Repellent Activation of Glutamatergic Inputs Evokes a Long-Lasting Excitation in Rat Globus Pallidus Neurons In Vitro

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INTRODUCTION

The rodent external globus pallidus (GPe) is the homolog of the external pallidal segment in the primate. The level and pattern of GPe activity change with the development of basal ganglia disorders such as Parkinson’s disease and hemiballism (Boraud et al. 2002; Filion and Tremblay 1991; Nini et al. 2000). Among these, the group I mGluRs, which consist of mGluR1 and mGluR5, are highly localized postsynaptically in the rodent and primate GPe (Hanson and Smith 1999; Poisik et al. 2000). It was previously shown that activation of mGluR1 by group I mGluRs agonist depolarized GPe neurons (Poisik et al. 2003). Thus it is possible that synthetically released glutamate may activate mGluR1 and contribute to the control of GPe neuronal activity, although this possibility has not been tested before.

Neurons in the STN, a major origin of glutamatergic inputs to the GPe, exhibit irregular firing in normal resting conditions but discharge in bursts in relation to movement-related events (Bergman et al. 1994; Matsumura et al. 1992; Wichmann et al. 1998), although little is known about the effect of burst activity of STN neurons on GPe neuronal activity. In other brain regions, repetitive stimulation, which mimics burst activity, evokes mGluR-mediated responses presumably arising from accumulation of glutamate released from synaptic terminals (Bengtson et al. 2004; Kim et al. 2003). Thus the aim of the present study was to investigate whether synthetically released glutamate with repetitive stimulation of STN axons can activate mGluRs in the GPe using rat brain slice preparations.

METHODS

Slice preparation

This study was performed in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Sprague-Dawley juvenile rats (15–19 days old; 20–42 g) of both sexes were anesthetized with an intraperitoneal injection of a mixture of Ketamine (85 mg/kg) and Xylazine (15 mg/kg) and then were

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decapitated. The brains were rapidly removed and blocks containing the GPe were obtained. Parasagittal slices (300–350 μm thick) were cut from the blocks on a Leica VT1000S slicer (Leica Microsystems, Nussloch, Germany), in ice-cold oxygenated artificial cerebrospinal fluid (ACSF) containing (in mM): 126 choline chloride, 3 KCl, 1.24 NaH₂PO₄, 26 NaHCO₃, 0.5 CaCl₂, 6.3 MgSO₄, 0.2 thiourea, 0.2 ascorbic acid, and 20 nM-glucose (pH 7.4). The slices were then incubated in ACSF containing (in mM): 126 NaCl, 3 KCl, 1.24 NaH₂PO₄, 26 NaHCO₃, 2.4 CaCl₂, 1.3 MgSO₄, and 10 nM-glucose (pH 7.4) at 33°C for ≥1 h before recording.

**Electrophysiological recordings**

The slices were transferred to a recording chamber with oxygenated ACSF continuously superfused at a flow rate of 1–2 ml/min. The temperature of the recording chamber was kept at 33 ± 1°C. Whole cell patch-clamp and cell-attached recording pipettes with a tip diameter of about 1.5 μm were pulled from 1.5-mm, thin-wall borosilicate glass capillaries on a horizontal electrode puller (P-97; Sutter Instruments, Novato, CA). The whole cell pipettes were filled with an electrolyte containing (in mM): 135 K-gluconate, 5 KCl, 10 HEPES, 10 mV, and/or had action potentials with an amplitude exceeding twice the root mean square (rms) of the membrane potential fluctuations at the baseline. The duration of an sDEPO was determined as a period between the stimulus onset and the time point in which the membrane potential returned to the baseline. Under the voltage-clamp recording, the inward currents induced by repetitive stimulation with 20 pulses were very small, usually <10 pA (e.g., Fig. 3, E and G), and only rarely exceeded twice the rms of the baseline fluctuations. Thus current recordings were filtered using a boxcar filter, which replaces each point in a trace with the average of the surrounding 4,000 points. In some experiments, it was also necessary to use 50 repetitive stimulus pulses to evoke currents large enough to be analyzed (e.g., Fig. 5, C–F). The magnitudes of long-lasting excitations recorded in the cell-attached mode were obtained from the mean firing rate over 5 s starting 1 s after the onset of the stimulation divided by the mean firing rate over 5 s preceding the stimulus. In 22 neurons, the membrane time constant was estimated by fitting a hyperpolarizing response, which was a ~10 pA, 600-ms current injection, to dual-exponential curves, of which the longer one represents the membrane time constant. All group data were expressed as means ± SE and were analyzed statistically using Student’s t-test and nonrepeated or repeated-measures ANOVA with a post hoc Bonferroni test.

**Chemicals**

CGP55845, (S)-(+)-α-amino-4-carboxy-2-methylbenzenecacetic acid (LY367385), 7-hydroxymethylcyclopropan[b]chromen-1-carboxylic acid ethyl ester (CPCCOEt), (2R)-1-(3-hydroxyphenyl)sulfonyl-2-[2-(4-methyl-1-piperidinyl)ethyl]pyrrolidine hydrochloride (SB269970), 6-methyl-2-(phenylethynyl)pyrrolidine hydrochloride (MPEP), di-threo-β-benzyloxyaspartate (TBOA), and 7-nitroindazole monosodium salt (7-NINA) were obtained from Tocris Cookson (Ellisville, MO). CPP, gabazine (SR-95531), NBQX, SCH23390, SKF96365, and sulpiride were obtained from Sigma–Aldrich RBI (St. Louis, MO). QX-314 was obtained from Alomone Labs (Jerusalem, Israel).

**Histology**

After recording, the slices were fixed overnight with a mixture of 4% paraformaldehyde and 0.2% picric acid. The fixed slices were rinsed several times with buffered saline, incubated overnight with avidin–biotin–HRP complex (1% in buffered saline with 0.4% Triton-X 100), rinsed again, and then reacted with diaminobenzidine. The slices were postfixed with 0.5% osmium tetroxide, infiltrated with Epon-Araldite, and mounted on glass slides. The stained neurons were drawn using a BH2 microscope (Olympus, Tokyo, Japan) equipped with a drawing tube and a ×40 dry objective lens.

**RESULTS**

**Type of neurons recorded**

The neurons included in this report had membrane potentials more negative than −50 mV and/or had action potentials with...
the amplitudes >60 mV. The rates of spontaneous repetitive firings were <20 Hz. Depolarizing current injections triggered repetitive firing without prominent spike accommodation and hyperpolarizing currents induced either prominent or moderate sags arising from inwardly rectifying current \( I_h \). These neurons included both type A and type B of Cooper and Stanford (2000) or the type I and type II neurons of Poisik et al. (2003) and consisted of most rodent GPe neurons (Kita and Kitai 1991). Intracellular staining with Neurobiotin revealed that these neurons were of medium size and had oval- or polygonal-shaped somata with spine-free dendrites (Fig. 2).

sDEPOs induced by repetitive IC stimulation

GPe neurons examined in a current-clamp mode were continuously hyperpolarized between −64 and −72 mV during data acquisition to prevent spontaneous firing. Repetitive IC stimulation with standard parameters (20 pulses, 200-μs duration, at 50 Hz) evoked a series of fast excitatory postsynaptic potentials (EPSPs), of which large EPSPs triggered action potentials (Fig. 1Aa). The EPSPs were followed by an sDEPO lasting for 10–20 s (Fig. 1Ba, \( n = 32 \)), a small and slow hyperpolarization that was sensitive to the GABA\(_B\) receptor antagonist CGP55845 (\( n = 15 \), data not shown; see Kaneda and Kita 2005), or no detectable level of slow responses (\( n = 118 \)).

Bath application of the GABA\(_A\) receptor antagonist gabazine (10 \( \mu \)M) increased the amplitude of the EPSPs and the number of the accompanying action potentials (Fig. 1Ab). Gabazine also slightly but significantly enhanced the amplitude of sDEPOs (Fig. 1, Bb and C). An additional application of CGP55845 (3 \( \mu \)M) appreciably enhanced the amplitude of sDEPOs (Fig. 1, Bc and C). CGP55845 also increased the number of the action potentials triggered from EPSPs (Fig. 1Ac). In the presence of gabazine and CGP55845, repetitive IC stimulation with the standard parameters evoked sDEPOs with detectable level of amplitude in 47 of 165 neurons. The neurons evoking slow responses appeared to have longer membrane time constants than that of others. To test this possible relationship, the time constant of the membrane response to 10-pA hyperpolarizing current steps was measured for 11 neurons with sDEPOs and for 11 neurons with no response. The time constants of the former neurons were significantly longer.

**FIG. 1.** Responses of external globus pallidus (GPe) neurons to repetitive internal capsule (IC) stimulation (20 pulses, at 50 Hz). A and B: fast and slow traces show effects of gabazine and (2S)-3-[(1S)-1-(3,4-dichlorophenyl)ethyl]amino-2-hydroxypropyl](phenylmethyl)phosphinic acid (CGP55845) on the responses of a neuron to repetitive stimulation. Neuron was current clamped at about −65 mV. In the control, the stimulation evoked a series of excitatory postsynaptic potentials (EPSPs) with an action potential (Aa) followed by a slow depolarizing response (sDEPO) (Ba). Bath application of gabazine (10 \( \mu \)M) increased the number of action potentials triggered from EPSPs, suggesting that the amplitude of the EPSPs was increased even though the increase was masked by large spike afterhyperpolarizations (Ab). Gabazine increased only slightly the amplitude of the sDEPO (Bb). An additional application of CGP55845 (3 \( \mu \)M) appreciably augmented the sDEPO (Bc) and increased the number of action potentials triggered by the EPSPs (Ac). Pre-stimulus hyperpolarizing deflections in the slow traces in this and the following figures represent responses to hyperpolarizing square pulses (−10 pA, 300 ms) that were used to monitor the input resistance of neurons. Action potentials were truncated. C: summary graph shows that gabazine slightly but significantly increased the amplitude of the sDEPOs. \( D: \) effects of the 1,2,3,4-tetrahydro-6-nitro-2,3-dioxobenzo[f]quinoxaline-7-sulfonamide (NBQX, 10 \( \mu \)M)/(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid (CPP, 30 \( \mu \)M) mixture on the sDEPOs recorded in the presence of gabazine (10 \( \mu \)M) and CGP55845 (3 \( \mu \)M). NBQX/CPP mixture did not affect either the amplitude or the duration of the sDEPOs. Data are presented as means ± SE.
longer than those of the latter ones (38.4 ± 7.0 vs. 21.6 ± 2.6 ms, P < 0.05, t-test). There was no significant difference in the input resistance (351.1 ± 61.5 MΩ for sDEPO-positive and 248.0 ± 24 MΩ for sDEPO-negative neurons, P > 0.05). The hyperpolarization-induced sags, arising from inwardly rectifying current \(I_h\), were not distinct between the two groups of neurons. Two neurons without the appreciable sag evoked sDEPOs.

Intracellular staining revealed no significant difference in somatic morphology between the two groups of neurons. The responsive neurons had oval \((n = 8)\) or polygonal \((n = 3)\) somatic shapes and the unresponsive ones were all oval \((n = 11)\). The short and long diameters of the responsive neurons were 11.5 ± 1.5 and 21.5 ± 4.7 μm, respectively, and those of unresponsive neurons were 10.2 ± 1.5 and 21.3 ± 3.6 μm, respectively. The responsive and unresponsive groups had 2.8 ± 0.9 and 2.9 ± 0.7 primary dendrites, respectively. The two groups of neurons were recorded from various parts of the GPe and appeared not to be location specific (Fig. 2A). The dendrites of both groups of neurons were free of spines and arbor were oriented roughly parallel to the border between the GPe and the Str. A noticeable difference was that the responsive neurons often had more complicated dendritic tips than did the unresponsive ones \((3.1 ± 1.0 \text{ vs. } 0.4 ± 0.7, P < 0.0001, \text{ANOVA})\) (Fig. 2, B and C).

Possible contributions of ionotropic glutamate receptors to the generation of sDEPOs were tested with bath application of a mixture of the AMPA/kainate receptor antagonist NBQX (10 μM) and the NMDA receptor antagonist CPP (30 μM), referred to hereafter as the NBQX/CPP mixture, in the presence of gabazine and CGP55845. The NBQX/CPP mixture completely blocked the induction of EPSPs but had no effect on the generation of sDEPOs (Fig. 1D). Thus most of the following experiments were performed in the presence of gabazine and CGP55845 both with and without the NBQX/CPP mixture.

**Stimulus conditions needed to evoke sDEPOs**

The amplitude and duration of the sDEPOs recorded under the presence of gabazine (10 μM) and CGP55845 (3 μM) were dependent on the number and frequency of stimulus pulses. When the number of pulses was increased stepwise from five to 50, with the standard stimulus frequency of 50 Hz and intensity of 100 μA, the induction of a distinct sDEPO required a minimum of 10 stimulus pulses. In addition, as the pulse number increased, the amplitude and duration of the responses increased (Fig. 3, A and B). Stimulation with >20 pulses often elicited large sDEPOs with a large number of action potentials (Fig. 3A). When the stimulus frequency was increased stepwise from 25 to 200 Hz, while maintaining the pulse number at 20, the maximum amplitude of the sDEPOs was observed with stimulus frequencies at 50 Hz for two neurons and 100 Hz for two neurons (Fig. 3, C and D).

To minimize the involvement of regenerative potentials in the assessment of the sDEPO amplitude, voltage-clamp recordings were performed in the presence of the NBQX/CPP mixture, gabazine, and CGP55845. The amplitude of the slow inward currents recorded at a holding potential of −60 mV was small (<10 pA), under the standard repetitive stimulation condition of 20 pulses with 100 μA at 50 Hz. The amplitude of the slow inward currents showed stimulus condition dependencies similar to those of sDEPOs. A minimum of 10 stimulus pulses were required to evoke slow inward currents and the amplitude increased as the pulse number increased. By increasing the number of pulses to 50, slow inward currents with 20–30 pA could be evoked (Figs. 3E and 5C). The maximum slow inward currents were observed with 50-Hz repetitive stimulation for three neurons and 100 Hz for two neurons (Fig. 3, G and H).

**mGluR1s partially mediate sDEPOs**

The possible involvement of mGluR1 in sDEPOs was examined with the mGluR1 selective antagonist LY367385. Bath application of LY367385 (10 μM) reversibly and significantly, but incompletely, decreased the amplitude of sDEPOs (Fig. 4, A and B). Application of a tenfold higher concentration of LY367385 failed to show additional blocking action, indicating that mGluR1 only partially mediated the sDEPOs (Fig. 4B).

To find other receptors that might mediate the LY367385-insensitive sDEPOs, the following experiments were performed. Because mGluR5, which is also expressed in the GPe...
and acts as a modulator of mGluR1 responses (Poisik et al. 2003), effects of the mGluR5 selective antagonist MPEP (10 μM) were tested; however, MPEP had no effect on the sDEPOs (Fig. 4B). MPEP applied after the application of LY367385 also failed to affect LY367385-insensitive sDEPOs (Fig. 4C). Because the GPe receives dense serotoninergic innervations (Vertes 1991) and serotonin depolarizes GPe neurons through the activation of 5-HT₇ receptors (Hashimoto and Kita 2002), the 5-HT₇ receptor selective antagonist SB269970 (10 μM, n = 2) was tested. However, SB269970 also had no effect on LY367385-insensitive sDEPOs (Fig. 4C). A nitric oxide synthase (NOS) inhibitor 7-NINA (200 μM), which was previously reported to suppress the depolarization induced by NO in the striatal medium spiny neurons (West and Grace 2004), also failed to inhibit LY367385-insensitive sDEPOs (Fig. 4C). The involvement of dopaminergic receptors was also shown to be unlikely because application of the mixture of the D1 antagonist SCH23390 (10 μM) and the D2 antagonist sulpiride (10 μM) failed to affect the amplitude of CPCCOEt (another selective mGluR1 antagonist)-insensitive sDEPOs (Fig. 4C). We also observed that neither the nicotinic receptor antagonist mecamylamine (10 μM, n = 2) nor the muscarinic receptor antagonist scopolamine (10 μM, n = 2) affected LY367385-insensitive sDEPOs (data not shown). Application of tetrodotoxin (TTX, 1 μM) completely blocked sDEPOs (n = 2, data not shown), confirming that the response was a result of synaptic transmission(s) or action potential generation. Moreover, none of the neurons recorded with pipettes containing QX-314 (3 mM) exhibited sDEPOs (n = 29, data not shown).
The membrane potential dependency of sDEPOs was examined to explore the ionic mechanisms. When neurons were treated with gabazine (10 μM) and CGP55845 (3 μM) and current clamped at −60 mV, large sDEPOs with a number of action potentials could be evoked (Fig. 5A). Hyperpolarization of the neurons decreased the amplitude of the sDEPOs and decreased the number of action potentials (Fig. 5A). However, the amplitude was slightly increased by hyperpolarization beyond −80 or −85 mV (Fig. 5, A and B).

The measurements of the amplitude of the slow inward currents resulted in a slightly different outcome. Because the amplitude of slow inward currents evoked by the standard stimulus protocol (20 pulses at 50 Hz) was very small, as shown in Fig. 2, we applied 50 pulses at 50 Hz in this series of experiments. We examined membrane potential dependency of slow inward currents from four neurons in control and after application of CPCCOEt (100 μM). The amplitudes of slow inward currents were similar at different holding potentials (Fig. 5, C and D). CPCCOEt decreased the amplitudes of slow inward currents at −60 and −80 mV. However, the decrease became less clear as the membrane potentials were hyperpolarized (Fig. 5, C and E). The CPCCOEt-insensitive currents were largest at a holding potential of −100 mV (Fig. 5, C and E). Subtractions of the responses evoked in the control while using CPCCOEt were made in each holding potential to calculate the voltage dependency of the CPCCOEt-sensitive current (Fig. 5C). The amplitudes of the CPCCOEt-sensitive current were largest at −60 mV and decreased as the membrane potential was hyperpolarized (Fig. 5F).

**Voltage dependency of sDEPOs**

To assess the effects of sDEPOs on the firing activity of GPe neurons, cell-attached extracellular recordings from spontaneously active neurons were performed in the presence of the NBQX/CPP mixture, gabazine (10 μM), and CGP55845 (3 μM). Under this control condition, repetitive IC stimulation evoked a long excitation lasting for over 25 s in nine of ten neurons (Fig. 6Aa). Bath application of mGluR1-selective antagonists LY367385 (10 μM) or CPCCOEt (100 μM) significantly reduced the response (Fig. 6, A–C) without significantly altering the basal firing activity (data not shown). The effects of LY367385 and CPCCOEt were not additive (Fig. 6, A–C). These results confirmed the results of whole cell recordings that repetitive IC stimulation activated mGluR1 and other unidentified receptors and that each of the two mGluR1 antagonists alone at the given concentrations can totally block mGluR1-mediated responses.

The possible involvement of the transient receptor potential (TRP) channels, which mediate mGluR1 responses in other brain areas (Bengtson et al. 2004; Kim et al. 2003), was tested using the selective TRP channel blocker SKF96365. SKF96365 (50 μM) did not affect the magnitude of excitations evoked by repetitive stimulation (Fig. 6, D and E) and spontaneous firing rates (data not shown).
Effects of glutamate uptake inhibitor and mGluR1 agonist on the long-lasting excitations

Previous studies in other brain areas showed that a blockade of glutamate transporters appreciably augments mGluR1-mediated responses (Brasnjo and Otis 2001; Dzubay and Otis 2002; Heuss et al. 1999; Huang et al. 2004). Therefore we tested effects of the glutamate uptake inhibitor TBOA in slices treated with the NBQX/CPP mixture, gabazine (10 µM), and CGP55845 (3 µM). TBOA dose-dependently and significantly increased the basal firing activity of GPe neurons (Fig. 7C). TBOA (100 µM) diminished the repetitive stimulation-induced long-lasting excitations (Fig. 7). Additional application of CPCCOEt (100 µM) reversed the TBOA-induced increase in spontaneous firing (Fig. 7). These results suggest a possibility that TBOA increased the level of ambient glutamate, activated mGluR1, and increased firing activity of GPe neurons. To examine this possibility further, we applied a low concentration of the selective group I mGluRs agonist (S)-3,5-dihydroxyphenylglycine (DHPG, 1 µM) to mimic an increased level of mGluR1 stimulation. Like TBOA, DHPG decreased responses to repetitive stimulation (Fig. 8, A and C) and significantly increased the spontaneous firing rate, which could be reversed with an additional application of CPCCOEt (Fig. 8B). These results suggested the possibility that the reduction of the excitatory responses after TBOA application results from occlusion of the mGluRs. To test this, we applied TBOA first and then DHPG. In the presence of 100 µM TBOA, both 1 and 10 µM DHPG failed to increase the spontaneous firing rate (Fig. 8D). We also examined the effect of a low concentration of glutamate (1 µM). Unlike DHPG, glutamate application did not affect the basal firing rate (Fig. 8F), presumably a result of the rapid glutamate uptake by transporters. On the other hand, glutamate showed a slight but nonsignificant enhancement of the excitation (Fig. 8, E and G). An additional application of TBOA significantly increased the rate of spontaneous firing (Fig. 8F).

Repetitive IC stimulation increased frequency of spontaneous EPSCs

The duration of the sDEPO and the excitation evoked by repetitive IC stimulation was much longer than that of mGluR1-mediated responses reported in other brain regions (e.g., Bengtson et al. 2004; Kim et al. 2003). To investigate the possible mechanism(s) of this long excitation, we recorded spontaneous EPSCs from five QX-314–injected neurons, which were voltage clamped at −65 mV in the presence of...
gabazine (10 μM) and CGP55845 (3 μM). We counted the number of spontaneous EPSCs with amplitude >5 pA. EPSCs with multiple small (<5 pA) peaks were counted as one EPSC. The results revealed that spontaneous EPSCs before the repetitive stimulation were relatively low and the stimulation significantly increased the frequency for several seconds in four of the five neurons tested (Fig. 9, A and B).

**DISCUSSION**

The main findings of this study were as follows: 1) repetitive IC stimulation induced long-lasting excitations in GPe neurons, 2) the excitations were a result of the activation of mGluR1 and other unidentified mechanisms, 3) the excitations were TTX sensitive, and 4) a blockade of glutamate transporter increased the basal firing activity of GPe neurons but did not augment the repetitive stimulation-evoked excitations. These results suggest that glutamate released during burst synaptic activation stimulated postsynaptic mGluR1 but only partially contributed to the long-lasting excitation of GPe neurons.

The data that 90% of neurons responded with long-lasting excitations under cell-attached recording suggested that most GPe neurons, including some of those lacking Ih, exhibited long-lasting excitations. In whole cell recording, roughly 30% of neurons showed sDEPOs. Intracellular staining revealed that responsive neurons often had more complicated dendritic endings than did unresponsive ones, although the somatic morphology of the two groups was similar. Thus both the physiological and morphological characteristics were uncorrelated with the type A and type B neurons of Cooper and Stanford (2000) or the type I and type II neurons of Poisik et al. (2003). Rather, the present physiological and morphological observations are consistent with the suggestion that sDEPO is generated at distal dendrites and that the complicated endings provided larger areas of generation sites.

**Effects of GABAergic inputs on EPSPs and sDEPOs**

As expected, a mixture of ionotropic glutamate receptor blockers completely eliminated the EPSPs evoked by repetitive
IC stimulation but did not suppress sDEPOs (Kita 1992, 1994; Kita and Kita 1991; Parent and Hazrati 1995). The sDEPOs were sensitive to TTX, suggesting they were synaptically induced. The IC stimulation might have activated glutamatergic axons and also striatal and/or pallidal GABAergic axons, evoking both GABA_A- and GABA_B-receptor–mediated responses that were overlapped by glutamatergic responses (Kaneda and Kita 2005). Application of the GABA_B antagonist CGP55845 considerably augmented the amplitude of sDEPOs. This effect may be attributable not only to the distinct slow kinetics of the GABA_B response, which are similar to those of sDEPOs, but also to the interaction of intracellular mechanisms at the level of second messengers or effector channels, given that both GABA_B responses and sDEPOs are mediated, at least partly, by metabotropic receptors. For instance, mGluR1 stimulation has been reported to inhibit G-protein–gated inwardly rectifying potassium channels, whereas GABA_B receptor stimulation results in activation of the channels (Sharon et al. 1997). It is unlikely that suppression of presynaptic GABA_B receptors located at asymmetric synaptic terminals (Chen et al. 2004a) increased glutamate release because synaptically released GABA did not modulate the release of glutamate under similar experimental conditions in the GPe (Kaneda and Kita 2005).

**Pharmacological mechanisms of sDEPO induction**

Experiments with mGluR1 antagonists suggested that mGluR1 only partially mediated sDEPOs and long-lasting excitations. The large sDEPOs with multiple spikes evoked at a holding potential of around −60 mV seemed to be augmented by the recruitment of persistent sodium channels and/or voltage-dependent calcium channels (Hanson and Smith 2002; Hanson et al. 2004). Two results support this possibility: the inward currents recorded in the somata were small and sDEPOs were abolished by intracellular XQ-314, which is known to block both sodium and calcium channels (Crill 1996; Talbot and Sayer 1996).

The sDEPOs and excitations in the GPe had much longer durations than the mGluR1-mediated responses recorded in other brain regions (Bengtson et al. 2004; Kim et al. 2003). Although the mechanisms underlying the long duration are unknown, one possibility is an increase in spontaneous glutamate release after repetitive stimulation, an effect observed in this study. This result is compatible with the previous finding that high-frequency STN stimulations evoked a long-lasting increase in the extracellular glutamate level in the rat GPe (Windels et al. 2000).

In contrast to the effect of the selective mGluR1 antagonist LY367385, the selective mGluR5 antagonist MPEP had no effect on sDEPOs. These results are in agreement with previous studies showing that a depolarization induced by the group I mGluRs agonist DHPG was solely mediated by mGluR1 in GPe neurons (Matsui and Kita 2001; Poisik et al. 2003). However, our results are inconsistent with the previously observed synergistic interaction between mGluR1 and mGluR5, in which mGluR5 blockade enhanced the activity of mGluR1 in some GPe neurons (Poisik et al. 2003). The reason for this discrepancy is unknown at present.

Voltage-clamp recordings revealed that the amplitude of the CPCCOEt-sensitive slow inward current was larger at a holding membrane potential of −60 than at −100 mV. These results were consistent with previous results that G-protein–coupled metabotropic receptors, including mGluR1, suppress leaking or background potassium currents (Guérin et al. 1994; Kettunen et al. 2003; Mannaioni et al. 2001; Takeshita et al. 1996). TRP channels have been suggested for the mGluR1–mediated slow EPSCs in other brain areas (e.g., Bengtson et al. 2004; Kim et al. 2003). However, the channels may not be involved in the generation of the mGluR1–mediated responses observed here because the TRP channel blocker SKF96365 did...
not affect the long-lasting excitations. Moreover, TRP-channel-mediated currents were not affected by QX-314 (Bengston et al. 2004), whereas none of the GPe neurons recorded with QX-314 containing electrodes exhibited sDEPOs.

The pharmacological and ionic natures of the mGluR1 antagonist-insensitive component of sDEPOs and long-lasting excitations were not completely revealed in the present study. So far, we have examined antagonists to the 5-HT7 receptor, D1 and D2 dopamine receptors, nicotinic and muscarinic receptors, and a NOS inhibitor, although none of these affected the response. Another putative transmitter of sDEPOs may be neurotensin or substance P (Chen et al. 2004b; Stanfield et al. 1985). It is also possible that repetitive stimulation activated many neurons and axons and increased the level of extracellular potassium.

**Glutamate transporters regulate level of extracellular glutamate**

The result that the glutamate uptake blocker TBOA did not augment long-lasting excitations to repetitive IC stimulation is in contrast to previous studies in other brain areas showing that a blockade of glutamate transporters substantially enhanced mGluR1-mediated responses (Brasnjo and Otis 2001; Dzubay and Otis 2002; Heuss et al. 1999; Huang et al. 2004). On the other hand, TBOA significantly increased the spontaneous...
firing rate of GPe neurons. The mGluR1-selective antagonists blocked this increase. TBOA has no direct effect on mGluR1 (Shimamoto et al. 1998) and also the ionotropic glutamate receptors were blocked in these experiments. Thus under our experimental conditions, the significant increase of extracellular glutamate by TBOA seems to have tonically activated mGluR1 and occluded or partially desensitized the mGluR1-mediated response. We tested these possibilities further by bath application of DHPG and glutamate. DHPG activates mGluR1 and is not taken up by glutamate transporters, and thus it mimics the increased extracellular glutamate by TBOA application. On the other hand, application of DHPG in the presence of TBOA failed to increase the firing rate, suggesting occlusion of mGluR1s. Bath application of glutamate did not change spontaneous firing rates or long-lasting excitations to repetitive stimulation. These results suggested that glutamate transporters took up bath-applied glutamate. Coapplication of glutamate and TBOA increased the spontaneous firing and decreased excitations to repetitive stimulation; this agrees with the results of the DHPG application experiments. These results were consistent with the idea that the background activation of mGluR1 suppresses the long-lasting excitations.

Functional implications for the sDEPO

We found that repetitive IC stimulation evoked sDEPOs or long-lasting excitations in GPe neurons. An induction of an sDEPO required repetitive stimulation with >10 pulses. This observation was consistent with the results of the monkey unit recording study where repetitive stimulation of the STN with 10 pulses at 100 Hz did not evoke appreciable NBQX/CPP-insensitive excitation (Kita et al. 2005). Although STN neurons have been observed to exhibit high-frequency burst firings during movement (Bergman et al. 1994; Matsumura et al. 1992; Wichmann et al. 1994), the strength and duration of the behavioral event-related burst firing may not be sufficient to evoke strong slow responses in the GPe. Strong and long-lasting bursts of STN neurons were reported in some parkinsonian patients and animals (Bergman et al. 1994; Hutchison et al. 1998). A continuous high-frequency stimulation of the STN, which has been used increasingly in recent years to ameliorate symptoms of Parkinson’s disease (Benazzouz et al. 1993; Hutchison et al. 1998; Limousin et al. 1995), also induced strong, coincidental, long-lasting bursts in the STN (Garcia et al. 2003). Such strong, coincidental, long-lasting activity of STN neurons might induce an abundant release of glutamate and evoke a volume transmission in the GPe. This possibility is consistent with the observation that high-frequency STN stimulation in rats increased the extracellular level of glutamate in the GPe (Windels et al. 2000). Furthermore, high-frequency STN stimulation increased the firing rate of some GPe neurons in vivo (Benazzouz et al. 1995; Hashimoto et al. 2003; Kita et al. 2005).

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