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Phosphoinositide Second Messenger System Is Enriched in Striosomes: Immunohistochemical Demonstration of Inositol 1,4,5-Trisphosphate Receptors and Phospholipase C β and γ in Primate Basal Ganglia

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The neurochemical organization of the basal ganglia has been studied extensively with respect to neurotransmitters, neuropeptides, and their receptors. The chemoarchitecture of the striatum has been found particularly striking, because it distinguishes many substances by their relative distributions within the striosome and matrix compartments of the striatum. Very little is yet known about the differential distribution of second messenger systems in the basal ganglia, however, and no information is available about whether the distribution of second messenger systems is related to the prominent neurochemical compartmentalization of the striatum. We have examined the distribution of the phosphoinositide second messenger system in the primate basal ganglia and substantia nigra, as detected with polyclonal antisera against the inositol 1,4,5-trisphosphate receptor (IP_3R), and monoclonal antisera against phospholipase C β (PLCβ) and phospholipase C γ (PLCγ).

In the striatum, immunostaining for each of the three proteins was present predominantly in medium-sized neuronal perikarya and in the neuropil. Circumscribed zones of enhanced IP_3R, PLCβ, and PLCγ immunoreactivity appeared in a background of generally weaker staining, and these zones corresponded to striosomes as identified by calbindin D_{28K} and substance P immunostaining in adjacent sections. Thus, the richest representation of the phosphoinositide system in the primate striatum appears to be in striosomes.

In the substantia nigra pars compacta, neurons and neuropil were immunopositive, but in the substantia nigra pars reticulata and in each segment of the globus pallidus, immunostaining was mainly confined to the neuropil. Perikaryal PCLγ immunoreactivity in the absence of detectable PLCβ or IP_3R immunolabeling was found in the magnocellular neurons embedded in the medullary layer between the putamen and the globus pallidus.

These observations demonstrate that the phosphoinositide second messenger system is selectively enhanced in neuronal subsystems of the basal ganglia, including striosomes, and suggest that signaling by phosphoinositide pathways elicits discrete effects on input–output processing by the basal ganglia.

[Key words: phosphoinositide, phospholipase C, striatum, striosome, primate]

An important organizational principle of the basal ganglia involves differentiation of the neostriatum into compartments referred to as striosomes and matrix (Graybiel and Ragsdale, 1978). Neurons in striosomes and matrix differ in their ontogeny as well as in their afferent and efferent connections. Neurons in striosomes mature earlier than those in the matrix, and the striosomal system receives dense inputs from prefrontal orbitoinsular parts of the cortex, whereas the matrix input predominantly originates in other regions including sensorimotor cortical areas (Fishell and van der Kooy, 1982; Gerfen, 1984; Ragsdale and Graybiel, 1988, 1990; Flaherty and Graybiel, 1991). Striosomal neurons appear to receive their dopamine-containing input mainly from densocellular islands in ventral parts of the substantia nigra pars compacta (SNc) and to project mainly back to medial nigral regions. Neurons of the striatal matrix, by contrast, receive their dopamine-containing inputs mainly from other neurons of the nigral complex and project mainly to the external (GPe) and internal (GPi) segments of the globus pallidus (GP) and to the substantia nigra pars reticulata (SNr) (Graybiel et al., 1979; Gerfen, 1984; Donoghue and Herkenham, 1986; Gerfen et al., 1987; Jiménez-Castellanos and Graybiel, 1987, 1989; Giménez-Amaya and Graybiel, 1991).

Striosomes and matrix differ also in their content of neurotransmitters and receptors. For instance, striosomes are selectively enriched in limbic-associated membrane protein (LAMP) (Chesselet et al., 1991), whereas the matrix is enriched in calbindin D_{28K}, AChE, ChAT, somatostatin, and tyrosine hydroxylase (TH). Opiate and dopamine D_1-like receptors are enriched in striosomes, whereas dopamine D_2-like and some muscarinic cholinergic receptors predominate in the matrix (Pert et al., 1976; Herkenham and Pert, 1981; Graybiel et al., 1986; Joyce et al., 1986; Besson et al., 1988; for a review, see Graybiel, 1990). There are also regional variations in these striosome/

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matrix distributions for individual transmitter-related markers (Graybiel and Ragsdale, 1978; Besson et al., 1990; Martin et al., 1991), and species differences as well.

This differential distribution of multiple neurotransmitter-related elements in striosome and matrix compartments suggests functional specificity, but it is difficult to relate them to specific extrapyramidal functions (Albin et al., 1989). Neurotransmitters, however, act through only a few second messenger systems, with the cAMP and phosphoinositide (PI) systems predominating, so what seems complex at the neurotransmitter level may be systematically ordered at the second messenger level. PI markers, such as the inositol 1,4,5-trisphosphate (IP_3) receptor (IP_3R) and protein kinase C (PKC), and markers of the cAMP system, such as ^{3}H-forskolin binding, are differentially distributed in the brain with a number of complementary localizations (Worley et al., 1986a,b, 1987, 1989). In the extrapyramidal system, PI markers occur in high densities in the corpus striatum and the SNr. The neuroanatomical distributions of phospholipase C (PLC) (Gerfen et al., 1988), PKC (Huang et al., 1988; Saito et al., 1988; Yoshihara et al., 1991), and IP_3R (Nakanishi et al., 1991; Sharp et al., in press) have been described in the rat brain, but these messenger distributions have not been mapped in primate, in which the striatum and substantia nigra are highly differentiated as compared to these regions in rat. In particular, the relationship of the PI system to the striosome and matrix systems has not been characterized. In the present study we analyzed the distribution of immunoreactivity for IP_3R and PLCβ and PLCγ in the primate basal ganglia and substantia nigra. We report that, in addition to a broad distribution of these PI markers in the basal ganglia, there is a selective enrichment of PI markers in striosomes of the striatum.

Methods

Tissue preparation. Six rhesus monkeys (*Macaca mulatta*), two cynomolgus monkeys (*Macaca fascicularis*), and two squirrel monkeys (*Saimiri sciureus*) were perfused transcardially, under deep anesthesia, with 0.1 M phosphate-buffered saline (PBS), pH 7.4, followed by 4% paraformaldehyde (PF) in 0.1 M PBS with or without 5% sucrose. The brains were removed immediately, blocked in coronal planes, postfixed overnight in 4% PF in 0.1 M PBS, and cryoprotected in 20% glycerol, 0.1 M PBS for 24–48 hr. Blocks containing the basal ganglia and substantia nigra were frozen and cut on a sliding microtome at 40 μm into 50 mM Tris-buffered saline (TBS; pH 7.4). Serial floating sections were processed for immunohistochemistry.

Antisera. Affinity-purified polyclonal IP_3R antisera were produced in rabbits and in a goat immunized with purified IP_3R, as described elsewhere (Supattapone et al., 1988; Peng et al., 1991; Sharp et al., in press). Mouse monoclonal antibodies for PLCβ and PLCγ were gifts from Dr. S. G. Rhee, and those for calbindin D_{28K}, for TH, and for substance P were purchased from Sigma, Boehringer Mannheim, and Sera Lab, respectively. Antiserum against met-enkephalin was kindly contributed by Dr. R. P. Elde or purchased from Incstar. For both Western blot analysis and immunohistochemistry, the following dilutions of antisera were used: IP_3R, 1:500; PLCβ and PLCγ, 1:200; calbindin D_{28K}, 1:1000; TH, 1:200; substance P, 1:1000; met-enkephalin, 1:2000.

Western blotting. To test the selectivity of the IP_3R, PLCβ, and PLCγ antisera in the monkey brain, Western blot analysis was carried out. Briefly, samples of frozen monkey forebrain were homogenized, and their protein content was assayed using Pierce BCA reagent. Equal quantities of protein were subjected to SDS-PAGE using 7.5% polyacrylamide gels, and the separated proteins were transferred to Immobilon-P membranes. The blots were pretreated with 3% bovine serum albumin (BSA) in TBS for 2 hr before an overnight incubation at 4°C with IP_3R, PLCβ, and PLCγ antisera (dilutions: 1:500, 1:200, 1:200, respectively) with 3% BSA in TBS. The blots were then washed three times for 20 min each with 3% BSA in TBS, and were incubated with horseradish peroxidase–linked secondary antibodies (1:1500) for 1 hr at room temperature. After three washes of 10 min each in TBS, the blots were developed using 4-chloro-1-naphthol as the substrate.

Immunohistochemistry. All steps were carried out in TBS and immunostaining for each antiserum was performed under the following conditions. Serial transverse sections were permeabilized in 0.4% Triton X-100 for 30 min and pretreated for an additional 30 min in 4% normal goat serum (NGS) or, for sections to be incubated in goat IP_3R antiserum, in normal rabbit serum (NRS) or, for sections to be incubated in PLCβ, PLCγ, or calbindin D_{28K}, in normal horse serum (NHS) containing 0.1% Triton X-100. They were then incubated overnight in primary antiserum (see above for dilutions) containing 2% NGS (or 2% NRS for IP_3R or 2% NHS for PLCβ, PLCγ, or calbindin D_{28K}), 0.1% Triton, and 0.02% sodium azide at 4°C.

After three washes of 10 min each in TBS, sections were incubated with biotinylated secondary anti-goat or anti-rabbit antibodies (1:200) (ABC elite kits of Vector Laboratories, Burlingame, CA) in 0.1% Triton X-100 at room temperature for 1 hr. Sections were washed 3 × 10 min in TBS and incubated in an avidin-biotin-peroxidase complex (1:50) for 45 min. Finally, they were washed (3 × 10 min) and developed in the presence of 0.02% hydrogen peroxide with diaminobenzidine as the chromogen. Sections were rinsed thoroughly and mounted on subbed glass slides, dehydrated, and coverslipped with Permount. Striosomes were identified as zones with few neurons expressing calbindin D_{28K} or enkephalin, or by enrichment in substance P–like immunoreactivity.

Controls for immunocytochemical staining for the IP_3R antibody were prepared by preadsorbing with excess (100-fold) IP_3R protein. Control sections for antisera to PLCβ, PLCγ, calbindin D_{28K}, TH, substance P, and met-enkephalin were prepared by incubating sections in the appropriate pre-immune serum, respectively.

The density of immunostaining for IP_3R, PLCβ, and PLCγ was arbitrarily assigned value based on visual inspection of sections from all immunostaining experiments, with the highest densities being assigned a value of 5 and the lowest densities a value of 0.

Results

Characterization of immunoreactivity for IP_3R, PLCβ, and PLCγ. For immunohistochemical localization of IP_3R, we employed two antisera, raised in rabbit and in goat, against IP_3R protein purified from rat cerebellum (Supattapone et al., 1988; Sharp et al., in press). Both of these antisera have been shown to react selectively and with considerable potency with IP_3R protein in a range of vertebrate species including salamander, rat, guinea pig, monkeys, and humans (Peng et al., 1991). PLCβ and PLCγ were detected with murine monoclonal antibodies prepared against the two forms of PLC purified from bovine brain (Ryu et al., 1987). These antibodies have been shown to bind selectively to PLCβ and PLCγ in rat brain (Gerfen et al., 1988).

In Western blots of rhesus monkey forebrain extracts, the goat antiserum to IP_3R stained one band at about 260 kDa, corresponding to the known molecular weight of IP_3R monomers (Supattapone et al., 1988) (Fig. 1). Similar staining with the rabbit antiserum marked an identical 260 kDa band (data not shown). The monomers for PLCβ and PLCγ are about 145–150 kDa (Ryu et al., 1987). Western blot analysis of the same monkey forebrain extracts with monoclonal antibodies against these two enzymes showed single prominent bands at about 145–150 kDa.

To establish the validity of the IP_3R immunohistochemical staining, we incubated some sections with IP_3R antiserum preadsorbed with IP_3R protein. The preadsorption eliminated immunostaining almost completely (Fig. 2*B*). Immunostaining of rat brain with the monoclonal antibodies to PLCβ and PLCγ employed here has been shown to be eliminated by preadsorption with the corresponding proteins (Gerfen et al., 1988). We observed no immunoreactivity for PLCβ and PLCγ control in sections incubated in preimmune mouse serum (Fig. 2*D,F*).

IP_3R, PLCβ, and PLCγ distributions in the striatum. Im-

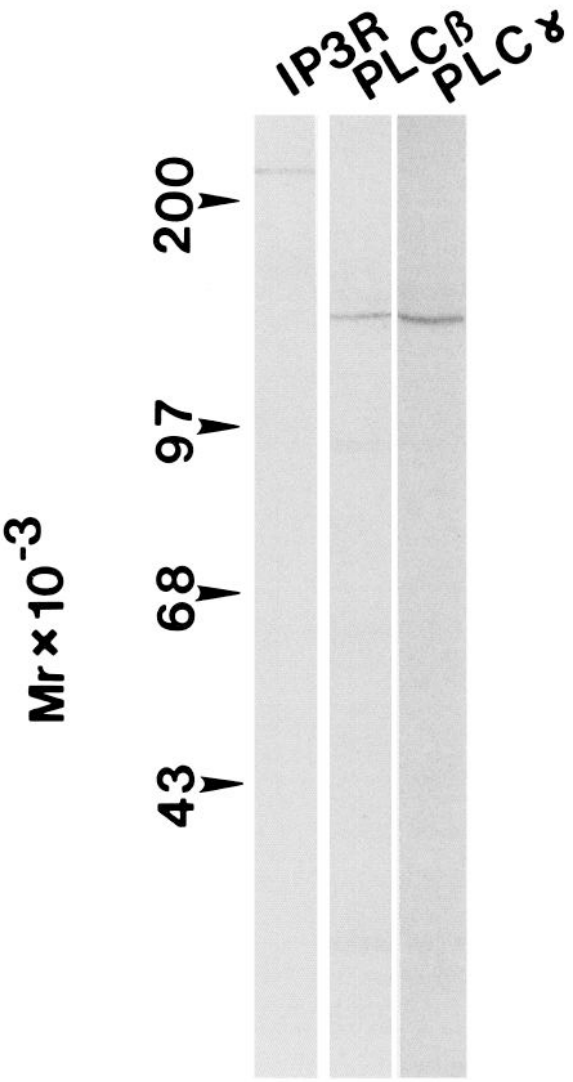

Figure 1. Characterization of immunoreactivity for IP_3R, PLCβ, and PLCγ by Western blot analysis of rhesus monkey forebrain extracts. Antisera against IP_3R, PLCβ, and PLCγ recognize single bands with approximate molecular weights of 260 and 150 kDa, respectively, corresponding to molecular weights of IP_3R and PLC proteins.

munostaining for IP_3R, PLCβ, and PLCγ appeared in the caudate nucleus, putamen, and ventral striatum in each of the monkey brains. Immunostaining with each of the antisera appeared in both the neuropil and cell bodies (Figs. 3, 4; Table 1). The abundance and appearance of the IP_3R-, PLCβ-, and PLCγ-immunoreactive cells suggested that these proteins were mainly in medium-sized neurons of the striatum. Immunostaining for PLCβ tended to be stronger than that for PLCγ (Figs. 2, 3), as noted also by Gerfen et al. (1988) in the rat. The ventral striatum expressed higher levels of immunoreactivity for each of the proteins than did the dorsal striatum (data not shown). In some monkeys, the immunostaining within the caudate nucleus tended to be highest medially (data not shown).

In the caudate nucleus especially, immunostaining with all three antisera was distinctly inhomogeneous (Figs. 2–4). Variably shaped patches of elevated staining were distributed through rostral and dorsal parts of the nucleus. Comparisons among nearly adjacent sections showed that the zones of heightened immunostaining for IP_3R, PLCβ, and PLCγ were spatially aligned

Table 1. Distribution of immunoreactivity for IP_3R, PLCβ, and PLCγ in perikarya and neuropil of the primate basal ganglia

	IP_3R		PLCβ		PLCγ	
	Perikarya	Neuropil	Perikarya	Neuropil	Perikarya	Neuropil
Striatum	2–4	4	5	5	3	3
GPe and GPi	0	4	0	4	0	4
SNc	4	2	4	2	4	2
SNr	0	4	0	4	2	4
Lateral medullary lamina	0	0	0	0	2	0

Data show a distinct pattern of distribution of IP_3R, PLCβ, and PLCγ immunoreactivity in the primate basal ganglia. In striatum and SNc, IP_3R, PLCβ, and PLCγ are present in both perikarya and neuropil. With the exception of the presence of PLCγ immunoreactivity in cell bodies of SNr, the immunoreactivity for these three proteins is limited primarily to the neuropil of striatal target nuclei, GPe, GPi, and SNr. Also, PLCγ, but not PLCβ or IP_3R, immunoreactivity occurs in magnocellular neurons of the intralaminar layer between striatum and GP (see Fig. 8). Throughout the basal ganglia, PLCβ immunoreactivity is denser, in any given area, than IP_3R and PLCγ, while PLCγ immunoreactivity is weaker, though more widespread, than IP_3R and PLCβ. In this table, based on visual inspection of all immunostaining experiments considered together, the highest and lowest densities of stainings are arbitrarily assigned values of 5 and 0, respectively.

with each other (Fig. 2). These zones of enriched immunostaining were most reliably identified with the PLCβ antiserum, with which immunostaining was most robust (Figs. 2, 4). Immunostaining for IP_3R, PLCβ, and PLCγ was also inhomogeneous in the putamen and ventral striatum, although the zones of heightened immunoreactivity were less discrete than those observed in the caudate nucleus.

To determine whether the zones enriched in these PI immunomarkers were aligned with striosomes in the caudate nucleus, we compared their locations with those of calbindin-poor zones (rhesus monkeys), substance P–enriched zones (rhesus monkeys), or enkephalin-poor zones (cynomolgus monkey, squirrel monkeys; data not shown) known to correspond to striosomes (Graybiel and Chesselet, 1984; Gerfen et al., 1985; Martin et al., 1991). As demonstrated in Figures 3 and 4 (and data not shown), there was close spatial correspondence between the striosomes and the regions expressing enhanced IP_3R, PLCβ (Figs. 3, 4), and PLCγ (data not shown) immunoreactivity. This was true both in the more medial parts of the caudate nucleus and in the middle and lateral parts of the nucleus. For some of the zones, the IP_3R immunostaining seemed more intense in the peripheral parts of the striosomes. Some of the dorsal PI-enriched zones were bordered by thin rims of reduced staining (Fig. 2). These variations were not always noticeable, but the stronger neuropil staining of the striosomes compared to nearby matrix was always pronounced in well-stained sections.

IP_3R, PLCβ, and PLCγ immunostaining appeared in numerous neuronal cell bodies of medium size (Figs. 4*C*, 5), including large numbers of medium-sized spiny neurons. The number of immunostained cell bodies appeared similar in the striosome and matrix compartments with the IP_3R, PLCβ (Figs. 3, 4), and PLCγ (data not shown) antisera. The relative enrichment of IP_3R, PLCβ, and PLCγ staining within the striosomes seemed primarily associated with staining of the neuropil. With this general enhancement of the staining intensity, we could not determine whether, in addition, the immunostaining of individual perikarya was also enhanced.

IP_3R, PLCβ, and PLCγ immunostaining in the substantia

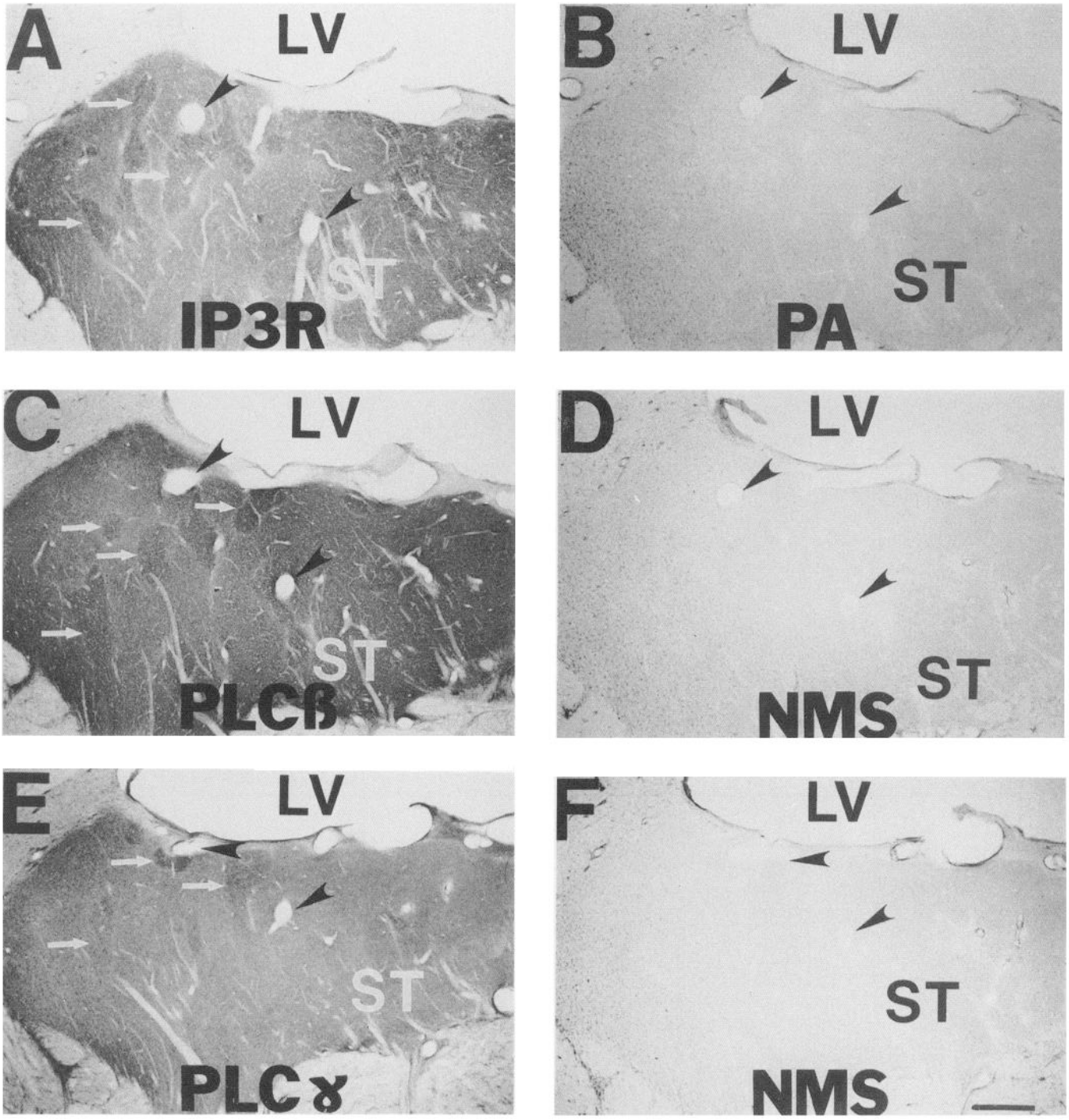

Figure 2. Specificity of immunostaining with IP_3R, PLCβ, and PLCγ antisera. *A, C,* and *E* illustrate similar patterns of immunostaining with IP_3R (*A*), PLCβ (*C*), and PLCγ (*E*) antisera in near-serial sections through the caudate nucleus (*ST*) of a macaque monkey. Note patchy zones of dense staining (examples at *Arrows*). Immunostaining is absent in near-adjacent control sections treated with IP_3R antiserum preabsorbed with IP_3R protein (PA; *B*) or with normal mouse serum (NMS; *D, F*). *Arrowheads* mark blood vessels used as landmarks. *LV,* lateral ventricle. The medial aspect of the caudate is by the lateral ventricle and the dorsal aspect of the caudate is on the right side of the figure. Scale bar, 200 μm.

nigra and GP. In the substantia nigra, IP_3R immunoreactivity was dense in the neuropil of both SNc and SNr (Fig. 6). In SNc, weak IP_3R-like immunoreactivity was also visible in many perikarya (Fig. 7). The distribution and sizes of the IP_3R-positive cells were similar to those of neurons immunostained for TH (Fig. 7). These observations suggest that IP_3R may occur in all or most all of the dopamine-producing neurons of the SNc, as well as in nigra neuropil, especially in SNr (Table 1).

Earlier immunohistochemical localization of PLCβ and PLCγ in the rat's substantia nigra demonstrated staining for both enzymes in neural perikarya in SNc and in fibers in SNr. Some neurons in SNr were stained for PLCγ but not PLCβ (Gerfen et al., 1988). In the primate substantia nigra, large numbers of neurons in SNc were immunopositive for PLCβ (Figs. 5–7, Table 1), but staining for PLCγ was only slightly above background (data not shown). In SNr, immunostaining for both enzymes

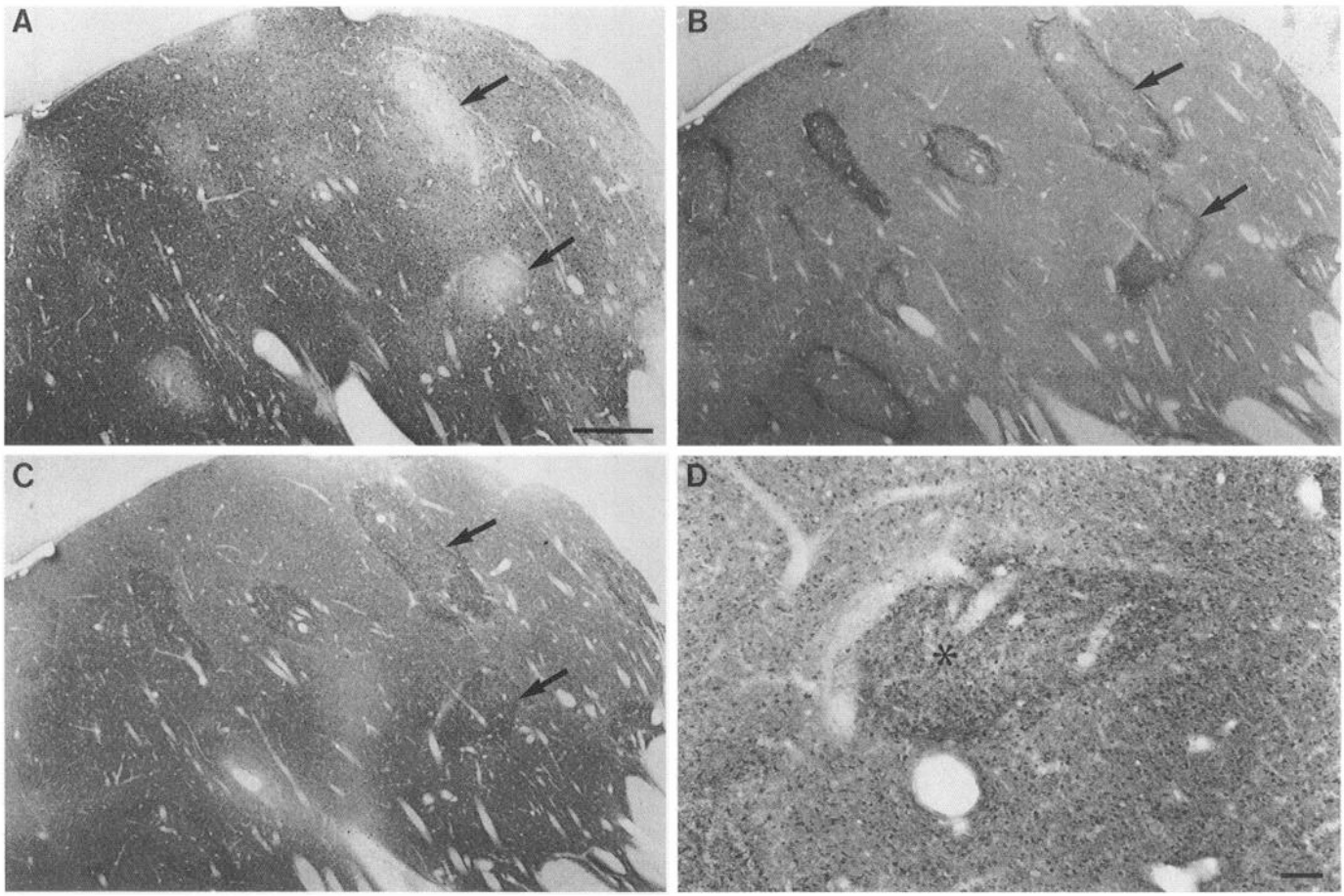

Figure 3. Near-adjacent coronal sections through the head of the caudate nucleus in rhesus monkey demonstrate the striosomal enrichment of IP_3R. *A* and *B*, Striosomal and matrix compartments were identified by the distributions of calbindin (*A*) and substance P (*B*) immunoreactivities. *Arrows* point to two striosomes. *C*, IP_3R immunoreactiviity is relatively enriched in striosomes (*arrows*) as compared to the surrounding matrix. *D*, Both striosomal and matrix compartments contain numerous IP_3R-immunoreactive neuronal cell bodies in apparently equivalent abundances within the two compartments. The relative enrichment of IP_3R is associated with diffuse staining within the neuropil of striosomes (*asterisk*). Scale bars: *A–C*, 583 μm; *D*, 70 μm.

was abundant in the neuropil, but evidence for weak cellular immunostaining was only seen with the PLCβ.

In the GP, staining for IP_3R, PLCβ, and PLCγ was present in both segments and largely confined to the neuropil (Fig. 8, Table 1). Although immunostaining within GP appeared similar with the three antisera, the fiber lamina separating GP from striatum showed different staining for PLCβ, PLCγ, and IP_3R (Fig. 8, Table 1). Very little staining in cells or neuropil was apparent in sections stained for IP_3R or PLCβ (Fig. 7*A,B*). By contrast, the fibrous layer contained abundant PLCγ-immunopositive large neurons and neuropil. There were no PLCγ-positive cells detectable in the fiber layer between GPe and GPi.

Discussion

The major finding of this study lies in the differential striosome–matrix localizations of markers of the PI system, IP_3R and two isoforms of PLC. These three proteins, as judged by immunohistochemistry, occur in both striosomes and matrix, but are more strongly expressed in striosomes than in most parts of the matrix. The differences in staining intensities mainly seem to reflect differences in the neuropil, which may reflect an enrichment of these proteins in subsets of dendritic spines and/or presynaptic axonal terminals. This compartmental difference in the representation of a major second messenger system in striosomes and matrix strongly reinforces the idea that these compartments are distinct functional entities in the striatum.

The general cellular patterns of immunostaining for PLCβ, PLCγ, and IP_3R in the primate basal ganglia appear similar to those in the rat; that is, these proteins are mainly within medium-sized neurons. They also are in accord with the distributions of mRNAs for these proteins in the rat brain (Ross et al., 1989; C. A. Ross et al., unpublished observations). PLCβ and IP_3R tend to be strongly represented, whereas PLCγ is detectable in lesser amounts (Gerfen et al., 1988; Ross et al., 1989). The main difference between monkey and rat in the distributions of these PI cycle markers lies in the striosomal compartmentalization of these proteins in the monkey striatum. No such compartmentalization has been detected in the rat brain (Gerfen et al., 1988; Sharp et al., in press; M. Fotuhi, T. M. Dawson, and S. H. Snyder, unpublished observations). A similar sharpening of neurochemical compartments from rat to monkey (and to human) has been reported previously for many striatal transmitters and receptors (a notable exception being μ-opiate receptors as detected by ligand binding) and may reflect the increased specialization of subgroups of striatal neurons with respect to their connections and/or functions (Graybiel and Ragsdale, 1983; Graybiel, 1990; Martin et al., 1991).

Regional variations in a number of other neurotransmitter-

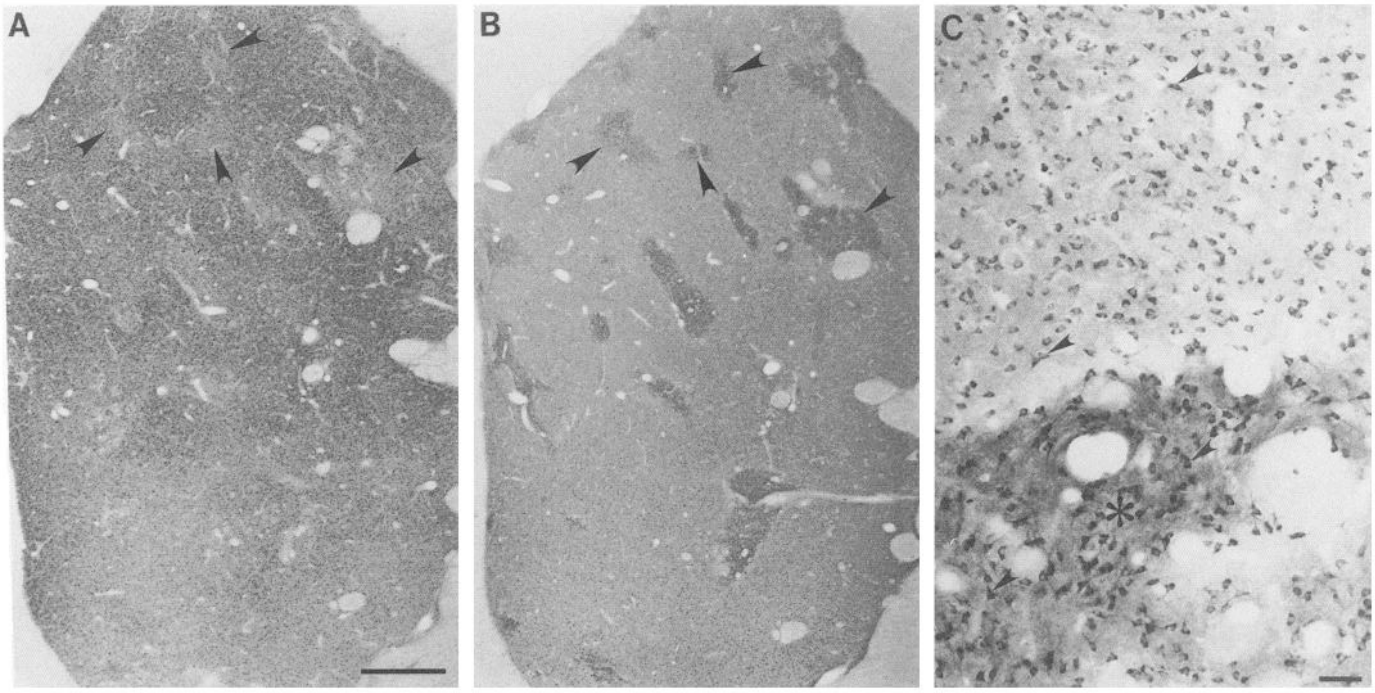

Figure 4. Evidence that striosomes in rhesus monkey caudate nucleus are enriched in PLCβ. *A* and *B*, The heightened PLCβ immunostaining of striosomes is illustrated in near-adjacent sections stained for calbindin D_{28K} (*A*) and PLCβ (*B*). *Arrowheads* identify examples of corresponding striosomes. *C*, High-magnification photomicrograph showing that in striosomal and matrix compartments, most medium-sized neurons are PLCβ-positive examples (*arrowheads*), whereas the neuropil within striosomes (*asterisk*) has more intense PLCβ immunoreactivity than the surrounding matrix. Scale bars: *A* and *B*, 563 μm; *C*, 40 μm.

related substances in the striatum have been observed previously in many species (see, e.g., Graybiel and Ragsdale, 1983; Besson et al., 1988; Chesselet et al., 1991; Martin et al., 1991). In some monkeys we also noted regional variations, in that the medial and ventral parts of the striatum showed substantially greater intensities of immunostaining for IP_3R, PLCβ, and PLCγ than the lateral parts.

What might be the functional significance of the selective distribution of the PI system in the striatum? The largest neuronal input to the striatum is composed of fibers from the cerebral cortex, which release glutamate or a closely related amino acid. Glutamate can interact with metabotropic receptors linked to the PI system and with ionotropic receptors of NMDA and AMPA/kainate subtypes (see Miller, 1991, for a review). Conceivably, the greater density of immunoreactivity for PI markers in striosomes reflects an association with postsynaptic metabotropic glutamate receptors or other G-protein–coupled receptors. Interestingly, a difference in ionotropic glutamate receptor representation in striosomes and matrix has recently been reported on the basis of ligand binding autoradiography (Dure et al., 1992). Autoradiographic localization of the metabotropic glutamatergic receptors is not readily performed by direct ligand binding, and the molecular cloning of this receptor has only recently been published (Houamed et al., 1991; Masu et al., 1991), so the striosome–matrix disposition of its corresponding mRNA or immunoreactivity remains unknown. The striatal regions with heightened immunoreactivity for the PI system could also coincide with specializations in cortical or other regions projecting especially strongly to the striosomes.

The differential representation of PI markers in the striosomes clearly could also reflect the distribution of nonglutamatergic receptors. More than 20 different cell membrane receptors are known to be linked to the PI system (Chuang, 1989). Many such neurotransmitter and neuropeptide receptors including substance P are abundant in the striatum and have compartmentalized and regionally graded distributions (Graybiel, 1990). In fact, considering the variety of PI-linked receptors in the striatum, it is of particular interest that PI markers are not uniformly distributed. Their differential representation suggests that the actions of a number of receptor types may converge on the same second messenger system.

These compartmental patterns of distribution of PI markers may also be significant in relation to the distribution of other second messenger systems. In the rat, complementary localizations in the brain have been noted for adenylyl cyclase monitored by ^{3}H-forskolin binding, for PKC by ^{3}H-phorbol ester binding, and for IP_3R by ^{3}H-IP_3 binding (Worley et al., 1986a). Recently, we have localized adenylyl cyclase catalytic activity by a novel histochemical stain, and have observed selectively high concentrations in the matrix (M. Fotuhi, T. M. Dawson, A. Verma, and S. H. Snyder, unpublished observations). Thus, the cAMP and PI systems may both be differentially distributed across the striosome–matrix system of the striatum, and their distributions may in part be complementary.

The expression of PI-associated proteins by neurons and processes in the striatum and substantia nigra may in part reflect interconnections between these regions. Neurons in striosomes are thought to project mainly to SNc, or at least to the medial part of the substantia nigra. The axon terminals of striosomal cells may contribute to the limited amount of neuropil staining for IP_3R, PLCβ, and PLCγ present in SNc. Neurons of the matrix project preferentially to GP and SNr, and it is likely that

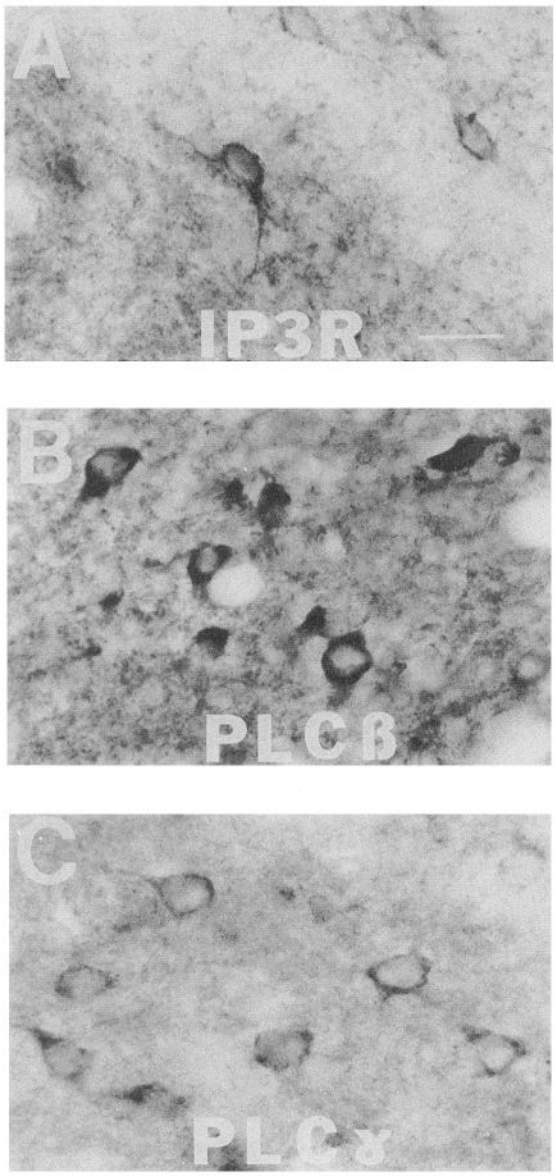

Figure 5. Cellular distribution of immunoreactivity for IP_3R, PLCβ, and PLCγ (*A, B,* and *C,* respectively) appear abundantly in medium-sized striatal neurons. The immunostaining with each antiserum is also present in the neuropil. PLCβ staining (*B*) is substantially more intense than that of IP_3R or PLCγ. Scale bar, 20 μm.

the PI marker–rich neurons of the matrix are associated with the IP_3R-, PLCβ-, and PLCγ-positive terminals fields in these regions. Finally, the immunostained neurons in SNc are likely to be dopamine-containing cells, as their appearance is similar to that of TH-immunostained cells in adjacent sections. Terminals from these neurons presumably could contribute to the neuropil in the striatum that is immunopositive for IP_3R, PLCβ, and PLCγ.

In most of the regions we examined, the relative patterns of staining for IP_3R, PLCβ, and PLCγ were quite similar. The one striking exception was in the fiber layer between the putamen and external pallidum, where substantial staining for PLCγ appeared in magnocellular neurons, but where there was only negligible staining for PLCβ or IP_3R. This difference was particularly notable, because the intensity of PLCβ staining exceeded

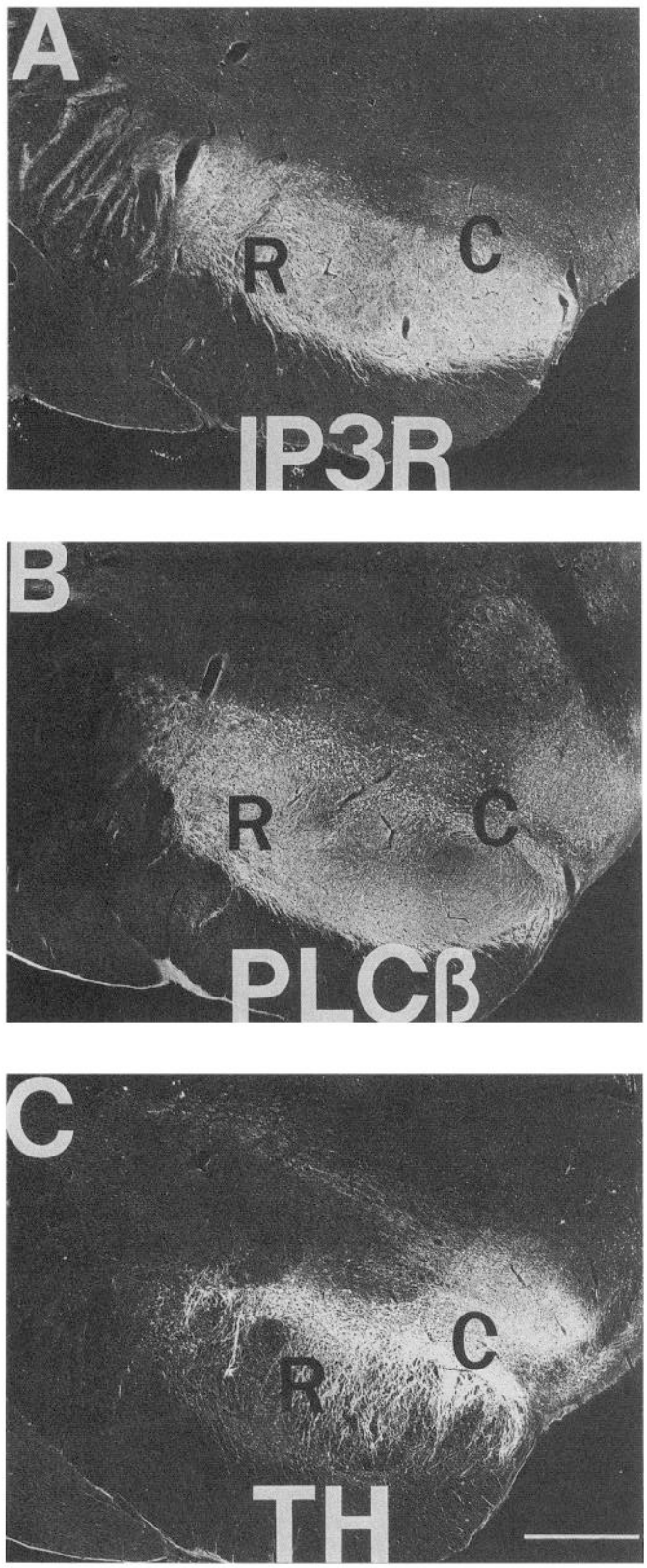

Figure 6. IP_3R and PLCβ immunostaining in substantia nigra. In SNc, IP_3R and PLCβ are present in virtually all neurons, and these have distributions similar to those of TH-positive neurons in a nearby section (*C*). However, the characteristic ventrally extending TH-positive dendrites of SNc neurons lack appreciable staining in sections treated with IP_3R and PLCβ antisera (*A* and *B,* respectively). *C,* SNc; *R,* SNr. Scale bar, 100 μm.

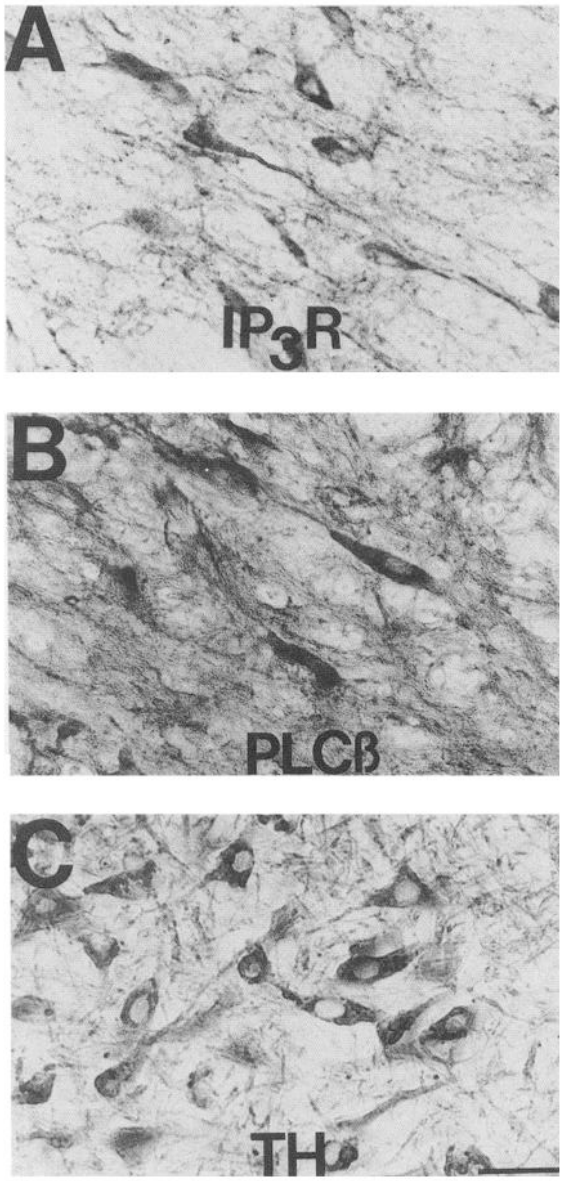

Figure 7. Cellular distribution of IP_3R and PLCβ immunostaining in SNc. PLCβ immunoreactivity (*B*) appears denser than that of IP_3R (*A*) but weaker than that of TH staining (*C*) in sections treated by similar protocols. Scale bar, 100 μm.

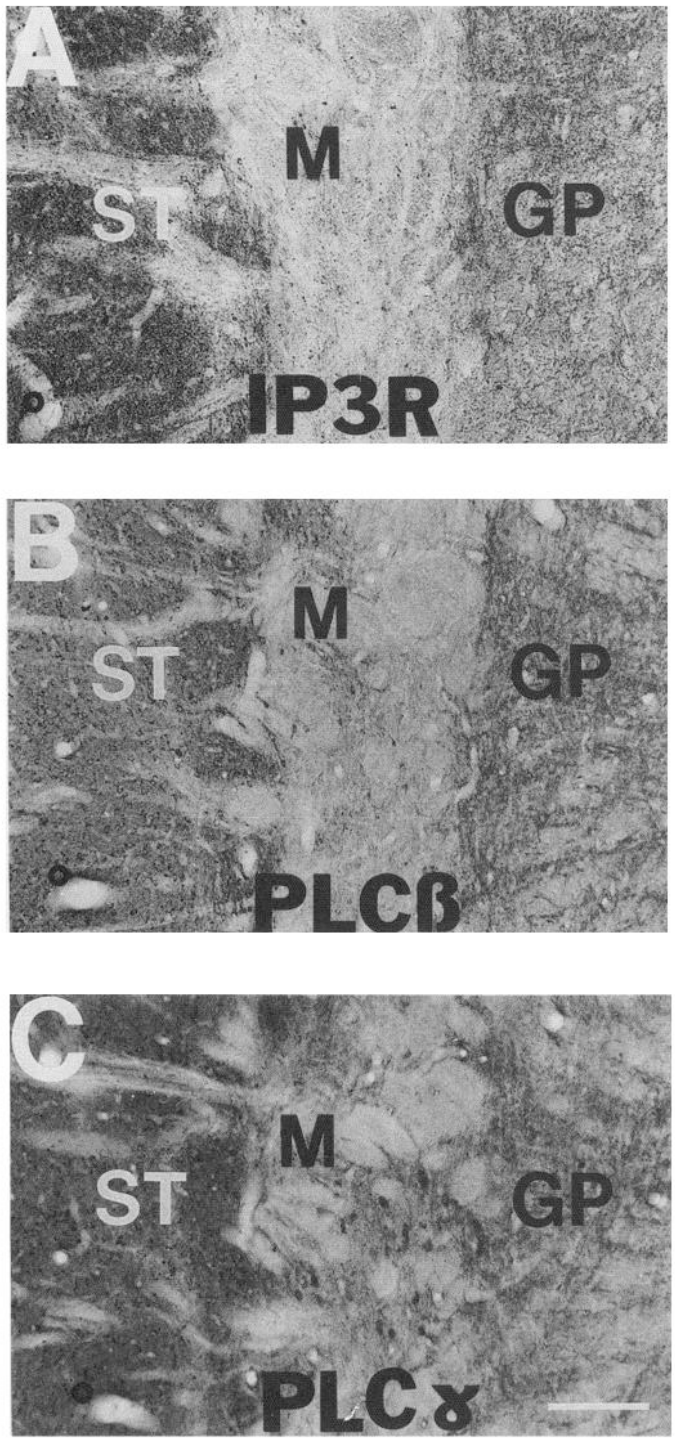

Figure 8. Evidence that PLCγ immunostaining does occur in the absence of detectable IP_3R and PLCβ immunostaining. Magnocellular neurons in the medullary lamina (*M*) separating the putamen (*ST*) from GP express PLCγ immunoreactivity (*C*), but lack IP_3R (*A*) or PLCβ (*B*) immunoreactivity. Also note that striatal labeling for all three epitopes is enriched in both perikarya and neuropil, whereas staining in *GP* is limited to the neuropil. Scale bar, 200 μm.

that of PLCγ in almost all regions of the basal ganglia proper. The discrepancy highlights the heterogeneity within the extrapyramidal PI second messenger system. Previous immunohistochemical studies in the rat have displayed marked regional differences in the distribution of subtypes of PKC (Huang et al., 1988; Yoshihara et al., 1991) and some differences between patterns of PLCβ and PLCγ immunostaining (Gerfen et al., 1988). Subtypes of IP_3R derived from alternative splicing (Danoff et al., 1991; Nakagawa et al., 1991) or from distinct genes (Sudhof et al., 1991; Ross et al., 1992) are not equally represented in all brain regions. It will be interesting to learn whether different subtypes of IP_3R also are differentially distributed in the basal ganglia and allied nuclei. If so, some of the selective staining patterns we have observed could be related to such differences in receptor subtype expression.

References

Albin RL, Young AB, Penney JB (1989) The functional anatomy of basal ganglia disorders. Trends Neurosci 12:366–375.

Besson MJ, Graybiel AM, Natsuk M (1988) SCH 23390 ligand binding to D_1 dopamine receptors in the basal ganglia of the cat and primate:

delineation of striosomal compartments and pallidal and nigral subdivision. Neuroscience 26:101–119.
Besson MJ, Graybiel AM, Quinn B (1990) Coexpression of neuropeptides in the cat's striatum: an immunohistochemical study of substance P, dynorphin B and enkephalin. Neuroscience 39:33–58.
Chesselet M-F, Gonzales C, Levitt P (1991) Heterogeneous distribution of the limbic system–associated membrane protein in the caudate nucleus and substantia nigra of the cat. Neuroscience 40:725–733.
Chuang D-M (1989) Neurotransmitter receptors and phosphoinositide turnover. Annu Rev Pharmacol Toxicol 29:71–110.
Danoff SK, Ferris CD, Donath C, Fischer G, Munemitsu S, Ullrich A, Snyder SH, Ross CA (1991) Inositol 1,4,5-trisphosphate receptors: distinct neuronal and non-neuronal forms derived by alternative splicing differ in phosphorylation. Proc Natl Acad Sci USA 88:2951–2955.
Donoghue JP, Herkenham M (1986) Neostriatal projections from individual cortical fields conform to histochemically distinct striatal compartments in the rat. Brain Res 365:397–403.
Dure LS IV, Penny JB, Young AB (1992) Compartmentalization of excitatory amino acid receptors in human striatum. Proc Natl Acad Sci USA 89:7688–7692.
Fishell G, van der Kooy D (1987) Pattern formation in the striatum: developmental changes in the distribution of striatonigral neurons. J Neurosci 7:1969–1978.
Flaherty AW, Graybiel AM (1991) Corticostriatal transformations in the primate somatosensory system. Projections from physiologically mapped body-part representations. J Neurophysiol 66:1249–1263.
Gerfen CR (1984) The neostriatal mosaic: compartmentalization of corticostriatal input and striatonigral output systems. Nature 311:461–464.
Gerfen CR, Baimbridge KG, Miller JJ (1985) The neostriatal mosaic: compartmental distribution of calcium binding protein and parvalbumin in the basal ganglia of the rat and monkey. Proc Natl Acad Sci USA 82:8780–8784.
Gerfen CR, Baimbridge KG, Thibault J (1987) The neostriatal mosaic: III. Biochemical and developmental dissociation of patch-matrix mesostriatal systems. J Neurosci 7:3935–3944.
Gerfen CR, Choi WC, Suh PG, Rhee SG (1988) Phospholipase C I and II brain isozymes: immunohistochemical localization in neuronal systems in rat brain. Proc Natl Acad Sci USA 85:3208–3212.
Giménez-Amaya JM, Graybiel AM (1991) Modular organization of projection neurons in the matrix compartment of the primate striatum. J Neurosci 11:779–791.
Graybiel AM (1990) Neurotransmitters and neuromodulators in the basal ganglia. Trends Neurosci 13:244–254.
Graybiel AM, Chesselet MF (1984) Compartmental distribution of striatal cell bodies expressing met-enkephalin–like immunoreactivity. Proc Natl Acad Sci USA 81:7980–7984.
Graybiel AM, Ragsdale CW Jr (1978) Histochemically distinct compartments in the striatum of human, monkey and cat demonstrated by acetylcholinesterase staining. Proc Natl Acad Sci USA 75:5723–5726.
Graybiel AM, Ragsdale CW Jr (1983) Biochemical anatomy of the striatum. In: Chemical neuroanatomy, pp 427–504. New York: Raven.
Graybiel AM, Ragsdale CW, Moon Edley S (1979) Compartments in the striatum of the cat observed by retrograde cell-labeling. Exp Brain Res 34:189–195.
Graybiel AM, Baughman RW, Eckenstein F (1986) Cholinergic neuropil of the striatum observes striosomal boundaries. Nature 323:625–627.
Herkenham M, Pert CB (1981) Mosaic distribution of opiate receptors, parafascicular projections and acetylcholinesterase in rat striatum. Nature 291:415–418.
Houamed KM, Kuijper JL, Gilbert TL, Haldeman BA, O'Hara PJ, Mulvihill ER, Almers W, Hagen FS (1991) Cloning, expression, and gene structure of a G protein–coupled glutamate receptor from rat brain. Science 252:1318–1321.
Huang FL, Yoshida Y, Nakabayashi H, Young WS III, Huang K-P (1988) Immunocytochemical localization of protein kinase C isozymes in rat brain. J Neurosci 8:4734–4744.
Jiménez-Castellanos J, Graybiel AM (1987) Subdivisions of dopamine-containing A8-A9-A10 complex identified by their differential mesostriatal innervation of striosomes and extrastriosomal matrix. Neuroscience 23:223–242.
Jiménez-Castellanos J, Graybiel AM (1989) Compartmental origins of striatal efferent projections in the cat. Neuroscience 32:297–321.
Joyce JN, Sapp DW, Marshall JF (1986) Human striatal dopamine receptors are organized in compartments. Proc Natl Acad Sci USA 83:8002–8006.
Martin LJ, Hadfield MC, Dellovade TC, Price DL (1991) Striatal mosaic in primates: patterns of neuropeptide immunoreactivity differentiate the ventral striatum from dorsal striatum. Neuroscience 43:397–417.
Masu M, Tanabe Y, Tsuchida K, Shigemoto R, Nakanishi S (1991) Sequence and expression of a metabotropic glutamate receptor. Nature 349:760–765.
Miller JM (1991) Metabotropic excitatory amino acid receptors reveal their true colors. Trends Pharmacol Sci 12:365–367.
Nakagawa T, Okano H, Furuichi T, Aruga J, Mikoshiba K (1991) The subtypes of the mouse inositol 1,4,5-trisphosphate receptor are expressed in tissue-specific and developmentally specific manner. Proc Natl Acad Sci USA 88:6244–6248.
Nakanishi S, Maeda N, Mikoshiba K (1991) Immunohistochemical localization of an inositol 1,4,5-triphosphate receptor, P_{400}, in neural tissue: studies in developing and adult mouse brain. J Neurosci 11:2075–2086.
Peng Y-W, Sharp AH, Snyder SH, Yau K-W (1991) Localization of the inositol 1,4,5-trisphosphate receptor in synaptic terminals in the vertebrate retina. Neuron 6:525–531.
Pert CB, Kuhar MJ, Snyder SH (1976) Opiate receptor: autoradiographic localization in rat brain. Proc Natl Acad Sci USA 73:3729–3733.
Ragsdale CW, Graybiel AM (1988) Fibers from the basolateral nucleus of the amygdala selectively innervate striosomes in the caudate nucleus of the cat. J Comp Neurol 269:506–522.
Ragsdale CW, Graybiel AM (1990) A simple ordering of neocortical areas established by the compartmental organization of their striatal projections. Proc Natl Acad Sci USA 87:6196–6199.
Ross CA, MacCumber MW, Glatt CE, Snyder SH (1989) Brain phospholipase C isozymes: differential mRNA localizations by *in situ* hybridization. Proc Natl Acad Sci USA 86:2923–2927.
Ross CA, Danoff SK, Schell MJ, Snyder SH, Ullrich A (1992) Novel inositol 1,4,5-trisphosphate receptors: molecular cloning and differential localization in brain and peripheral tissues. Proc Natl Acad Sci USA 89:5265–5269.
Ryu SH, Cho KS, Lee K-Y, Suh P-G, Rhee SG (1987) Purification and characterization of two immunologically distinct phosphoinositide-specific phospholipase C from bovine brain. J Biol Chem 262:12511–12518.
Saito N, Kikkawa U, Nishizuka Y, Tanaka C (1988) Distribution of protein kinase C–like immunoreactive neurons in rat brain. J Neurosci 8:369–382.
Sharp AH, Dawson TM, Ross CA, Fotuhi M, Mourey RJ, Snyder SH (in press) Inositol 1,4,5-triphosphate receptors: immunohistochemical localization to discreet areas of rat brain. Neuroscience, in press.
Sudhof TC, Newton CL, Archer BT III, Mignery GA (1991) Structure of a novel $InsP_3$ receptor. EMBO J 10:3199–3206.
Supattapone S, Worley PF, Baraban JM, Snyder SH (1988) Solubilization, purification and characterization of an inositol trisphosphate receptor. J Biol Chem 263:1530–1534.
Worley PF, Baraban JM, DeSouza EB, Snyder SH (1986a) Mapping second messenger systems in the brain: differential localizations of adenylate cyclase and protein kinase C. Proc Natl Acad Sci USA 83:4053–4057.
Worley PF, Baraban JM, Snyder SH (1986b) Heterogeneous localization of protein kinase C in rat brain: autoradiographic analysis of phorbol ester receptor binding. J Neurosci 6:199–207.
Worley PF, Baraban JM, Colvin JS, Snyder SH (1987) Inositol trisphosphate receptor localization in brain: variable stoichiometry with protein kinase C. Nature 325:159–161.
Worley PF, Baraban JM, Snyder SH (1989) Inositol 1,4,5-trisphosphate receptor binding: autoradiographic localization in rat brain. J Neurosci 9:339–346.
Yoshihara C, Saito N, Taniyama K, Tanaka C (1991) Differential localization of four subspecies of protein kinase C in the rat striatum and substantia nigra. J Neurosci 11:690–700.