Control of the Subthalamic Innervation of the Rat Globus Pallidus by D<sub>2/3</sub> and D<sub>4</sub> Dopamine Receptors

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Hernández, Adán, Osvaldo Ibáñez-Sandoval, Arturo Sierra, René Valdiosera, Dagoberto Tapia, Verónica Anaya, Elvira Galarraga, José Bargas, and Jorge Aceves. Control of the subthalamic innervation of the rat globus pallidus by D<sub>2/3</sub> and D<sub>4</sub> dopamine receptors. J Neurophysiol 96: 2877–2888, 2006. First published August 9, 2006; doi:10.1152/jn.00664.2006. The effects of activating dopaminergic D<sub>2/3</sub> and D<sub>4</sub> receptors during activation of the subthalamic projection to the globus pallidus (GP) were explored in rat brain slices using the whole cell patch-clamp technique. Byocitin labeling and both orthodromic and antidromic activation demonstrated the integrity of some subthalamopallidal connections in in vitro parasagittal brain slices. Excitatory postsynaptic currents (EPSCs) that could be blocked by CNQX and AP5 were evoked onto pallidal neurons by local field stimulation of the subthalamopallidal pathway in the presence of bicuculline. Bath application of dopamine and quinpirole, a dopaminergic D<sub>2</sub>-class receptor agonist, reduced evoked EPSCs by about 35%. This effect was only partially blocked by sulpiride, a D<sub>2/3</sub> receptor antagonist. The sulpiride-sensitive reduction of the subthalamopallidal EPSC was associated with an increase in the paired-pulse ratio (PPR) and a reduction in the frequency but not the mean amplitude of spontaneous EPSCs (sEPSCs), indicative of a presynaptic site of action, which was confirmed by variance–mean analysis. The sulpiride-resistant EPSC reduction was mimicked by PD 168,077 and blocked by L-745,870, selective D<sub>4</sub> receptor agonist and antagonist, respectively, suggesting the involvement of D<sub>4</sub> receptors. The reduction of EPSCs produced by PD 168,077 was not accompanied by changes in PPR or the frequency of sEPSCs; however, it was accompanied by a reduction in mean sEPSC amplitude, indicative of a postsynaptic site of action. These results show that dopamine modulates subthalamopallidal excitation by presynaptic D<sub>2/3</sub> and postsynaptic D<sub>4</sub> receptors. The importance of this modulation is discussed.

INTRODUCTION

The globus pallidus (GP) plays a critical role in the control of movement exerted by the basal ganglia (DeLong et al. 1985; Mitchell et al. 1987; Törmönen et al. 1998) through its projections to the subthalamic nucleus (STN) and to other basal ganglia structures that are inhibited by GABAergic synaptic inputs (Beckstead 1983; Gandía et al. 1993; Kita and Kita 2001; Kita and Kitai 1991, 1994; Parent et al. 2000; Sato et al. 2000a; Shinonaga et al. 1992; Windels et al. 2005).

In turn, GP neurons receive and integrate several entries. Their main inhibitory afferents come from the striatum (Cooper and Stanford 2001; Engler et al. 2006; Fornum et al. 1978; Gimenez-Amaya and Graybiel 1990; Hazrati and Parent 1992; Kawaguchi et al. 1990; Pan and Walters 1988; Parent et al. 1984; Shin et al. 2003; Tremblay and Filion 1989) and from axon collaterals that interconnect GP neurons themselves (Kita et al. 2004; Parent et al. 2000; Shin et al. 2003), although their main excitatory inputs come from the STN (Hazrati and Parent 1992; Kita and Kitai 1991; Nambu et al. 2000; Sato et al. 2000b; Smith et al. 1990) and from thalamic intralaminar nuclei (Mouroux et al. 1997; Yasukawa et al. 2004). Connections between STN and GP form the principal site of reciprocally innervated excitatory and inhibitory neurons in the basal ganglia (Bevan et al. 2002b). When inhibitory and excitatory neuronal populations are reciprocally innervated, they could form the basis of a central pattern generator (Grillner 2003; Terman et al. 2002; Yuste et al. 2005) that may transform excitatory drives into spatiotemporal patterns of oscillatory activity for the generation of motor programs (Bevan et al. 2002b; Plenz and Kitai 1999).

The above-mentioned afferents converging onto pallidal neurons follow a structured topographical arrangement: the GP network (Hazrati and Parent 1992; Kaneda et al. 2002; Shink et al. 1996; Yasukawa et al. 2004). These inputs are integrated by pallidal neurons with the important contribution of a distinct dopaminergic innervation from neurons of the substantia nigra pars compacta (Debeir et al. 2005; Gauthier et al. 1999; Lindvall and Bjorklund 1979; Prens and Parent 2001; Ruskin and Marshall 1997). The way in which dopaminergic innervation modulates the GP network is a matter of study (e.g., Costall and Naylor 1972; Galvan et al. 2001; Hauber and Lutz 1999; Kreiss et al. 1997; Nakanishi et al. 1985; Querejeta et al. 2001). For example, a presynaptic modulation of the striatopallidal projection through D<sub>2</sub>-class receptors (Cooper and Stanford 2001; Floran et al. 1997) was previously described. In addition, a postsynaptic modulation of inhibitory inputs through D<sub>4</sub>-receptor activation (Ariano et al. 1997; Mauger et al. 1998; Mrzljak et al. 1996; Shin et al. 2003) was also proposed.

Because it was recently shown that subthalamic projections to substantia nigra pars reticulata are presynaptically modulated by dopamine (Ibañez-Sandoval et al. 2006), this work investigated the possibility that the subthalamopallidal connections were also modulated by dopamine. Correspondingly, it is...
shown here that D<sub>2/3</sub> and D<sub>4</sub> receptor activation modulates the subthalamopallidal input onto GP neurons. A preliminary report of these results was previously presented in abstract form (Hernández et al. 2004).

**METHODS**

**Preparation of slices**

All procedures were carried out in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals and were approved by the Institutional Animal Care Committees of the CINVESTAV and UNAM. The experiments were performed on brain slices obtained from Wistar rats [postnatal day (PD) 14 to PD 21]. The rats were anesthetized and decapitated. The brain was rapidly immersed for 1 min in cold oxygenated saline (about 4°C; 95% O<sub>2</sub>-5% CO<sub>2</sub>) of the following composition (in mM): 124 NaCl, 2.5 KCl, 1.3 MgCl<sub>2</sub>, 1.2 NaH<sub>2</sub>PO<sub>4</sub>, 2.4 CaCl<sub>2</sub>, and 10 glucose. The same saline but with choline chloride (124 mM) instead of NaCl was used during the slicing procedure. Parasagittal slices (300 μm) containing both GP and STN (see Fig. 1) were cut on a vibroslicer (Lancer, Technical Products International, St. Louis, MO) and transferred to the saline with NaCl (above). The slices were left for equilibration for at least 1 h in oxygenated saline at room temperature (about 25°C). After equilibration, a single slice was transferred to a recording chamber placed on the stage of an upright microscope and was continuously superfused (2–3 ml min<sup>-1</sup>) with oxygenated saline at room temperature.

**Whole cell recordings**

Recordings were made at room temperature (about 25°C) from neurons located inside the GP boundaries as seen in the parasagittal slice (Fig. 1). Neurons were visualized using infrared differential interference videomicroscopy with a ×40 water-immersion objective (Hamamatsu C2400-50, Hamamatsu Photonics Systems USA and Axioscop, Carl Zeiss, Oberkochen, Germany.). Micropipettes for whole cell recordings were pulled (Sutter Instrument, Novato, CA) from borosilicate glass tubes (1.5 mm outer diameter, WPI, Sarasota, FL) for a final resistance of 2–5 MΩ when filled with internal saline of the following composition (in mM): 120 KSO<sub>3</sub>CH<sub>3</sub>, 16 KCl, 2 MgCl<sub>2</sub>, 10 HEPES, 1.1 K<sub>2</sub>-EGTA, 1.1 ATP-Mg, 1.1 GTP-Na, and 5 mM QX-314 (pH 7.3 adjusted with KOH and 287–290 mOsm L<sup>-1</sup>). QX-314 was used to avoid contamination of synaptic responses by unclamped action currents. Voltage-clamp recordings were made with an Axopatch 200A amplifier (Axon Instruments, Foster City, CA). Liquid junction potentials (±5 mV) were not corrected.

Recordings were acquired at 10 kHz using a Digidata 1200 interface (Axon), Bessel filtered at 5 kHz, and analyzed with pCLAMP (v 7.0) (Axon). Access resistance (7–20 MΩ) was monitored continuously and experiments were abandoned if changes >20% occurred.

**Histochemistry**

As described elsewhere (see Ibañez-Sandoval et al. 2006; Vergara et al. 2003), a sharp micropipette (tip <0.5 μm) filled with external saline and 5% biocytin (Sigma-Aldrich, St. Louis, MO) was placed inside the STN borders (near the middle) and left there for 1 h while the slice was constantly superfused. Thereafter the pipette was retired and the slice was superfused with oxygenated saline for another 6 h. The slice was then fixed overnight in 0.1 M phosphate-buffered saline (PBS; pH = 7.4; 4°C) with 4% paraformaldehyde and 1% picric acid. Afterward, slices were infiltrated with 30% sucrose and cut on a vibratome (Ted Pella, Reading, CA) into 60-μm sections. After washing with Tris-buffered saline (TBS) containing Triton X-100 and avidin–biotin–peroxidase complex (1:100; Vector Laboratories, Burlingame, FL) for a final resistance of 2–5 MΩ when filled with internal saline of the following composition (in mM): 120 KSO<sub>3</sub>CH<sub>3</sub>, 16 KCl, 2 MgCl<sub>2</sub>, 10 HEPES, 1.1 K<sub>2</sub>-EGTA, 1.1 ATP-Mg, 1.1 GTP-Na, and 5 mM QX-314 (pH 7.3 adjusted with KOH and 287–290 mOsm L<sup>-1</sup>). QX-314 was used to avoid contamination of synaptic responses by unclamped action currents. Voltage-clamp recordings were made with an Axopatch 200A amplifier (Axon Instruments, Foster City, CA). Liquid junction potentials (±5 mV) were not corrected.

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**FIG. 1.** Connectivity between globus pallidus (GP) and subthalamic nucleus (STN). A: field stimulation inside the STN (see inset) evoked an antidromic action potential in a GP neuron (top). Arrowhead signals stimulus artifact. An action potential was evoked in the same GP neuron with a depolarizing current step (bottom). Arrowhead signals stimulus artifact of field stimulus in STN after this response; collision blocked the antidromic spike. Note absence of synaptic response after the artifact. B: field stimulation inside the GP (see inset) evoked an antidromic action potential in a STN neuron (top). Arrowhead signals stimulus artifact. An action potential was evoked in the same STN neuron with a depolarizing current step (bottom). Arrowhead signals stimulus artifact of field stimulus in STN after this response; collision blocked the antidromic spike. C: site of biocytin instillation within the confines of the STN. Note several filled STN neurons. D: numerous fibers leaving the STN and reaching the GP were observed in the parasagittal slice. E: an axon (white arrow heads) emerging from a labeled STN neuron (black arrow) is shown entering the internal capsule in route to the GP. F: some GP neurons were also filled retrogradely, with example shown. This neuron was located at the site marked by the rectangle in D. NSt, neostriatum; ic, internal capsule. S indicates the stimulation electrode.
Evoked synaptic currents

Synaptic currents were evoked in GP neurons by field stimulation with concentric bipolar tungsten electrodes (50 μm at the tip, 1 kΩ DC resistance; FHC, Bowdoinham, ME). The stimulating electrode was placed either on the STN itself or on the internal capsule approaching the caudoventral part of the GP (Ogura and Kita 2000), where subthalamopallidal projections were seen to run in histochemistry experiments (see Fig. 1D). The likelihood of attaining an excitatory connection was one out of 10 cases when field stimulation was in the STN itself. The probability increased to at least eight out of 10 cases when field stimulation was in the internal capsule, but decreased to zero when the stimulus was located a few microns outside this path (Fig. 1, C and D) (Hanson and Jaeger 2002). The frequency of stimulation was 0.1 Hz and the strength of the pulses was adjusted to obtain roughly 70% of the maximal excitatory post synaptic current (EPSC) amplitude. The location of dopamine receptors involved in the modulation of the subthalamopallidal transmission was assessed by three different methods: paired-pulse ratio (PPR), frequency and amplitude of spontaneous EPSCs (sEPSCs), and variance–mean analysis (see following text). Glutamatergic EPSCs were isolated by recording the currents in the presence of 30 μM bicuculline to avoid contamination with GABAergic responses (Kita and Kitai 1991; Kita et al. 2005; Nambu et al. 2000). Unless stated otherwise, a point in the time-course graphs is the average of six records, at 0.1 Hz (above), which is one trace per 10 s and one point every minute. However, illustrated records of paired-pulse responses are the average of 25 consecutive events during steady state. sEPSCs were also recorded in the presence of bicuculline (30 μM) and tetrodotoxin (TTX, 1 μM). Traces of sEPSCs were filtered at 2 kHz and digitized at 10 kHz. Time intervals of recorded events during 1.5 min were analyzed (Minianalysis, Jaein Software, Leonia, NJ) in each experimental condition. The glutamatergic nature of sEPSCs was assessed by their complete elimination by CNQX (30 μM). The N-methyl-d-aspartate (NMDA)–receptor antagonist AP5 (50 μM) was also added in some experiments but no qualitative difference was found at this holding potential (~80 mV) with experiments that used only CNQX (cf. Ibañez-Sandoval et al. 2006).

When the paired-pulse protocol was performed with field stimulation, interpulse intervals of about 50 ms were used. Changes in the paired-pulse ratio (PPR) of EPSC responses (PPR = second EPSC/first EPSC) are known to be linearly proportional to the probability of release and the paired-pulse protocol was demonstrated to detect presynaptic actions of transmitters (e.g., Baldelli et al. 2005; Bolshakov and Siegelbaum 1995; Dunwiddie and Hass 1985; Guzman et al. 2003; Ibañez-Sandoval et al. 2006; Kamiya and Zucker 1994; Rajakumar et al. 1994; Salgado et al. 2005; Zucker 1999). Nonstationary fluctuations analysis was performed from 60 consecutive 50 Hz trains evoked at 0.1 Hz to perform variance–mean analyses in which mean amplitudes of evoked EPSCs (corrected for basal lines from previous EPSCs) were plotted against their peak variance: variance–mean (V–M) plots (Clements and Silver 2000; Ibañez-Sandoval et al. 2006; Koos et al. 2004; Salgado et al. 2005). Then, a parabola of the form

\[ y = Ax - Bx^2 \]  

was fitted with a Marquardt algorithm (Fig. 9), where y represents evoked EPSC peak variance (ordinates), x represents EPSC mean peak amplitude (abscissae), and A and B are free parameters. Basically, parameter A indicates the initial slope of the parabola and parameter B depends on the width of the parabola. From this fit, a weighted average of the quantal amplitude \( Q_w \) was obtained

\[ Q_w = A/(1 + CV^2) \]  

where \( CV \) is the coefficient of variation of EPSC amplitudes. In addition, the approximate number of release sites (N) and the average probability of release \( P_r \) across release sites (assuming a binomial distribution) can be approximated by

\[ N = 1/B \]  

\[ P_r = x(B/\alpha)(1 + CV^2) \]  

Statistics

All data are given as means ± SE unless stated otherwise. Significance of the effect of drugs as well as the significance of the differences in paired-pulse ratios was tested with nonparametric statistics: the Wilcoxon t-test or Mann–Whitney’s U test, depending on paired or unpaired samples. When the same sample had more than one treatment Friedman’s statistics with post hoc Student–Newman–Keuls test was used. Statistical differences of fitted functions were assessed by comparing the obtained parameters and their estimation errors with Student’s t-test.

Effects on sEPSCs were estimated by Kolmogorov–Smirnov two-sample test from cumulative distribution amplitudes or cumulative distributions of interevent intervals (Minianalysis).

Drugs

Drugs were stored in the freezer as dry aliquots and stock solutions were prepared just before each experiment and added to the perfusion solution in the final concentration. Bicuculline methiodide, 3,4-dihydroxyphenethylamine (dopamine), trans-(−)-4-[4-(4-chlorophenyl)piperazin-1-yl]methyl]-2-methylbenzamide (sulpiride), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), 3-aminosulfonyl-N-[1-ethyl-2-pyridinyl]-N-methyl-2-methoxybenzamide (quinpirole), 5,8-dihydropyridine acid (AP5), N-[(2-cyanoethyl)-1-piperazinyl]-1-methyl-3-ethyl-benzamide maleate (PD 168,077), 5,8-dihydropyridine acid (AP5), N-[1-ethyl-2-pyridinyl]-1-piperazinyl]-1-methyl-3-ethyl-benzamide maleate (PD 168,077), 5,8-dihydropyridine acid (AP5), N-[(2-[4-chlorophenyl]piperazin-1-yl)-1-methyl]-1-ethyl-2-pyridinyl(2,3-bpypyrine hydrochloride (L-745,870), lidocaine N-ethyl bromide (QX-314), and tetrodotoxin (TTX) were obtained from Sigma (St. Louis, MO). Drugs were diluted into the bath solution.

RESULTS

Anatomical connectivity of the GP and STN neurons

All recordings were made from neurons situated inside the GP borders, during field stimulation of the subthalamopallidal path (see METHODS). It is known that there are different populations of neurons in the GP (Cooper and Stanford 2000; Kita and Kitai 1994; Nambu and Linhas 1997). They can express GAD, calbindin, parvalbumin, or enkephalin (Hoover and Marshall 1999; Kita and Kita 2001; Rajakumar et al. 1994; Voorn et al. 1999). Field stimulation inside the STN borders or in the internal capsule (see METHODS) could evoke antidromic action potentials in some GP neurons, sometimes without evoking an excitatory postsynaptic potential (EPSP) with an orthodromic action potential (Fig. 1A, top; stimulus artifact is signaled with an arrowhead). This antidromic action potential could collide with an action potential evoked with a depolarizing current injection [Fig. 1A, bottom; artifact of antidromic stimulation (arrowhead) can be seen inside the refractory
period of the direct spike; note the absence of synaptic response or autorreceptive spike] (Kita and Kitai 1991). On the other hand, field stimulation inside the GP borders could evoke antidromic action potentials in some STN neurons, sometimes without evoking an inhibitory postsynaptic potential (IPSP) (Fig. 1B, top; stimulus artifact is signaled with an arrowhead). This antidromic action potential could collide with an action potential evoked with a depolarizing current injected on a STN neuron (Fig. 1B, bottom; arrowhead signals the artifact of antidromic stimulation). Staining after anterograde transport of biocytin injected inside the STN borders (Fig. 1C; see labeled STN neurons around the site of injection) (see Ibáñez-Sandoval et al. 2006) can disclose subthalamic-pallidal fibers in a parasagittal brain slice that includes both STN and GP (Fig. 1D). Axons emerging from STN neurons had a varicose appearance and could be followed entering the internal capsule toward the GP with other labeled fibers (Fig. 1E; white arrows). In some cases, retrogradely labeled GP neurons could also be observed (Fig. 1F), with morphology similar to that described previously (Kita and Kita 2001; Kita and Kitai 1994; Ogura and Kita 2000), i.e.: variable (oval or triangular) shape with three to five primary dendrites. Thus abundant anterograde labeling and some retrograde labeling, together with antidromic activation in either way, suggested that some reciprocal connections between the GP and STN are preserved in a parasagittal brain slice of the present characteristics (see METHODS) (Ogura and Kita 2000). Taken together, these results (n = 6 slices) suggested that field stimulation in the present conditions is mostly the product of the orthodromic activation of the subthalamic-pallidal pathway in vitro (see also Hanson and Jaeger 2002).

Glutamatergic synaptic responses recorded in GP neurons after stimulation of the subthalamic-pallidal pathway

The electrophysiological properties of GP neurons were thoroughly characterized in previous studies (e.g., Chan et al. 2004; Cooper and Stanford 2000; Kita and Kitai 1991; Nambu and Llinas 1994; Overton and Greenfield 1995). During the present work, neurons of previously described classes were recorded. For example, voltage records of a representative neuron (without QX-314) that responded to subthalamic-pallidal stimulation are depicted in Fig. 2A. As in the case of this neuron (Fig. 2A), many neurons recorded inside the GP borders exhibited a characteristic slowly developing voltage- and time-dependent rectification when injected with hyperpolarizing current pulses fired spontaneously, could fire at high frequencies, and showed a rebound firing after hyperpolarizing pulses (Chan et al. 2004; Cooper and Stanford 2000; Nambu and Llinas 1994). However, QX-314 was used in most cases to avoid contamination of the synaptic response with orthodromic unclamped active currents (see METHODS) (Kita and Kitai 1991; Kita et al. 2005; Nambu et al. 2000). Therefore in most cases, we did not characterize, electrophysiologically, the type of postsynaptic neuron recorded. Second, the criterion to accept an experiment was the synaptic response to subthalamic-pallidal pathway stimulation (see above). For these reasons, our sample could be biased toward some neuronal classes, i.e., those that preferentially receive STN inputs (Hoover and Marshall 1999; Kita and Kita 2001; Rajakumar 1994). The dopaminergic modulation of the synaptic responses described below did not appear to differ from one experiment to the other once the connection was stable.

Synaptic currents onto another neuron (in the presence of QX-314; see METHODS), evoked by stimulating the subthalamic-pallidal pathway, are illustrated in the inset of Fig. 2B. These currents were recorded at different holding potentials in the presence of bicuculline (30 μM) and AP5 (50 μM). The current–voltage relationship (I–V plot) for the peak EPSC amplitude had a linear relationship with a reversal potential around 0 mV (Fig. 2B). This I–V plot is typical of synaptic currents flowing through α-amino-3-hydroxy-5-methyl-4-isoxazolopropionic acid (AMPA)/kainate receptor channels (e.g., Jonas et al. 1993). In fact, most EPSC was carried by AMPA/kainate receptors in these conditions as confirmed by CNQX (10 μM) blockage (Fig. 2C), which practically abolished most of the synaptic current when evoked at a holding potential of −80 mV. Subsequent addition of AP5 (50 μM), a selective blocker of NMDA receptor channels, eliminated a minimal residual current (Fig. 2C, circles) (cf. Ibáñez-Sandoval et al. 2006). The actions of both blockers were reversible (Fig. 2C). When no blockers were added, currents were seen to remain stable for ≥30 min with these stimulation parameters (Fig. 2C, triangles). In the present conditions, an EPSC of these characteristics is expected, given the previous information about the subthalamic-pallidal connection (see Kita and Kitai 1991; Kita et al. 2005; Nambu et al. 2000).
Dopaminergic modulation of subthalamopallidal excitation

Dopamine (10 µM) reversibly reduced peak EPSC amplitude by 34%, from 62 ± 9 pA in the control to 42 ± 8 pA after dopamine (Fig. 3A and histogram in Fig. 3B; \(n = 7; P < 0.0002\); Wilcoxon’s \(t\)-test); representative records at the bottom are numbered according to the illustrated time course. To determine the type of receptor involved in the action of dopamine, the slices were exposed to dopamine in the presence of sulpiride, a selective D2/3 receptor antagonist, and then, to dopamine alone, after washing sulpiride off. As shown in the time course depicted in Fig. 3C, sulpiride could not block all dopamine action. A sulpiride-resistant reduction of the EPSC by dopamine was evident. Dopamine significantly reduced EPSCs by 29% in the presence of sulpiride: from 96 ± 9 pA in the control to 68 ± 6 pA in the presence of dopamine plus sulpiride (\(n = 7; P < 0.001\); Friedman’s nonparametric ANOVA with pairwise comparisons using Student–Newman–Keuls test). Figure 3, C and D also shows that when sulpiride was washed off, an additional sulpiride-sensitive reduction was revealed. EPSC reduction was enhanced from 29 to 41%, down to 56 ± 6 pA (Fig. 3D; \(n = 7; P < 0.001\); Friedman’s nonparametric ANOVA with pairwise comparisons using Student–Newman–Keuls test). In summary, the results showed that dopamine can modulate the subthalamopallidal EPSC by reducing it and that this reduction has sulpiride-resistant and sulpiride-sensitive components, that is, D2/3 receptors are only partially responsible for the dopaminergic modulation.

To be sure that D2/3 receptors are not the only ones involved in this action, we studied the effects of quinpirole (1 µM), a potent dopaminergic receptor agonist that activates all three receptors of the D2-class (D2, D3, and D4-type receptors), also in the presence and absence of sulpiride (1 µM). The time course in Fig. 4A shows that quinpirole also has an effect in the presence of sulpiride (Fig. 4A). The sulpiride-resistant action was a 17% reduction of the EPSC, from 104 ± 8 pA in the control to 86 ± 7 pA in the presence of both quinpirole and sulpiride (Fig. 4, A and B; \(n = 11; P < 0.01\); Friedman’s nonparametric ANOVA with pairwise comparisons using Student–Newman–Keuls test). After sulpiride removal, a sulpiride-sensitive reduction of EPSC (from 17 to 39%) by quinpirole was also revealed, down to 63 ± 4 pA (Fig. 4, A and B; \(n = 11; P < 0.01\); Friedman’s nonparametric ANOVA with pairwise comparisons using Student–Newman–Keuls test). The locus of quinpirole actions was investigated by measuring the paired-pulse ratio (PPR) after a paired-pulse protocol (e.g., Baldelli et al. 2005; Bolshakov and Siegelbaum 1995; Dunwiddie and Hass 1985; Guzman et al. 2003; Ibañez-Sandoval et al. 2006; Kamiya and Zucker 1994; Salgado et al. 2005; Zucker 1999) (see Methods). As shown in Fig. 4, C and D, the depression of EPSC amplitude that was sulpiride-resistant did not modify PPR (NS); suggesting that this action is postsynaptic (see following text). On the other hand, EPSC reduction after sulpiride removal (sulpiride-sensitive) was associated with a significant increase in PPR (Fig. 4D; \(n = 10; P < 0.02\); Friedman’s nonparametric ANOVA with pairwise comparisons using Student–Newman–Keuls test), suggesting that it was the result of a presynaptic location of the D2/3-type receptor.

Postsynaptic EPSC reduction is the result of a D2-type receptor

D2-type receptors are expressed in GP neurons (Ariano et al. 1997; Mauger et al. 1998; Mrzljak et al. 1996; Shin et al. 2003). Therefore a logical hypothesis would imply such receptors to explain the sulpiride-resistant reduction of the subthalamopallidal EPSC by dopamine and quinpirole. To test this hypothesis, we explored whether the blockade of D2-type receptors prevented the sulpiride-resistant reduction of the subthalamopallidal EPSC by quinpirole. Experiments were done in the presence of sulpiride to eliminate the participation of D2/3 receptors. As seen in Fig. 5, L-745,870 (1 µM), a D2-type receptor antagonist 2,000- and 5,000-fold more selective for the D2 over D3- and D4-type receptors, respectively (Kulagowski et al. 1996; Patel et al. 1997), completely blocked the sulpiride-resistant action induced by quinpirole. The EPSC amplitude was 85 ± 11 pA in the control and 88 ± 11 pA in quinpirole plus L-745,870, in the presence of sulpiride (\(n = 7; \text{NS}; \text{Friedman’s test}\)). Subsequent removal of the D2 antagonist allowed a reduction of the EPSC by quinpirole (16%) in the continuous presence of sulpiride, to 72 ± 8 pA (Fig. 5B; \(n = 7; P < 0.006\); Wilcoxon’s \(t\)-test), thus confirming the presence of a sulpiride-resistant action in GP cells.
PD 168,077 (1 μM), a selective D4-type receptor agonist with 300-fold selectivity over D3- and 400-fold selectivity over D2-type receptors (Glase et al. 1997), was also tested to confirm the participation of D4-type receptors in the dopaminergic modulation of subthalamopallidal EPSCs. In this case, too, the participation of D2/3 receptors was prevented by sulpiride (1 μM). The effect of the D4 agonist was studied first, in the presence and then in the absence of the selective D4 antagonist L-745,870 (1 μM). As shown in Fig. 6, A and B, the EPSC amplitude was 100 ± 11 pA in the control and 105 ± 11 pA after addition of D4 agonist PD 168,077 in the presence of D4 antagonist L-745,870 and D2/3 antagonist sulpiride (Fig. 6, A and B; n = 7; NS; Friedman’s test). However, the agonist PD 168,077 reduced the EPSC after the antagonist L-745,870 was removed from the bath in the continuous presence of sulpiride. Reduction of EPSC was about 26%, to 74 ± 7 pA (Fig. 6, A and B; n = 7; **P < 0.03; Wilcoxon’s t-test). The effect of PD 168,077 was reversible. EPSC reduction induced by PD 168,077 was not accompanied by any change in PPR (Fig. 6, C and D; n = 6; NS; Wilcoxon’s t-test), consistent with a postsynaptic site of action.

Activation of D2/3 receptors reduces the frequency but not the amplitude of sEPSCs

Taken together, experimental evidence suggested that D2/3 receptors have a presynaptic locus, whereas D4 receptors have a postsynaptic one. To further test this hypothesis, the actions of D2-class dopamine agonists were tested on spontaneous synaptic currents (sEPSCs) recorded on GP neurons in the presence of TTX (1 μM). Participation of γ-aminobutyric acid type A (GABA_A) and D4-type receptors was excluded by the

FIG. 4. Different types of D2-class receptors explain EPSC modulation. A: trans-(-)-4AR-4,4a,5,6,7,8,8a,9-octahydro-5-propyl-1H-pyrazolo(3,4-g)quinoline (quinpirole, 1 μM), a D2-class receptor agonist, reduces the EPSC in the presence of sulpiride (1 μM), a D2/3 receptor antagonist, thus revealing a sulpiride-resistant reduction of the EPSC. On sulpiride wash, a sulpiride-sensitive reduction induced by quinpirole is also revealed. **Bottom traces: representative records taken at the indicated numbers. B: histogram summarizing the results of the experimental sample (*P < 0.01 vs. control; **P < 0.01 vs. quinpirole + sulpiride). C: representative traces of quinpirole actions showing changes in the paired-pulse ratio (PPR). D: plot summarizing the results of 11 experiments. Note that EPSC reduction in the presence of quinpirole and sulpiride is not accompanied by significant changes in PPR. However, after sulpiride removal, the reduction in EPSC amplitude is accompanied by a significant enhancement of PPR (P < 0.02).

PD 168,077 (1 μM), a selective D4-type receptor agonist with >300-fold selectivity over D2- and >400-fold selectivity over D2-type receptors (Glase et al. 1997), was also tested to confirm the participation of D4-type receptors in the dopaminergic modulation of subthalamopallidal EPSCs. In this case, too, the participation of D2/3 receptors was prevented by sulpiride (1 μM). The effect of the D4 agonist was studied first, in the presence and then in the absence of the selective D4 antagonist L-745,870 (1 μM). As shown in Fig. 6, A and B, L-745,870 (agonist) blocked the effect of PD 168,077 (agonist) when administered together. EPSC amplitude was 100 ± 11 pA in the control and 105 ± 11 pA after addition of D4 agonist PD 168,077 in the presence of D4 antagonist L-745,870 and D2/3 antagonist sulpiride (Fig. 6, A and B; n = 7; NS; Friedman’s test). However, the agonist PD 168,077 reduced the EPSC after the antagonist L-745,870 was removed from the bath in the continuous presence of sulpiride. Reduction of EPSC was about 26%, to 74 ± 7 pA (Fig. 6, A and B; n = 7; **P < 0.03; Wilcoxon’s t-test). The effect of PD 168,077 was reversible. EPSC reduction induced by PD 168,077 was not accompanied by any change in PPR (Fig. 6, C and D; n = 6; NS; Wilcoxon’s t-test), consistent with a postsynaptic site of action.

Activation of D2/3 receptors reduces the frequency but not the amplitude of sEPSCs

Taken together, experimental evidence suggested that D2/3 receptors have a presynaptic locus, whereas D4 receptors have a postsynaptic one. To further test this hypothesis, the actions of D2-class dopamine agonists were tested on spontaneous synaptic currents (sEPSCs) recorded on GP neurons in the presence of TTX (1 μM). Participation of γ-aminobutyric acid type A (GABA_A) and D4-type receptors was excluded by the
mean frequency of sEPSCs was not significantly altered, from 1.4 ± 0.5 to 1.5 ± 0.4 Hz in the presence of PD 168,077 (Fig. 8, D and E).

Taken together, the results are consistent with a presynaptic location of D2/3-type receptors and a postsynaptic location of D4-type receptors.

Variance–mean analysis of dopaminergic synaptic modulation in the GP

Variance–mean analysis (Clements and Silver 2000; Reid and Clements 1999) was used as an independent test to assess the sites where dopaminergic receptors were exerting their modulatory actions on the subthalamopallidal EPSC (see Ibáñez-Sandoval et al. 2006; Salgado et al. 2005). Trains of 50 Hz each eliciting 10 EPSCs were evoked by stimulating the subthalamopallidal pathway during recording of GP neurons. Trains were evoked every 10 s (Fig. 9, A and C; see METHODS). With these parameters, the type of short-term dynamics recorded in control conditions was short-term depression (STD) even if the frequency used allowed some temporal summation (Fig. 9, A and C, top, control).

**FIG. 6.** Activation of D4-type dopamine receptors reduces subthalamopallidal EPSCs without changing the PPR. A: time course of action of the selective D4-type receptor agonist A-[4-(2-cyanophenyl)-1-piperazinyl]methyl]-3-methyl-benzamide maleate (PD 168,077, 1 μM), first in the presence and then in the absence of the selective D4-type receptor antagonist L-745,870 (1 μM). Sulpiride (1 μM) was continuously present in the superfusion to eliminate the participation of D2/3 receptors. L-745,870 blocked the action of PD 168,077. However, on L-745,870 removal, a sulpiride-resistant reduction of the EPSC was induced by PD 168,077. Bottom traces: records taken at the indicated numbers. B: histogram summarizing the results of 6 experiments (* nonsignificant difference vs. control; ** \( P < 0.03 \) vs. agonist plus antagonist). C: representative paired-pulse responses show no changes in PPR after D4-agonist. D: summary of PD 168,077 action on PPR in 6 independent experiments (NS).

addition of bicuculline (30 μM) and L-745,870 (1 μM) administered together in the bathing medium. In these conditions, quinpirole (1 μM) significantly reduced the frequency of sEPSCs, from 1.3 ± 0.4 Hz in the control to 0.9 ± 0.2 Hz in the presence of quinpirole (Fig. 7, A, D, and E; \( n = 7; \) \( P < 0.04; n = 8 \); Wilcoxon’s t-test). However, quinpirole in the same conditions did not significantly alter the mean amplitude of the sEPSCs: from 14 ± 1 pA in the control to 13 ± 2 pA in quinpirole (Fig. 7, B and C).

**FIG. 7.** Activation of D2/3 receptors reduces the frequency but not the amplitude of spontaneous sEPSCs. A: representative recordings of sEPSCs before (control) and during addition of quinpirole (1 μM). Experiments, since the control, were done in the presence of bicuculline (30 μM) to block GABA_A receptors, and TTX (1 μM) to block synaptic currents produced by neuronal firing. In these conditions, the D2-agonist PD 168,077 significantly reduced the amplitude of sEPSCs, from 11 ± 0.8 to 8 ± 0.7 pA (Fig. 8, B and C; \( n = 8; \) \( P < 0.002 \); Wilcoxon’s t-test). However, the

Activation of D4 receptors reduces the amplitude but not the frequency of sEPSCs

To study the activation of D4-type receptors on sEPSCs, sulpiride (1 μM) was added to the medium to rule out participation of D2/3-type receptors, together with bicuculline (30 μM) to block GABA_A receptors, and TTX (1 μM) to block synaptic currents produced by neuronal firing. In these conditions, the D4-agonist PD 168,077 significantly reduced the amplitude of sEPSCs, from 11 ± 0.8 to 8 ± 0.7 pA (Fig. 8, B and C; \( n = 8; \) \( P < 0.002 \); Wilcoxon’s t-test). However, the
The effect of activation of D2/3 receptors was studied administering the D2-class agonist quinpirole (1 μM) together with the selective D4 receptor antagonist L-745,870 (1 μM) in the presence of bicuculline (see above). Activation of D2/3 receptors reduced the amplitude of first events but not of secondary events (cf. Fig. 9C, top with Fig. 9C, middle and bottom). The variance–mean relationships (V–M plots) (Fig. 9B) (Clements and Silver 2000) show that most control (filled circles) and quinpirole data points (empty circles) cluster together at the initial part of the fitted parabolas. In fact, weighted quantal amplitudes ($Q_w$; Eq. 2) were 5 ± 0.6 pA for the control and 5 ± 0.3 pA after activation of D2/3-type receptors (range = 4–9 pA; n = 4 different experiments; NS; Student’s t-test), similar to quantal amplitudes found in the subthalamic pathway (Ibáñez-Sandoval et al. 2006) and other glutamatergic synapses of the CNS (Bolshakov and Siegelbaum 1995; Paulsen and Heggelund 1994). These results agree with those showing a lack of change in synaptic depression as the short-term dynamics of the subthalamicolpidal excitation at 50 Hz. Black trace: average of 60 consecutive trials. Gray traces: individual records illustrating amplitude variation in both columns (A and C). Top traces were taken in control conditions. Action of quinpirole (1 μM) in the presence of the selective D4-antagonist L-745,870 (1 μM) is shown in the middle. Bottom traces: superimposition of average traces at a higher magnification (control in black; quinpirole in gray). B: mean peak EPSC amplitudes of above data plotted against their mean peak variances in control (filled circles) and in the presence of quinpirole (empty circles) with D4 receptors blocked. Lines are the best-fitted parabolas (see METHODS) for each data set (control in black; PD 168,077 in gray). The V–M points (Fig. 9D) thus showed that fitted parabolas (control = filled circles and black line; PD 168,077 = empty circles and gray line) diverged in their initial slopes (Fig. 9D). Thus weighted quantal amplitudes for both data sets were $Q_w = 5 ± 0.3$ pA for the control and 4 ± 0.2 pA after D2-receptor activation (Fig. 9D; $P < 0.04$; Student’s t-test). These results agree with those found in experiments such as those in Fig. 8, A–C, which showed a change in mean sEPSCs arising from D2-receptor activation. Because the change in the width of fitted functions.
was accompanied with a change in initial slope, calculations of the presynaptic parameter \( N \) did not show any difference: 24 ± 2 in the control versus 23 ± 2 in PD 168,077 (Clements and Silver 2000). Further quantal analysis may be performed in the future to assess agonist effects on release probabilities because average probabilities (Eq. 4) did not show any difference with this analysis (e.g., Salgado et al. 2005). For the goals of the present work, however, variance–mean analysis was consistent with the view that dopaminergic modulation of subthalamo-pallidal transmission occurs at both presynaptic (D\(_{2/3}\) receptors) and postsynaptic sites (D\(_4\) receptors).

**DISCUSSION**

Reciprocal connections between STN and GP are a source of rhythmicity in neuronal firing (Bevan et al. 2002b; Plenz and Kitai 1999; Terman et al. 2002). In this work, biocytin anterograde and retrograde labeling, as well as antidromic activation, demonstrated that some reciprocal connections between GP and STN (Gandia et al. 1993;Hazrati and Parent 1992; Kita and Kita 1991, 2001; Kita et al. 2004; Nambu et al. 2000; Parent et al. 2000; Sato et al. 2000b; Shink et al. 1996; Shimonaga et al. 1992; Smith et al. 1990) can functionally be preserved in vitro slice preparations (Beurrier et al. 2006; Hansson and Jaeger 2002; Ogura and Kita 2000). Furthermore, moderate-strength orthodromic stimulation of the subthalamo-pallidal pathway in the presence of bicuculline to block inhibitory inputs and intracellular QX-314 to block unclamped action potentials in the recorded cell can isolate single excitatory postsynaptic currents (EPSCs) that, at −80 mV holding potential, were mostly the product of AMPA/kainate receptor activation (Ogura and Kita 2000). The main finding of this work was that dopamine in the globus pallidus, presynaptically and postsynaptically, modulates this excitatory subthalamo-pallidal input. Both D\(_{2/3}\) - and D\(_4\)-type receptors participate, thus decreasing the magnitude of the synaptic response. D\(_{2/3}\)-type receptors appear to be located presynaptically on subthalamo-pallidal afferents, whereas D\(_4\)-type receptors appear to be located postsynaptically on GP neurons. Physiologically, both receptors appear to act simultaneously to control the excitatory tone and the firing of GP neurons. Because STN also projects to the substantia nigra pars compacta (Iribe et al. 1999; Kanazawa et al. 1976; Smith and Grace 1992), the results then suggest that subthalamic afferents to the GP may autocontrol their output through the simultaneous activation of dopaminergic modulation.

**Presynaptic control of subthalamic inputs by D\(_{2/3}\) receptors**

Subthalamic neurons express both D\(_1\)-class and D\(_2\)-class receptors (Baufreton et al. 2003; Ciliax et al. 2000; Dawson et al. 1986; Flores et al. 1999; Hurd et al. 2001; Johnson et al. 1994; Svenningsson and Le Moine 2002), which mediate dopamine actions that modulate the firing of STN neurons (Baufreton et al. 2003; Kreiss et al. 1996, 1997; Mintz et al. 1986; Ni et al. 2001; Svenningsson and Le Moine 2002; Zhu et al. 2002). Both receptor classes may be transported to the synaptic terminals of axons arising from STN projection neurons, for example, to presynaptically modulate subthalamosomal inputs to the reticulata (Ibañez-Sandoval et al. 2006). This suggests that they could also be present in the terminals of the subthalamopallidal efferents.

In the present work we chose to study the subthalamopallidal modulation by D\(_2\)-class receptors only. This decision was influenced by the fact that GP neurons express D\(_4\)-type receptors (Ariano et al. 1997; Floran et al. 2004; Mauger et al. 1998; Mrzljak et al. 1996) and it was previously shown that D\(_4\) receptors located on GP neurons exert an inhibitory control on GABAergic inputs (Shin et al. 2003). Moreover, the GP also receives glutamatergic excitatory inputs from the thalamus (Mouroux et al. 1997; Yasukawa et al. 2004). Although thalamic afferents do not express D\(_{2/3}\) receptors, they may express D\(_5\)-type receptors (Meador-Woodruff et al. 1992), which may also be the most important type of D\(_1\)-class receptor present in STN neurons (Baufreton et al. 2003; Ciliax et al. 2000; Svenningsson and Le Moine 2002). Therefore modulation by D\(_1\)- or D\(_2\)-class receptors needs to be carefully separated to understand their actions in the GP.

This work reports that both dopamine and quinpirole, the D\(_2\)-class nonselective receptor agonist, reduced the subthalamopallidal-evoked EPSCs. In both cases this reduction was only partially blocked by the D\(_{2/3}\) receptor antagonist sulpiride, thus revealing a potentially important sulpiride-resistant action of dopamine D\(_2\)-class agonists. Even at high concentrations (e.g., 10 \( \mu \)M) sulpiride does not block D\(_4\) receptors (Price and Pittman 2001; Shin et al. 2003; Wang et al. 2002) and, because GP neurons express D\(_4\)-type receptors, it was logical to hypothesize that the sulpiride-resistant action was mediated by D\(_4\) receptors (see following text).

The sulpiride-sensitive action could then be mediated by D\(_{2/3}\) receptors located in the subthalamopallidal pathway (Cooper and Stanford 2001; Ibañez-Sandoval et al. 2006). The present work supported this hypothesis with three kinds of experimental evidence. First, the sulpiride-sensitive reduction of synaptic currents was associated with a change in the paired-pulse ratio, consistent with a presynaptic site of action (e.g., Baldelli et al. 2005; Dunwiddie and Haas 1985; Guzman et al. 2003; Ibañez-Sandoval et al. 2006; Kamiya and Zucker 1994; Price and Pittman 2001; Salgado et al. 2005; Zucker 1999). Second, selective activation of D\(_{2/3}\) receptors changed the frequency but not the amplitude of sEPSCs (Cooper and Stanford 2001; Koga and Momiyama 2000; see also Mann-Metzer and Yarom 2002; Sahara and Takahashi 2001). Finally, variance–mean analysis (Clements and Silver 2000; Ibañez-Sandoval et al. 2006; Koos et al. 2004; Reid and Clements 1999; Salgado et al. 2005) suggested that selective activation of D\(_{2/3}\) receptors did not change the quantal amplitude. Instead, the parameter that was different in the fitted function (\( N \)) was consistent with a presynaptic change (Eq. 3; see METHODS).

**Postsynaptic control of subthalamic inputs by D\(_4\) receptors**

The hypothesis that the sulpiride-resistant action was mediated by D\(_4\) receptors located postsynaptically on GP neurons was supported by three types of experiments. First, selective activation of D\(_4\) receptors reduced subthalamopallidal EPSCs without changing the paired-pulse ratio. Second, D\(_4\) receptor activation reduced the mean amplitude of sEPSCs without affecting the frequency. Finally, variance–mean analysis revealed a significant reduction in quantal amplitude without affecting any presynaptic parameter.
To conclude, both D_{2/3} and D_{4} type receptors modulate the subthalamopallidal response. Nevertheless, D_{2/3}-type receptors exert their modulation acting presynaptically, whereas D_{4}-type receptors modulate the inputs from the postsynaptic side.

Possible physiological consequences

Dopamine actions in the GP are necessary to achieve motor control (Pan and Walters 1988; Raz et al. 2000). Bilateral infusions of D_{1}- or D_{2}-class receptor antagonists into the GP induce akinesia and cataplexia (Costall and Naylor 1972; Hauber and Lutz 1999). However, the infusion of dopamine in the same nucleus causes amelioration of motor deficits in hemiparkinsonian rats (Galvan et al. 2001). It is then important to know the actions of dopamine in the GP.

The GP and the STN assemble a reciprocally innervated network of excitatory and inhibitory neurons (Bevan et al. 2002b; Gandia et al. 1993; Hazrati and Parent 1992; Kita and Kita 1991, 2001; Kita et al. 2004; Nambu et al. 2000; Parent et al. 2000; Sato et al. 2000a,b; Shink et al. 1996; Shionaga et al. 1992; Smith et al. 1990). Because of the intrinsic properties of GP and STN neurons (Beurrier et al. 1999, 2000; Bevan and Wilson 1999; Bevan et al. 2002a; Chan et al. 2004; Cooper and Stanford 2000; Hallworth et al. 2003; Kita and Kita 1991; Kita et al. 2004; Nambu and Llinás 1994; Overton and Greenfield 1995), this network forms a robust central pattern generator (CPG) in the basal ganglia (Grillner 2003; Terman et al. 2002; Yuste et al. 2005). Diverse spatiotemporal patterns of rhythmic and synchronous oscillatory activity can arise when the levels of inhibitory or excitatory inputs impinging onto this network are varied (Bevan and Wilson 1999; Bevan et al. 2002b; Kita et al. 2004; Overton and Greenfield 1995; Plenz and Kita 1999; Terman et al. 2002; Urbain et al. 2000). The present and previous (Cooper and Stanford 2001; Floran et al. 1997) findings showed that the levels of inhibitory and excitatory inputs impinging onto GP neurons depend on dopaminergic modulation. Therefore it is logical to infer that the variety of spatiotemporal patterns of oscillatory activity that this network exhibits to produce motor organization may be a product of dopaminergic modulation. Perhaps, a detailed somatotopic organization of dopaminergic neurons in the compacta determines, according to the afferents active during a motor task, which inputs increase and which decrease their strength. If dopamine modulation is lost, however, a dramatic reduction in spatiotemporal patterns is obtained. The system then becomes locked in a few extreme and robust set of variations (Bergman et al. 1994; Brown et al. 2001; Levy et al. 2002; Magill et al. 2001; Magnin et al. 2000; Nini et al. 1995; Raz et al. 2000).

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