

G_o, a guanine nucleotide-binding protein: Immunohistochemical localization in rat brain resembles distribution of second messenger systems

(immunohistochemistry/GTP-binding regulatory proteins/phosphatidylinositol/protein kinase C/adenylate cyclase)

PAUL F. WORLEY*[†], JAY M. BARABAN*[‡], CORNELIS VAN DOP[§], EVA J. NEER[¶],
AND SOLOMON H. SNYDER*[‡]||**

Departments of *Neuroscience, [¶]Pharmacology and Experimental Therapeutics, [†]Neurology, [‡]Psychiatry and Behavioral Sciences, and [§]Pediatrics, The Johns Hopkins University School of Medicine, 725 North Wolfe Street, Baltimore, MD 21205; and [¶]Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115

Contributed by Solomon H. Snyder, January 6, 1986

ABSTRACT We have localized a guanine nucleotide-binding protein, G_o, in rat brain by immunohistochemistry with a selective polyclonal antiserum to the α_{39} subunit of G_o. Specific staining is widely distributed, abundant in neuropil, absent from neuronal cell bodies, and displays regional heterogeneity. Staining is enriched in cerebral cortex, particularly the molecular layer, neuropil of the hippocampal formation, striatum, substantia nigra pars reticulata, molecular layer of the cerebellum, substantia gelatinosa of the spinal cord, and posterior pituitary. High density staining in the substantia nigra reflects a G_o-containing striatonigral pathway since striatal lesions reduce ipsilateral immunostaining in the pars reticulata. Confirming immunostaining, quantitative [³²P]ADP-ribosylation of nigral membranes with pertussis toxin indicates a 66% \pm 11% (mean \pm SEM) reduction of G_o ipsilateral to striatal lesions. G_o may be associated with Purkinje cells in the cerebellum since membranes from mutant mice (Nervous), which postnatally lose Purkinje cells, are markedly depleted in pertussis toxin substrate. The localizations of G_o correspond in many areas with those of protein kinase C, a component of the phosphatidylinositol cycle, suggesting a major role for G_o in the brain related to regulation of the phosphatidylinositol cycle.

Guanine nucleotide-binding regulatory proteins couple hormonal, neurotransmitter, and light stimuli to second messenger systems (1). Adenylate cyclase is linked to receptors by means of the GTP-binding regulatory proteins G_s and G_i, which stimulate and inhibit, respectively, cyclase activity (2). The phosphatidylinositol (PtdIns) cycle is another major second messenger system in which receptors stimulate the phosphodiesteratic cleavage of phosphatidylinositol bisphosphate to inositol trisphosphate and diacylglycerol (3). GTP-binding proteins may play a role in the PtdIns cycle, since GTP stimulates PtdIns turnover in membrane preparations and permeabilized cells (4, 5). Furthermore, pertussis toxin, which ADP-ribosylates several GTP-binding proteins, also inhibits the PtdIns cycle (6–10). In brain, pertussis toxin ADP-ribosylates G_i and another protein referred to as G_o (11–13). Although G_o is five times as plentiful as G_i, its function is unknown. Like G_s and G_i, G_o is a membrane-associated heterotrimer composed of an α subunit of molecular mass 39 kDa (α_{39}), which is distinct from the 41-kDa α subunit of G_i (α_{41}), and a $\beta\gamma$ dimer, which is functionally indistinguishable from the $\beta\gamma$ dimer of G_i and G_s. Also like G_s and G_i, the α_{39} subunit possesses GTPase activity and dissociates from $\beta\gamma$ in the presence of guanine nucleotides. Further indication that G_o functions as a regulatory protein is

provided by ligand–receptor binding studies. The guanine nucleotide dependence of agonist binding to muscarinic and γ -aminobutyric acid B (GABA_B) receptors is blocked by pertussis toxin and restored by the addition of either purified G_i or G_o (14, 15). Since guanine nucleotides stimulate and pertussis toxin blocks PtdIns breakdown, a guanine nucleotide-binding regulatory protein is thought to couple membrane receptors to the PtdIns system. Accordingly, G_o, which is a pertussis toxin substrate, could perform this function (13).

Purified α_{39} has been used to produce polyclonal rabbit antibodies (13). This immune serum specifically recognizes α_{39} , cross-reacting only weakly with α_{41} and not at all with the α subunit of G_s or the $\beta\gamma$ subunit. To help clarify the function of G_o, we have used this immune serum with selectivity for α_{39} to map its distribution in rat brain.

MATERIALS AND METHODS

Tissue Preparation and Immunohistochemical Staining. Male Sprague–Dawley rats (200–250 g) were anesthetized with pentobarbital and perfused with room temperature buffers at a rate of 50 ml/min with a peristaltic pump (Watson–Marlow, Falmouth, Cornwall, U.K.) through the ascending aorta with 100 ml of 100 mM NaCl/50 mM Tris·HCl, pH 7.7, followed by 500 ml of 4% formaldehyde freshly depolymerized from paraformaldehyde (Electron Microscopy Sciences, Fort Washington, PA) in 0.1 M sodium phosphate at pH 7.5. Brains were left *in situ* for 30 min at room temperature and then removed, embedded in brain paste, and rapidly frozen on dry ice. Sections (10 μ m) were cut at -15°C and thaw-mounted on gelatin-coated slides. The slides were desiccated at room temperature for 2 hr and stored at -20°C . For immunohistochemical studies, brain sections were preincubated at room temperature for 2 hr in 50 mM Tris·HCl (pH 7.7), 100 mM NaCl, 0.05% Triton X-100, and 2.5 mg of bovine serum albumin (RIA grade; Sigma) per ml (buffer A) to which 1:10 normal goat serum (NGS; Vector, Burlingame, CA) was added. The preincubation solution was replaced with buffer A containing 1:10 NGS and 1:100 through 1:800 dilutions of the immune serum, and the sections were incubated overnight at 4°C . Sections were then washed in buffer A at room temperature (all subsequent steps were done at room temperature) three times for 5 min each and once in buffer A containing 1:10 NGS for 30 min.

Abbreviations: PtdIns, phosphatidylinositol; G_i, inhibitory GTP-binding regulatory protein of adenylate cyclase; G_o, GTP-binding protein; PKC, protein kinase C; G_s, stimulatory GTP-binding regulatory protein of adenylate cyclase.

**To whom reprint requests should be addressed.

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.

Sections were then incubated 90 min in buffer A containing 1:10 NGS and 1:200 biotinylated goat anti-rabbit serum (Vectastain ABC Kit; Vector) followed by three 5-min washes in buffer A. They were next incubated 60 min in buffer A containing 1:200 avidin-biotin-peroxidase complex prepared 30 min before use. Following three 5-min washes in 50 mM Tris-HCl, pH 7.7/100 mM NaCl (buffer B), the sections were incubated 15 min in buffer B containing 0.8 mM diaminobenzidine (grade II; Sigma) and 0.025% H₂O₂ (Superoxol; Baker) followed by two 30-min washes in buffer B and dried. Sections were not osmicated. Adjacent sections were stained with cresyl violet.

Immunohistochemical specificity was assessed in parallel studies on adjacent sections with normal rabbit serum (Vector), preimmune serum, and preadsorbed immune serum that had been treated with purified α_{39} bound to nitrocellulose.

Lesion Studies. Quinolinic acid (200 nmol in 2 μ l of saline, Sigma) was injected unilaterally into the striata of adult male Sprague-Dawley rats (200–250 g) (16). The animals were sacrificed 4 days later and used either for immunohistochemical staining or for regional biochemical quantitation of pertussis toxin-stimulated ADP-ribosylation.

Pertussis Toxin-Stimulated ADP-Ribosylation. Fresh rat or mouse brain regions were homogenized (2 mg/ml) in a solution containing 50 mM Tris-HCl (pH 8), 5% sucrose, 6 mM MgCl₂, 1 mM EDTA, 3 mM benzamide, 1 mM dithiothreitol, and 1 μ g of soybean trypsin inhibitor per ml. The homogenates were stored at -70°C.

Pertussis toxin was activated as described (17). The ADP-ribosylation reaction mixture (50 μ l) containing 100 mM Tris-HCl (pH 8), 2.5 mM ATP, 2 mM GTP, 5 μ M [³²P]NAD (specific activity, 50 Ci/mmol; 1 Ci = 37 GBq), 10 mM thymidine, 10 mM isoniazid, 4% sucrose, 4.8 mM MgCl₂, 0.8 mM EDTA, 2.4 mM benzamide, 2.8 mM dithiothreitol, 0.8 μ g of soybean trypsin inhibitor per ml, 1.6 mg of tissue per ml, and 20 μ g of activated pertussis toxin per ml was incubated at 25°C for 45 min. The membranes were then separated by centrifugation (12,000 \times g for 15 min) and washed with the homogenization buffer. Membrane proteins were size-fractionated on a 12.5% polyacrylamide/Na-DodSO₄ gel (18) and the dried gel was exposed to Kodak XAR-5 film with intensifying screens. The relative incorporation of ³²P was determined by using two-dimensional densitometric measurements (19) of bands with molecular masses of 39–41 kDa on the autoradiograms. Pertussis toxin was purchased from List Biochemicals (Campbell, CA) and [³²P]NAD was obtained from New England Nuclear.

RESULTS

Characterization of Immunohistochemical Staining of α_{39} in Rat Brain. The immune serum produces selective staining at dilutions of 1:100–1:400. Immunohistochemical specificity was assessed in parallel studies on adjacent sections. No staining is observed when any of the immunoreagents are omitted. Preimmune serum or normal rabbit serum at concentrations as high as 1:50 dilution provide no detectable staining. Purified α_{39} (13) bound to nitrocellulose was used to remove specific antibody batchwise from the immune serum. This preadsorbed serum at concentrations as high as 1:100 dilution produces no staining. Electrophoretic transfer blot analysis previously revealed high concentrations of α_{39} in rat and bovine brain, much lower levels in liver, and even less in renal cortex (13). Similar to these results with electrophoretic transfer blot analysis, we find histochemically detectable α_{39} -like immunoreactivity at 1:400 dilution in liver but not in spleen, renal cortex, testis, or epididymis. The correlation between regional electrophoretic transfer blot and immunohistochemical assays as well as the absence of staining with preadsorbed immune, preimmune, and normal rabbit serum

controls indicate that the histochemical staining represents specific antibody recognition of an α_{39} -like antigen. Though the antiserum has 10% cross-reactivity with α_{41} , concentrations of this protein in the brain are <20% that of α_{39} , so only 2% of the overall staining should represent binding to α_{41} . Preliminary immunohistochemical studies with 40- μ m vibratome brain sections reveal intense specific staining with a primary antibody dilution of 1:8000, which is blocked by addition of 10 μ g of purified α_{39} per ml to the incubation solution. The pattern of staining appears identical with either the cryostat- or vibratome-cut brain sections.

Immunohistochemical Localization of α_{39} in Rat Brain. A general feature of α_{39} -like immunoreactivity is its high concentration in synaptic-rich neuropil and absence from neuronal cell bodies (Figs. 1 and 2). For instance, in the

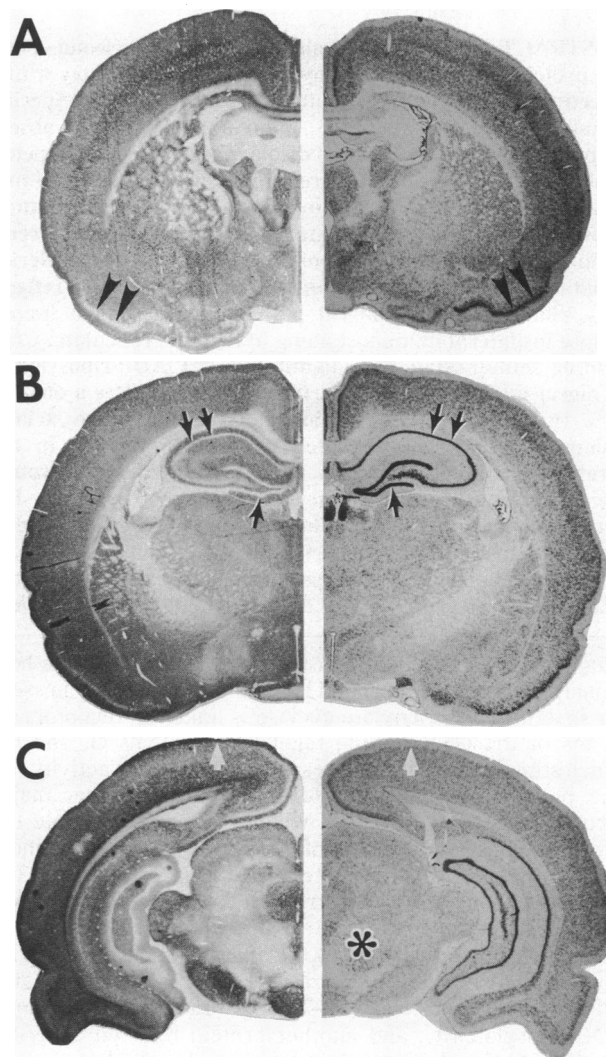


FIG. 1. Light micrographs of α_{39} immunohistochemical staining in coronal sections of rat brain. ($\times 6$.) Immunohistochemical staining is shown on the left and cresyl violet-stained sections are shown on the right. (A) Immunostaining is present in the neocortex, caudate nucleus, globus pallidus, and septal nuclei but is absent over clustered neurons of the primary olfactory cortex (arrowheads) and white matter. (B) The hippocampus is immunostained except for the pyramidal and granule cell layers (arrows). Immunostaining is present in the thalamus and hypothalamus, most prominent medially in both structures. (C) Arrows point to intensely immunostained molecular layer of the neocortex. Substantia nigra, interpeduncular nucleus, central grey, grey layers of the superior colliculus, and lateral geniculate are all intensely immunostained. By contrast, the area of the red nucleus (asterisk) is weakly immunostained.

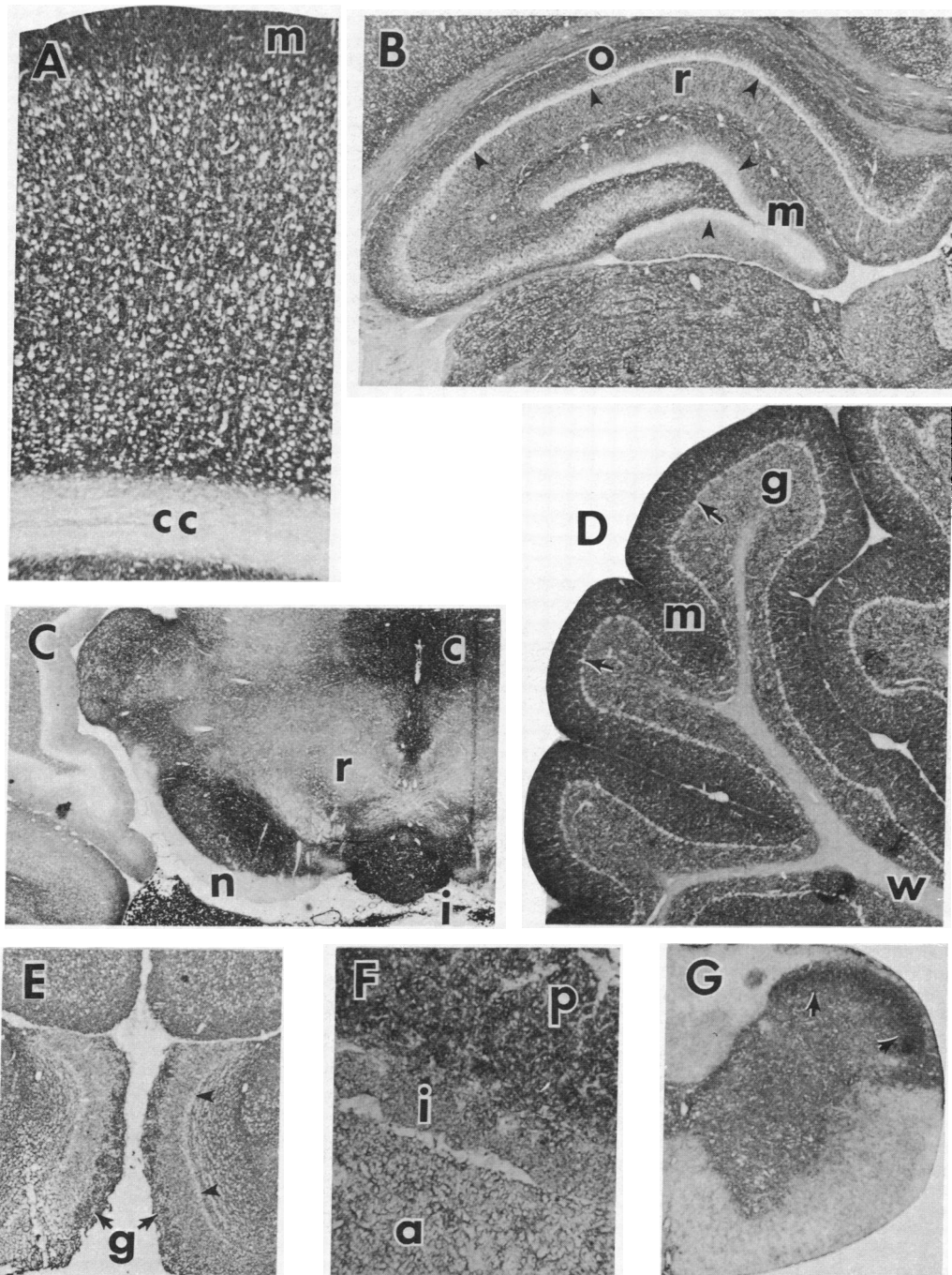


FIG. 2. Light micrographs of α_{39} immunohistochemical staining. (A) Frontal cortex. ($\times 35$.) Intense immunoreactivity is present in the molecular layer (m) but is absent from pyramidal cell bodies and corpus callosum (cc). (B) Dorsal hippocampus. ($\times 30$.) Immunoreactivity is seen in the strata oriens (o) and radiatum (r) as well as the molecular layer of the dentate gyrus (m). Arrowheads point to absence of immunoreactivity over the pyramidal and granule cells. (C) Midbrain. ($\times 10$.) Intense immunoreactivity is present in the substantia nigra (n) most prominent along the dorsomedial aspect, in the interpeduncular nucleus (i), and central grey (c). By contrast, the red nucleus (r) has weak immunoreactivity. (D) Cerebellum. ($\times 20$.) The molecular layer (m) is more intensely stained than the granule cell layer (g). Unstained are the Purkinje cells (arrows) and white matter (w). (E) Olfactory bulb. ($\times 20$.) The glomerular layer (g) is intensely stained. Arrowheads point to the mitral cell layer, which is faintly stained. (F) Pituitary. ($\times 45$.) The posterior lobe (p) is intensely stained, whereas the intermediate (i) and anterior (a) lobes are lightly stained. (G) Spinal cord. ($\times 25$.) Arrows point to the intensely stained substantia gelatinosa.

hippocampus staining is absent in the pyramidal and granule cell layers, but the adjacent neuropil stains intensely. Staining in cerebral cortex is most intense in the molecular layer, confined to neuropil and absent from neuronal cell bodies. Lamination is also apparent in the primary olfactory cortex with heavy staining in the superficial and deeper layers, whereas the interposed neuronal cell body layer is devoid of staining. In the olfactory bulb, the most intense staining is in the glomerular layer and it is absent from the mitral cell layer. In the caudate and globus pallidus, staining is fairly homogeneous but spares the large white matter bundles. In the thalamus and hypothalamus, there is moderate staining more prominent medially. Again, staining in these areas is confined to neuropil.

In the midbrain, the substantia nigra displays α_{39} -like immunoreactivity most intense in the dorsomedial region of the zona reticulata (Fig. 2). Unilateral striatal lesions produced with the neuron-selective toxin quinolinic acid reduce

α_{39} -like immunoreactivity in the ipsilateral substantia nigra, indicating an association of G_o with synaptic terminals of the striatonigral projection (Fig. 3). The interpeduncular nucleus is also densely stained with more intense immunoreactivity in lateral than in medial areas (Fig. 3). In contrast to the very heavy staining in the substantia nigra and interpeduncular nucleus, the red nucleus, which is immediately dorsal to these two areas, shows much less staining. Other areas in the midbrain with prominent α_{39} -like immunoreactivity include the periaqueductal grey and the superficial and intermediate grey layers of the superior colliculus.

In the cerebellum, staining is most dense in the molecular layer, with substantially less staining in the granule cell layers and no staining at all over the Purkinje cell bodies. Staining is apparent in the grey matter but not the white matter of the spinal cord and is most intense in a dorsal band corresponding to the substantia gelatinosa. Within the pituitary gland staining is intense in the posterior pituitary with much less

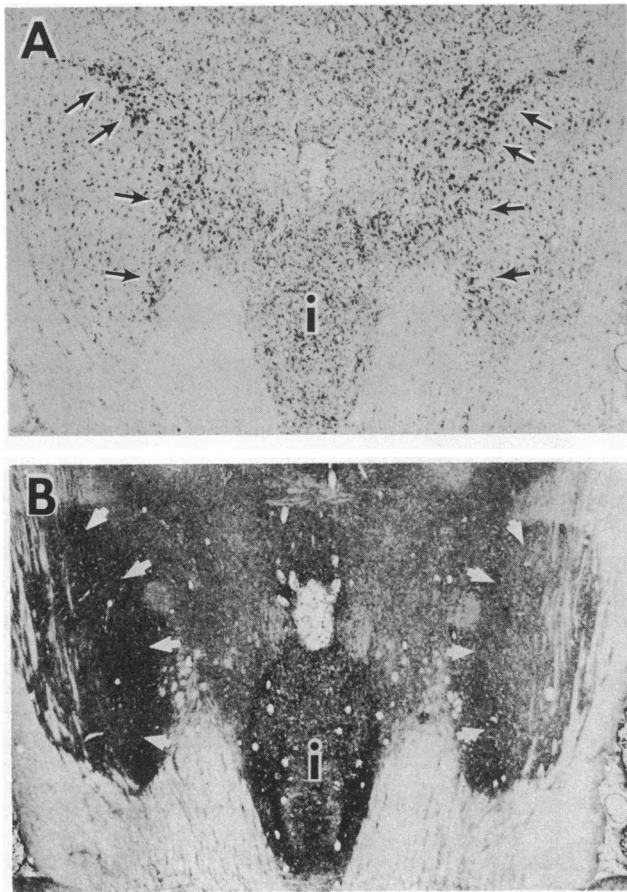


FIG. 3. Localization of α_{39} to striatonigral pathway. Unilateral lesions of the rat striatum with quinolinic acid (200 nmol) lower α_{39} -like immunoreactivity in the ipsilateral substantia nigra. (A) Cell stain of a horizontal section through the substantia nigra. Cells in zona compacta are bilaterally symmetric (arrows), indicating that the lesion is specific for afferent terminals. (B) α_{39} Immunohistochemistry of adjacent section demonstrates decreased reactivity ipsilateral to the lesion (right). Arrows delineate medial aspect of nigra. Staining of the interpeduncular nucleus (i), which is more prominent on its lateral borders, is symmetric, indicating that the nigra is selectively affected by the lesion.

staining in the intermediate and anterior lobes. The pineal is lightly stained (not shown).

Pertussis Toxin-Stimulated ADP-Ribosylation: Comparison with Immunohistochemistry. Pertussis toxin catalyzes the NAD-dependent ADP-ribosylation of G_0 and G_i (11, 12). We have monitored levels of pertussis toxin-stimulated ADP-ribosylation in membranes from various brain areas as a biochemical assay to corroborate immunohistochemical data. High levels of pertussis toxin-stimulated ADP-ribosylation of α_{39} and α_{41} are detected in hippocampus, cerebral cortex, cerebellum, and substantia nigra. Histochemical experiments show lower levels of α_{39} in the red nucleus than in the adjacent substantia nigra. Pertussis toxin-stimulated ADP-ribosylation is likewise $41\% \pm 7\%$ (mean \pm SEM; $n = 3$) lower in the red nucleus than in the nigra. Quinolinic acid lesions of the caudate decrease immunohistochemically detected antigen in the ipsilateral substantia nigra (Fig. 3). In identically lesioned rats, pertussis toxin-stimulated ADP-ribosylation is also reduced in the ipsilateral substantia nigra by $66\% \pm 11\%$ (mean \pm SEM; $n = 3$; Fig. 4).

Previously, we showed that protein kinase C (PKC) in the cerebellum is associated with Purkinje cell dendrites, based on autoradiographic experiments with lesioned rats and Nervous mice, which postnatally lose Purkinje cells (20).

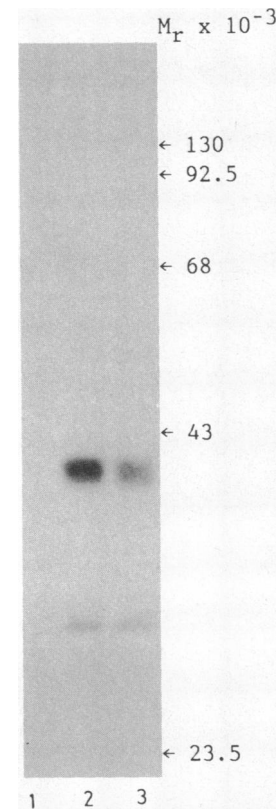


FIG. 4. Autoradiograms of pertussis toxin-stimulated ADP-ribosylation in substantia nigra following striatal lesion. Rats were lesioned unilaterally in the striatum by stereotaxic injections of quinolinic acid (200 nmol) and sacrificed 4 days later. The substantiae nigrae ipsilateral (lane 3) and contralateral (lanes 1 and 2) were removed, and homogenates (1.6 mg of tissue per ml) were incubated using [32 P]NAD without (lane 1) or with (lanes 2 and 3) activated pertussis toxin.

Similarly, pertussis toxin-stimulated ADP-ribosylation is decreased between 50% and 70% ($n = 2$) in the cerebella of Nervous mice relative to normally developing heterozygotes, indicating an association of G_0 with Purkinje cells.

DISCUSSION

In the present study we have localized the α_{39} subunit of the GTP-binding protein G_0 with a view to clarifying its function. The selective enrichment of G_0 in synaptic zones indicates a role in neurotransmission. Since the GTP-binding proteins G_s and G_i are known to be components of the adenylate cyclase second messenger system, G_0 might conceivably be involved in regulating adenylate cyclase or another second messenger system such as the PtdIns cycle.

Previously, we mapped components of the adenylate cyclase and PtdIns second messenger systems autoradiographically in rat brain (20, 21). [3 H]Forskolin binds with high affinity to adenylate cyclase in the presence of activated G_s and provides a marker for receptor-stimulated adenylate cyclase (22, 23). 3 H-labeled 4 β -phorbol 12,13-dibutyrate binds with high affinity to PKC, a component of the PtdIns cycle (24, 25). Accordingly, we can compare the immunohistochemical localization of α_{39} or G_0 with these second messenger system markers. The localization of α_{39} -like immunoreactivity closely resembles that of PKC but clearly differs from that of adenylate cyclase (Table 1). In the hippocampus, PKC and α_{39} -like staining are present throughout the entire hippocampus, sparing only cell body layers, whereas adenylate cyclase is primarily localized to granule

Table 1. Comparative localizations of α_{39} , PKC, and adenylate cyclase

Region	α_{39}	PKC	Adenylate cyclase
Hippocampus	Throughout neuropil	Throughout neuropil	Granule cell dendrites and mossy fibers
Corpus striatum	Equal to cortex	Equal to cortex	5× cortex
Cerebral cortex	↑ Molecular layer	↑ Molecular layer	All layers similar
Substantia nigra	↑ Striatonigral terminals	↑ Striatonigral terminals	↑ Striatonigral terminals
Cerebellum	↑ Purkinje cells	↑ Purkinje cells	↑ Granule cells
Pituitary	↑ Posterior lobe	↑ Posterior lobe	↑ Intermediate lobe
Olfactory bulb	↑ Glomerular layer	↑ External plexiform layer	↑ Glomerular layer

Data for PKC and adenylate cyclase derive from autoradiographic localizations of ^3H -labeled 4β -phorbol 12,13-dibutyrate and ^3H forskolin, respectively (20, 21). The \uparrow indicates highest levels in the designated area.

cell dendrites and mossy fibers (20, 21). ^3H Forskolin-binding sites are markedly enriched in the corpus striatum relative to the cerebral cortex or thalamus, whereas densities of α_{39} -like immunoreactivity and PKC are more evenly distributed between these structures. In the neocortex, α_{39} -like immunoreactivity and PKC are enriched in the molecular layer, whereas adenylate cyclase lacks prominent lamination. In cerebellum, α_{39} -like immunoreactivity and PKC are largely associated with Purkinje cells, whereas ^3H forskolin-binding sites are associated with granule cell axons and terminals. In the pituitary, α_{39} -like immunoreactivity and PKC are concentrated in the posterior or neural lobe. By contrast, adenylate cyclase is highest in the intermediate lobe. A clear exception to the similar distribution of α_{39} -like immunoreactivity and PKC occurs in the olfactory bulb, where α_{39} and adenylate cyclase are concentrated in the glomerular layer, whereas PKC is most enriched in the subjacent external plexiform layer.

A link between G_o and the PtdIns cycle has been hypothesized (13, 26). The similar distribution in the great majority of brain areas of α_{39} -like immunoreactivity and PKC, a component of the PtdIns system, supports a major role for G_o in coupling receptors to the PtdIns cycle. In addition, the similar distribution of G_o and ^3H forskolin binding in the olfactory bulb may reflect the ability of G_o to modulate adenylate cyclase by means of the $\beta\gamma$ subunit (13), as has been proposed for G_i (27). Recent evidence also implicates GTP-binding regulatory proteins that are pertussis toxin substrates, such as G_o , in coupling receptors directly to ion channels (28, 29). In brain homogenate, G_o comprises >1% of the membrane protein and is at least five times more plentiful than either G_i or G_s . Its selective localization to neuropil areas indicates even higher concentrations in synaptic zones. Although our knowledge of the functional repertoire of G_o is incomplete, it seems likely that G_o plays a major role in signal transduction.

We thank Naomi Taylor and Virginia Wilson for technical assistance, Rita Hollingsworth for secretarial assistance, and Drs. Karen Braas and Mark Molliver for valuable advice. This work was supported by Public Health Service Grant MH-18501, Grant DA-00266, Research Scientist Award DA-00074 (to S.H.S.), Physician Scientist Award AG-00256 (to P.F.W.), Grant AM-36085 (to C.V.D.), and Grant GM-36259 (to E.J.N.). J.M.B. is a Lucille P. Markey Scholar and this work was supported in part by a grant from the Lucille P. Markey Charitable Trust and a grant of the Laboratories for Therapeutic Research.

- Gilman, A. G. (1984) *Cell* **36**, 577-579.
- Schramm, M. & Selinger, Z. (1984) *Science* **225**, 1350-1356.
- Berridge, M. J. & Irvine, R. F. (1984) *Nature (London)* **312**, 315-321.
- Litosch, I., Wallis, C. & Fain, J. N. (1985) *J. Biol. Chem.* **260**, 5464-5471.
- Cockcroft, S. & Gomperts, B. D. (1985) *Nature (London)* **314**, 534-536.
- Brandt, S. J., Dougherty, R. W., Lapetina, E. G. & Niedel, J. E. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 3277-3280.
- Okajima, F. & Ui, M. (1984) *J. Biol. Chem.* **259**, 13863-13871.
- Nakamura, T. & Ui, M. (1985) *J. Biol. Chem.* **260**, 3584-3593.
- Volpi, M., Naccache, P. H., Molski, T. F., Shetyck, J., Huang, C. K., Marsh, M. L., Munoz, J., Becker, E. L. & Shaati, R. I. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 2708-2712.
- Smith, C. D., Lane, B. C., Kusaka, I., Verghese, M. W. & Snyderman, R. (1985) *J. Biol. Chem.* **260**, 5875-5878.
- Neer, E. J., Lok, J. M. & Wolf, L. G. (1984) *J. Biol. Chem.* **259**, 14222-14229.
- Sternweis, P. C. & Robishaw, J. D. (1984) *J. Biol. Chem.* **259**, 13806-13813.
- Huff, R. M., Axton, J. M. & Neer, E. J. (1985) *J. Biol. Chem.* **260**, 10864-10871.
- Florio, V. A. & Sternweis, P. C. (1985) *J. Biol. Chem.* **260**, 3477-3483.
- Asano, T., Ui, M. & Ogasawara, N. (1985) *J. Biol. Chem.* **260**, 12653-12658.
- Schwarz, R., Whetsell, W. D., Jr., & Mangano, R. M. (1983) *Science* **219**, 316-318.
- Van Dop, C., Yamanaka, G., Steinberg, F., Sekura, R. D., Manclark, C. R., Stryer, L. & Bourne, H. R. (1984) *J. Biol. Chem.* **259**, 23-26.
- Laemmli, U. K. (1970) *Nature (London)* **227**, 680-685.
- Kuhar, M. J., Whitehouse, P. J., Unnerstall, J. R. & Loats, H. (1984) *Neurosci. Abstr.* **10**, 558.
- Worley, P. F., Baraban, J. M. & Snyder, S. H. (1986) *J. Neurosci.* **6**, 1-9.
- Worley, P. F., Baraban, J. M., DeSouza, E. B. & Snyder, S. H. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 4053-4057.
- Seamon, K. B. & Daly, J. W. (1985) *Adv. Cyclic Nucleotide Res.* **19**, 125-135.
- Nelson, C. A. & Seamon, K. B. (1985) *Fed. Euro. Biochem. Soc.* **183**, 349-352.
- Nishizuka, Y. (1984) *Science* **225**, 1365-1370.
- Blumberg, P. M., Jaken, S., Konig, B., Sharkey, N. M., Leach, K. L. & Jeng, A. Y. (1984) *Biochem. Pharmacol.* **33**, 933-940.
- Joseph, S. K. (1985) *Trends Biochem. Sci.* **10**, 297-298.
- Northup, J. K., Sternweis, P. C. & Gilman, A. G. (1983) *J. Biol. Chem.* **258**, 11361-11368.
- Pfaffinger, P. J., Martin, J. M., Hunter, D. D., Nathanson, N. M. & Hille, B. (1985) *Nature (London)* **317**, 536-538.
- Breitwasser, G. E. & Szabo, G. (1985) *Nature (London)* **317**, 538-540.