produce an intracellular signal and change the behavior of the cell. Effectors may include ion channels, adenylyl cyclase, or phosphoinositide phospholipase C (Lochrie and Simon, 1988). Another class of GTP-binding proteins with lower molecular weight and regions of conserved amino acid sequences has been found. Two members of this family, *ypt1* and *sec4,* have been shown to be involved in the secretion pathway in yeast, and homologs of these genes may be involved in exocytosis in mammalian cells (Segev et al., 1988). While a great deal of diversity of G_{α} subunit proteins exists, several α-subunits are found to be either tissue- or cell-type specific. $G_{o\text{-}\alpha}$ shows tissue specificity and is localized predominantly in neural tissues such as the brain and the diffuse neuroendocrine system (Perry, 1982; Asano et al., 1988; Chang et al., 1988; Cortés et al., 1988; Gabrion et al., 1989; Péraldi et al., 1989). Cell-type specificity is found for the 2 forms of transducin; one is specific for rod photoreceptor cells ($T_{r\text{-}\alpha}$), and the other ($T_{c\text{-}\alpha}$) is specific for cone photoreceptors (Lerea et al., 1986; Rodrigues et al., 1987). G_{olf}, a homolog of the $G_{s\text{-}\alpha}$ subunit, is found only in olfactory neurons (Jones and Reed, 1989).

Recently, a cDNA for the G protein, $G_{z\text{-}\alpha}$, was isolated from a human retinal cDNA library by homology to common sequences with other G proteins (Fong et al., 1988). A corresponding human genomic clone and rat brain cDNA were also reported (Maksuoka et al., 1988). Northern blot analysis using bovine poly(A)$^+$ RNA revealed a major transcript of 3.0 kb, which was expressed most highly in retina, brain, and adrenal. Sequence analysis of the complete cDNA showed the absence of a typical pertussis toxin modification site (Fong et al., 1988; Maksuoka et al., 1988), suggesting that it may mediate transduction in signaling systems, such as phospholipase C, that are not blocked by the toxin. To characterize this nervous system-enriched G protein further, specific antibodies to $G_{z\text{-}\alpha}$ synthetic peptides were made and used for immunocytochemically localizing $G_{z\text{-}\alpha}$ protein at the cellular and subcellular level in human, bovine, and murine tissues.

Materials and Methods

Production of specific antisera. Gz3 (CRQSSEEKEAARRSRR), Gz111 (CTGPAESKGEITPELL), and Gαcommon (CGAGESGKSTIVKQMK) peptides were synthesized with an Applied Biosystems automated peptide synthesizer (by Suzanna Horvath, Caltech) and conjugated to keyhole limpet hemocyanin. An emulsion of 500 μg of conjugate in equal volume of complete Freund's adjuvant was injected subcutaneously into female New Zealand white rabbits. After 3 weeks, the rabbits were boosted with 250 μg of conjugate in incomplete Freund's adjuvant, and after boosting again at 5 weeks, they were bled every 10–14 d. Antibodies were affinity-purified by immunoadsorption to their cognate peptides conjugated to Affi-Gel 10 (Bio-Rad). Antisera P-961 (Casey et al., 1990) was prepared against an N-terminal peptide identical to Gz3 and has the same specificity on immunoblot.

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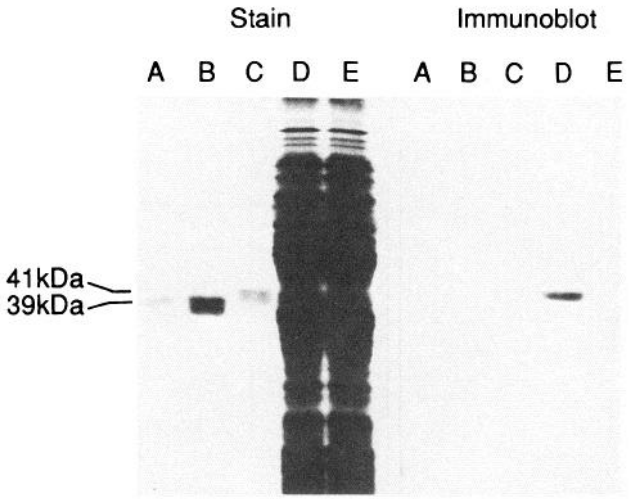


Figure 1. Specificity of B-$G_{z\text{-}3}$ antisera for $G_{z\text{-}\alpha}$ as tested by immunoblotting. Purified G proteins and *E. coli* extracts were electrophoresed on SDS-polyacrylamide gels and stained with Coomassie brilliant blue (*left*) or transferred to nitrocellulose for immunoblot reactions (*right*). The blot was processed with affinity-purified B-$G_{z\text{-}\alpha}$ antibody and with anti-rabbit IgG (Fc) conjugated to alkaline phosphatase using nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate substrates. *Lane A,* partially purified transducin from bovine rod outer segments, 1 μg; *lane B,* bovine brain α-39, 1 μg; *lane C,* bovine brain α-41, 1 μg; *lane D,* extract of *E. coli* C600 cells transformed with pGD-G_z2, a bacterial G_z expression vector; *lane E,* extract of control *E. coli* C600 cells, 30 μg.

Determination of antibody specificity. Plasmids for expression of $G_{z\text{-}\alpha}$ in *E. coli* were constructed by subcloning the entire coding region of G_z cDNA into pGD108, a 2-cistron expression vector (kindly provided by Dr. Gloria MacFarland, Caltech). The bacterial G_z expression vector, pGD-Gz2, was used to transform a protease-deficient strain of C600 cells. An overnight culture of transformed cells was diluted 1:50 and grown to OD_{600} of 1.0. IPTG (1 mM) was added, and the cells were incubated for another hour. One milliliter of cells was centrifuged, resuspended, and extracted into 100 μl SDS-PAGE sample loading buffer. Purified bovine brain α40 ($G_{o\text{-}a}$) and α41 ($G_{i\text{-}\alpha}$) were prepared by established procedures (Casey et al., 1989). Purified G proteins and *E. coli* extracts were electrophoresed on SDS-polyacrylamide gels and transferred to nitrocellulose for immunoblot reactions. The blot was incubated with affinity-purified antibody for 2 hr at room temperature. The binding of the primary antibody was detected using anti-rabbit IgG (Fc) secondary antibody conjugated to alkaline phosphatase. The immunoblot assay was developed with nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate substrates.

Subcellular fractionation studies. Bovine adrenal medulla was dissected and homogenized in 0.3 M sucrose/0.1 M NaCl/15 mM Tris Cl, pH 7.5, and the 800 × *g* supernatant centrifuged at 26,000 × *g*. The pellet (membrane) fraction was layered onto 0.9 M (5 ml), 1.2 M (5 ml), 1.4 M (5 ml), 1.6 M (5 ml), 1.8 M (7.5 ml) sucrose (all + 15 mM Tris, pH 7.5) step gradients, and centrifuged at 100,000 × *g* for 2 hr, and 12 fractions were collected from the top of the gradient. The 26,000 × *g* supernatant was centrifuged again at 100,000 × *g* for 1 hr and the supernatant (S) and pellet (P) fractions were also analyzed. Samples (25 μg protein from each fraction) were subjected to SDS-PAGE, transferred to nitrocellulose, and immunoblotted with antisera P-961 (equivalent to antisera Gz3) (Casey et al., 1990). Acetylcholinesterase was assayed by the method of Ellman et al. (1961). Glucose-6-phosphatase was assayed as described by Baginski et al. (1974), with the following modifications: glucose phosphate was used at 10 mM, the reaction mixture was incubated for 1 hr, and the molybdate reaction was performed at 0°C for 20 min.

Immunohistochemistry on tissue sections. Human tissues were obtained at autopsy from patients (ages 30–76) with no known neurological disease. Postmortem intervals ranged from 6–12 hr. Human sympathetic ganglion tissue was a gift from Dr. Pat Reynolds (Childrens Hospital, Los Angeles). Bovine tissues were obtained within 1 hr of slaughter. In each case, tissue blocks 1 × 1 × 0.4 cm were dissected from adrenal, cervical spinal cord, and a variety of brain regions (prefrontal cortex, hippocampus, basal ganglia, cerebellum) and fixed for 2 hr at 4°C in 4% buffered paraformaldehyde. For eye tissues, the retinas with attached choroid were dissected from intact globes, fixed as above, and cut into 3-mm-wide strips. The fixed tissues were infiltrated with 1 mM sucrose in PBS overnight at 4°C. The tissue blocks were then frozen, sectioned at 6 μm on a cryostat, and thaw-mounted onto chrom-alum-coated glass slides. Murine brain and eyes were dissected whole from anesthetized C57/BL6J mice, frozen in liquid nitrogen-cooled isopentane, and sectioned on a cryostat. Sections were incubated with the primary affinity-purified antibody diluted 1:200 in PBS at 4°C overnight after a 5 min preincubation in 0.1% Triton X-100 in PBS. Detection of immunoreactivity was performed using the Streptavidin–biotin immunoperoxidase method (Zymed, South San Francisco, CA) with aminoethylcarbazole (0.04%) reacted with H_2O_2 (0.015%) as the chromagen. Controls included use of preimmune serum instead of primary antibody, omission of the primary antibody, and use of a primary antibody previously absorbed with the corresponding peptide in 10× molar excess for 1 hr.

Ultrastructural immunocytochemistry. C57/BL6J mice were anesthetized and then perfused through the heart with 4% paraformaldehyde for 10 min. The cerebellum was dissected and immersion-fixed for 1 hr. Fifty-micron-thick sections were cut on a Vibratome (Technical Products International, Inc., St. Louis, MO) and washed in PBS. Sections were reacted as above except that the chromagen was diaminobenzidine (0.05%). Sections were washed with PBS and reacted with 2% osmium tetroxide for 30 min. After 2 washes in PBS, sections were postfixed for 15 min in buffered 2% glutaraldehyde. The sections were dehydrated and flat-embedded in Eponate (Ted Pella, Inc., Redding, CA). After an initial semi-thin section was cut, ultrathin plastic sections were obtained, and viewed on a Zeiss EM-10 electron microscope.

Results

Characterization of antibody against $G_{z\text{-}\alpha}$

The antisera raised against the synthetic Gz3 and Gz111 peptides, designated Gz3/B and Gz111/A, each recognized their cognate peptide on immunoblot (results not shown). Gz3/B was also assayed by immunoblotting for its ability to react with recombinant $G_{z\text{-}\alpha}$ protein that was produced in *E. coli.* The affinity-purified antibody, Gz3/B, specifically recognized the 40 kDa recombinant $G_{z\text{-}\alpha}$ product but not the α-subunit of rod transducin, nor the α39- and α41-subunits of bovine brain G proteins (Fig. 1). The antiserum against Gαcommon recognizes several G protein α-subunits, including $G_{s\text{-}\alpha}$, $G_{i\text{-}\alpha}$, $G_{o\text{-}\alpha}$, and $G_{i\text{-}\alpha}$, but not $G_{z\text{-}\alpha}$ (Mumby et al., 1986; Casey et al., 1990). Characterization of antisera P-961 has been reported (Casey et al., 1990).

Subcellular fractionation

All of the immunoreactive $G_{z\text{-}\alpha}$ was detected in the 26,000 × *g* and 100,000 × *g* pellets with absence of reactivity in the 100,000 × *g* supernatant (Fig. 2), indicating that $G_{z\text{-}\alpha}$ is found in association with membrane and not with cytosol. Fractionation of the 26,000 × *g* pellet showed that the predominant reactivity is in the fractions that also show plasma membrane-associated acetylcholinesterase activity. The lower levels of reactivity observed in the other fractions cannot exclude the possibility that a smaller fraction of the immunoreactive $G_{z\text{-}\alpha}$ is associated with intracellular membranes, typified by the distribution profile of the endoplasmic reticulum associated marker glucose-6-phosphatase (Fig. 2).

Immunocytochemical localization of $G_{z\text{-}\alpha}$ in brain and retina

A wide distribution of $G_{z\text{-}\alpha}$ reactivity was observed in murine, bovine, and human nervous systems. The pattern of reactivity

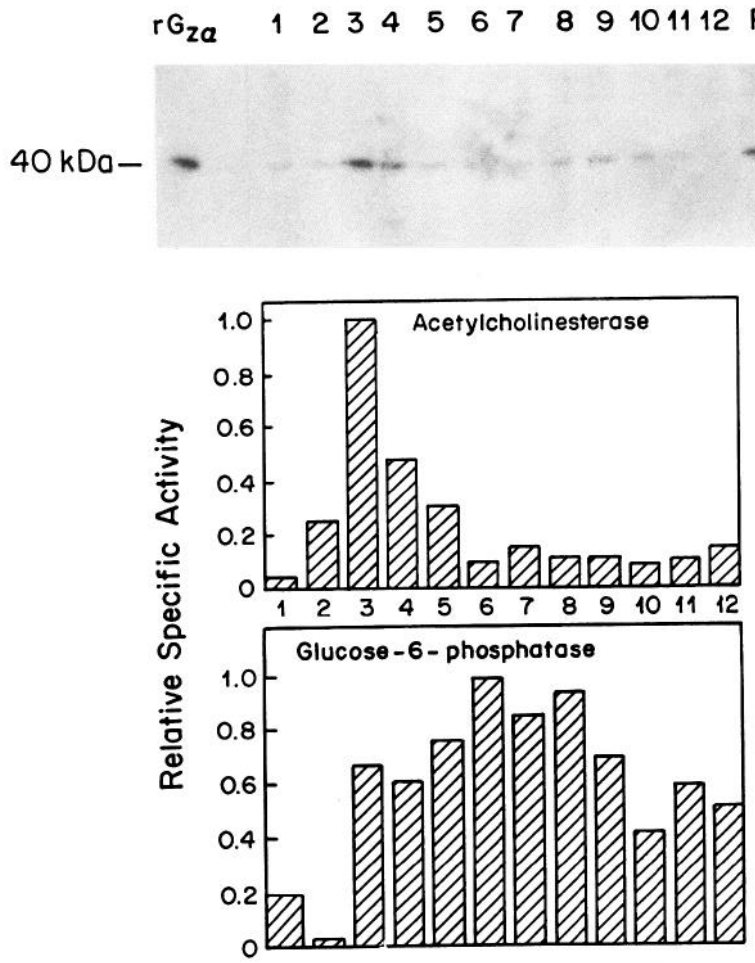


Figure 2. Sucrose gradient fractionation of membranes derived from bovine adrenal medulla. The 26,000 × *g* membrane fraction from the homogenate of adrenal medulla tissue was layered on a sucrose step gradient and centrifuged, and 12 fractions were collected, as described under Materials and Methods. The 26,000 × *g* supernatant fraction was subjected to an additional centrifugation at 100,000 × *g* for 1 hr, and the supernatant (*S*) and pellet (*P*) fractions from this step were also analyzed. *Upper panel,* Samples (25 μg of protein from each fraction) were subjected to SDS-PAGE and transferred to nitocellulose; immunoblots were processed using affinity-purified P-961 as the primary antibody. The recombinant G_z (rG_z) lane shows the signal obtained using 25 ng of purified recombinant G_z. *Center & lower panels,* Marker enzyme activity in the various fractions. Acetylcholinesterase (plasma membrane) and glucose-6-phosphatase (*ER*) were assayed as described in Materials and Methods. Activities in the respective sucrose gradient fractions are expressed relative to that determined in the peak fraction, being 26.4 and 1.05 μmol/hr/mg protein for acetylcholinesterase and glucose-6-phosphatase, respectively.

was similar in all 3 species and in all regions was confined to neurons. Gz3/B and Gz111/A reacted in an identical pattern in cerebellum and retina. Gz3/B reactivity, however, was more intense and was used for the remainder of the study. By light microscopy, the staining was always most intense in the cytoplasm of the perikaryon and proximal dendrites (Fig. 3*f*), except the ganglion cells of the retina, in which staining was equally intense in the proximal axons (Fig. 4*a, b*). The adjacent neuropil usually showed weak reactivity (Fig. 3*a–d*). The intracellular staining obtained with Gz3/B was in marked contrast to the intense membrane-associated staining found using the antiserum made against Gαcommon (results not shown). All control preparations yielded negative immunostaining (Fig. 3*e*), suggesting a high antibody specificity for the $G_{z\text{-}\alpha}$ antigen in tissue sections. Omission of detergent in the procedure resulted in a similar but much weaker staining pattern. Parallel experiments using 0.01% saponin, 0.1% saponin, and 0.1% Triton X-100 detergent washes prior to the primary antibody showed that increasing detergent extraction of the sections led to a marked increase in immunoreactivity (results not shown).

In neocortical regions, immunoreactivity was confined to neurons (Fig. 3*a*) and was most intense in the large neurons of layers 3 and 5; glia were consistently negative. The reactivity was granular in appearance and was distributed throughout the cytoplasm of perikaryon, dendrites, and proximal axons. Axonal staining did not extend to white matter tracts. In the hippocampus, neurons of the dentate gyrus, and pyramidal neurons of Ammon's horn were intensely positive (Fig. 3*b*). Examination of deep cerebral nuclei showed strong reactivity of neurons in the basal ganglia and thalamus, with absence of staining in the adjacent white matter tracts (Fig. 3*c*).

Within the cerebellar cortex, reactivity was localized predominantly in Purkinje cells (Fig. 3*d*), with reaction product present within the cytoplasm of the perikaryon and dendrites (Fig. 3*f*). Axonal staining was seen only rarely and was weak in intensity (Fig. 5*a*). Weak neuronal staining was present in the molecular and granular cell layers, but was negligible after further dilution of the antibody.

Within the bovine and human retina, reactivity was localized to the neurons of the ganglion cell layer (Fig. 4*a*). Staining was identified in the perikaryon and proximal dendrites of these cells but was most prominent in the ganglion cell axons as they coursed through the nerve fiber layer (Fig. 4*b*). As the axons penetrate the optic nerve head to enter the optic nerve, staining abruptly stopped (Fig. 4*c*). This region, termed the lamina cribrosa, coincides with the site at which myelination of the nerve fibers usually begins. It is of particular interest that most, but not all, neurons of the ganglion cell layer were reactive with Gz3/B (Fig. 4*b*). Occasional cells within the inner nuclear layer were also weakly positive; however, these could not be consistently identified as being of any one cell type. Photoreceptor cells were never reactive.

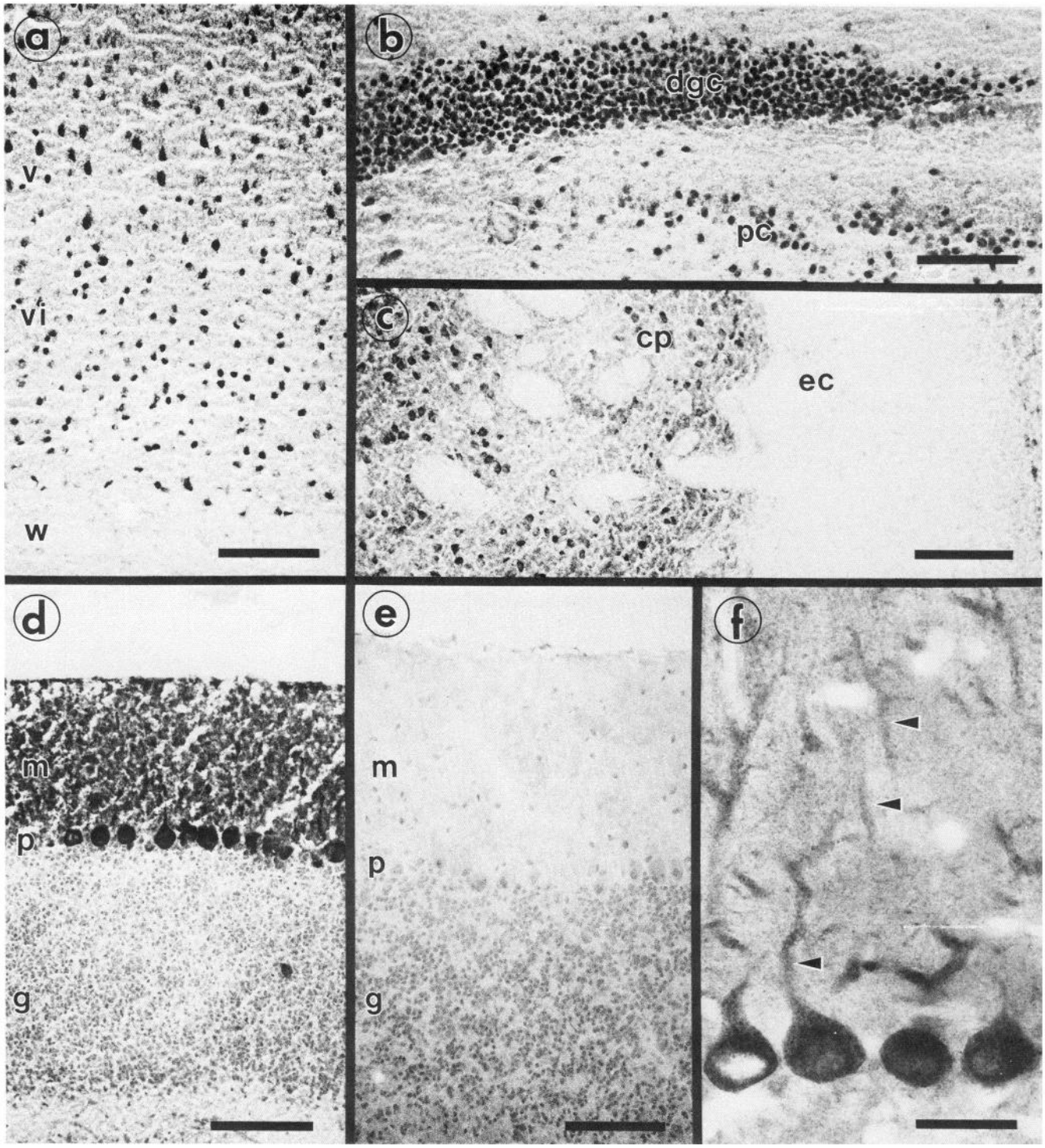
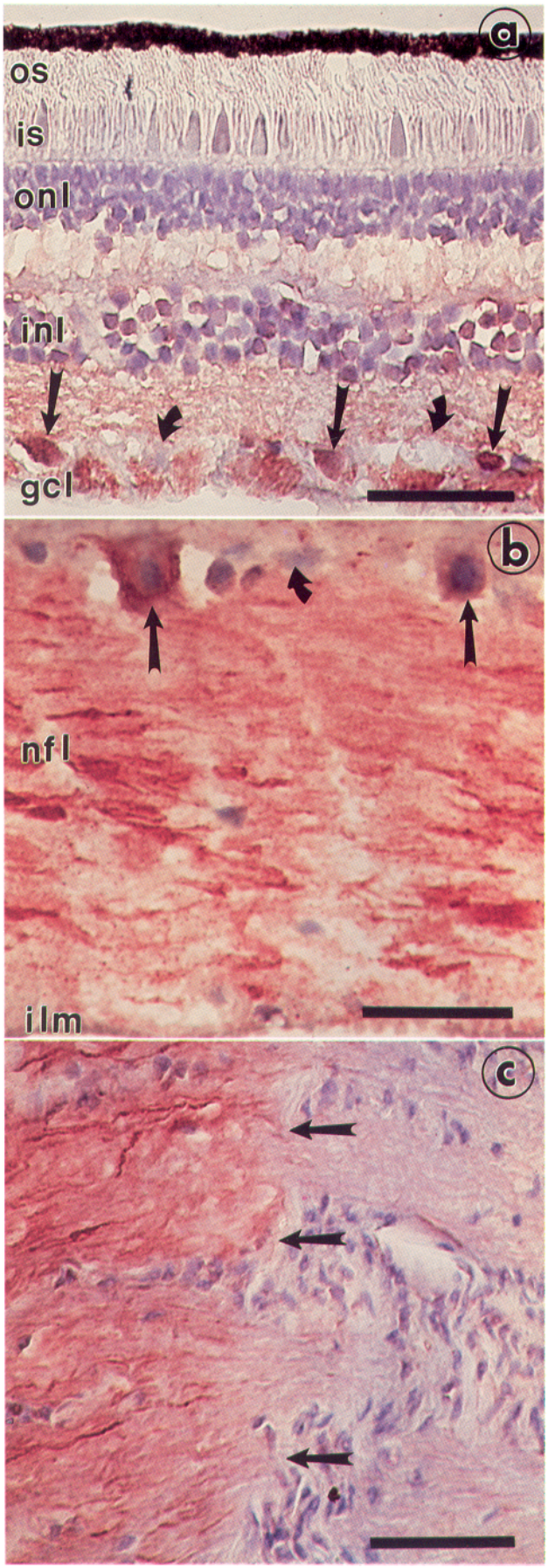


Figure 3. Immunoreactivity of murine brain tissues for Gz3/B. The brain of a C57/black mouse was snap frozen and sectioned at 6 μm. Sections were fixed with 4% paraformaldehyde and treated with 0.1% Triton X-100 for 5 min. The sections were stained by a Streptavidin immunoperoxidase method using Gz3/B as the primary antibody at a dilution of 1:200 and with a weak hematoxylin counterstain. In *a*, the section of layers V and VI of prefrontal cortex shows strong immunoreactivity within neurons, particularly the large neurons of layer V. Reactivity extends into the adjacent neuropil in layer V. The underlying white matter (*w*) is nonreactive. Scale bar, 100 μ. In *b*, the section of hippocampus shows strong immunoreactivity of the neurons of the dentate granule cell layer (*dgc*), and the adjacent pyramidal cells (*pc*). Scale bar, 100 μ. In *c*, the section includes caudate/putamen (*cp*) and the adjacent external capsule (*ec*). Note the intense reactivity of neurons of the striatum as well as the weaker staining of the neuropil. Scale bar, 100 μ. In *d*, the section of cerebellar cortex shows intense reactivity in the Purkinje cells (*p*) and their dendrites in the molecular cell layer (*m*). The granular cell layer (*g*) is only weakly positive. Scale bar, 100 μ. In *e*, an adjacent section of cerebellar cortex is stained after preabsorption of the primary antibody with Gz3 peptide. There is no immunoreactive staining; only the weak hematoxylin counterstain is seen. Scale bar, 100 μ. In *f*, a mouse was perfused through the heart with 4% paraformaldehyde, and the cerebellum was sectioned at 50 μ on a Vibratome. The sections were stained in a manner identical to the previous sections except that diaminobenzidine was used as the substrate. Note the intense cytoplasmic reactivity of Purkinje cells and their dendrites (arrows). Scale bar, 25 μ.



Sections of spinal cord showed strong reactivity in most of the neurons of the anterior horn and their proximal nerve roots. Within the adrenal gland, $G_{z\text{-}\alpha}$ was localized to neurons of the medulla; the cortex was consistently negative. Sympathetic ganglia sections showed strong immunoreactivity of ganglion cells within the cytoplasm of their perikaryon and proximal processes. Sections of kidney, lung, and skeletal muscle were all negative (results not shown).

Ultrastructural localization of $G_{z\text{-}\alpha}$ in cerebellum

One micron sections of plastic-embedded immunostained Vibratome sections of murine cerebellum (Fig. 5*a*) showed intense Gz3/B reactivity in Purkinje cells, with lesser reactivity in the adjacent neurons of molecular and granular cell layers. The staining was present both in the cytoplasm and in association with the plasma membrane. The cytoplasmic staining exhibited a prominent granularity that was most easily seen in the proximal dendrite. Ultrastructural studies showed that the diaminobenzidine reaction product was, for the most part, confined to the cytoplasmic compartment; the nucleus was consistently negative (Fig. 5*b*). At higher magnifications, the granular nature of the staining observed by light microscopy was explained by its focal localization to the external aspects of the nuclear membrane (Fig. 5*c*), endoplasmic reticulum (ER) of the Nissl body (Fig. 5*c*), and the internal aspect of the plasma membrane (Fig. 5*d*). Nuclear staining was not in association with the nuclear pores. The plasma membrane-associated staining was not continuous but was often adjacent to ER reactivity and had no apparent association with synaptic contacts. Golgi, mitochondria, and cytosol were consistently negative. Within the proximal dendrite the reaction product was also seen in ER, but more distal reactivity was in association with small granules that decorated the microtubules (Fig. 5*e*).

Discussion

$G_{z\text{-}\alpha}$ is immunocytochemically localized to populations of neurons of human, bovine, and murine brain and retina. In cerebral cortex and hippocampus, $G_{z\text{-}\alpha}$ is found in most neurons; however, in several other sites, including retina and cerebellum, it is preferentially localized to subpopulations of large neurons. The tissue- and cell-specific pattern of reactivity resembles previously characterized G proteins, particularly $G_{o\text{-}\alpha}$ (Worley et al., 1986; Terashima et al., 1987a, b; Asano et al., 1988; Chang

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Figure 4. Immunocytochemical reactivity of Gz3/B with human retina. Immunoperoxidase stains of human frozen retina using Gz3/B at a dilution of 1:200, aminoethyl carbazole as the chromagen and hematoxylin as the counterstain. In *a,* the antibody reacts predominantly with the perikarya of neurons in the ganglion cell layer (*gcl, arrowheads*); however, not all cells are stained (*curved arrows*). Immediately below the neurons, cross sections of axons in the nerve fiber layer are stained. Note the weak reactivity of some neurons in the inner nuclear layer (*inl*). There was no reactivity of the outer nuclear layer (*onl*) or of the inner segment (*is*) or outer segment (*os*) of photoreceptor cells. The pigmented epithelium is at the top. Scale bar, 80 μ. In *b,* in a section of retina near the optic nerve head, the strong reactivity of ganglion cell axons in the nerve fiber layer (*nfl*) is seen. Perikaryal staining in neurons of the ganglion cell layer is also present (*arrowheads*), but not all cells are stained (*curved arrow*). Note the lack of reactivity of the inner limiting membrane (*ilm*). Scale bar, 50 μ. In *c,* at the level of the lamina cribrosa (*arrows*), the ganglion cell axonal reactivity markedly decreases in intensity as the axons pass into the optic nerve. Scale bar, 50 μ.

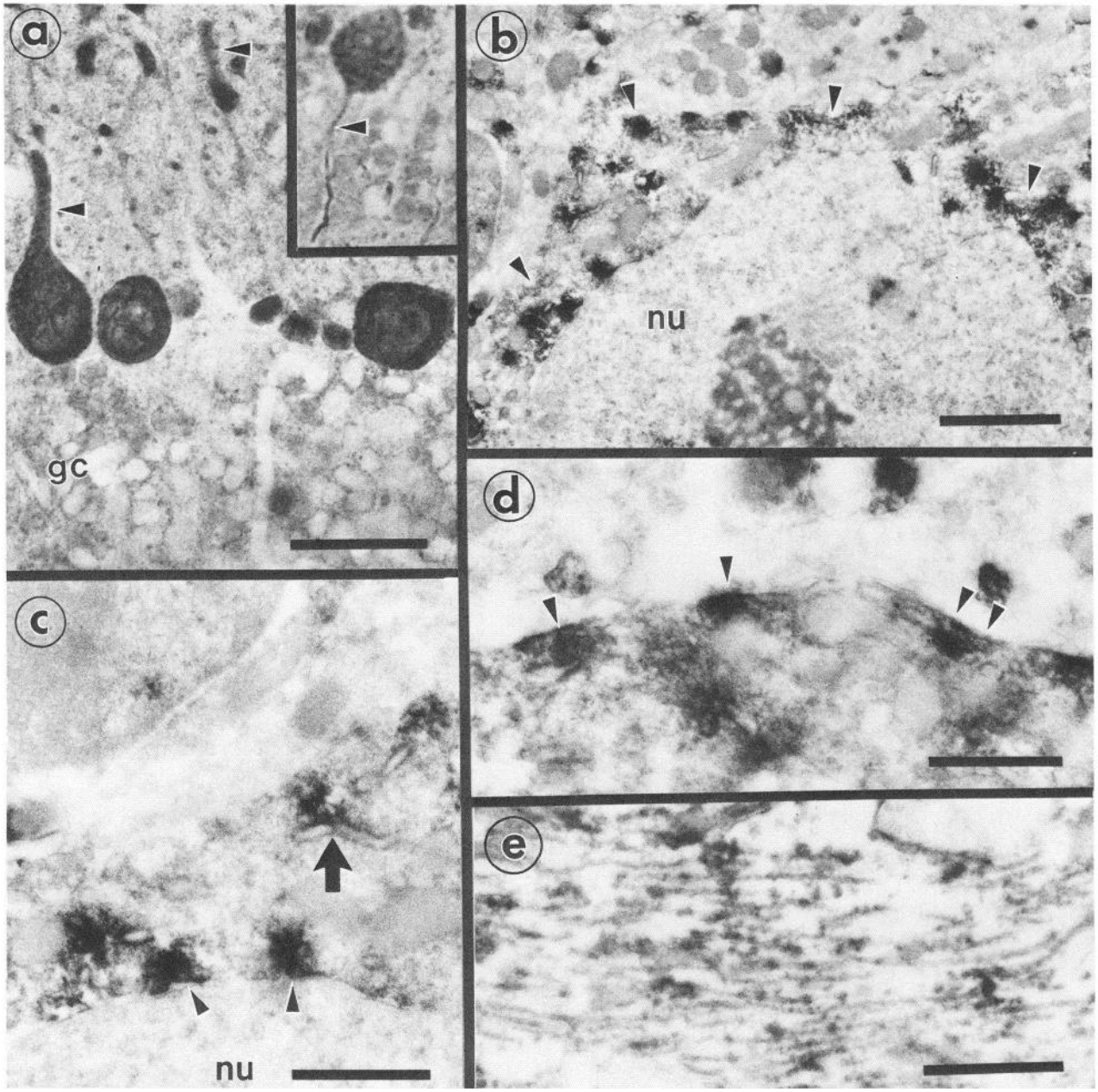

Figure 5. Ultrastructural localization of $G_{z\text{-}3}$ immunoreactivity in murine Purkinje cells. Vibratome sections of mice perfused with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100 were reacted with Gz3/B overnight. Reactivity was visualized using the Streptavidin immunoperoxidase method and diaminobenzidine as the substrate. Sections were embedded in Epon and cut at 1 μ and at 80 nm. In *a,* the 1 μ section shows strong granular immunoreactivity in the Purkinje cell cytoplasm and extending into the proximal dendrites (*arrows*). The neurons of the granular cell layer (*gc*) show weak immunoreactivity. In the inset, a Purkinje cell shows axonal reactivity (*arrowhead*). Scale bar = 30 μ. In *b,* the thin section of a Purkinje cell shows the absence of staining in the nucleus (*nu*) and nucleolus. Numerous dense deposits of reaction product are seen within the cytoplasm and extending from the nuclear membrane to the plasma membrane (*arrowheads*). Scale bar, 2 μ. In *c,* the reaction product is identified in a punctate manner in association with the nuclear membrane (*arrowheads*) and the endoplasmic reticulum (*arrow*). The nucleus (*nu*) is not stained. Scale bar, 1 μ. In *d,* the reaction product is identified on the inner aspect of the plasma membrane in a focal pattern (*arrows*). Scale bar, 0.7 μ. In *e,* a section through the proximal dendrite shows punctate reactivity (*arrowheads*) adjacent to microtubules. Scale bar, 0.7 μ.

et al., 1988); however, localization of the protein within the cell, under similar conditions, appears to be quite different (Worley et al., 1986; Terashima et al., 1987a, b; Asano et al., 1988; Chang et al., 1988; Gabrion et al., 1989).

Several lines of evidence suggest that the observed immunocytochemical localization of $G_{z\text{-}\alpha}$ represents its true distribution. First, in Western immunoblots, affinity-purified antibody reacts only with $G_{z\text{-}\alpha}$ protein, and not with purified α-39 or α-41. Second, there is concordance in staining patterns using antisera made against peptides from different regions of the protein. Third, immunocytochemical staining with omission of primary antibody, or after preabsorption of antibody with specific peptide, shows lack of significant background staining. Finally, Gαcommon, an antiserum directed against other Gα sub-

units and which does not react with $G_{z\text{-}\alpha}$ (Mumby et al., 1986), shows a different pattern of cellular and subcellular staining.

The retina and cerebellum, CNS regions that are well defined morphologically and biochemically, consist of a variety of cell types. Within the retina, $G_{z\text{-}\alpha}$ is localized to the cytoplasm of ganglion cells and their axons in the nerve fiber layer, but not all cell bodies of the ganglion cell layer are reactive with $G_{z\text{-}\alpha}$. This may indicate that either a subpopulation of ganglion cells is reactive with this antibody or that other nonreactive cell types are present within this layer. The latter is more likely since a large proportion of amacrine cells, termed displaced amacrine cells, are present in the ganglion cell layer in several vertebrate species (Perry, 1982). Studies are currently underway to address this question by using retrograde double-labeling techniques. $G_{z\text{-}\alpha}$ is not found in photoreceptors; in contrast, $G_{t\text{-}\alpha}$ is specifically localized to photoreceptor cells and is thought to play an integral role in transduction (Lochrie and Simon, 1988). $G_{o\text{-}\alpha}$ is identified in major synaptic regions, including the inner plexiform layer, the outer plexiform layer, and, to a lesser extent, the neuropil of the ganglion cell layer and inner nuclear layer (Lad et al., 1987; Terashima et al., 1987a, b). Several studies have shown absence of $G_{o\text{-}\alpha}$ staining within nerve cell bodies (Worley et al., 1986; Terashima et al., 1987a, b; Asano et al., 1988; Chang et al., 1988). The extensive axonal staining for $G_{z\text{-}\alpha}$ found in the retinal ganglion cell was in marked contrast to the minimal axonal reactivity found in most other neurons. The ganglion cell axons were reactive along the length of the nerve fiber layer, but reactivity dramatically ceased at the lamina cribrosa. This region is well recognized as a transition point in the axon, both morphologically and biochemically; at the lamina cribrosa myelination usually begins, and neurofilament proteins shift from nonphosphorylated to phosphorylated forms (Sloan and Stevenson, 1987).

In cerebellum, $G_{z\text{-}\alpha}$ was localized predominantly to the cytoplasm of Purkinje cells; only weak reactivity was found in the neurons of adjacent molecular and granular cell layers. In contrast, $G_{s\text{-}\alpha}$ transcripts have been found mainly in the granular cell layer, with absent signal in Purkinje cells (Largent et al., 1988). $G_{o\text{-}\alpha}$ antibodies also react in the granular cell layer neuropil; however, *in situ* hybridization studies show the presence of specific RNA transcripts in Purkinje cells (Largent et al., 1988). In the hippocampus, $G_{z\text{-}\alpha}$ antibody reacts with large pyramidal cells and dentate granule neurons in a similar pattern to the *in situ* hybridization signal described for $G_{o\text{-}\alpha}$ (Largent et al., 1988).

Although the predominant localization of $G_{z\text{-}\alpha}$ at the light microscopic level was intracellular, the necessity for detergent extraction to obtain satisfactory immunocytochemistry suggested that it was found in association with membrane and not cytosol. To determine the subcellular distribution or compartmentalization of $G_{z\text{-}\alpha}$ in the neuron, 2 independent methods were used. Immunoblots of sucrose density-gradient fractions showed that most $G_{z\text{-}\alpha}$ was found in association with the plasma membrane; however, a smaller component appears to be associated with intracellular membranes. No cytosolic $G_{z\text{-}\alpha}$ was detected. Immunoelectron microscopic studies demonstrated focal prominent reactivity in association with the outermost aspect of the nuclear membrane, the ER, and the inner aspect of the plasma membrane in the perikaryon, and in association with microtubules in the proximal dendrites. Other organelles such as Golgi apparatus and mitochondria were nonreactive.

In contrast to our results, Spicher et al. (1988), using an antibody made against a synthetic peptide from a different region of $G_{z\text{-}\alpha}$ sequence, found that in bovine brain, $G_{z\text{-}\alpha}$ was present solely within cytosol fractions. Although the subcellular fractionation was performed in the present study on adrenal, Casey et al. (1990) recently confirmed the association of $G_{z\text{-}\alpha}$ with membrane by their purification of $G_{z\text{-}\alpha}$ from bovine brain membranes; they were unable to detect any of the protein in the soluble fraction.

Little attention has been paid to the subcellular localization of G proteins to sites other than plasma membrane. Subcellular fractionation studies of human neutrophils have shown that pertussis toxin substrate can be localized either to plasma membrane and specific granules (Rotrosen et al., 1988) or to plasma membrane and cytosol (Bokoch et al., 1988). G_{rer}, a G protein found by study of ADP ribosylation by pertussis toxin, is present in rough ER fractions but not in plasma membrane fractions of canine pancreas (Audigier et al., 1988). Very few studies have attempted immunocytochemical localization of G proteins at the ultrastructural level (Rodrigues et al., 1987; Gabrion et al., 1989; Péraldi et al., 1989); however, G_o has been found to be localized within different neural cell types to either the plasma membrane alone or both plasma membrane and cytosol (Gabrion et al., 1989; Péraldi et al., 1989).

The function of $G_{z\text{-}\alpha}$, both in its plasma membrane and intracellular locations, is unknown. The intracellular membrane-associated component of the protein may represent its biosynthetic pathway, although this is not the case with other G proteins. It may also function as a translocatable pool, used to regulate receptor activity, although if this is the case, association with vesicles, as has been reported for $G_{i\text{-}\alpha}$ in neutrophils, would be more likely (Rotrosen et al., 1988). The confinement of $G_{z\text{-}\alpha}$, in most neurons to the perikaryon and dendrites, and in retinal ganglion cells also to the extensive unmyelinated portion of the axon, indicates that functions different than those found in myelinated axons may be involved. Recently, Carlson et al. (1989) reported that $G_{z\text{-}\alpha}$ may be phosphorylated by protein kinase C in platelets, pointing to the possibility of a unique role for this protein. The immunolocalization of type 1 protein kinase C in cerebellum is quite similar to that of $G_{z\text{-}\alpha}$; reactivity is present in Purkinje cells both on plasma membrane and within the cytoplasm (Kose et al., 1988). The possible association of $G_{z\text{-}\alpha}$ with microtubules in dendrites suggests that G_z may function to carry a signal from the perikaryon to the more peripheral dendritic arborizations. Casey et al. (1990) have shown that $G_{z\text{-}\alpha}$ has unique functional properties with an extremely slow intrinsic GTPase activity and very slow guanine nucleotide exchange. Thus, once the $G_{z\text{-}\alpha}$ subunit binds GTP, it may remain activated for a very long time, barring the involvement of other ancillary proteins. $G_{z\text{-}\alpha}$ may therefore be capable of functioning in a *trans*-acting manner as an intracellular transduction molecule in specialized subsets of neural cells.

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