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G_{olf} and G_s in Rat Basal Ganglia: Possible Involvement of G_{olf} in the Coupling of Dopamine D_1 Receptor with Adenylyl Cyclase

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Using specific antibodies and cDNA probes, we have investigated, in rat basal ganglia, the distribution and the regulation of the expression of the α subunits of G_s and G_{olf}, two GTP-binding proteins (G-proteins) that stimulate adenylyl cyclase. We confirmed that $G_{olf}\alpha$ is highly expressed in caudate-putamen, nucleus accumbens, and olfactory tubercle, whereas $G_s\alpha$ is less abundant in these areas than in the other brain regions. Intrastriatal injections of quinolinic acid decreased dramatically the levels of $G_{olf}\alpha$ protein in the striatum and the substantia nigra, and those of $G_{olf}\alpha$ mRNA in the striatum. Retrograde lesions of striatonigral neurons with volkensin reduced markedly the levels of D_1 dopamine (DA) binding sites, as well as those of $G_{olf}\alpha$ protein and mRNA in the striatum, without altering D_2 binding sites. In contrast, both types of lesions increased the levels of $G_s\alpha$ protein in the striatum and substantia nigra. Immunocytochemistry showed the presence of $G_{olf}\alpha$ protein in striatal medium-sized neurons and in several other neuronal populations. These results demonstrate that striatonigral neurons contain high levels of $G_{olf}\alpha$ and little, if any, $G_s\alpha$, suggesting that the coupling of D_1 receptor to adenylyl cyclase is provided by $G_{olf}\alpha$. The levels of $G_{olf}\alpha$ were five- to sixfold higher in the striatum than in the substantia nigra, indicating a preferential localization of $G_{olf}\alpha$ in the somatodendritic region of striatonigral neurons and providing a basis for the low efficiency of D_1 receptor coupling in the substantia nigra. Six weeks after 6-hydroxydopamine lesions of DA neurons, an increase in $G_{olf}\alpha$ (+53%) and $G_s\alpha$ (+64%) proteins was observed in the striatum. This increase in $G_{olf}\alpha$ levels may account for the DA-activated adenylyl cyclase supersensitivity, without change in D_1 receptors density, that follows destruction of DA neurons. Fine regulation of the levels of $G_{olf}\alpha$ in physiological or pathological situations may be a critical parameter for the efficiency of DA neurotransmission.

[Key words: G-protein, $G_{olf}\alpha$, $G_s\alpha$, D_1 receptor, striatum, substantia nigra, basal ganglia, dopamine, signal transduction, adenylyl cyclase, receptor supersensitivity]

Receptors with seven putative transmembrane domains are known to stimulate adenylyl cyclase via two homologous heterotrimeric G-proteins, G_s and G_{olf}, composed of α, β, and γ subunits and differing by their α subunits (Simon et al., 1991). Whereas G_s is found in numerous cell types (Gilman, 1987), G_{olf} is thought to mediate specifically the stimulation of type III adenylyl cyclase by odorant signal receptors in the olfactory neuroepithelium (Jones and Reed, 1989; Bakalyar and Reed, 1990; Menco et al., 1992). However, the recent demonstration of the presence of $G_{olf}\alpha$ mRNA in rat basal ganglia (Drinnan et al., 1991) suggests its possible involvement in the signal transduction cascade initiated by neurotransmitter-triggered receptors. One of the aims of the present study was to substantiate this possibility by studying the localization of $G_{olf}\alpha$ in striatonigral neurons, which express high levels of dopamine (DA) D_1 receptors (Gerfen et al., 1990; Harrison et al., 1990; Le Moine et al., 1991; Sibley and Monsma, 1992) (EEDQ).

Several lines of evidence suggest that the coupling efficiency of D_1 receptors with adenylyl cyclase could be a factor regulating the function of these receptors. In rat striatum, experiments with an irreversible D_1 blocker, *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline, have shown that 40% of D_1 receptors are "spare" receptors, not coupled to adenylyl cyclase, and have suggested that coupling could represent a limiting step in the D_1 receptor–dependent activation of adenylyl cyclase (Hess et al., 1987). Accordingly, long-lasting interruption of DA neurotransmission may increase the coupling efficiency of D_1 receptors in the striatum, since lesions of nigrostriatal DA neurons or chronic treatment with reserpine increases DA-stimulated adenylyl cyclase without changing the D_1 receptor density (Savasta et al., 1988; Hervé et al., 1989; Missale et al., 1989; Cowburn et al., 1991). The comparison of D_1 receptor densities and DA-sensitive adenylyl cyclase activities in several cerebral regions suggests also the existence of a regional variability in the coupling efficiency between these two proteins (Andersen et al., 1990). For instance, the DA-stimulated adenylyl cyclase activity is sevenfold higher in the striatum than in the substantia nigra, whereas the density of D_1 receptors labeled by ^{3}H-SCH23390 is similar in both structures (Hervé et al., 1992). Moreover, the density of D_1 receptors in high-affinity state for agonists, which is thought to correspond to the form associated with the G-protein (DeLean et al., 1980; Leff and Creese, 1985), is much lower in the substantia nigra than in the striatum (Hervé et al., 1992). These observations could be explained by variations in the levels of stimulatory G-proteins, which would be lower in the substantia nigra than in the striatum, and which would increase following long-lasting interruption of DA neurotransmission. In the present study, using cDNA and antibody probes specific for $G_s\alpha$ and $G_{olf}\alpha$, we demonstrate that $G_{olf}\alpha$ is enriched in striatonigral neurons, contrasting with $G_s\alpha$, which does not seem to be expressed in these neurons. We also show that $G_{olf}\alpha$ levels are

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severalfold higher in the striatum than in the substantia nigra, and that they increase following chronic lesions of DA neurons.

Materials and Methods

Animals. Male Sprague–Dawley rats (Charles River, Rouen, France) weighing 250–300 gm were used for this study. They were kept in five-animal cages in a stable 12:12 hr light/dark cycle with the temperature maintained at 22°C and the humidity at 60%.

Lesions. Chemical lesions with 6-hydroxydopamine (6-OHDA) and volkensin were carried out under ketamine anesthesia (Imalgene, Ifa-Mérieux; 150 mg/kg). The anesthetic was pentobarbital (Sanofi; 50 mg/kg) for quinolinic acid microinjections. Animals were positioned in a David Kopf stereotaxic apparatus (DK900) with the incisor bar placed 3.4 mm above the interaural line, and the various neurotoxic solutions were injected locally at a rate of 0.3 μl/min using a stainless steel cannula (0.4 mm diameter) implanted into the brain and connected to a Braun-Melsungen pump. Striatal neuronal cell bodies were lesioned by two unilateral injections of quinolinic acid (120 nmol in 1 μl of phosphate buffer, 50 mM, pH 7.4) in the anteromedial and the posterolateral parts of the neostriatum (Beal et al., 1986). Volkensin (kindly provided by Dr. F. Stirpe, University of Bologna; 1–2 ng in 0.2 μl of phosphate-buffered saline) was injected unilaterally into the substantia nigra, 3.6 mm caudal and 1.8 mm lateral to the bregma, 8.8 mm under the surface of the skull (Harrison et al., 1990). The nigrostriatal DA neurons were destroyed unilaterally by the injection of 6-OHDA (6 μg in 1.5 μl of solvent) into the medial forebrain bundle, 2.2 mm caudal and 1.6 mm lateral to the bregma, 8.4 mm under the surface of the skull (Hervé et al., 1989).

Adenylyl cyclase assays. Rat brains were frozen on a microtome stage refrigerated at −7°C, and sectioned into coronal 500-μm-thick slices. Tissue microdisks (32 μg of protein each) were punched out from the anteromedian striatum and from the substantia nigra, using a stainless steel cylinder (0.9 mm diameter; Hervé et al., 1989), and homogenized (1 mg protein/ml) in 2 mM Tris-maleate (pH 7.2) containing 2 mM EGTA and 300 mM sucrose, in a Potter Elvehjem apparatus. Adenylyl cyclase activity was measured at 30°C by the conversion of α-^{32}P-ATP into cyclic ^{32}P-AMP for 7 min (Bockaert et al., 1977). The assay medium (final volume, 40 μl) contained 10 μl of homogenate, Tris-maleate (25 mM, pH 7.2), ATP (0.5 mM), $MgSO_4$ (1 mM), GTP (0.5 μM), theophylline (10 mM), creatine phosphate (10 mM), creatine kinase (0.2 mg/ml), α-^{32}P-ATP (2 μCi), and cyclic ^{3}H-AMP (2 nCi) as an internal standard. When adenylyl cyclase activity was tested in the presence of various concentrations of $MnCl_2$, EGTA and $MgSO_4$ were omitted from the incubation medium. The cyclic ^{32}P-AMP formed was separated according to Salomon et al. (1974) and adenylyl cyclase activity was expressed in picomoles of cyclic AMP produced per minute per milligram of protein.

Antibodies. Rabbit antiserum RM/1 (batch number PB-042, New England Nuclear Research Products) was directed against a synthetic decapeptide (RMHLRQYELL) corresponding to the carboxy-terminus of $G_s\alpha$ and $G_{olf}\alpha$ proteins (Jones and Reed, 1989; Simonds et al., 1989). Specific antibodies against $G_{olf}\alpha$ and $G_s\alpha$ were obtained in the laboratory by immunizing rabbits against two synthetic peptides, CKTA-EDQGVDEKERREA-$_{CONH2}$ (serum SL6) and CKTEDQRNEE-KAQREA-$_{CONH2}$ (serum SL8), derived from the published sequence of $G_{olf}\alpha$ and $G_s\alpha$, respectively (Jones and Reed, 1989), with an added cysteine at the amino-terminal end, and glutaraldehyde-conjugated to keyhole limpet hemocyanin. The anti-peptide antibodies were purified by affinity chromatography (Girault et al., 1989a) on columns of Sulfolink gel (Pierce) to which the corresponding peptide was coupled using the extra amino-terminal cysteine.

Immunoblot analysis. Brains were frozen on dry ice after rapid dissection. Microdisk samples of the various cerebral areas were punched out from frozen tissue slices, as described above, and stored at −80°C until biochemical analysis. Micropunches were homogenized by sonication in 1% SDS at 100°C and boiled for 5 min. Protein concentrations were measured with a bicinchoninic acid–based method (Smith et al., 1985), using BSA as a standard. Equal amounts of protein (100–140 μg) were separated by SDS-polyacrylamide gel electrophoresis (PAGE; 10% acrylamide) and transferred electrophoretically to Hybond C membranes (Amersham). Transfer membranes were incubated with primary antibodies (RM/1, 1:1000; affinity-purified SL6, 4 μg/ml; affinity-purified SL8, 0.2 μg/ml) in Tris-buffered saline (Tris-HCl, 50 mM, pH 7.4; NaCl, 0.2 M) with 5% skimmed dry milk for 4 hr at room temperature. After washing, the fixed IgGs were revealed by ^{125}I-labeled protein A overlay (Girault et al., 1989b) and autoradiography, or with a peroxidase/chemiluminescence method (ECL, Amersham), according to the manufacturer's instructions, using MP autoradiographic film (Amersham) in both cases. For sequential immunoblotting, antibodies were eluted by incubation of the membranes in urea (8 M), 2-mercaptoethanol (0.1 M), and BSA (5 mg/ml) for 1 hr at 60°C (Erickson et al., 1982). Quantification of the bands intensity on autoradiographic films was achieved using a computer-assisted densitometer (Hervé et al., 1992).

cDNA probes. The $G_s\alpha$ probe was isolated from a murine cDNA library in the course of a differential screening procedure designed to identify developmentally regulated mRNAs (J. M. Studler, J. Glowinski, and M. Lévi-Strauss, unpublished observations). Sequence analysis indicated that the cDNA insert corresponded to the most 3′ distal 550-base pair (bp) fragment of the murine cDNA sequence described by Sullivan et al. (1986) (data not shown). $G_{olf}\alpha$ sequences were amplified from rat striatal mRNAs, using reverse transcriptase and PCR. The primers used for PCR corresponded to nucleotides 89–106 and 541–522 of the published rat $G_{olf}\alpha$ cDNA sequence (Jones and Reed, 1989). The 453-bp-long DNA fragment was cloned into a pBluescript plasmid vector (Stratagene), using Eco R1 linkers. Sequence analysis of this cDNA fragment indicated that it corresponded to the published sequence (Jones and Reed, 1989) except at four base positions. These differences, which created only two modifications in the deduced protein sequence (isoleucine 94 changed to valine and isoleucine 90 changed to leucine), could result from DNA polymorphism between different rat strains, or from PCR-induced mutations.

Northern blots. Total RNA was extracted by the guanidinium thiocyanate/phenol-chloroform method (Chomczynski and Sacchi, 1987) from fresh tissue or tissue microdisks prepared as described above. RNA (5–10 μg) was resolved on 1.2% agarose gels containing 3.7% formaldehyde and transferred by capillarity to a nylon membrane (Hybond N, Amersham), using standard procedures (Sambrook et al., 1989). The ethidium bromide-stained 18S and 28S ribosomal RNAs were photographed on the filter, under UV light, in order to evaluate the amounts of RNA loaded in each lane and to provide molecular weight markers. cDNA inserts were labeled by random priming (specific activity, 10^9 cpm/μg) with α-^{32}P-dCTP (Sambrook et al., 1989). The filters were exposed successively to $G_{olf}\alpha$ and $G_s\alpha$ probes, with a dehybridization step between the two hybridizations. Hybridization conditions were 16 hr at 65°C in the presence of 10^6 cpm/ml of probe, 5× SSC (1× SSC: 150 mM NaCl, 15 mM sodium citrate), 5× Denhardt's solution (1× Denhardt's solution: 0.02% Ficoll, 0.02% polyvinylpyrrolidone, 0.02% BSA), 200 μg/ml denaturated salmon sperm DNA, 50 mM sodium phosphate (pH 6.5), and 0.4% SDS. Membranes were washed in increasing stringency conditions to reach 0.1 × SSC and 0.1% SDS at 65°C and apposed to autoradiographic films (MP, Amersham) with intensifying screens at −80°C, for several days.

Binding of ^{3}H-SCH23390 and ^{125}I-sulpride to forebrain sections. After decapitation and dissection, the brains of four volkensin-treated rats were frozen by immersion for 1 min in isopentane maintained at −30°C with dry ice. Coronal sections (15 μm thick) were cut with a cryostat (Bright, Hundingdon, UK) at the rostral level of the neostriatum and mounted onto gelatin-coated glass slides. The remaining brain blocks were stored at −70°C for preparation of micropunches for Western and Northern blot analysis. Slide-mounted sections were incubated for 60 min at room temperature, in the presence of either ^{3}H-SCH23390 (2.5 nM; specific activity, 74 Ci/mmol) or ^{125}I-sulpride (0.2 nM; specific activity, 1000 Ci/mmol) in a buffer solution containing Tris-HCl (50 mM, pH 7.4), NaCl (120 mM), KCl (5 mM), $CaCl_2$ (1 mM), $MgCl_2$ (1 mM) (Savasta et al., 1986; Bouthenet et al., 1987). In order to prevent the binding of ^{3}H-SCH23390 to 5-HT_2 receptors, the incubation medium included 10 nM spiroperidol. The sections were washed five times for 2 min each in cold Tris-HCl buffer (50 mM, pH 7.4, 4°C) and dried under an air stream. The exposure times for autoradiography on Ultrofilm ^{3}H films (Reichert-Jung) were 2 d for ^{125}I-sulpride and 12 d for ^{3}H-SCH23390.

Immunohistochemistry. Rats were perfused through the aorta, under ketamine anesthesia, with a solution containing 4% paraformaldehyde and 0.12 M sodium phosphate (pH 7.4). After dissection, brains were postfixed for 2 hr in the same solution. Coronal sections (40 μm thick) were collected at the level of the striatum using a Vibratome apparatus (Oxford). The free-floating sections were incubated with affinity-purified anti-$G_{olf}\alpha$ antibodies (SL6, 4 μg/ml) and the labeling was revealed with a steptavidine-biotin-peroxidase method (Amersham) (Febvret et al., 1991). Diaminobenzidine (0.15 mg/ml) was used as chromogen, with nickel ammonium sulfate (0.6%) intensification.

Results

Comparison of adenylyl cyclase activities in the striatum and the substantia nigra

Since in the substantia nigra, a high density of D_1 receptors is paradoxically associated with low DA-stimulated adenylyl cyclase activity (Hervé et al., 1992), the levels of adenylyl cyclase were compared in the substantia nigra and the striatum. The enzyme activity in the presence of various concentrations of Mn^{2+}, which stimulates the enzyme independently of G-proteins (Mickevicius et al., 1986), was very similar in the substantia nigra and the striatum (Fig. 1, left panel). These results indicate the presence of similar amounts of adenylyl cyclase in both structures. In contrast, a much higher activity was observed in the striatum than in the substantia nigra, at every tested concentration of forskolin (Fig. 1, right panel). Since the adenylyl cyclase activation by forskolin is highly potentiated in the presence of stimulatory G-proteins (Darfler et al., 1982), different amounts of these G-proteins could account for the difference between the two cerebral structures.

Comparison of the concentrations of α subunits of stimulatory G-proteins in the striatum and the substantia nigra

The levels of α subunits of stimulatory G-proteins were measured in the striatum and the substantia nigra by immunoblotting, and the results were compared to those obtained in the globus pallidus, another major projection structure for the striatal neurons. Using RM/1 antiserum described by Simonds et al. (1989), which reacts with the α subunits of both G_s and G_{olf}, two immunoreactive bands were detected in the substantia nigra, the intensity of the high-molecular-weight band (~48 kDa) being weaker than that of the ~42 kDa band (Fig. 2*A*). Only the 42 kDa band was observed in the striatum, where it was much more intensely labeled than in the substantia nigra (Fig. 2*A*). When quantified by densitometry, the immunoreactivity at the level of the 42 kDa band varied linearly with the amount of protein up to an optical density of 0.8 (Fig. 2*B*). The immunoreactivity in the 42 kDa band was 5.4-fold higher in the striatum than in the substantia nigra. In the globus pallidus, as in the substantia nigra, two immunoreactive protein bands were detected, with a predominence of the low-molecular-weight protein. The concentration of the 42 kDa protein was 3.3-fold lower in the globus pallidus than in the striatum (Fig. 2*B*).

Comparative distribution of $G_s\alpha$ and $G_{olf}\alpha$ proteins in the CNS

As previously observed (Cooper et al., 1990; Drinnan et al., 1991), the nonselective RM/1 antiserum labeled two bands of variable intensity depending on the cerebral structures (Fig. 3*A*). The short form of $G_s\alpha$, which results from an alternative splicing of the $G_s\alpha$ mRNA (Bray et al., 1986; Robishaw et al., 1986; Kozasa et al., 1988), and $G_{olf}\alpha$, whose mRNA has been recently detected in the striatum (Drinnan et al., 1991), exhibit very similar molecular weights. The 42 kDa band labeled with RM/1 antiserum could correspond to either of these two proteins. To address this issue, specific antibodies were produced in rabbits, using synthetic peptides derived from a region of $G_s\alpha$ and $G_{olf}\alpha$, which exhibit little sequence homology (Jones and Reed, 1989). The specific antibodies showed that the high-molecular-weight band corresponded to $G_s\alpha$ and the low-molecular-weight band mostly to $G_{olf}\alpha$.

SL6 antibodies, which were developed against a $G_{olf}\alpha$ peptide,

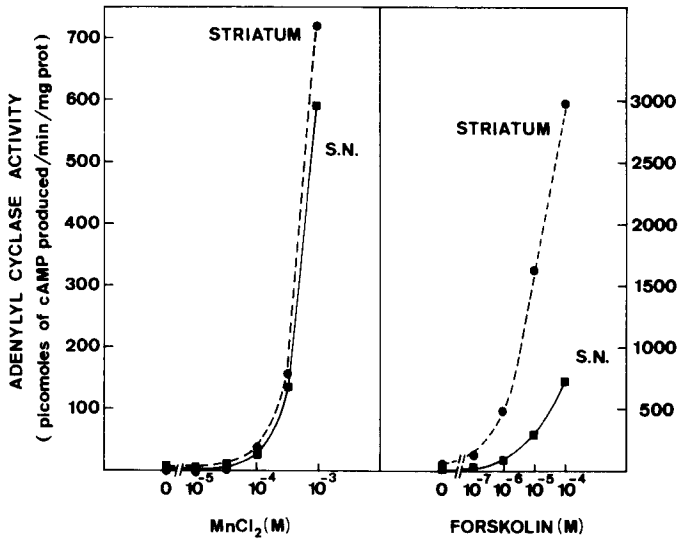

Figure 1. Stimulation of adenylyl cyclase activity by Mn^{2+} ions and forskolin in the striatum and the substantia nigra. Adenylyl cyclase activity was measured in triplicate in the presence of various concentrations of $MnCl_2$ or forskolin, in tissue homogenates from anteromedial striatum or substantia nigra (*S.N.*). For both areas, the assays contained ~10 μg of protein. Mg^{2+} ions were absent from the incubation medium when the effects of Mn^{2+} ions were tested.

labeled only one band in the olfactory epithelium (Fig. 3*B*, lane 9), exhibiting exactly the same migration as the lower band labeled by RM/1 antiserum (Fig. 3*B*). In brain, high concentrations of $G_{olf}\alpha$ were detected in the olfactory bulb (Fig. 3*B*, lane 10) and lower levels in the olfactory tubercle (lane 4), the nucleus accumbens (lane 5), and the caudate-putamen (lane 6). An immunoreactive band was detected also in the substantia nigra with a longer exposure time. In the other cerebral regions, no significant labeling could be observed. This topographical distribution of $G_{olf}\alpha$ matched that of the lower-molecular-weight protein labeled with nonspecific RM/1 antiserum.

SL8 antibodies, which were raised against a $G_s\alpha$ peptide, labeled a 48 kDa band exactly at the level of the high-molecular-weight band labeled by RM/1 (Fig. 3*C*). In all cerebral structures studied, a much weaker immunoreactivity was observed at the level of the short form of $G_s\alpha$ (not visible on the exposure shown in Fig. 3*C*). High levels of $G_s\alpha$ were observed in brain, except in the olfactory tubercle (Fig. 3*C*, lane 4), the nucleus accumbens (lane 5), and the caudate-putamen (lane 6). SL8 antibodies cross-reacted with two other brain-specific proteins with apparent molecular masses of 65 kDa and 51 kDa (Fig. 3*C*). These cross-reacting bands, whose intensity was somewhat variable from one experiment to the other, did not interfere with the analysis of $G_s\alpha$ since they were clearly resolved on SDS-PAGE.

Comparative distribution of $G_s\alpha$ and $G_{olf}\alpha$ mRNAs

Northern blot analysis of mRNA extracted from various areas of the brain and from other tissues were performed with cDNA probes for $G_s\alpha$ and $G_{olf}\alpha$ (Fig. 4). The $G_{olf}\alpha$ probe labeled three transcripts in the olfactory epithelium (lane 1). The two long forms may correspond to the $G_{olf}\alpha$ transcripts of 3.5 and 2.7 kilobases (kb) described by Jones and Reed (1989). A shorter $G_{olf}\alpha$ species was also detected with a size similar to that of $G_s\alpha$ mRNAs. However, this labeling was not due to a cross-hybridization with $G_s\alpha$ mRNA, since, under the high-stringency con-

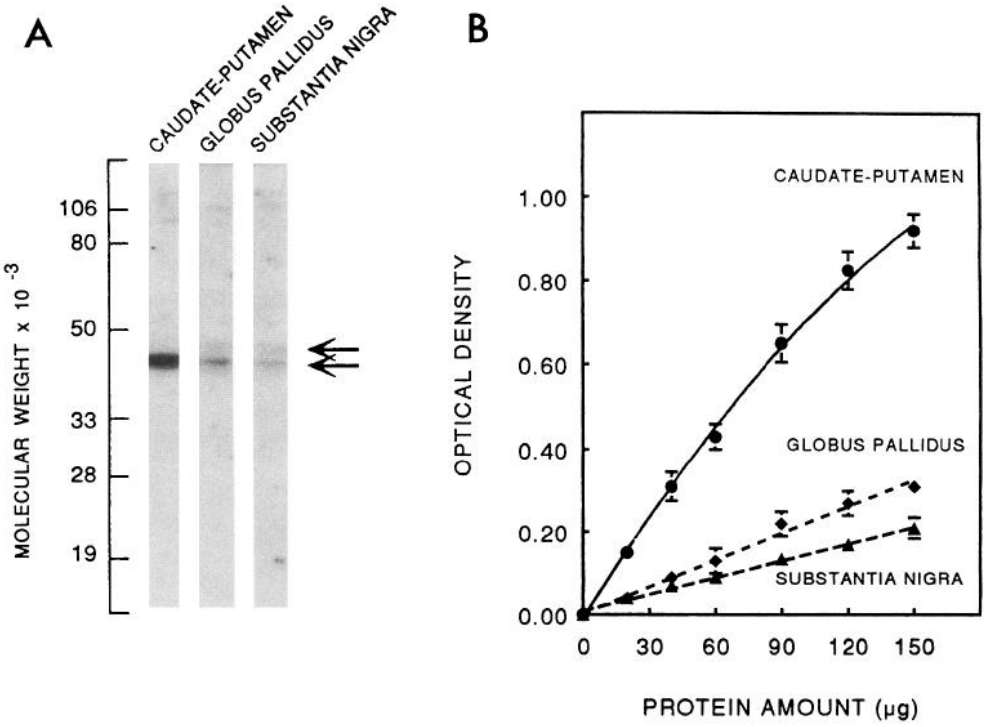

Figure 2. Immunoblot analysis of stimulatory G-protein α subunits in the caudate-putamen, globus pallidus, and substantia nigra. Microdisks of tissue were punched out from frozen 500-μm-thick coronal brain slices and sonicated in boiling 1% SDS. Increasing amounts of tissue homogenate (20–150 μg of protein) were analyzed by immunoblotting, using RM/1 antiserum, which reacts with both $G_s\alpha$ and $G_{olf}\alpha$, and revealed by ^{125}I-labeled protein A overlay. *A*, The autoradiograms correspond to lanes loaded with 120 μg of protein. In globus pallidus and substantia nigra, two immunoreactive bands are observed (*arrows*), whereas only the one with a lower molecular weight was detected in the caudate-putamen. *B*, The optical density was measured on the autoradiograms at the level of the band of low molecular weight. Each point represents the mean ± SEM of four different samples.

ditions used (0.1 × SSC, 65°C), the $G_{olf}\alpha$ probe did not produce any significant labeling in adrenal gland, which expresses high levels of $G_s\alpha$ mRNA (lane 2). RNA blot analysis confirmed the expression of $G_{olf}\alpha$ in the neostriatal areas: nucleus accumbens (lane 10), caudate-putamen (lane 11), and olfactory tubercle (lane 15). $G_{olf}\alpha$ was expressed at moderate levels in the olfactory bulb (lane 16), and at low levels in the other cerebral structures. Except for the very low levels seen in testis, no significant expression of $G_{olf}\alpha$ mRNA could be detected in non-neural tissues. In the neostriatal areas (lanes 10, 11, 15), three $G_{olf}\alpha$ mRNA species were found with the same apparent sizes as those observed in the olfactory epithelium (lane 1), although the relative proportion of the three forms was not identical: in the olfactory epithelium the 2.7 kb mRNA predominated, whereas in neostriatal areas the shorter mRNA was more abundant. In addition, a fourth minor species, slightly larger than the shortest form, appeared selective for the striatal areas.

The distribution of $G_s\alpha$ transcripts in various tissues was compared to that of $G_{olf}\alpha$ mRNAs (Fig. 4). In contrast with the results obtained for $G_{olf}\alpha$ mRNAs, only low expression of $G_s\alpha$ was observed in neostriatal areas (lanes 10, 11, 15) as compared to the high levels found in the other cerebral structures (cerebellum, lane 9; substantia nigra, lane 12; cerebral cortex, lanes 13 and 14; olfactory bulb, lane 16). Interestingly, these latter cerebral structures were very rich in $G_s\alpha$ transcripts since the adrenal gland (lane 2) was the only tested non-neural tissue exhibiting higher levels.

Effects of intrastriatal injections of quinolinic acid on $G_s\alpha$ and $G_{olf}\alpha$ in the striatum and the substantia nigra

The presence of $G_s\alpha$ and $G_{olf}\alpha$ transcripts in the striatum demonstrated the expression of both proteins in cells with perikarya in this structure. In order to investigate their neuronal or glial expression, $G_{olf}\alpha$ and $G_s\alpha$ contents were evaluated in the striatum following intrastriatal microinjections of quinolinic acid, which spare axon terminals of afferent neurons, but destroy the neuronal cell bodies (Schwarcz et al., 1983; Beal et al., 1986). The efficacy of our lesions was demonstrated by the pronounced reduction in the striatal levels of DARPP-32 (data not shown and Girault et al., 1992), a marker of the medium-sized spiny neurons (Hemmings and Greengard, 1986). Quinolinic acid lesions induced also a marked glial reaction indicated by a severalfold increase in the striatal levels of glial fibrillary acid protein (data not shown). These lesions produced opposing effects on the striatal levels of $G_{olf}\alpha$ and $G_s\alpha$: $G_{olf}\alpha$ was no longer detectable in homogenates from lesioned striatum, whereas $G_s\alpha$ levels were increased in the same samples (Fig. 5*A*). The long and short forms of $G_s\alpha$ were affected differently: the increase was more pronounced for the short form of $G_s\alpha$ than for the long form. The intrastriatal quinolinic acid lesions produced also a degeneration of nerve terminals of striatonigral neurons in the substantia nigra. The low levels of $G_{olf}\alpha$ in substantia nigra disappeared completely following this treatment, while the $G_s\alpha$ content was increased (Fig. 5*A*). As in the striatum, the long form of $G_s\alpha$ was less affected than the short one in the substantia nigra. Northern blot analysis indicated that the lesion produced a dramatic reduction in the $G_{olf}\alpha$ mRNA levels without major alteration in those of $G_s\alpha$ (Fig. 5*B*).

Effects of volkensin injections into the substantia nigra on $G_{olf}\alpha$ and $G_s\alpha$ in the striatum

Volkensin is a plant lectin with ribosomal toxicity, which is taken up by axon terminals and transported to the neuronal somas by the retrograde axonal flow (Stirpe et al., 1985; Wiley and Stirpe, 1988). When injected into the substantia nigra, this neurotoxin destroys the cells in the vicinity, and, distally, the neurons sending projections toward the substantia nigra, such as the striatonigral GABAergic neurons (Harrison et al., 1990). In the present study, the destruction of this subpopulation of neurons was confirmed by the decrease in D_1 receptors density,

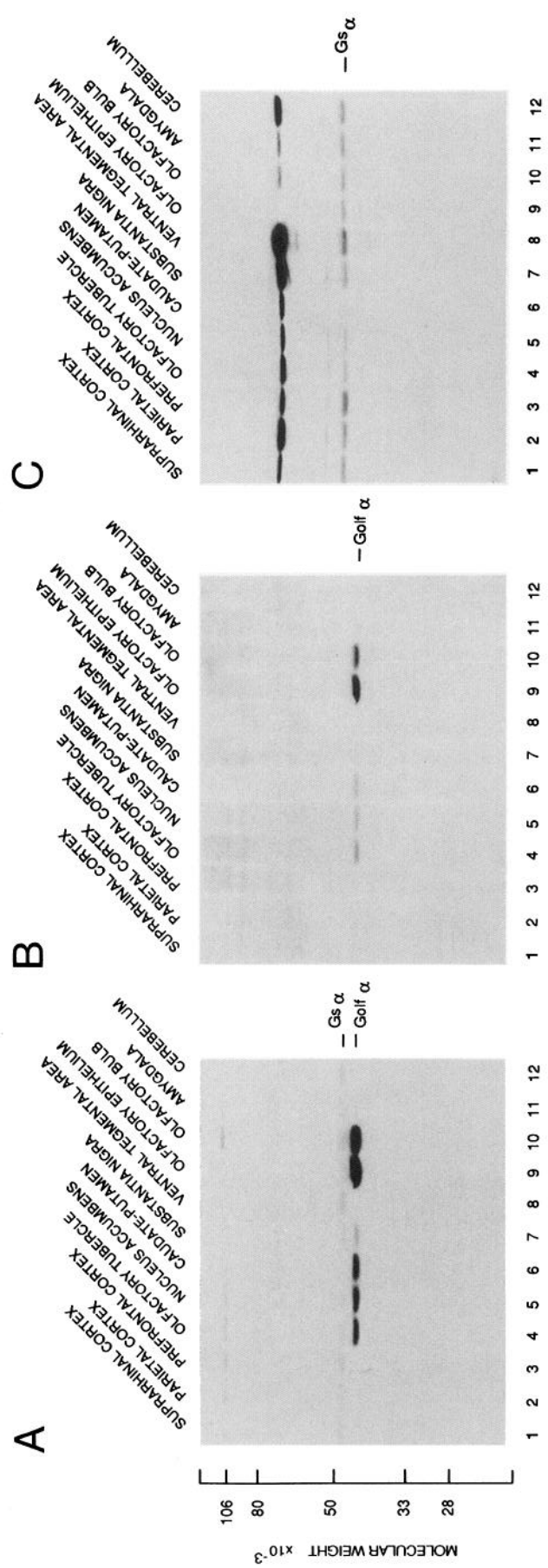

Figure 3. Distribution of $G_{olf}\alpha$ and $G_s\alpha$ proteins in brain and olfactory epithelium. Rat tissue samples (130 μg of protein) were subjected to SDS-PAGE (10% acrylamide), and $G_{olf}\alpha$ and $G_s\alpha$ proteins were revealed by sequential immunoblotting of the same transfer membrane using RM/1 (reacting with both $G_{olf}\alpha$ and $G_s\alpha$), SL6 (reacting with $G_{olf}\alpha$), and SL8 (reacting with $G_s\alpha$) antibodies. The immunoreactivity was revealed by a peroxidase/chemiluminescence detection method. Antibodies were eluted after each immunoblotting experiment. The positions of the migration of $G_{olf}\alpha$ and of the large form of $G_s\alpha$ are indicated.

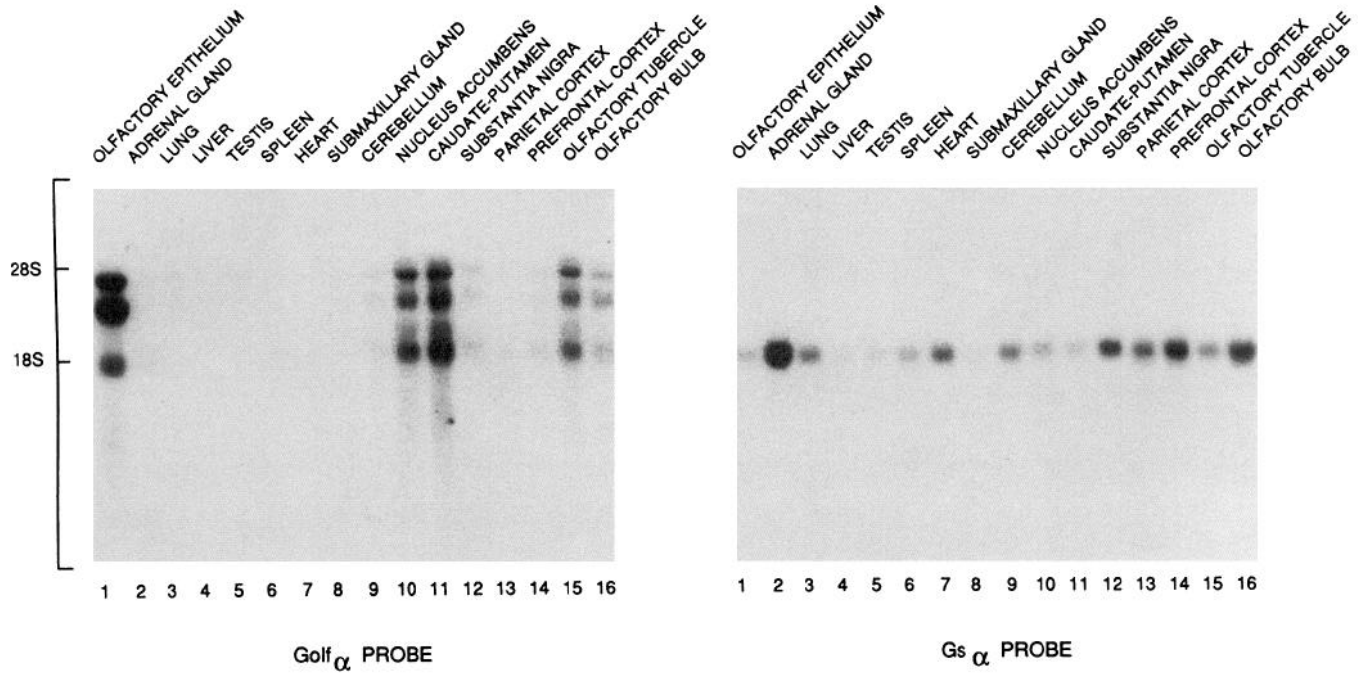

Figure 4. Distribution of $G_{olf}\alpha$ and $G_s\alpha$ mRNA in brain and other tissues. Total RNA (6 μg), extracted from the various tissues, was resolved on 1% agarose/formaldehyde gel and transferred to a nylon membrane. The membrane was exposed successively to ^{32}P-labeled cDNA probes for $G_{olf}\alpha$ and $G_s\alpha$. After hybridization, the membrane was washed in high-stringency conditions (0.1 × SSC, 65°C) and apposed to autoradiographic film. A dehybridization step eliminated the first probe before incubation with the other. The positions of the 18S and 28S ribosomal RNAs are indicated.

as previously reported by Harrison et al. (1990). ^{3}H-SCH23390 binding decreased substantially in the laterodorsal part of the ipsilateral striatum when compared to similar areas in the contralateral side (−65 ± 7%, mean ± SEM; $p < 0.01$, Student's t test; $n = 3$; Fig. 6*A*). ^{3}H-SCH23390 binding, however, was not decreased in the medial and ventral parts of the striatum and in the nucleus accumbens (Fig. 6*A*). The intranigral injection of volkensin did not affect significantly the binding of ^{125}I-iodosulpride, a specific ligand for D_2 receptors (−2 ± 3%; Fig. 6*B*). In the same animals, the destruction of the striatonigral neurons reduced the $G_{olf}\alpha$ content by 56 ± 8% ($p < 0.01$; $n = 3$) in the ipsilateral laterodorsal striatum as compared to the contralateral side (Fig. 6*C*). On the other hand, $G_s\alpha$ levels were increased in the same areas (Fig. 6*C*). Similar opposing variations of $G_{olf}\alpha$ and $G_s\alpha$ were observed in RNA blot analysis, since the lesion of striatonigral neurons diminished markedly the levels of $G_{olf}\alpha$ mRNA, but not those of $G_s\alpha$ (data not shown).

Effects of unilateral lesions of nigrostriatal DA neurons on $G_{olf}\alpha$ and $G_s\alpha$ in the striatum

In a previous study (Hervé et al., 1989), we had shown that the destruction of the nigrostriatal DA neurons increases DA-sensitive adenylyl cyclase activity in the striatum with little variation in D_1 receptor density. In order to investigate the possible role of stimulatory G-proteins in this denervation supersensitivity, the levels of $G_{olf}\alpha$ and $G_s\alpha$ proteins, as well as those of

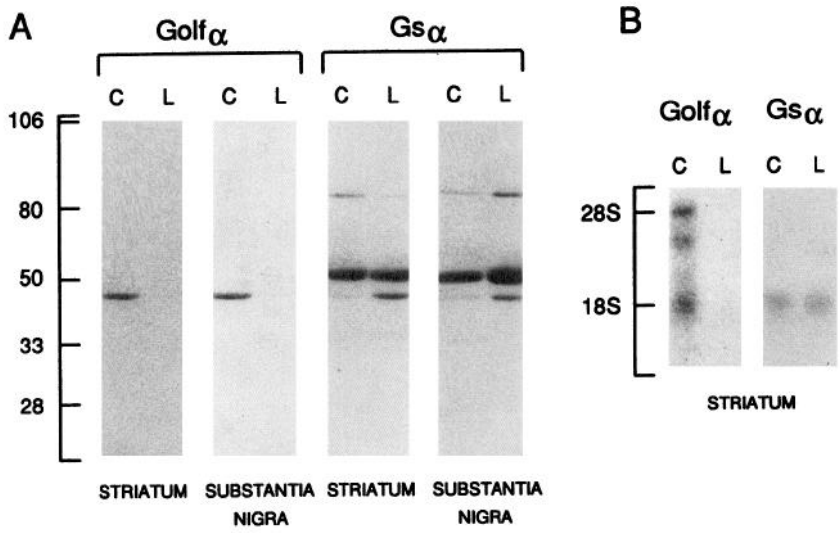

Figure 5. Effects of intrastriatal quinolinic acid injections on the levels of $G_{olf}\alpha$ and $G_s\alpha$ proteins and mRNAs in the striatum and the substantia nigra. Two unilateral injections of quinolinic acid (120 nmol in 1 μl) were performed, into the anteromedial and posterolateral parts of the striatum of pentobarbital-anesthetized rats. Twenty days later, tissue microdisks were punched out from the striatum and substantia nigra, on the lesioned (*L*) or contralateral (*C*) sides. *A*, $G_{olf}\alpha$ and $G_s\alpha$ proteins were analyzed by immunoblotting using SL6 and SL8 antibodies, respectively. *B*, Total RNA contained in microdisks punched from control and lesioned striatum was extracted and resolved on agarose gel. $G_{olf}\alpha$ and $G_s\alpha$ mRNAs were detected by hybridization blot analysis as described in the Figure 4 caption.

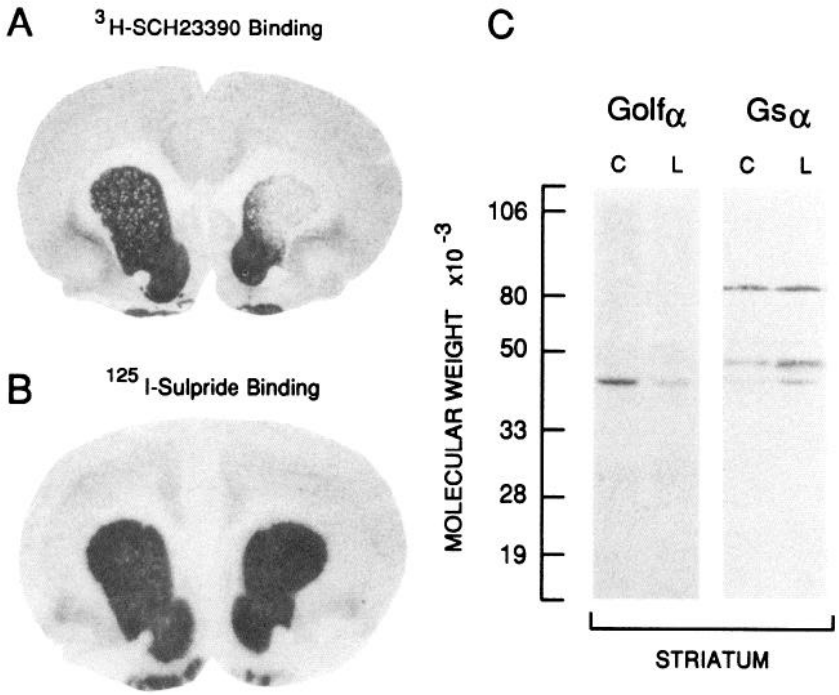

Figure 6. Effects of volkensin microinjections into the substantia nigra, on D_1 and D_2 receptor densities and on $G_{olf}\alpha$ and $G_s\alpha$ concentrations in the striatum. Volkensin (1–2 ng in 0.2 μl) was injected unilaterally into the substantia nigra of ketamine-anesthetized rats. Seventeen days later, the brains were frozen rapidly in isopentane at −30°C. Sections (15-μm-thick) were cut at the anterior levels of the striatum and micropunches were dissected out from the remaining striatum. Sections were incubated in the presence of ^{3}H-SCH23390 to label D_1 receptors (*A*) or ^{125}I-sulpride to label D_2 receptors (*B*). In *A* and *B*, sections of the same animal are shown, and the lesioned side is on the *right*. In *C*, $G_{olf}\alpha$ and $G_s\alpha$ were detected in homogenates from lesioned (*L*) and contralateral (*C*) striata by immunoblotting.

their corresponding mRNAs, were measured in the striatum 6 weeks after unilateral 6-OHDA microinjections into the medial forebrain bundle. These lesions destroyed striatal DA innervation, as indicated by the decrease (>90%) in the levels of DA (data not shown). The amounts of $G_{olf}\alpha$ protein increased in the striatum ipsilateral to 6-OHDA injections (Table 1). The increase in $G_{olf}\alpha$ protein was not associated with enhanced levels of $G_{olf}\alpha$ mRNA (Table 1). This lack of variation was not due to an effect of lesion on the concentration of actin mRNA that was measured for normalizing the RNA amounts, since the evaluation of RNA content using a mitochondrial cytochrome oxidase cDNA probe, led to analogous results (data not shown). In contrast, the levels of $G_s\alpha$ mRNA increased significantly in the ipsilateral striatum (Table 1).

Table 1. Effects of unilateral 6-OHDA lesions on striatal $G_{olf}\alpha$ and $G_s\alpha$

	Contralateral side (%)	Ipsilateral side (%)	*n*
$G_{olf}\alpha$ protein	99 ± 5	153 ± 6*	21
$G_s\alpha$ protein	115 ± 6	164 ± 8*	23
$G_{olf}\alpha$ mRNA	100 ± 7	103 ± 8	13
$G_s\alpha$ mRNA	100 ± 5	174 ± 9*	13

$G_{olf}\alpha$ and $G_s\alpha$ proteins and mRNAs were studied in the anteromedian region of rat striatum, 6 weeks after a unilateral injection of 6-OHDA into the median forebrain bundle. $G_{olf}\alpha$ and $G_s\alpha$ proteins were analyzed by immunoblotting. Results are expressed as percentage of levels of these proteins in samples from unlesioned rats studied on the same transfer membranes. $G_{olf}\alpha$ and $G_s\alpha$ mRNAs were analyzed by Northern blot, and results are expressed as percentage of the mean of levels in the control side of the brain. Filters were subsequently hybridized with an actin probe in order to correct the $G_{olf}\alpha$ and $G_s\alpha$ mRNAs amounts for the variations of total RNA. Quantification was achieved by computer-assisted densitometry of autoradiograms. Values are mean ± SEM for the indicated number of animals (*n*). Statistical comparisons between ipsilateral and contralateral sides were done with Student's t test (*, $p < 0.01$).

Immunohistochemical localization of $G_{olf}\alpha$

The immunohistochemical study of $G_{olf}\alpha$ localization confirmed the biochemical studies. Intense labeling was found throughout the neostriatal areas (caudate-putamen, nucleus accumbens, and olfactory tubercle) and in the piriform cortex (Fig. 7). At low magnification, islands of slightly denser staining were apparent in the caudate-putamen (Fig. 8*A*). At higher magnification, the somas of the vast majority of striatal medium-sized neurons were immunoreactive (Fig. 8*B*). In addition, the presence of labeling in some large neurons indicates that other neuronal populations contain $G_{olf}\alpha$-like immunoreactivity (Fig. 8*B*). A diffuse staining was observed within the striatal neuropil, but

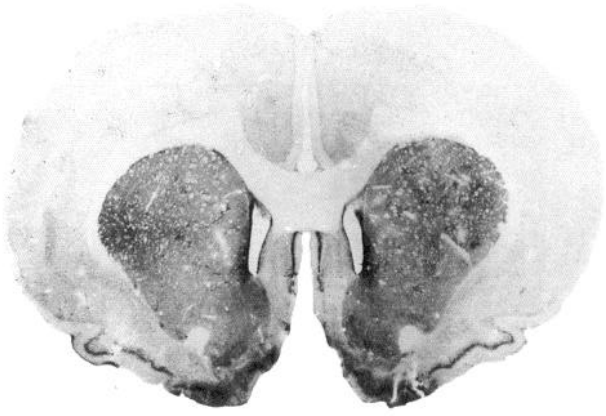

Figure 7. Distribution of $G_{olf}\alpha$-like immunoreactive cells in rat forebrain. A coronal section of rat forebrain (40 μm thick) was incubated with anti-$G_{olf}\alpha$ antibodies. Immunoreactivity was revealed with peroxidase-coupled secondary antibodies, using a nickel-enhanced procedure for diaminobenzidine staining. Intense labeling is present in the caudate-putamen, the nucleus accumbens, and the olfactory tubercle; note also the labeling of the piriform cortex.

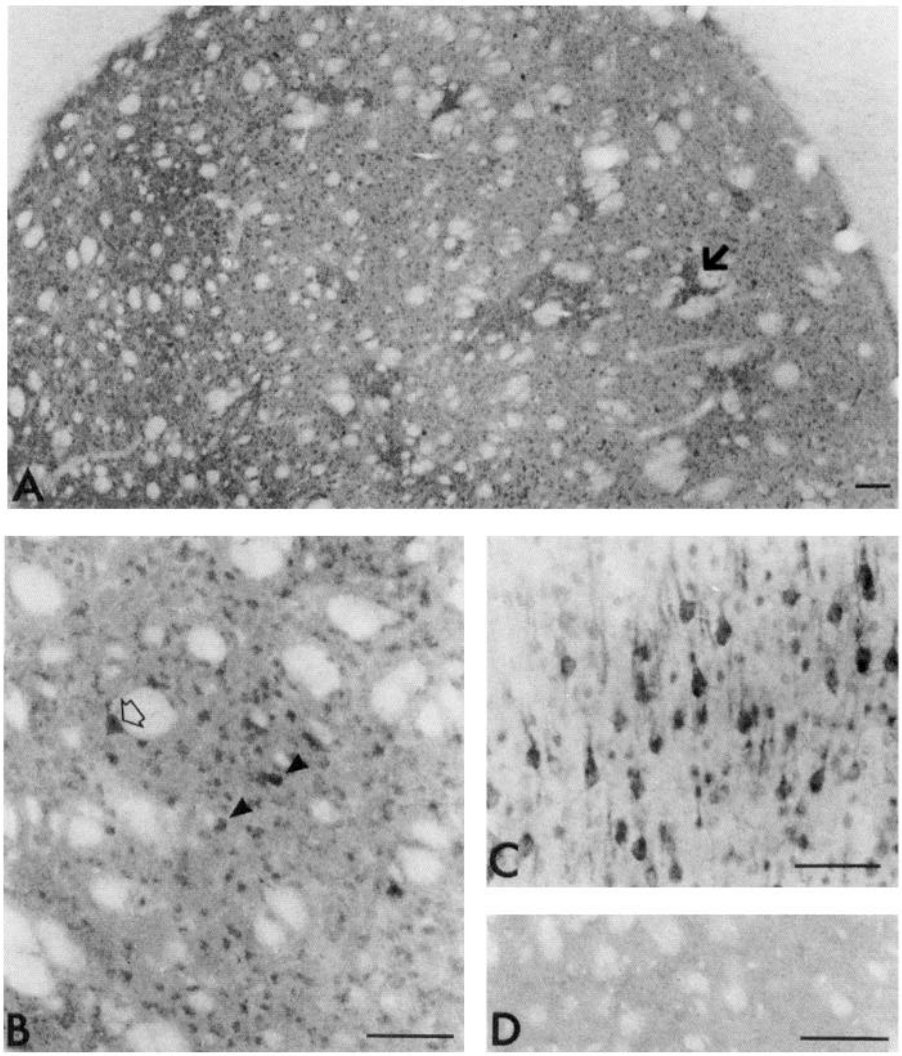

Figure 8. Immunocytochemical study of $G_{olf}\alpha$ in rat forebrain. Coronal sections were processed as described in the Figure 7 caption. *A*, Low magnification of the dorsal part of the caudate-putamen. Note the heterogeneity of the immunoreactivity with islands of more intense labeling (*arrow*). *B*, At higher magnification, the majority of the labeled neurons in the dorsal caudate-putamen have medium-sized cell bodies (*arrowheads*) but a few large perikarya are also immunoreactive (*open arrow*). *C*, High magnification at the level of layer V in sensorimotor cortex shows intensely labeled pyramidal cells. *D*, The specificity of the immunoreactivity is demonstrated by the absence of labeled perikarya in a caudate-putamen section (as in *B*) incubated in the presence of an excess (1 mg/ml) of the peptide used for immunization. Scale bars, 100 μm.

the resolution of the method did not allow to attribute this labeling to a cell element. The labeling was not restricted to the striatum, since immunoreactive cell bodies were found also in the septum, the tenia tecta, and the cerebral cortex, particularly in layer V of the sensorimotor cortex (Fig. 8*C*) and in the pyramidal layer of the piriform cortex (Fig. 7). The specificity of the labeling was demonstrated by the disappearance of immunoreactivity in the presence of an excess of the peptide used for immunization (Fig. 8*D*).

Discussion

Regional distribution of G_{olf} and G_s in rat brain

Following immunoblotting with RM/1 antiserum or ADP ribosylation by cholera toxin, two types of $G_s\alpha$-like proteins with different apparent molecular weights have been observed in rat brain (Nestler et al., 1989; Cooper et al., 1990; Drinnan et al., 1991). The low-molecular-weight species predominate in the striatum, whereas most other cerebral structures contain the high-molecular-weight species (Nestler et al., 1989; Cooper et al., 1990; Drinnan et al., 1991). These two types of proteins could result from the translation of four different transcripts derived from the $G_s\alpha$ gene by alternative splicing, two short forms and two long ones containing additional exon encoding peptides 15 or 16 amino acids long (Bray et al., 1986; Kozasa et al., 1988). In the present study, using anti-$G_s\alpha$ antibodies without cross-reactivity with $G_{olf}\alpha$, we found that the low-molecular-weight species of $G_s\alpha$ are minor components throughout the brain, including the striatum. Moreover, with selective anti-$G_{olf}\alpha$ antibodies, an intensely immunoreactive band was observed exactly at the level of the "short" form of $G_s\alpha$-like protein in the striatum. This indicates that the low-molecular-weight species observed in the caudate-putamen, the nucleus accumbens, and the olfactory tubercle correspond mostly to $G_{olf}\alpha$. These results are consistent with previous studies showing low amounts of $G_s\alpha$ mRNA and high amounts of $G_{olf}\alpha$ mRNA in the striatal areas, as compared to other cerebral structures (Largent et al., 1988; Drinnan et al., 1991). Assuming that RM/1 antiserum reacts equally well with $G_{olf}\alpha$ and $G_s\alpha$, $G_{olf}\alpha$ appears substantially more abundant than $G_s\alpha$ in the striatal areas, in agreement with the proposal by Drinnan et al. (1991) that striatal $G_{olf}\alpha$ mRNA levels are 10-fold higher than those of $G_s\alpha$ mRNA. By Western blot analysis, $G_{olf}\alpha$ was only observed in the olfactory epithelium, the olfactory bulb, the neostriatal areas, and the substantia nigra. By Northern blot analysis, however, the distribution of $G_{olf}\alpha$ in the brain appeared more widespread and immunohistochemistry showed the presence of neuronal cell bodies labeled with various intensities in many other cerebral areas, including the cerebral cortex.

The $G_{olf}\alpha$ cDNA probe hybridized with four different transcripts in rat brain and only three in the olfactory epithelium. The two longest species that were detected in the olfactory epithelium, as well as in the cerebral structures, have the same apparent lengths (3.5 and 2.7 kb) as those described by Jones and Reed (1989) in olfactory sensory neurons. These two mRNAs were shown to differ by utilization of alternative polyadenylation sites (Jones and Reed, 1989). In addition, we detected a short form (~2 kb), which was minor in the olfactory epithelium, whereas it was the major mRNA species in brain. A nonconventional polyadenylation site, TATAAA, used in the mRNA coding for hepatitis B virus surface antigen (Simonsen and Levinson, 1983) was found at position 1878 of the sequence published by Jones and Reed (1989). The utilization of this signal could generate the 2 kb transcript, but further investigation will be necessary to demonstrate it. An additional minor species, with a size intermediate between the 2.7 and 2 kb transcripts, was also detected selectively in the brain. Despite the existence of several $G_{olf}\alpha$ mRNA species, we have found no evidence for multiple isoforms of $G_{olf}\alpha$ proteins, although the possibility of different polypeptides with similar molecular weights cannot be excluded. It must also be pointed out that our antibodies would not react with isoforms of $G_{olf}\alpha$ in which an alternative splicing event would eliminate the peptide used for immunization.

Cellular localization of G_{olf} and G_s within the striatum

Quinolinic acid injections into the striatum led locally to a complete loss of $G_{olf}\alpha$ protein and mRNA demonstrating the expression of $G_{olf}\alpha$ within neuronal cell bodies in the striatum, and its absence from the glial cells and axon terminals of afferent neurons. Immunocytochemical studies revealed that $G_{olf}\alpha$ is contained in the majority of striatal neurons. D_1 receptors are localized in at least 50% of striatal neurons, mostly in those projecting toward the substantia nigra and containing GABA, substance P, and dynorphin (Gerfen et al., 1990; Le Moine et al., 1991; Weiner et al., 1991). In agreement with these data, the volkensin injections into the substantia nigra, which destroyed striatonigral neurons, induced a pronounced reduction in the D_1 receptor density in the striatum as described previously by Harrison et al. (1990). The selectivity of the destruction was attested by the relative sparing of neurons bearing D_2 receptors that belong mostly to the striatopallidal system and to the interneuron category (Gerfen et al., 1990; Le Moine et al., 1990a,b). In volkensin-treated rats, an important decrease in the levels of $G_{olf}\alpha$ protein and mRNA was observed in the striatum, implying that $G_{olf}\alpha$ and D_1 receptors are both expressed in the striatonigral neurons. On the other hand, the low levels of $G_s\alpha$ protein and mRNA in the striatum and the lack of decrease of $G_s\alpha$ in the striatum and in the substantia nigra following striatal quinolinic acid lesions indicate that striatonigral neurons contain very little, if any, $G_s\alpha$. Taken together, these observations suggest that G_{olf} couples D_1 receptors to adenylyl cyclase in the striatonigral neurons. Indeed, besides its role in the odorant signal transduction, G_{olf} is able to interact with neurotransmitter receptors since Jones et al. (1990) have shown that the transfection of $G_{olf}\alpha$ cDNA in cyc^- cells, deficient in stimulatory G-protein, restores the activation of adenylyl cyclase by β-adrenergic receptors. Since antibodies against $G_s\alpha$ have been reported to inhibit adenylyl cyclase activation (Simonds et al., 1989), we have examined the effects of selective antibodies against $G_s\alpha$ and $G_{olf}\alpha$ (SL8 and SL6, respectively) on DA-sensitive cyclase in striatal membranes (D. Hervé and J.-A. Girault, unpublished observations). Unfortunately, the small amplitude of the inhibitory effects observed did not allow definite demonstration of the involvement of $G_{olf}\alpha$ in the coupling between D_1 receptors and adenylyl cyclase, and suggested that the corresponding epitopes are not functionally important in this coupling.

The regional distribution of $G_{olf}\alpha$ is not restricted, however, to DA target neurons expressing D_1 receptors and may play a role in the transduction cascade of other types of receptors. For instance, low, but significant, amounts of $G_{olf}\alpha$ mRNA were detected in the cerebellum, an area lacking DA innervation (Björklund and Lindvall, 1984). Moreover, $G_{olf}\alpha$-like immunoreactivity was observed in neurons containing little or no D_1 receptors (Savasta et al., 1986; Fremeau et al., 1991; Le Moine et al., 1991; Mansour et al., 1992), including large-sized, presumably cholinergic, striatal neurons, and pyramidal neurons in the layer V of sensorimotor cortex and in the piriform cortex.

The effects of striatal quinolinic acid–induced lesions demonstrated the expression of $G_s\alpha$ in glial cells of the striatum, as previously described by Feinstein et al. (1992) in astrocytes in culture. $G_s\alpha$ could be linked to β-adrenergic or prostaglandin E_1 receptors in these cells (Minneman et al., 1978).

Somatodendritic localization of $G_{olf}\alpha$

$G_{olf}\alpha$ appears to be highly enriched in the striatum, as compared to the substantia nigra and the globus pallidus. This enrichment does not result from the preferential localization of $G_{olf}\alpha$ in striatal cell populations without long extrinsic projections: the striatal glial cells or afferent fibers do not contain $G_{olf}\alpha$, and the number of interneurons, which correspond to about 4% of the striatal neurons (Heimer et al., 1985), is too small to account for both the high striatal concentration of $G_{olf}\alpha$ and the large population of neurons labeled by $G_{olf}\alpha$-selective antibodies in immunohistochemistry. Therefore, the relative enrichment of $G_{olf}\alpha$ in the striatum is likely to reflect its preferential localization in the perikarya and dendrites of striatonigral and striatopallidal neurons. This localization contrasts with that of several proteins enriched in striatonigral neurons, which are found in similar amounts in the striatum and the substantia nigra: D_1 receptors (Boyson et al., 1986; Dawson et al., 1986), adenylyl cyclase (Worley et al., 1986a), and DARPP-32 (Hemmings and Greengard, 1986). The preferential somatodendritic localization of $G_{olf}\alpha$ contrasts also with that of another GTP-binding protein, G_o, which was found at high levels in the nerve terminals of striatonigral neurons (Worley et al., 1986b). These observations suggest the possibility of an intracellular targeting of G-proteins in neurons. Such a targeting has been reported in a renal epithelial cell line, which maintains a functional polarity similar to that observed *in vivo*, and in which the G_i protein α subunits $G_i\alpha 2$ and $G_i\alpha 3$ are localized to the basolateral and the Golgi/apical membranes, respectively (Ercolani et al., 1990). The nigral deficit in $G_{olf}\alpha$ could also be due to a lesser stability of $G_{olf}\alpha$ in nerve terminals, which would contrast with the slower turnover rate of D_1 receptors in the substantia nigra than in the striatum (Giorgi et al., 1991).

Comparison of DA-sensitive adenylyl cyclase activity in the striatum and the substantia nigra

Using forskolin as a stimulant, a much lower adenylyl cyclase activity was measured in the substantia nigra than in the striatum, whereas the adenylyl cyclase concentrations estimated by

^{3}H-forskolin binding (Gehlert et al., 1985) or by Mn^{2+}-stimulated enzymatic activity (present results) are similar in both areas. Since the presence of stimulatory G-proteins has been shown to increase dramatically the effects of forskolin on adenylyl cyclase activity (Darfler et al., 1982), the regional differences observed with forskolin may be due to the lower levels of $G_{olf}\alpha$ in the substantia nigra than in the striatum. A quantification of $G_{olf}\alpha$, using RM/1 antiserum, revealed five- to sixfold lower levels of $G_{olf}\alpha$ in the substantia nigra than in the striatum, in agreement with the marked nigral deficit in forskolin-induced cyclase activity. This relative deficit in G_{olf} of the substantia nigra could also explain the fact that DA-induced activation of adenylyl cyclase is sevenfold lower in the substantia nigra than in the striatum, despite the similar densities of D_1 receptors in both structures (Hervé et al., 1992).

Role of G_{olf} and G_s in the denervation supersensitivity of D_1 receptor in the striatum

In the striatum, the destruction of DA afferent fibers increased both $G_{olf}\alpha$ and $G_s\alpha$ protein contents 6 weeks after the lesion. Contrary to the levels of $G_{olf}\alpha$ protein, the concentrations of $G_{olf}\alpha$ mRNA did not vary significantly in 6-OHDA–lesioned rats. This could indicate that increased levels of $G_{olf}\alpha$ protein result from a slower degradation rate of $G_{olf}\alpha$ protein in the DA-denervated cells. Alternatively, the destruction of DA innervation could induce only a transient overexpression of $G_{olf}\alpha$ mRNA that would increase the levels of $G_{olf}\alpha$ protein for a longer time.

By contrast, the enhancement in $G_s\alpha$ protein levels in the striatum following 6-OHDA–induced lesion was associated with increased mRNA levels. Because of the presence of $G_s\alpha$ in glial cells, these effects could be, in part, related to the gliosis induced by the degeneration of DA fibers. However, intrastriatal quinolinic acid injections, which induced a much more intense glial reaction (data not shown), did not lead to more pronounced effects on the striatal amounts of $G_s\alpha$. This suggests that gliosis may not account for all the variations of $G_s\alpha$ following 6-OHDA lesions, and that DA neurons may exert an inhibitory influence on the expression of $G_s\alpha$ in the striatum. Further studies are needed to determine the glial or neuronal nature of the cells displaying the increased $G_s\alpha$ levels.

6-OHDA lesions of the nigrostriatal neurons were shown previously to produce an approximately 50% increase in DA-stimulated adenylyl cyclase activity (Hervé et al., 1989) with only a 10–15% increase in the ^{3}H-SCH23390 binding to D_1 receptors in the anteromedial striatum (Hervé et al., 1992). The demonstration, in the present study, of a 50–60% increase in the amounts of both $G_{olf}\alpha$ and $G_s\alpha$ suggests that the enhanced response of adenylyl cyclase to DA in 6-OHDA–lesioned striatum is essentially due to effects on stimulatory G-proteins. In a previous study, the variations of DA-sensitive adenylyl cyclase activity, following 6-OHDA–induced lesion, were less pronounced in the laterodorsal striatum than in the anteromedial striatum (Hervé et al., 1989). Interestingly, in the present study, the increases in $G_{olf}\alpha$ levels were less pronounced in the laterodorsal striatum than in the anteromedial striatum, whereas the $G_s\alpha$ concentrations were enhanced similarly in both areas (data not shown). These observations indicate that the effects on DA-sensitive adenylyl cyclase correlate better with the variations of $G_{olf}\alpha$ levels than with those of $G_s\alpha$ levels and that the levels of $G_{olf}\alpha$ may represent a limiting factor in the coupling efficiency of D_1 receptors.

Conclusions

In the present study, we show that $G_{olf}\alpha$ is abundantly expressed in striatonigral neurons and we suggest that G_{olf} couples D_1 receptors to adenylyl cyclase in these neurons. The functional implications of the preferential expression of $G_{olf}\alpha$ in the striatal neurons are unknown. Although $G_{olf}\alpha$ and $G_s\alpha$ were found to couple β-adrenergic receptors to adenylyl cyclase in transfected cells with relatively similar efficiencies (Jones et al., 1990), it will be interesting to compare their efficiencies for the coupling of D_1 receptors. G_s was also proposed to modulate other effectors, including calcium and sodium channels (Yatani et al., 1987; Schubert et al., 1989), and G_{olf} could have different influences on such effectors. Interestingly, the minor role of $G_s\alpha$ in striatal neurons is consistent with the absence of extrapyramidal neurological symptoms in Albright and McCune–Albright syndroms that are associated with mutations of $G_s\alpha$ gene (Weinstein et al., 1990; Schwindinger et al., 1992).

Within the striatonigral neurons, $G_{olf}\alpha$ is preferentially located in the perikarya and dendrites, and low $G_{olf}\alpha$ concentrations are observed at the level of nerve terminals in the substantia nigra. This may account for the fact that the DA-stimulated adenylyl cyclase activity is relatively low in the substantia nigra in comparison with what could be expected from the high densities of D_1 receptors and adenylyl cyclase molecules. Moreover, following the destruction of DA ascending pathways, the increased levels of $G_{olf}\alpha$ could explain the enhancement of the DA-stimulated adenylyl cyclase activity observed in the striatum. The comparison between the striatum and the substantia nigra, as well as the effects of DA neurons lesions, suggest that modulation of stimulatory G-proteins could play a key role in regulating the amplitude of the response to D_1 receptor stimulation. Interestingly several pharmacological treatments, including cocaine, morphine, lithium salts, and glucocorticoids, have been shown to alter the expression of various G-proteins in specific brain regions (Attali and Vogel, 1989; Nestler et al., 1989, 1990; Saito et al., 1989; Colin et al., 1991). Our study emphasizes further the physiological importance of regulation of stimulatory G-protein expression in the functional responses of neurotransmitter receptors in the brain.

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