Activation of Nigral and Pallidal Dopamine D1-Like Receptors Modulates Basal Ganglia Outflow in Monkeys

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INTRODUCTION

The basal ganglia are a group of subcortical structures that interact with the cerebral cortex and thalamus (Alexander and Crutcher 1989). According to the traditional model of basal ganglia connectivity and function (Albin et al. 1989; Alexander and Crutcher 1990; Nambu et al. 1996), cortical information reaches the basal ganglia primarily through the striatum and is then conveyed to the basal ganglia output structures, the internal pallidal segment (GPI), and the substantia nigra pars reticulata (SNr), by monosynaptic GABAergic projections, as well as polysynaptic routes. Basal ganglia output provides inhibitory input to the thalamus and brain stem. These circuits are highly topographic in their organization (Middleton and Strick 2002; Wichmann and Delong 2006). At the level of the basal ganglia output circuitry, movement-related circuits predominately pass through GPI, whereas associative and limbic circuits traverse the SNr.

Dopamine is thought to modulate neuronal activity throughout this system. Released in the striatum from terminals of the nigrostriatal projection (Bernheimer et al. 1973; Hornykiewicz and Kish 1987), dopamine is known to alter the activity of striatal projection neurons (Gerfen 1995), resulting downstream in an overall reduction of inhibitory basal ganglia output from GPI and SNr (Wichmann and Delong 2006). Dopamine may also act in the extrastriatal basal ganglia (Bernheimer et al. 1973; Cheramy et al. 1981; Geffen et al. 1976; Robertson et al. 1991b; Schneider and Rothblat 1991; Smith and Bolam 1989; Whone et al. 2003b). These actions of dopamine, specifically within SNr and GPI, are less well explored. Dopamine is supplied to GPI by the nigropallidal projection, which arises from a population of substantia nigra pars compacta (SNc) neurons different from that giving rise to the nigrostriatal projection in primates (Jan et al. 2000; Smith et al. 1989), whereas in the SNr, dopamine is released from dendrites of SNc neurons that descend dorsally and arborize profusely along the basis of the SNr (Arsenault et al. 1988; Bjorklund and Lindvall 1975; Nicoulon et al. 1978). It is unclear whether the neurons that provide dopamine to the SNr by dendritic release are the same as those that provide dopamine to the striatum or GPI.

Dopamine acts at D1-like receptors (D1LRs, including D1- and D5-receptors; Clark and White 1987; Neve 1997) and D2-like receptors (D2LRs, including D2-, D3-, and D4-receptors; Neve 1997). Receptor binding studies in monkeys have demonstrated that GPI and SNr contain predominantly D1LRs (Richfield et al. 1987). Most of these receptors are located on presynaptic axons and axon terminals of GABAergic striopallidal and striatonigral projections (Barone et al. 1987; Fremeau et al. 1991; Kliem et al. 2006; Levey et al. 1993; Mengod et al. 1991; Yung et al. 1995). The aim of the present study was to explore the effects of dopamine receptor activation and of locally released dopamine on the rate and patterns of neuronal spiking in the nonhuman primate GPI and SNr. Changes in spontaneous neuronal discharge were examined before, during, and after local infusions of selective D1LR ligands into GPI or SNr in awake monkeys. Microdialysis methods were used to assess whether the local administration of these agents alters γ-aminobutyric acid (GABA) concentrations.
METHODS

Animals

Five rhesus monkeys (Macaca mulatta, 3–5 kg) were used for these studies. They were housed under conditions of protected contact housing, with free access to standard primate chow and water. Before the other procedures, the animals were trained to sit in a primate chair, to adapt to the laboratory, and to permit handling by the experimenter. The experimental protocols were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (ILAC/OLAW 1996) and the PHS Policy on Humane Care and Use of Laboratory Animals (amended 2002), and were approved by the Animal Care and Use Committee of Emory University.

Surgical procedures

After completion of behavioral training, two stainless steel chambers for chronic access (ID, 16 mm) were stereotactically positioned over trephine holes under aseptic conditions and anesthesia with isoflurane (1–3%). A chamber directed at the pallidum was placed at an angle of 40° from the vertical in the coronal plane (A = 12, L = 10, D = 4; Horsley–Clarke coordinate system) and a chamber aimed at the SN was placed at an angle of 25° posterior to the vertical in the sagittal plane (A = 9, L = 5, D = 0). The chambers were affixed to the skull with dental acrylic. Metal head holders were also embedded into the acrylic cap to permit head stabilization during the recording and microdialysis procedures. The animals were allowed to recover for 1 wk after the surgery. They were awake throughout all of the subsequent experiments.

Electrophysiologic recording experiments

Electrophysiologic recording techniques were used to generate maps and to explore the effects of locally applied drugs on the spiking activities of neurons in SNr and Gpi.

MAPPING PROCEDURES. For initial electrophysiologic mapping, the neuronal activity in Gpi and SNr was recorded extracellularly with tungsten microelectrodes (impedance 0.5–1.0 MΩ at 1 kHz; FHC, Bowdoinham, ME). A microdrive (MO-95B; Narishige, Tokyo, Japan) was used to lower the electrodes through the dura into the brain, using a 20-gauge guide tube (Small Parts, Miami Lakes, FL). The microdrive carried an X–Y stage that allowed us to determine the penetration, with free coordinates inside the recording chamber. In addition, the microdrive was equipped with a linear potentiometer, used to generate digital depth readouts, reflecting the position of the tip of the electrode with 10-μm accuracy. The electrical neuronal signal was amplified (DAM-80 amplifier; WPI, Sarasota, FL), filtered (400–10,000 Hz; Krohn-Hite, Brockton, MA), displayed on a digital oscilloscope (DL1640; Yokogawa, Tokyo, Japan), and made audible by an audio amplifier. Gpi and SNr neurons were identified by their characteristic high-frequency discharge rates. Surrounding nuclei have distinctly different firing rates and patterns (Soares et al. 2004; Starr et al. 1999).

INJECTION/RECORDING EXPERIMENTS. We used combined microelectrode recording-injection probes (Klem and Wichmann 2004) to examine the effects of D1LR-selective ligands on SNr and Gpi neuronal activity in three monkeys. A standard polyimide-coated tungsten microelectrode (see above) alongside fused silica tubing (ID, 40 μm; OD, 102 μm; Polymicro Technologies, Phoenix, AZ) was enclosed by a protective polyimide sleeve (OD, 0.5 mm; MicroLumen, Tampa, FL) and held in place with epoxy glue. The tip of the electrode extended about 75 μm beyond the tip of the silica tubing (range 50–100 μm). At the proximal end of the silica tubing, a 10-mm 23-gauge stainless steel tube (Small Parts) was placed over the fused silica and glued in place with epoxy. The metal tubing provided a leak-free connection to a micro-T connector (CMA, Solna, Sweden) through a “flexible connector” (Tygon tubing; ID, 0.020 in.; Saint-Gobain, Akron, OH). The inlets of the micro-T were connected to 1-ml gastight syringes (CMA), driven by a remotely controlled dual-syringe infusion pump (CMA/102) for pressure infusion of submicroliter quantities of drug or vehicle solutions.

To test the integrity of the injection system, it was flushed with artificial cerebrospinal fluid [aCSF, consisting of (in mM): 143 NaCl, 2.8 KCl, 1.2 CaCl2, 1.2 MgCl2, 1 NaHPO4 (pH 7.2–7.4)] at 5 μl/min before insertion into the brain. Before the experimental sessions, the selective D1LR agonist (+)-6-chloro-7,8-dihydroxy-3-allyl-1-phenyl-2,3,4,5-tetrahydro-1-phenyl-1H-3-benzazepine hydrobromide (SKF82958, 3 μg/μl; Sigma–Aldrich, St. Louis, MO) and the D1LR antagonist R(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepin-7-ol(Z)-2-butenedioate (SCH23390, 5 μg/μl; Sigma–Aldrich) were dissolved in aCSF and 0.1% ascorbic acid solution, followed by sonication. All solutions were filtered before infusion (pore size = 0.2 μm; Fisher Scientific, Hampton, NH).

In a typical experiment, one syringe was filled with aCSF, whereas a second one was filled with the drug solution or vehicle (for control injections). The system was then lowered into the brain through a 20-gauge guide tube. Each cell encountered with the system’s microelectrode was recorded for ≥1 min preinfusion (pump off), during the infusion (pump on, running at 0.25 μl/min for 2 min), and for ≥4 min thereafter (pump off). The amplified signal, as described earlier, was stored to computer disk using the CED data acquisition system (Spike2, CED, Cambridge, UK). Each animal underwent several recording-injection procedures, separated by ≥24 h. Neurons throughout both nuclei were sampled. In most experimental sessions, single drug injections were carried out, although in a few, a second injection was done, spatially separated from the first by ≥500 μm, and injected ≥30 min apart. In our experience, drug effects (with the injection volume chosen here) rarely extend more than 200–300 μm beyond the tip of the recording electrode (personal observation and Kita et al. 2004), and cumulative drug effects were not seen in these studies.

Microdialysis experiments

Microdialysis experiments were carried out to assess the effects of D1LR ligands on GABA release in the SNr and Gpi in two monkeys. The dialysis probes were custom-made (length, 135 mm; CMA). They consisted of a cuprophane membrane (length, 2 mm; outer diameter, 0.24 mm; cutoff molecular weight, 6 kDa). The diameter of the outer steel shaft was 0.38 mm. The inflow line of the microdialysis system was connected to a 1-m1 gastight glass syringe (CMA). Before use, the probes were flushed in succession with 70% ethanol, distilled water, and aCSF (all delivered at a rate of 5 μl/min).

Each animal underwent several microdialysis procedures, separated by ≥24 h. For each microdialysis session, a new probe track was used to sample undisturbed tissue. Before insertion, a metal tube with a fitting stylet was lowered to the appropriate stereotaxic coordinates through a guide tube into the brain. The distal end of the tube was adjusted, according to depth data obtained through electrophysiologic mapping experiments, so that the tube ended 1 mm above the target. The stylet was removed and the microdialysis assembly was inserted into the inner tube, advanced into the target area. Before the start of sample collections, the probes were perfused with aCSF for 2 h at a rate of 2 μl/min for stabilization of GABA levels. Two 10-min microdialysate samples were then collected into glass centrifuge vials. These samples were used to establish the baseline GABA levels. The microdialysate samples were then collected into glass centrifuge vials. The dialysis probes were custom-made (length, 135 mm; CMA). They consisted of a cuprophane membrane (length, 2 mm; outer diameter, 0.24 mm; cutoff molecular weight, 6 kDa). The diameter of the outer steel shaft was 0.38 mm. The inflow line of the microdialysis system was connected to a 1-m1 gastight glass syringe (CMA). Before use, the probes were flushed in succession with 70% ethanol, distilled water, and aCSF (all delivered at a rate of 5 μl/min).

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experiment. In these experiments, a high concentration of potassium (80 mM) was added to the drug/vehicle solution for the duration of sample 5 to assess the ability of the tissue to release GABA when stimulated. During high-potassium exposure, the concentration of NaCl was lowered to maintain osmolality (67.8 mM NaCl, 80 mM KCl, 1.2 mM MgCl\textsubscript{2}, and 1.2 mM CaCl\textsubscript{2}). At the end of each microdialysis session, the probe was removed and placed into aCSF containing a known concentration of GABA (10 ng/ml) to determine transmitter recovery. The recovery values clustered closely around 15–20%. The values reported here were not further adjusted for recovery.

All samples were immediately placed on dry ice and later stored at −80°C until the time of analysis. The concentration of GABA in the dialysate samples was determined by high-performance liquid chromatography with fluorometric detection as described previously (Murphy and Maidment 1999). Briefly, automated precolumn α-phthalaldehyde (OPA) derivatization of amino acids was achieved by adding 30 μl of derivatization reagent, containing OPA (3 μg) and β-mercaptoethanol (0.125% vol/vol) in 0.125 M boric acid buffer (pH 10), to each sample 2 min before injection onto a C18 reverse-phase column (150 × 3.0 mm; 3 μm particle size; Thermo-Fisher). GABA was eluted with an acetonitrile/methanol gradient. The limit of detection for GABA was 5 fmol (3:1 signal-to-noise ratio).

**Histology**

At the conclusion of the experiments, the monkeys were sedated with ketamine (10 mg/kg, administered intramuscularly) and then injected with an overdose of sodium pentobarbital (25 mg/kg, intravenous), followed by transcardial perfusion with 500 ml of saline and 4% paraformaldehyde/0.1% glutaraldehyde fixative in 0.1 M phosphate buffer (PB, pH 7.2). The brains were removed, cryoprotected in a 30% sucrose solution in 0.1 M PB, cut at 50 μm with a freezing microtome, collected serially, and stored at −20°C in an antifreeze solution. Tissue blocks containing GPi were sectioned in the coronal plane, whereas those containing the SNr were sectioned in the parasagittal plane. One of every four sections was stained with cresyl violet. These sections were later used to verify probe and electrode locations.

**Data analysis**

**ELECTROPHYSIOLOGIC DATA.** For the electrophysiologic analysis, data from three monkeys (F, I, and A) were examined. Only those recordings in which the reconstruction of the location of recorded neurons, based on probe position measurements during the experimental sessions and postmortem histological analyses, showed that they were located in GPi or SNr were used. Forty-five neurons were included in this study (monkey F, 10 neurons; monkey I, 31 neurons; monkey A, 4 neurons). There were no clear differences in neuronal activity or the responses to drug infusions between the animals and the data were pooled for subsequent analysis. A template-matching spike-sorting routine with subsequent principal component analysis (Spike2) was used for off-line measurements of interspike intervals (ISIs). ISI distribution histograms and autocorrelograms were carefully examined for evidence of erroneous sampling of multiple cells, or inclusion of noise in the data. If such evidence was found, the data records were either re-sorted or discarded.

All of the subsequent analysis steps were carried out, using custom-written algorithms in the Matlab software environment (The MathWorks, Natick, MA). The ISI data were binned in 1-s intervals to generate frequency readouts. The median of these values in the preinfusion data segment was defined as the baseline firing rate of the neuron. An injection was considered to have an effect if the rate of discharge significantly differed from baseline for a minimum duration of 60 s, with an onset within 240 s from the beginning of drug injection (see following text). The parameters chosen to define a drug effect were based on a previous study using the same recording-injection device (Kliem and Wichmann 2004). The “effect period” was defined as the time period in which the discharge rate of a neuron was maximally affected by the injection. This epoch consisted of a 60-s data segment that started 30 s before and ended 30 s after the maximal effect. In most cases, drug effects lasted much longer than the minimum of 60 s, usually until the end of the record. For neurons in which the injection had no effect on the firing rate, a 60-s data segment starting 120 s after the beginning of the injection was used as a surrogate.

To examine whether drugs affected the activity of the cells within a given experimental group (e.g., SNr cells tested after injection of the D1LR agonist), the discharge rates during the effect epoch were statistically compared with the respective median baseline discharge rates (see following text). In each case, the median discharge rate during the effect epoch was also expressed as a percentage of the baseline discharge rate. Medians, as well as 25th and 75th percentiles of these percentages, are reported in RESULTS.

In addition, burst indices were calculated for the baseline and effect periods. Bursts were detected using the Poisson “surprise” method developed by Legendy and Salcman (1985). For the present analysis, a “surprise” value of 3 was used (Aldridge and Gilman 1991; Wichmann and Soares 2006) and burst indices were calculated as the ratio between the number of spikes found in bursts and the total number of spikes in the record. Finally, changes in oscillatory firing of SNr and GPi cells were assessed with power spectral methods. As described in previous publications (Soares et al. 2004), we converted the ISI data into frequency readouts and calculated power spectra. The algorithm was implemented in Matlab (Welch’s method, mean detrending). For each cell, the raw spectra were integrated in the following Hz ranges: 1–3, 3–8, 8–15, and >15, and normalized to the total power in the spectrum. Although the evaluation of slow oscillations would also have been of interest (Ruskin et al. 1999; Wichmann et al. 2002), this type of analysis was not possible because the duration of the evaluated response periods was too short.

**ANALYSIS OF MICRODIALYSIS DATA.** In each experiment, the basal level of GABA was calculated as the average of microdialysis samples 1 and 2. The magnitudes of drug (or vehicle) effects on GABA measurements were calculated by subtracting the basal level (as defined earlier) from sample 3. A similar calculation was performed to assess changes in GABA levels after stimulation with a high concentration of potassium. For this, the basal GABA level was subtracted from sample 5. The changes in GABA levels were expressed as a percentage change from baseline for each experiment.

**STATISTICS.** Nonparametric statistics were used throughout this study. Within individual electrophysiologic drug-injection experiments, Mann–Whitney tests were used to compare the frequency values in the baseline period with those in the drug effect period. The Wilcoxon signed-rank test was used to compare paired recording or microdialysis data collected before and after drug injections. P values <0.05 were accepted as indicators of significance.

**RESULTS**

**Electrophysiologic effects of D1LR ligand injections**

We examined the effects of local microinjections of D1LR-selective ligands on the neuronal activity of 22 SNr neurons and 23 GPi neurons in the immediate vicinity of the injection sites. The median firing rate for all recorded SNr cells at baseline was 37.6 spikes/s (30.6–47.1 spikes/s, 25th–75th percentiles), whereas the median rate for all GPi cells at baseline was 64.4 spikes/s (44.2–81.6). Control injections of aCSF in SNr (n = 6 cells) or GPi (n = 5) had no effect on the neuronal activity (data not shown). Although we tested neurons...
throughout the extent of both nuclei, the relatively small sample size does not allow us to examine whether the drug effects, reported below, were regionally specific within GPi or SNr.

**EFFECTS OF D1LR ACTIVATION.** The infusion of the selective D1LR agonist SKF82958 (3 μg/μl) reduced the discharge rates of the majority of recorded neurons in SNr (10/13, Fig. 1) and GPi (8/13), whereas a minority of cells in both structures (2/13 in SNr, 1/13 in GPi) responded with an increase in neuronal discharge. Only a few cells did not respond (1/13 in GPi, 4/13 in GPi) to the drug injections. The median discharge rate of SNr cells tested decreased from 38.3 spikes/s (30.1–60.1) at baseline to 12.4 spikes/s (2.3–46.5) after drug exposure. Infusions of the D1LR agonist reduced the firing of SNr cells by a median of 61% (37–93%; P < 0.05, Wilcoxon signed-rank test; Fig. 2). The maximal effect was seen in the SNr with a median latency of 357.8 s (198.4–520.3). In GPi, the median discharge rate was 75.5 spikes/s (59.2–96.9) at baseline and 56.1 spikes/s (29.5–93.7) after exposure to the D1LR agonist. Infusion of the D1LR agonist reduced the firing of GPi cells by a median of 13% (5–59%; P < 0.05, Wilcoxon signed-rank test; Fig. 2). In this case, the maximal effect was seen at a median latency of 296.2 s (146.8–376.0). In most SNr cells (6/10), the reduced neuronal discharge was preceded by a brief increase in neuronal discharge as shown in Fig. 1A (arrow). Such biphasic effects were not seen in GPi.

Analysis of burst discharges (Fig. 3) revealed that the median proportion of spikes within bursts increased in SNr from 12.3% (4.5–18.5) to 18.5% (6.1–34.5) and in GPi from 12.8% (9.4–16.0) to 13.3% (7.4–21.4). Compared with the cell’s baseline burst index, the median increase amounted to 39.0% (–5.2 to 155.0) in SNr and 15.4% (0–34.7) in GPi. These changes achieved significance only in GPi (P < 0.05, Wilcoxon signed-rank test).

Statistical comparisons of the normalized integrated power spectra showed that in SNr, the median power in the 3- to 8-Hz band increased with the exposure to the D1LR agonist from 3.0% (2.1–4.2) to 4.2% (3.0–4.8). Compared with the cell’s baseline power in this band, the drug exposure produced a median increase of 27.9% (12.3–42.4, P < 0.05). The median power in the 8- to 15-Hz band increased from 4.5% (2.7–5.6) to 6.7% (3.3–6.8). Compared with the cell’s baseline power in the 8- to 15-Hz band, the drug exposure produced a median increase of 17.8% in this band (9.4–72.8, P < 0.05). There was a concomitant decrease in the frequencies >15 Hz, from 89.9% (87.6–93.0) to 86.4% (85.7–90.3). In GPi, the proportional distribution of power was qualitatively similar to that in SNr, but did not reach significance (Fig. 3).

**EFFECTS OF D1LR BLOCKADE.** The infusion of the D1LR antagonist SCH23390 had different effects on SNr and GPi neurons. The responses of SNr neurons to the administration of this drug varied. Although a proportion of recorded SNr cells (3/9) showed an increase in firing after the injections, the median change in firing rates from 36.2 spikes/s (28.9–45.9) at baseline, to 47.5 spikes/s (25.9–55.7) after drug application, was not significant when compared with the cell’s baseline firing rate. Two SNr cells showed a reduction of activity and four did not respond to drug application (Fig. 2). The median latency of the drug effect was 486.7 s (356.4–525.9) after the start of the

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**Fig. 1.** Responses of a substantia nigra pars reticulata (SNr) and a internal pallidal segment (GPi) neuron to infusions of dopamine D1-like receptor (D1LR) ligands. Figure shows changes in neuronal discharge rates of an SNr neuron (A) after infusion of a D1LR agonist (SKF82958, 3 μg/μl) and a GPi neuron (B) after infusion of a D1LR antagonist (SCH23390, 5 μg/μl). Both drugs were infused for 2 min at 0.25 μl/min. Thick solid line represents the median increase of 27.9% (12.3–42.4, P < 0.05). The mean change from baseline for each experiment. Medians of these percentages are reported here along with the 25th and 75th percentiles (bottom and top of boxes) and minima and maxima (bottom and top of error bars). *P < 0.05, Wilcoxon signed-rank test.

**Fig. 2.** Changes in discharge rate of SNr and GPi neurons to infusions of D1LR ligands. For each cell in an experimental group, the discharge rate during the effect epoch was calculated and compared with the baseline discharge rate. Changes in discharge rate were expressed as a percentage change from baseline for each experiment. Medians of these percentages are reported here along with the 25th and 75th percentiles (bottom and top of boxes) and minima and maxima (bottom and top of error bars). *P < 0.05, Wilcoxon signed-rank test.
drug infusion. The majority of GPi neurons (7/10) showed a significant increase in their discharge rate after SCH23390 administration (Fig. 1), whereas two cells showed a reduction in discharge and one cell did not respond. The median discharge rate increased from 44.9 spikes/s (24.8–69.3) to 69.5 spikes/s (28.8–91.4; Fig. 2). Compared with the cell's baseline firing rate, the median change amounted to an increase of 30.7% (1.8–62.4, P < 0.05). The median latency between the beginning of the injection and the maximal effect was 182.6 s (160.5–325.0).

Analysis of burst discharges (Fig. 3) revealed that the median proportion of spikes within bursts in SNr was not significantly changed, whereas it decreased from 19.9% (17.4–23.1) to 11.8% (4.4–22.2) in GPi. Compared with each cell's baseline burst index, the median reduction amounted to a decrease of 37.8% (66.1–8.0, P < 0.05) in GPi.

As shown in Fig. 3, the median integrated power in the 3- to 8-Hz band changed from 3.9% (3.5–4.7) to 3.7% (3.1–4.4) in GPi. Compared with the cell's baseline power in this band, the drug exposure produced a median reduction of 7.8% (3.7–15.5, P < 0.05). The median power in the 8- to 15-Hz band did not change. There was no significant change in the proportional distribution of power in the SNr.

Microdialysis experiments

The median basal levels of GABA were 0.2 μM in SNr (0.12–0.40, n = 26) and 0.35 μM in GPi (0.25–0.79, n = 26). Exposure of the tissue to 80 mM K+ was done to assess the viability of the tissue and resulted in at least a threefold increase in GABA efflux in all cases tested (data not shown). As shown in Fig. 4, reverse microdialysis of SKF82958 (0.411 μg/μl) induced a significant increase in the GABA concentration in GPi (P < 0.05; Wilcoxon test) and SNr (P < 0.05; Wilcoxon test). The median increase in GABA levels was 16.8% (5.7–40.1; n = 9) in SNr and 12.0% (4.3–20.3; n = 10) in GPi. Infusion of SCH23390 (0.01 μg/μl; SNr, n = 11; GPi, n = 10) or of vehicle (SNr, n = 6; GPi, n = 5) had no significant effect on GABA concentrations in GPi and SNr.

Discussion

Our results demonstrate that the activation of D1LRs reduces neuronal discharge rates in SNr and GPi, along with changes in oscillatory and burst discharge patterns. The microdialysis data suggest that at least some of these effects could be secondary to increased GABA release from terminals of the striatogniral and striato-GPi projections. The effects of local infusion of the D1LR antagonist provide evidence that activation of D1LRs by endogenous dopamine acts to reduce GPi activity.

Technical considerations

Although the methods used in this report are well established in the literature, several technical issues need to be addressed. One concern is that the pharmacologic effects seen with the combined injection/recording device are more variable in terms of the maximal amplitude and the latency of the observed effects than those seen with in vitro methods (see also Galvan et al. 2005; Kita et al. 2004, 2006; Kliem and Wichmann 2004). This is explained in part by the greater biological complexity of the in vivo system, but also by technical constraints, such as differences in the exact dimensions of the injection system, the variable extent or direction of drug diffusion, or the presence of drug diffusion barriers (see also Galvan et al. 2005; Kita et al. 2004). To minimize the variability of our results, we kept the configuration of the recording-injection device, the rate and volume of drug delivery, and the drug concentrations constant throughout our studies. Although it is not feasible to generate detailed quantitative dose–response curves with this system, qualitative distinctions between responding and nonresponding cells are possible, and quantitative assessments of differences between the responses of pools of neurons to fixed drug concentrations can be performed (see Galvan et al. 2005).
A second issue is that in vivo sampling of tissue concentrations of GABA with microdialysis is an imperfect technique, due to its low time resolution, and because it is not clear what proportion of the measured GABA concentrations is derived from synaptic sources (Westerink and de Vries 1989). Despite this shortcoming, however, our results demonstrate that the method is sensitive enough to detect changes in ambient GABA levels after local administration of D1LR ligands in Gpi and SNr. Because most D1LRs are located on axons or terminals of GABAergic neurons in these structures, it is likely that the measured changes in GABA levels, in fact, reflect changes in transmitter release at synaptic sites. The basal levels of GABA measured in SNr and Gpi in our study were similar to those reported in previous rodent and nonhuman primate experiments (Galvan et al. 2005; Matuszewich and Yamamoto 1999; Robertson et al. 1991b; Trevitt et al. 2002; Windels et al. 2005).

It is also important to consider the specificity of the D1LR active compounds that were used in this study. SKF82958 binds with much higher affinity to D1LRs than to D2LRs (Neumeyer et al. 2003; Toll et al. 1998), but does not reliably distinguish between D1 and D5 receptors. It has low affinities at serotonin- and α2-adrenergic receptors (Neumeyer et al. 2003; Toll et al. 1998). Likewise, SCH23390 binds with high affinity to D1LRs, but does not distinguish between D1 and D5 receptors. Its affinity for adrenergic alpha receptors (Bogeso et al. 1995; Neumeyer et al. 2003), D2LRs, or serotonin 5-HT1 or 5-HT3 receptors is low (Markstein et al. 1986; Neijt et al. 1988; Waebier et al. 1988). It binds, however, with relatively high affinity to 5-HT2A (Neumeyer et al. 2003) and 5-HT2C receptors (Briggs et al. 1991; Millan et al. 2001; Woodward et al. 1992). Because 5-HT2A receptors are present only in relatively low density in the monkey Gpi, SNr, and Snc, the affinity of SCH23390 does not represent a significant problem (Lopez-Gimenez et al. 2001). The situation is different for 5-HT2C receptors. These receptors are expressed in the rat SNr and Gpi (Eberle-Wang et al. 1997), and mRNA for them has been found in lateral portions of the monkey SN (not in Gpi) (Lopez-Gimenez et al. 2001). SCH23390 acts as a partial or full agonist at 5-HT2C receptors (Hoyer et al. 1989; Millan et al. 2001) and could therefore increase pallidal or nigral activity through postsynaptic 5-HT2C receptor activation. In our experiments, SCH23390 effects at 5-HT2C receptors in Gpi would be particularly important because the drug acted to increase firing in this area. However, in distinction to rodent studies where activation of postsynaptic 5-HT2C receptors increases SNr firing (Di Giovanni et al. 2006a,b; Fox and Brochtie 2000a,b; Fox et al. 1998; Invernizzini et al. 2007; Rick et al. 1995), a recent study of serotonin effects on neuronal activity of pallidal neurons in awake primates did not find evidence for significant postsynaptic 5-HT2 receptor-mediated effects (Kita et al. 2007). Other studies have failed to show mRNA for 5-HT2C receptors in the primate Gpi (Lopez-Gimenez et al. 2001). Because of these findings, it seems most likely that SCH23390 in our study acted by blockade of D1LRs rather than as a 5-HT2C receptor agonist.

Another concern related to the drugs used in this study is the possibility that D1LR activation may have led to desensitization of D1 receptors. This is not likely to be a significant confounding factor, however, because the selective D1LR ligands were infused over a short time period, followed by a gradual diffusion of ligand, limiting the degree of desensitization (Lewis et al. 1998).

**D1LR agonist effects**

D1LRs are strongly expressed in SNr and Gpi (Barone et al. 1987; Fremeau et al. 1991; Richfield et al. 1987), supporting the hypothesis that dopamine released at these locations may activate D1LRs. Electron microscopic studies have shown that in the rat SNr and entopeduncular nucleus (rodent homologue of monkey Gpi) D1 receptors are predominantly located presynaptically on myelinated axons and axon terminals (Yung et al. 1995). These receptors are expressed in putative GABAergic terminal boutons that form synaptic synapses on dopaminergic dendrites arising from nearby SNc neurons, or from dendrites of nondopaminergic SNr neurons (Caille et al. 1996). In both SNr and Gpi, the labeled terminals show ultrastructural features typical for striatal GABAergic boutons, suggesting that the striatum is the main source of the D1-containing GABAergic terminals of basal ganglia output nuclei in rodent (Levey et al. 1993; Yung et al. 1995) and monkey (Caille et al. 1996).

The effects of dopamine receptor activation in the SNr and, specifically, the effects of D1LR activation have been extensively evaluated in rodents. These studies have resulted in conflicting evidence—very likely arising from the use of different experimental techniques and different pharmacologic approaches. In addition, the in vivo studies on this topic differ in the use of anesthetics.

One approach has been to study GABA release in response to either dopamine or D1LR ligands. Dopamine was shown to increase GABA release from rat brain slices in early studies (Reubi et al. 1977), or to induce biphasic effects (van der Heyden et al. 1980), perhaps explained by the fact that D1LR and D2LR receptors are activated by dopamine. Other studies of GABA release from brain slices showed that D1LR activation potentiates evoked GABA efflux in the SNr (Aceves et al. 1995; Floran et al. 1990; Starr 1987), although this was not true in other studies (Mayfield et al. 1999). Published reports on the effects of D1LR activation on GABA release in vivo, studied in awake rats with microdialysis techniques, have consistently demonstrated that D1LR activation increases GABA levels in the SNr (Matuszewich and Yamamoto 1999; Rosales et al. 1997; Timmerman and Westerink 1995; Trevitt et al. 2002; You et al. 1994) and entopeduncular nucleus (the rodent homologue of the primate Gpi; Ferre et al. 1996).

In vivo electrophysiologic studies reported that dopamine acts to increase the activity (Ruffieux and Schultz 1980) and/or to attenuate the inhibitory effects of GABA (Waszczak and Walters 1983, 1986) on SNr neurons. At least some of these effects may have been mediated by D2LRs (Waszczak 1990). More recent in vivo studies in which the electrophysiologic effects of D1LR-specific ligands were investigated have shown that SNr neurons are inhibited by endogenous dopamine, released by application of amphetamine in anesthetized (Timmerman and Averbrombe 1996) or in awake unrestrained rats (Windels and Kiyatkin 2006). This effect was blocked by SCH23390 and was thus likely due to D1LRs activation effects.
receptors may induce direct activation of basal ganglia output (Timmerman and Abercrombie 1996; Windels and Kiyatkin 2006).

Brain slice recording studies have also led to inconsistent results. Although D1LR-mediated excitatory effects on SNr neurons were seen in some studies (Martin and Waszczak 1994; Miyazaki and Lacey 1998), most of the in vitro recording studies demonstrated the opposite effect, i.e., an increase in GABAergic transmission in response to D1LR activation (Fioran et al. 2002; Misgeld 2004; Radnikow and Misgeld 1998). Although there is substantial evidence that most D1LRs are located on striatonigral terminals, a recent study has suggested that portions of the D1LR-mediated modulation of SNr activity may involve D1LR effects on glutamate release from the STN-SNr projection (Ibáñez-Sandoval et al. 2006).

There is obviously no clear consensus on the effects of D1LR activation in the rodent SNr, but most of the studies are compatible with the notion that D1LR activation results in increased GABA release from striatonigral terminals and in subsequent inhibition of SNr neurons. Our electrophysiologic and microdialysis studies are in line with this view.

The exact mechanism by which D1LR activation increases ambient GABA levels in the primate GPe and SNr, and leads to a reduction of firing in these brain areas, remains unclear, however. Under rest conditions, such as those chosen for our study, the striatal output neurons that give rise to these monosynaptic pathways have a low intrinsic activity (Kimura 1992), so that D1LR effects on action-potential (AP)-induced GABA release would be small. It is therefore likely that a substantial component of the ambient GABA levels measured with microdialysis in the SNr and GPe originates from other sources, such as projections from GPe (Parent and Hazrati 1995; Windels et al. 2005). Local axon collaterals of neurons intrinsic to GPe or SNr may also play a role, although the anatomy and the extent of such axon collaterals have not been conclusively studied in the primate GPe or SNr. The activity of GPe projections would not be expected to change with activation of D1LRs in GPe or SNr.

Given the predominant anatomical location of D1 receptors on striatal afferents to SNr and GPe, it is possible that the modulation of GABA levels through D1LR activation at rest may have occurred largely through AP-independent mechanisms, for instance through an influence of the D1LR agonist SCH23390 exerted its frequency-increasing effects through activation of postsynaptic 5-HT2C receptors rather than modulation of GABA levels in this structure. Because the available evidence is in favor of a predominant action of this drug at D1LRs rather than 5-HT2 receptors (see earlier part of discussion), we will consider the drug effects only in terms of its known effects on dopaminergic transmission.

In behavioral experiments in rodents, unilateral intranigral injections of SCH23390 inhibit amphetamine-induced behaviors (Timmerman and Abercrombie 1996; Yurek and Hipkins 1993) and impair performance on a rod-balancing task (Bergquist et al. 2003), whereas bilateral injections diminish lever-pressing behavior and locomotor activity (Trevitt et al. 2001). Several studies have confirmed the presence of dopamine in rat (Cobb and Abercrombie 2003; Heeringa and Westerink 1995; Robertson et al. 1991a; Santiago and Westerink 1991) and monkey SNr (Gerhardt et al. 2002) as well as monkey GPe (Pifl et al. 1990). The question whether dopamine has a physiologic role in the regulation of activity in GPe and SNr is particularly relevant because it has been suggested that dopamine depletion at these sites may contribute to the development of some of the behavioral abnormalities in Parkinson's disease (Crocker 1995; Forno 1996; Gash et al. 1996; Gerhardt et al. 1999; Hemsley and Crocker 2001; Hornykiewicz 1998; Parent and Cossette 2001; Starr et al. 1999; Wichmann and DeLong 2003).

Our study provides evidence that many GPe cells may be tonically inhibited by ambient dopamine, whereas the responses of SNr neurons were more variable, perhaps because sources of endogenous dopamine in GPe and SNr differ substantially. GPe receives a direct projection from the SNr (Parent et al. 1990; Schneider and Dacko 1991; Smith et al. 1989), whereas dopamine in the SNr originates from dendritic release from SNr cells (Bjorklund and Lindvall 1975; Cheramy...
et al. 1981; Geffen et al. 1976; Wassef et al. 1981). Compared with GPi, the degree of tonic activation of D1LRs in the primate SNr appears to be low under physiologic conditions, either because less dopamine is released or because the released dopamine is rapidly cleared from the extracellular space (Suada-Chagny et al. 1995). The latter possibility is suggested by binding studies in human and monkey GPi and SNr that have demonstrated that dopamine uptake sites are present in higher concentrations in SNr compared with GPi (Gnanalingham et al. 1995; Marcusson and Eriksson 1988).

The behavior of the animal under study may also have a strong influence on the level of ambient dopamine in these structures. It is well established that the activity of dopaminergic neurons, and thus potentially also dopamine concentrations, changes during the performance of rewarded tasks (Hollerman and Schultz 1998; Ljungberg et al. 1992; Mirenowicz and Schultz 1994; Schultz 1998; Schultz et al. 1997; Waelti et al. 2001). The lack of effect of the D1LR antagonist in the SNr, measured during behavioral “idling,” as in our study, may not reflect the true behavioral importance of the neuromodulator. It remains to be seen whether locally injected dopamine receptor antagonists would interfere with behavioral performance or influence neuronal spiking during the performance of tasks, as was shown for D1LR antagonist injections into striatum and frontal cortex (Nakamura and Hikosaka 2006; Williams and Goldman-Rakic 1995).

**Activation of D1LRs influences discharge patterns of SNr and GPi neurons**

D1LR activation increased the proportion of spikes found in bursts in SNr and GPi, whereas blockade of these receptors had the opposite effect. We also found that in both structures, oscillations in the 3- to 8-Hz and 8- to 15-Hz bands were enhanced by D1LR activation, whereas D1LR blockade had the opposite effect in GPi (no effect was observed in the SNr). The changes in bursting may be explained by the fact that GABA, released after D1LR activation, may hyperpolarize SNr and GPi cells that, in turn, may increase their tendency to discharge in bursts. This phenomenon has been described for multiple brain regions, including GP, the rodent equivalent of the primate GPe, and STN (Beurrier et al. 1999, 2000; Bevan et al. 2002; Kass and Mintz 2006; Nambu and Llinas 1994; Overton and Greenfield 1995), but has not been described for GPi or SNr in rodents or primates. It is interesting that the increases in bursting and oscillatory activity in the basal ganglia output nuclei are seen not only after D1LR activation, but also in chronically dopamine-depleted animals and humans (Bergman et al. 1998; Levy et al. 2001; Murer et al. 1997; Wichmann and Soares 2006; Wichmann et al. 1999).

Our findings suggest therefore that reduced activation of D1LRs in GPi or SNr does not contribute to, and may even counteract, the parkinsonism-related differences in firing. In the parkinsonian state, network interactions between the striatum and other basal ganglia structures may be more important in determining the pattern abnormalities in GPi and SNr than the reduced activation of D1LRs by endogenous dopamine (see also Gatev et al. 2006).

**Functional and clinical implications**

Our study demonstrates both similarities and differences between GPi and SNr. Based on their position within the basal ganglia circuitry, similarities in their neuronal composition, and basic similarities in neuronal firing characteristics (Wichmann et al. 1999), these nuclei are often seen as homologous, with GPi subserving motor functions and SNr subserving in large part associative and limbic functions. Some of our results, such as the finding of an inhibitory response to D1LR activation in most neurons in both GPi and SNr support this view. However, the examination of dopaminergic functions in these nuclei also demonstrates clear differences between them that are apparent in our results, including the lack of D1LR agonist effects in SNr, the finding that SNr neurons often showed biphasic responses after infusion of D1LR agonists (an initial increase followed by a decrease), and the more pronounced (median) inhibition seen after D1LR infusion in SNr. These differences may be related to one another, for instance through the possibility that low levels of background activation may result in (moderate) desensitization of D1LRs in GPi, whereas no such desensitization is at work in the SNr. Although not explained at a mechanistic level, the different responses of GPi and SNr neurons to D1LR activation may also have behavioral consequences. The motor circuit of the basal ganglia that passes through the primate GPi may be under tighter (and perhaps more precisely timed) dopaminergic control than the limbic and associate circuits that are prominent in the SNr.

The behavioral effects of D1LR activation in SNr or GPi have not been extensively studied. The available evidence suggests that D1LR activation in the SNr results in behavioral activation in rats (Timmerman and Abercrombie 1996) and may be involved in startle responses (Meloni and Davis 2004). Consistent with these results, D1LR antagonist injections in the SNr impair motor activities in rats (see above; Bergquist et al. 2003; Trevitt et al. 2001). It remains an open question whether dopamine depletion in GPi and SNr contributes to the development of parkinsonism and the changes in firing rates and patterns seen in the basal ganglia output nuclei that have been identified in parkinsonism (see above). Combined microdialysis and behavioral studies using a rod-balancing task have indicated that partial depletion of dopamine in the SNr significantly impairs motor functions and that dopamine release in the SNr can compensate for some of the impairments induced by striatal dopamine loss (Andersson et al. 2006). Further evidence for the potential role of local dopamine loss at the level of the SNr in the generation of abnormal basal ganglia output in the dopamine-depleted state comes from studies in which electrophysiologic abnormalities in SNr activity were identified in brain slices that included the SN (but not the striatum) in reserpinized rats (Wittmann et al. 2002). It has also been suggested that changes in the dopaminergic system in GPi may be involved because dopamine concentrations are lowered in GPi in the brains of patients with Parkinson’s disease, compared with controls (Bernheimer et al. 1973), and the finding of (presumably adaptive) changes in the uptake of the.
distribution and morphology of dopamine precursor 18F-dopa in Gpi in positron emission studies in parkinsonian patients (Whone et al. 2003a).

Our studies do not support the hypothesis that loss of nigral D1LR activation in Parkinson’s disease contributes significantly to firing abnormalities in the majority of basal ganglia output neurons. This does, however, not diminish the potential for a functional impact of dopamine replacement strategies aimed at these nuclei, specifically the SN. Infusions of glial-derived neurotrophic factor into the SN have been demonstrated to ameliorate parkinsonism in MPTP-treated primates, most likely through an increase in nigral dopamine levels (Gash et al. 1996), and dopaminergic mesencephalic cell grafts into the SN area substantially improve motor signs of parkinsonism in this animal model (Gerhardt et al. 1999; Starr et al. 1999).

The finding that drugs acting at D1LRs exert pharmacologic effects in SNr or Gpi also implies that some of the side effects of dopaminergic treatments may be explainable through actions at these sites. For instance, because the SNr is strongly involved in associative and limbic basal ganglia circuits (Haber and Fudge 1997; Middleton and Strick 2002; Parent and Hazrati 1994), some of the cognitive or emotional side effects of D1LR-active drugs may be mediated through activation of the nigral dopamine receptors.

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