Activation of Group I Metabotropic Glutamate Receptors Produces a Direct Excitation and Disinhibition of GABAergic Projection Neurons in the Substantia Nigra Pars Reticulata

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A pathological increase in excitatory glutamatergic input to substantia nigra pars reticulata (SNr) from the subthalamic nucleus (STN) is believed to play a key role in the pathophysiology of Parkinson’s disease. We present an analysis of the physiological roles that group I metabotropic glutamate receptors (mGluRs) play in regulating SNr functions. Immunocytochemical analysis at the light and electron microscopic levels reveal that both mGluR1a and mGluR5 are localized postsynaptically in the SNr. Consistent with this, activation of group I mGluRs depolarizes SNr GABAergic neurons. Interestingly, although both group I mGluRs (mGluR1 and mGluR5) are expressed in these neurons, the effect is mediated solely by mGluR1. Light presynaptic staining for mGluR1a and mGluR5 was also observed in some terminals forming symmetric synapses and in small unmyelinated axons. Consistent with this, activation of presynaptic mGluR1a and mGluR5 decreases inhibitory transmission in the SNr. The combination of direct excitatory effects and disinhibition induced by activation of group I mGluRs could lead to a large excitation of SNr projection neurons. This suggests that group I mGluRs are likely to play an important role in the powerful excitatory control that the STN exerts on basal ganglia output neurons.

Key words: substantia nigra pars reticulata; group I metabotropic glutamate receptors; movement disorders; slow excitatory postsynaptic potential; disinhibition; basal ganglia output nuclei

The basal ganglia are a richly interconnected group of subcortical nuclei involved in the control of motor behavior. The primary input nucleus of the basal ganglia is the striatum, and the primary output nuclei are the substantia nigra pars reticulata (SNr) and the internal globus pallidus (entopeduncular nucleus in nonprimates). The striatum projects to these output nuclei both directly, providing an inhibitory GABAergic input, and indirectly through the external globus pallidus and the subthalamic nucleus (STN). The STN provides excitatory glutamatergic input to the SNr. A delicate balance between this inhibition and excitation is believed to be critical for motor control, and disruptions in this balance are believed to underlie a variety of movement disorders (Wichmann and DeLong, 1997, 1998).

Although much effort has been directed at elucidating the connectivity of the direct and indirect pathways, less is known about the modulatory influence various transmitters may have on these pathways. Increasing evidence suggests that G-protein-coupled metabotropic glutamate receptors (mGluRs) play an important role in the regulation of basal ganglia functions. To date, eight mGluR subtypes (mGluR1–mGluR8) have been cloned and are classified into three major groups based on sequence homology, coupling to second-messenger systems, and agonist selectivity (for review, see Conn and Pin, 1997). Group I mGluRs (mGluR1 and GluR5) couple to Gq and phosphoinositide hydrolysis, whereas groups II (mGluR2 and mGluR3) and III (mGluR4, mGluR6, mGluR7, and mGluR8) couple to Gq/Go, and related effector systems such as inhibition of adenylate cyclase. These mGluRs are widely distributed throughout the CNS in which they play important roles in regulating cell excitability and synaptic transmission.

Previous studies have shown that mGluRs are expressed throughout the basal ganglia (Testa et al., 1994, 1998; Kernert et al., 1997; Kosinski et al., 1998, 1999; Bradley et al., 1999a,b) and play important roles in the regulation of synaptic transmission in the SNr. For example, activation of presynaptic group II and III mGluRs inhibits excitatory transmission at the STN–SNr synapse (Bradley et al., 2000; Wittmann et al., 2000). One of the major postsynaptic effects of mGluRs in many brain regions is a group I mGluR-mediated slow depolarization (Crepel et al., 1994; Guerinaeu et al., 1994, 1995; Gereau and Conn, 1995a; Miller et al., 1995). Because glutamatergic innervation of the SNr from the STN plays an important role in motor control, an understanding of the roles mGluRs play in modulating SNr GABAergic neurons could provide important insight into the mechanisms involved in the regulation of SNr firing in both physiological and pathological states. We now report that activation of group I mGluRs produces an excitation of the SNr by two distinct mechanisms. Activation of postsynaptic mGluR1 induces a pronounced excitation of SNr GABAergic neurons that is mimicked by stimulation of excitatory afferents. In addition, activation of both mGluR1 and mGluR5...
produce a decrease in inhibitory transmission in the SNR, resulting in increased excitability of this crucial basal ganglia output nucleus.

**MATERIALS AND METHODS**

[R-(R*,S*)]-6-(4-chlorophenyl)-2-hydroxypropyl-sulfonic acid (2-hydroxysaclofen), with was removed from the skull and stored in PBS (0.01 M, pH 7.4) before EDTA and 2 mM HEPES, pH 7.4. Cells were then homogenized by hand


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the stimulation electrode placed within the SNr rostrally or caudally to the recorded cell and recorded at a holding potential of \(-50\) mV. CNQX (10–20 \(\mu M\)) and 10–20 \(\mu M\) T-AP-5 were continuously added to the bath to block excitatory transmission. To study miniature IPSCs (mIPSCs), the 140 mM potassium gluconate in the internal solution were substituted with 140 mM CsCl to reduce postsynaptic mGluR effects and increase mIPSC amplitudes. Therefore, inward mIPSCs were recorded at a holding potential of \(-80\) mV in the presence 1 \(\mu M\) tetrodotoxin (TTX).

**Data analysis.** All curve fitting was performed using the Marquardt-Levenburg algorithm as implemented in the SigmaPlot software package (SPSS, Chicago, IL). To determine an accurate reversal potential from the \(I-V\) ramps presented in Figure 6, the current-voltage relationships were fit with an arbitrary higher-order polynomial function of the form \(I = I_o + (C_1V^3) + (C_2V^5) \ldots + (C_nV^n)\), where \(I\) is the whole-cell current, \(V\) is the command potential, \(I_o\) is an offset variable, and \(C_i\) are constants. It was found that a third-order polynomial \((i = 3)\) provided the best fit, with additional terms decreasing the error about the fit by <1%. Concentration-response curves were fit with a three-parameter Hill equation to obtain EC_{50} and Hill slope values. All values are reported as mean ± SEM.

**RESULTS**

**Antibody specificity**

To assess the specificity of the antibodies used in these studies, we performed immunoblot analysis on proteins isolated from cell lines and specific rat brain regions. As shown in Figure 1A, the anti-mGluR1 monoclonal antibody (PharMingen) specifically labels a band at \(-140\) kDa in lanes containing protein from cells expressing mGluR1 but not from cells expressing mGluR5. In addition, the mGluR1 antibody specifically labels a similar band in cerebellar homogenate, demonstrating a distribution consistent with previous reports (Martin et al., 1992; Shigemoto et al., 1992; Petralia et al., 1997). Similar results were observed with both mGluR1-selective antibodies used in these studies. In contrast to this, anti-mGluR5 polyclonal antibody specifically labels a similar band from cells expressing mGluR5 and, consistent with the known distribution of mGluR5, exhibits a broader labeling of brain homogenates in noncerebellar regions (Shigemoto et al., 1993; Romano et al., 1995) (Fig. 1B). In addition to these immunoblot studies, we also observed light level immunostaining for each antibody, consistent with previously reported distributions (data not shown).

**Localization of group I mGluRs in the SNr**

Previous studies have demonstrated the expression of both mGluR1 and mGluR5 in the SNr (Testa et al., 1994, 1998). However, these studies did not address the subcellular and subsynaptic localization of these receptors. To determine whether group I mGluRs are postsynaptically localized in the SNr, we performed immunocytochemical studies with antibodies selective for mGluR1a and mGluR5.

At the light microscopic level, the SNr exhibited labeling for both mGluR1a (Fig. 2A, B) and mGluR5 (Fig. 3A, B). To determine whether this immunoreactivity represents presynaptic or postsynaptic staining, we performed immunocytochemical analyses at the electron microscope level. Both antibodies primarily labeled dendritic processes that formed symmetric and asymmetric synapses with unlabeled terminals (Figs. 2C, 3C). In the case of presynaptic labeling for both group I mGluRs, the immunoreactivity was seen only in terminals forming symmetric synapses. A few glial processes were also labeled with both antibodies. Most immunoreactive dendrites were tightly surrounded by a large density of striatal-like terminals forming symmetric synapses (Figs. 2C, D; 3C–E), an ultrastructural feature typical of SNr GABAergic neurons (Smith and Bolam, 1991). In contrast, SNC dopaminergic neurons are much less innervated (Bolam and Smith, 1990). These data suggest that the majority of immunoreactive elements labeled with the two group I mGluR antibodies belong to SNr GABAergic neurons. Immunoreactive elements were counted in a random sample of SNr tissue to determine the relative frequency of group I mGluR-immunopositive elements. The relative distribution of mGluR1a immunoreactivity, expressed as a percentage of total labeled elements, was 64.2% dendrites, 34.3% axons, 0.4% somata, 0.8% terminals, and 0.4% glia. The relative distribution of mGluR5 immunoreactivity was 58.7% dendrites, 40.2% axons, 0.5% somata, and no observed labeling in terminals or glia.

**Electrophysiological identification of GABAergic neurons in the SNr**

For electrophysiological analysis of the roles of mGluRs in SNr GABAergic projection neurons, it is critical to differentiate between GABAergic neurons and the smaller population of dopaminergic neurons in this region. Fortunately, these two neuronal types exhibit distinct electrophysiological and morphological features. Therefore, we used electrophysiological criteria that were established previously to distinguish between dopaminergic neurons and GABAergic projection neurons (Nakanishi et al., 1987;...
Figure 2. mGluR1a immunoreactivity in the SNr. A, Low-power light micrograph of mGluR1a immunostaining in the SNr. B, High-power light micrograph of mGluR1a-immunoreactive processes in the SNr. Lightly labeled neuronal cell bodies are indicated by asterisks. C, Low-power electron micrograph of mGluR1a-immunoreactive dendrites and axons (Ax) in SNr. D, High-power electron micrograph of mGluR1a-immunoreactive dendrites that form asymmetric (arrowhead) and symmetric (arrow) synapses with unlabeled terminals. E, High-power electron micrograph showing an mGluR1a-immunoreactive terminal in contact with a small, labeled dendrite. Note also the presence of an immunoreactive glial process (Gl) surrounding an unlabeled terminal. Scale bars: A, 500 μm; B, 50 μm; C, 1 μm; D, E, 0.5 μm.

Hausser et al., 1995; Richards et al., 1997). GABAergic neurons exhibit a high rate of spontaneous repetitive firing, short-duration action potentials (half-amplitude duration, 1.7 ± 0.2 msec; n = 4), little spike accommodation, and a lack of inward rectification (Fig. 4). In contrast, dopaminergic neurons display no or low-frequency spontaneous firing, longer-duration action potentials (half-amplitude duration, 7.0 ± 0.5 msec; n = 4), strong spike accommodation, and a pronounced inward rectification (Fig. 4). Light microscopic examination of biocytin-filled neurons indicated that GABAergic neurons had extensive dendritic arborizations close to the cell body, whereas dopaminergic neurons had sparser dendritic structure (data not shown). All data presented in this study are from electrophysiologically identified GABAergic neurons.

**Activation of group I mGluRs depolarizes SNr GABAergic neurons**

Previous studies have demonstrated that all three groups of mGluRs are expressed in the SNr (Testa et al., 1994, 1998). We therefore used maximal concentrations of group-selective mGluR agonists to determine whether activation of these receptors has an effect on membrane properties of SNr GABAergic neurons. In the presence of 0.5 μM TTX, application of the group I mGluR-selective agonist DHPG induces a robust direct depolarization (300 μM DHPG, 16.1 ± 2.6 mV; n = 5) of SNr neurons that reverses during drug washout (Fig. 5A, C). This depolarization is accompanied by a significant increase in input resistance (pre-drug, 498 ± 70 MΩ, n = 4; 100 μM DHPG, 619 ± 89 MΩ, n = 4; p < 0.05; paired t test) (Fig. 5B), suggesting that a DHPG-induced decrease in membrane conductance underlies this effect. The concentration–response relationship for DHPG-induced depolarization of SNr GABAergic neurons exhibited a steep sigmoid shape and was fit with a Hill equation that gave an EC₅₀ of 37 μM and a Hill slope of 2.6 (Fig. 5D), consistent with an effect on group I mGluRs (Schoepp et al., 1994; Gereau and Conn, 1995a). In contrast to this group I mGluR-mediated depolarization, the group II-selective agonist LY354740 (Monn et al., 1997; Kingston et al., 1998) and the group III-selective agonist t-AP-4 (Conn and Pin, 1997) had no significant effect on resting membrane potential (Fig. 5A–C). Therefore, we focused on the physiology and pharmacology of the group I mGluR-mediated depolarization.

To determine the effect of group I mGluR activation on action potential firing in SNr GABAergic neurons, we applied the selective group I mGluR agonist DHPG in the absence of TTX. At the beginning of whole-cell recording, cells fire spontaneous action potentials (Fig. 4); however, within a few minutes, cells tend to hyperpolarize and do not fire spontaneously. Application of 100 μM DHPG induced a robust depolarization and a large increase in action potential firing (Fig. 5E). This DHPG-induced firing is completely blocked by injection of hyperpolarizing current to maintain a −65 mV membrane potential during drug...
application and is mimicked by direct depolarization of the cells to the same membrane potential (100 μM DHPG, 3.8 ± 0.3 Hz, n = 4; direct depolarization 3.2 ± 0.7 Hz, p > 0.05; Student’s t test). These data suggest that the increase in firing is solely attributable to the depolarization and that mGluR activation does not have other effects on membrane properties of SNr neurons to increase firing frequency.

In other neurons, activation of group I mGluRs has been demonstrated to depolarize the cells by inhibition of a leak potassium conductance (Guerineau et al., 1994) or by an increase in a nonselective cationic conductance (Guerineau et al., 1995; Miller et al., 1995). Our observation that DHPG causes an increase in input resistance suggests that inhibition of leak potassium conductance is the most likely mechanism underlying this effect. Consistent with this, voltage-clamp analysis revealed a DHPG-induced inward current underlying the depolarization (Fig. 6A). Voltage ramps between −40 and −120 mV (20 mV/sec) were used to establish a current–voltage relationship of the

Figure 4. Demonstration of the identification of SNr GABAergic neurons. A, Response of a GABAergic (left) and dopaminergic (right) neuron to depolarizing and hyperpolarizing current injections. Note the pronounced spike frequency adaptation and inward rectification exhibited by the dopaminergic cell that is absent in the GABAergic cell. B, Examples of spike activity from resting cells. GABAergic neurons (left) fire at high frequency, whereas dopaminergic neurons (right) exhibit lower frequency or no spontaneous activity. C, Comparison of single action potentials from a GABAergic (left) and dopaminergic (right) neuron. All data presented here are from electrophysiologically identified GABAergic neurons.

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Figure 3. mGluR5-immunoreactive subtype within the SNr. A, Low-power light micrograph of mGluR5 immunostaining in the SNc and SNr. B, High-power light micrograph of mGluR5-immunoreactive processes in the SNr. Labeled cell bodies are indicated by asterisks. C, Low-power electron micrographs of mGluR5-immunoreactive elements in the SNr.

D, E, High-power electron micrographs of mGluR5-immunoreactive dendrites (Den) and spines (Sp) that form asymmetric synapses (arrowheads) with unlabeled terminals. Note the presence of an immunoreactive glial process (Gl). Scale bars: A, 500 μm; B, 50 μm; C, 1 μm; D, E, 0.5 μm.

Note that the mGluR5 immunoreactivity is present in axonal (4x) and dendritic process. D, E. High-power electron micrographs of mGluR5-immunoreactive dendrites (Den) and spines (Sp) that form asymmetric synapses (arrowheads) with unlabeled terminals. Note the presence of an immunoreactive glial process (Gl). Scale bars: A, 500 μm; B, 50 μm; C, 1 μm; D, E, 0.5 μm.
DHPG-induced current. Application of 100 μM DHPG induced a change in the slope of the whole-cell current–voltage relationship (Fig. 6B). Subtracting the trace in the presence of DHPG from the predrug I–V trace reveals the I–V relationship for the DHPG-induced current. This current was best fit with a third-order polynomial function (see Materials and Methods) (Fig. 6C). The interpolated reversal potential of $-111.7 \pm 7.4$ mV ($n = 5$) is in good agreement with the calculated Nernst equilibrium potential for potassium ($-103.4$ mV). In experiments in which cesium was included in both the intracellular and extracellular solutions and the ACSF included 4-aminopyridine and tetraethylammonium to block potassium channels, the DHPG-induced current was eliminated (Fig. 6C,D). Together, these data suggest that the DHPG-induced depolarization of SNr GABAergic neurons is mediated by decreasing a leak potassium conductance.

The DHPG-induced excitation of SNr GABAergic neurons is mediated by mGluR1

Our findings that both mGluR1a and mGluR5 are postsynaptically localized in SNr projection neurons suggests that both of these receptors could be involved in the DHPG-induced depolarization. To determine the role each of these receptors plays in this effect, we used newly available pharmacological tools that distinguish between mGluR1 and mGluR5. CBPG, a partial agonist at mGluR5 that has antagonistic properties at mGluR1 (Mannaioni et al., 1999) failed to induce a depolarization at maximal concentrations (Fig. 7A,B), indicating that the depolarizing effect of DHPG is likely attributable to activation of mGluR1. Consistent with this, pretreatment with the highly selective, noncompetitive mGluR1 antagonist CPCCOEt (Annoura et al., 1996; Casabona et al., 1997; Litschig et al., 1999) or the highly selective, competitive mGluR1 antagonist LY367385 (Clark et al., 1997) produced a significant reduction in the DHPG-induced depolarization of SNr GABAergic neurons (Fig. 7A,B). Pretreatment with MPEP, a highly selective noncompetitive antagonist of mGluR5, had no significant effect at concentrations shown to be effective at blocking mGluR5 in other systems (Bowes et al., 1999; Gasparini et al., 1999) (Fig. 7A,B).

mGluR1 mediates a slow EPSP in SNr GABAergic neurons

The data presented thus far indicate that mGluR1 mediates direct excitation of SNr projection neurons. The SNr receives a sparse yet important glutamatergic innervation from the STN, and burst firing of the STN is known to play a key role in several neurological disorders, including Parkinson’s disease (PD) (Hollerman and Grace, 1992; Bergman et al., 1994; Hassani et al., 1996).
activation of glutamatergic afferents to the SNr release sufficient glutamate to activate mGluR1, the resulting excitation of SNr projection neurons could play an important role in these disease states. We tested this hypothesis by recording from SNr GABAergic neurons in the presence of ionotropic glutamate receptor and GABA receptor antagonists, as well as haloperidol to block dopamine receptors and strychnine to block glycine receptors. High-frequency stimulation of the afferents within the SNr produced a robust and reliable slow EPSP that reached threshold for action potential firing (Fig. 8A). Recent reports have demonstrated that, under carefully controlled conditions, synaptically released glutamate acting on group I mGluR1 can induce a hyperpolarizing response in midbrain dopamine neurons (Fiorillo and Williams, 1998). Under the conditions used in these studies, we were able to elicit a hyperpolarizing response in four of four dopaminergic neurons recorded from the SNc–ventral tegmental area (Fig. 8A). However, we never observed a hyperpolarizing response in SNr neurons (0 of 22 cells). This suggests that depolarization is the primary action of glutamate acting on group I mGluRs on SNr GABAergic neurons. Consistent with mediation by mGluR1, this slow EPSP was reversibly blocked by 300 μM LY367385 (predrug, 9.0 ± 1.2 mV; LY367385, 3.9 ± 0.7 mV; n = 6; p < 0.05; paired t test) (Fig. 8B, C), whereas the mGluR5-selective antagonist MPEP was without significant effect (predrug, 7.8 ± 1.0 mV; MPEP, 6.4 ± 0.9 mV; n = 6; p > 0.05; paired t test). Because it is possible that a small component of the slow EPSP is mediated by mGluR5, which is not detectable in the presence of the larger mGluR1-mediated component, we applied a combination of the two selective antagonists. This combination did not produce any inhibition greater than that observed with LY367385 alone (inhibition by LY367385, 44.8 ± 5.6%; n = 6; inhibition by LY367385 plus MPEP, 46.5 ± 4.2%, n = 4; p > 0.05; t test) (Fig. 8C). Interestingly, application of 1 μM tetrodotoxin fully blocked the slow EPSP (predrug, 8.0 ± 1.0 mV; TTX, 0.1 ± 0.3 mV; n = 3; p < 0.05; paired t test), suggesting that the LY367385-insensitive component of the slow EPSP is mediated by the action potential-dependent release of neurotransmitter acting on a receptor other than the group I mGluRs.

Figure 7. The group I mGluR-induced depolarization is mediated by mGluR1. A. Representative traces demonstrating that the DHPG-induced depolarization of SNr GABAergic neurons is not mimicked by the mGluR5-selective agonist CBPG. Furthermore, preincubation with the highly selective noncompetitive mGluR1 antagonist CPCCOEt or the highly selective competitive mGluR1 antagonist MPEP is without effect. *p < 0.01; Student’s t test.

Figure 8. mGluR1 mediates a slow EPSP in SNr GABAergic neurons. A. High-frequency stimulation of afferents in the SNr elicits a slow EPSP that exceeds action potential threshold and induces firing. Similar experiments in dopaminergic neurons of the SNC reveal a hyperpolarizing response; however, the only response observed in SNr GABAergic neurons is a depolarization. Representative traces (B) and mean ± SEM data (C) demonstrating the inhibition of the slow EPSP by the mGluR1-selective antagonist LY367385. MPEP alone or in the presence of LY367385 is without effect. *p < 0.05; t test. This slow EPSP is fully blocked by 1 μM TTX, suggesting that the residual slow EPSP in the presence of LY367385 is mediated by the release of some transmitter acting on a receptor other than a group I mGluR. Calibration in A has the same value as in B. Membrane potential in A was −50 mV. For experiments in B and C, membrane potential was manually held at −70 mV by current injection to avoid spiking and allow for accurate quantification.

Group I mGluRs decrease inhibitory transmission in the SNr

It was surprising that our immunocytochemical studies revealed presynaptic labeling in the SNr. In some other brain regions, mGluRs can act as heteroreceptors to reduce GABA release and...
inhibitory synaptic transmission. If activation of group I mGluRs decreases inhibitory transmission in the SNr, this combined with the direct excitatory effects described above would provide a mechanism whereby group I mGluR activation could exert a powerful excitatory influence on the SNr. We directly tested this hypothesis by recording IPSCs in SNR GABAergic projection neurons. IPSCs were evoked by stimulating within the SNr with bipolar stimulation electrodes (0.4–12.0 mA every 30 sec) and were recorded at a holding potential of −80 mV in the presence of AMPA (CNQX; 10–20 μM) and NMDA (d-AP-5; 10–20 μM) receptor antagonists to prevent excitatory synaptic transmission. Bicuculline (10 μM; n = 8) abolished evoked IPSCs in all cells tested, confirming that the evoked currents were GABA-A receptor-mediated responses. Short (3 min) bath application of the group I mGluR-selective agonist DHPG (100 μM) reduced the amplitude of evoked IPSCs in a reversible manner (Fig. 9A,B). Concentration–response analysis revealed that the inhibition of IPSCs by DHPG was concentration dependent. The relationship was fit with a Hill equation that gave an EC₅₀ value of 30 μM and Hill slope of 1.1. (Fig. 9C). This is consistent with the potency of DHPG on group I mGluRs.

Pharmacological studies of the DHPG-induced decrease in inhibitory transmission using subtype-selective antagonists were performed to determine which group I mGluR subtypes mediate this effect. The mGluR5-selective antagonist MPEP (10 μM) had a slight tendency to block the DHPG-induced effect, but the response to MPEP did not reach statistical significance (n = 8; p > 0.05; t test) (Fig. 10B,E). In contrast, the mGluR-selective antagonist CPCCOEt induced a significant reduction of the DHPG-induced suppression of IPSCs (n = 8; p < 0.05; t test) (Fig. 10C,E). However, the response to CPCCOEt was only a partial blockade of the response, and DHPG still induced a 20.9 ± 4.6% inhibition of IPSCs in the presence of this antagonist. Because neither antagonist was capable of completely blocking the response when added alone, we also determined the effect of a combination of both CPCCOEt and MPEP. The combination of antagonists completely blocked the ability of DHPG to reduce evoked IPSCs (n = 8; p < 0.01) (Fig. 10D,E), suggesting that both mGluR1 and mGluR5 may participate in regulation of IPSCs in SNr.

The group I mGluR-mediated decrease in inhibitory transmission occurs by a presynaptic mechanism

To determine whether the group I mGluR-mediated decrease in inhibitory transmission in the SNr is mediated through a presynaptic mechanism, we determined the effect of maximal concentrations of DHPG on frequency and amplitude of spontaneous mIPSCs. All mIPSC recordings were performed at a holding potential of −80 mV in the presence of CNQX (10–20 μM) and d-AP-5 (10–20 μM) to block glutamatergic synaptic currents and 1 μM TTX to block activity-dependent release of transmitter. mIPSCs were measured as inward currents with pipettes in which Cl− (140 mM) was the major anion in the internal solution.

Application of the group I selective agonist DHPG (100 μM) had no significant effect on mIPSC frequency or amplitude (Fig.
were made in the presence of CNQX (10 μM) and D-AP-5 (10–20 μM) with standard internal solution to allow measurement of outward IPSCs. IPSCs were evoked every 30 sec by paired stimulations of equal strength with a 50 msec interpulse interval. At these intervals, paired-pulse facilitation was observed in all recordings (60.2 ± 6.3%; n = 11). Only cells that showed an agonist-induced inhibition of the amplitude of the first IPSC of at least 25% were used for analysis. DHPG (30 μM) induced an increase in paired-pulse facilitation (Fig. 11D) in five of six cells examined. In those cells, the average increase in paired-pulse facilitation induced by DHPG was 56.1 ± 11.7% (p < 0.05; n = 5) over the facilitation seen in the absence of DHPG.

Together, these studies suggest that activation of the group I mGluRs mGluR1 and mGluR5 reduce inhibitory transmission in the SNr through a presynaptic mechanism. Furthermore, this decrease in GABAergic inhibition may combine with the direct postsynaptic excitatory effects of mGluR1 activation to produce a powerful excitation of this crucial basal ganglia output nucleus.

DISCUSSION

The data presented here demonstrate that activation of group I mGluRs produces an excitation of the SNr. Both mGluR1 and mGluR5 are found at postsynaptic sites in the SNr and are sparsely localized in unmyelinated axons and putative GABAergic axon terminals in this region. Activation of group I mGluRs produces an excitation of SNr neurons by two distinct mechanisms. Activation of postsynaptically localized group I mGluRs on SNr GABAergic neurons produces a robust depolarization that induces a marked increase in action potential firing. The depolarization is accompanied by a decrease in membrane conductance, and the underlying current has a reversal potential consistent with mediation by inhibition of a leak potassium channel. Furthermore, this effect is attributable to selective activation of mGluR1 and can be produced by synthetically released glutamate. Activation of group I mGluRs also induces a decrease in inhibitory transmission in the SNr. This effect is mediated by both mGluR1 and mGluR5 and occurs through a presynaptic mechanism.

Because the glutamatergic projection from the STN provides a large proportion of excitatory terminals on SNr GABAergic neurons, it is likely that the primary source of glutamate acting on group I mGluRs is released from STN afferents. However, several other regions, including the pedunculopontine nucleus (Charara et al., 1996) and the nucleus raphe (Corvaja et al., 1993), provide a sparse projection accounting for a small percentage of asymmetric terminals in the SNr. Therefore, group I mGluRs may also modulate these inputs. Interestingly, although both mGluR1 and mGluR5 are postsynaptically localized in SNr neurons, our pharmacological studies demonstrate that activation of mGluR1 is solely responsible for the group I-mediated depolarization. This is of interest because both mGluR1 and mGluR5 couple to phosphoinositide hydrolysis and are capable of inducing depolarization of other neuronal populations (for review, see Conn and Pin, 1997; Anwyl, 1999). A potential explanation of this may be provided by recent immunogold studies examining the subcellular localization of the group I mGluRs in SNr (Hubert et al., 2001). This study observed that mGluR1a immunoreactivity is predominately associated with the membrane. In contrast, >80% of mGluR5 immunoreactivity was localized to a cytoplasmic compartment. Thus, specificity of function may be produced by differences in subsynaptic localization or some other functional segregation of these receptors. It should be noted that, whereas mGluR1 plays the predominant role in mediating the group I mGluR-induced depolarization in the SNr, mGluR5 may play...
important physiological roles regulating cell properties that were not measured in the present study. For example, group I mGluRs are known to modulate NMDA receptor currents in a variety of brain regions, and it is possible that mGluR5 is involved in a similar modulation in SNr. Future studies on the role of mGluR5 in these cells may provide important insight into the distinct functional roles of closely related receptor subtypes within a single neuronal population.

In addition to the postsynaptic labeling of neurons in the SNr for both group I mGluR subtypes, we also detected presynaptic staining. Consistent with this, we found that activation of presynaptic group I mGluRs decreases inhibitory transmission. The results of both the paired-pulse experiments and the analysis of mIPSCs strongly suggest that the group I mGluR-mediated decrease in IPSCs has a presynaptic mechanism of action, yet the relatively sparse staining detected in inhibitory terminals appears unlikely to be sufficient to mediate this response. The more abundant axonal staining may represent group I mGluRs on preterminal axons of GABAergic neurons, which could mediate the observed decrease in inhibitory transmission. It should be noted that this distribution is reminiscent of previous reports of mGluR2/3 distribution in preterminal axons at sites distant from the synapse (Lujan et al., 1997). On the other hand, the finding that the decrease in inhibitory transmission has a presynaptic locus does not necessarily require that the receptor mediating this response is localized presynaptically. For example, in the CA1 region of the hippocampus, depolarization of CA1 pyramidal neurons induces the release of a putative retrograde transmitter that decreases inhibitory transmission through a presynaptic mechanism (Alger et al., 1996). Our current experiments do not allow us to distinguish between such a mechanism and an action of DHPG on a presynaptically localized receptor.

The finding that group I mGluRs both directly excite and disinhibit SNr neurons is of particular interest for understanding the role the STN plays in modulation of the SNr. The indirect pathway is composed of striatal projections through the globus pallidus and the STN, which constitute a large percentage of excitatory terminals on SNr GABAergic neurons (Smith et al., 1998). Although the glutamatergic input to the SNr is sparse, it plays a critical role in basal ganglia functions, as evidenced by the pronounced clinical effects of SNr lesions in PD (Guridi and Obeso, 1997). The STN also plays a key role in the pathological activity of the SNr. Transition of STN neurons from single-spike activity to burst-firing mode and resultant over excitation of the SNr has been implicated in the pathophysiology of PD (Hollerman and Grace, 1992; Bergman et al., 1994; Hassani et al., 1996), as well as some forms of epilepsy (Deransart et al., 1998). Furthermore, STN neurons exhibit extremely high firing rates and can typically exceed 25–50 Hz during burst-firing mode (Hollerman and Grace, 1992; Bergman et al., 1994; Wichmann et al., 1994; Beurrier et al., 1999; Bevan and Wilson, 1999). The robust excitatory effects of mGluR1 activation described here could play an important role in the powerful control exerted by the relatively sparse glutamatergic input to this nucleus from the STN.

Our current findings add to a growing body of literature suggesting that group I mGluRs play important roles in regulating functions of basal ganglia circuits (for review, see Smith et al., 2000, 2001; Conn et al., 2001; Rouse et al., 2001). For instance, mGluR5 is heavily expressed in the striatum and is also present at lower levels in the STN and the pallidal complex (Testa et al., 1994, 1995; Kerner et al., 1997; Tallaksen-Greene et al., 1998; Hanson and Smith, 1999). Although the levels of mGluR1 mRNA are more limited, this receptor is also found throughout the basal ganglia (Testa et al., 1994; Kerner et al., 1997; Tallaksen-Greene et al., 1998; Hanson and Smith, 1999). A number of studies suggest that agonists of group I mGluRs may act at several levels to increase the net activity of projection neurons in basal ganglia. For instance, activation of group I mGluRs potentiates NMDA receptor currents in striatal neurons (Colwell and Levine, 1994; Pisani et al., 1997). Furthermore, behavioral studies combined with studies of changes in 2-deoxyglucose uptake and Fos immunoreactivity suggest that injection of group I mGluR agonists in the striatum induces a selective activation of the indirect pathway from the striatum and thereby increases activity of the output nuclei (Kaatz and Albin, 1995; Kearney et al., 1997). In addition, recent physiological studies suggest that activation of group I mGluRs has profound excitatory effects on STN projection neurons (Abbott et al., 1997; Awad and Conn, 1999). These previous studies together with the present data suggest that group I mGluRs function at three major sites to increase overall output of the basal ganglia motor circuit.

**REFERENCES**


