Dynorphin Exerts Both Postsynaptic and Presynaptic Effects in the Globus Pallidus of the Rat

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Ogura, Mitsuhiro and Hitoshi Kita. Dynorphin exerts both postsynaptic and presynaptic effects in the globus pallidus of the rat. J Neurophysiol 83: 3366-3376, 2000. The opioids contained in striato-pallidal axons are thought to play a significant role in motor control. We examined postsynaptic effects of the kappa (κ)-receptor agonist dynorphin A (1–13) (DYN13) on the globus pallidus (GP) neurons in rat brain slice preparations using the whole cell recording method. DYN13 hyperpolarized and decreased the input resistance of approximately one-quarter of neurons examined. All of these DYN13-sensitive neurons had medium-sized somata, large aspiny dendrites, and generated repetitive firing without strong accommodation. The hyperpolarization was blocked by barium and was independent of intracellular chloride levels. The hyperpolarization was also selectively blocked by the κ-antagonist nor-binaltorphimine dihydrochloride but not by the mu- or delta-antagonists. These data suggested that DYN13 activates barium-sensitive potassium currents in some GP neurons. Low- and high-intensity stimulation of the neostriatum (Str) evoked long- and short-latency GABAergic responses, respectively. Previous data suggested that the long- and the short-latency responses were due to activation of the striato-pallidal axons and the local collaterals of pallido-striatal axons, respectively. DYN13 diminished the amplitude of both the short- and long-latency GABAergic responses in all the neurons tested. The effects of DYN13 on GABAergic postsynaptic responses were also selectively blocked by a κ-antagonist. To investigate whether the effects were pre- or postsynaptic, the effects of DYN13 on spontaneous inhibitory postsynaptic potentials (IPSPs) and TTX-independent miniature-inhibitory postsynaptic currents (mIPSCs) were examined. DYN13 decreased the frequency, but not the amplitude, of spontaneous IPSCs and calcium-dependent miniature-IPSCs. However, DYN13 did not alter the cadmium-insensitive miniature-IPSCs. These results suggested that DYN13 suppressed GABA release from presynaptic terminals. This possibility was tested using a paired-stimulation test. DYN13 reduced the probability of evoking IPSCs to the first stimulation and greatly increased the success probability to the second stimulus. The amplitude of successfully evoked IPSCs was not changed with DYN13. DYN13 did not affect the excitatory postsynaptic potentials (EPSPs) or the response to iontophoretically applied GABA and glutamate. Together, these results suggest that DYN released from striato-pallidal axons controls the activity of GP neurons 1) by directly hyperpolarizing a population of neurons and 2) by presynaptically inhibiting GABA release from strato-pallidal and intrapallidal terminals.

INTRODUCTION

The globus pallidus (GP) of the rodent is homologous to the external segment of the pallidum of the primate. The GP is a large nucleus located caudomedially to the neostriatum (Str). The existence of rich reciprocal connections between the GP and other basal ganglia nuclei implies that the GP plays a significant role in controlling the neuronal activity of the entire basal ganglia (reviews by Chesselet and Delfs 1996; Kita 1994a; Kita et al. 1999; Mink 1996; Smith et al. 1998). Anatomical and morphological studies suggest that the activity of GP neurons is controlled by three major inputs: Str inhibitory inputs, subthalamic excitatory inputs, and intrinsic collateral inhibitory inputs (Chesselet and Delfs 1996; Kita 1994a; Mink 1996). The striato-pallidal fibers form the largest number of terminals in the GP and terminate mainly on the dendritic shafts of GP neurons (Falls et al. 1983; Okoyama et al. 1987). Intracellular staining and immunohistochemical studies indicated that the striato-pallidal terminals could be classified into two major groups: approximately two-thirds of the terminals contain GABA and enkephalin and belong to the Str neurons projecting only to the GP; the other one-third contain GABA, dynorphin (DYN) and substance P and belong to the collateral axons of Str neurons projecting to the entopeduncular nucleus and the substantia nigra (Gerfen and Young 1988; Kawaguchi et al. 1990; Lee et al. 1997; Penny et al. 1986). The subthalamic axons form the second greatest number of terminals in the GP. These axons terminate on the somata and dendrites of GP neurons. Stimulation of the subthalamo-pallidal pathway evokes powerful excitatory responses in GP neurons (Kita 1994a; Kita and Kitai 1991). The third largest number of terminals in the GP are formed by the local-collateral axons of GABAergic GP projection neurons (Kita 1994b). Intracellular staining studies have shown that all GP projection neurons have local-collateral axons (Bevan et al. 1998; Kita and Kitai 1994; Nambu and Linas 1997). These collateral axons may strongly inhibit GP neurons because they terminate on the somata and proximal dendrites of GP neurons (Kita 1994b).

Several possible roles of opioids contained in the Str efferent fibers have been described recently. DYN and enkephalin are endogenous ligands of kappa (κ)- and mu (μ)-opioid receptors, respectively (Brookes and Bradley 1984; Chavkin et al. 1982; Raynor et al. 1994). Both types of receptors are found in the GP (Mansour et al. 1988; Morris and Herz 1986; Sharif and Hughes 1989). A recent study indicated that μ-opioid receptor agonists presynaptically inhibit GABAergic transmission in the GP of rat brain slice preparations (Stanford and Cooper 1999). Several studies suggested that DYN may also play a prominent role in controlling the neuronal activity of the GP. Intrapallidal injection of κ-selective agonists caused a slowing of the contralateral head turn movement evoked by Str elec-
trical stimulation (Slater and Longman 1980). Intrapallidal injection of κ-agonists decreased apomorphine-induced circling behavior of the rats that had received unilateral deafferentation of nigro-striatal dopaminergic fibers (Slater 1982). In rats with unilateral dopamine lesions, administration of apomorphine resulted in a marked increase in the expression of mRNA for DYN in the lesioned side of the Str (Gerfen et al. 1991). Systemic or iontophoretically applied morphine or DYN decreased unit activity of the GP in anesthetized rats (Huffman and Felpel 1981; Huffman and Frey 1989; Napier et al. 1983a,b). These behavioral and unit recording studies all suggest that DYN may play a significant role in controlling the activity of GP neurons.

The aim of this study was to explore the underlying mechanism of these κ-agonist actions cited above using whole cell recordings in brain slice preparations. Specifically, we examined the effects of the κ-agonist dynorphin A (1–13) (DYN13) on the neuronal membrane and GABAergic and glutamatergic synaptic transmissions in the GP. The results indicate that DYN13 exerts both post- and presynaptic effects in the GP. Preliminary accounts of these results have appeared previously (Ogura and Kita 1998).

METHODS

Slice preparations

Sprague-Dawley juvenile rats (15–21 days old, 28–45 g) of both sexes were used. The physiological responses to current injection and synaptic stimulation and the morphology of the juvenile GP neurons reported herein were very similar to those reported for adults GP (Bevan et al. 1998; Kita and Kitaï 1991, 1994; Nambu and Llinas 1997). Furthermore, the proprieties of immunohistochemical staining for enkephalin, glutamate decarboxylase, and parvalbumin in the GP of juvenile rats (H. Kita, unpublished observation) were similar to those of adult rat GP (Kita 1994b; Kita and Kitaï 1994). Animals were anesthetized (ip) with a mixture of Ketamine (85 mg/kg) and Xylazine (15 mg/kg) and were perfused through the heart with cold oxygenated artificial cerebrospinal fluid (ACSF). After decapitation, the brains were rapidly removed and blocks containing the GP were obtained. Parasagittal slices (300 μm thick) were cut on a Vibroslice® (Campden, UK) in ice-cold ACSF. The slices were incubated in ACSF at 37°C for 1 h before recording. The composition of ACSF (in mM) was 124 NaCl, 5.0 KCl, 1.24 KH2PO4, 26 NaHCO3, 2.4 CaCl2, 1.3 MgSO4, and 10 glucose.

Recording and electrical stimulation

The slices were transferred to a recording chamber with oxygenated ACSF continuously perfused at a flow rate of 2 ml/min. The temperature of the recording chamber was kept at 34°C. Whole cell patch 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-87, Sutter Instruments, Navato, CA). Two kinds of electrolytes were used to fill the pipettes. For inhibitory postsynaptic potential (IPSP) and inhibitory postsynaptic current (IPSC) recordings, the pipettes were filled with high-Cl electrolyte containing (in mM) 90 K-glucuronate, 50 KCl, 10 HEPES, 2 Mg-ATP, and 0.2 Na-GTP, 0.2% Neurobiotin, with pH adjusted to 7.2 with KOH. The chloride equilibrium potential of the cells was calculated to be –25 mV by the Nernst equation when the cytoplasm of the recorded cells was fully equilibrated with 50 mM chloride. For EPSP recordings, the pipettes were filled with low-Cl electrolyte containing (in mM) 135 K-glucuronate, 5 KCl, 10 HEPES, 2 Mg-ATP, and 0.2 Na-GTP, 0.2% Neurobiotin, with pH adjusted to 7.2. The resistance of these record-
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—horseradish peroxidase (HRP) complex (1% in buffered saline with 0.4% Triton-X 100), rinsed, and then reacted with diaminobenzidine. The slices were postfixed with 0.5% osmium tetroxide, infiltrated with a plastic resin, and mounted onto glass slides. The stained neurons were drawn under the microscope BH2 (Olympus, Tokyo, Japan) equipped with a drawing tube and a ×60 dry objective.

Statistics

All group data were expressed as a means ± SE, and analyzed statistically using a Student’s t-test or an ANOVA. The Kolmogorov-Smirnov test (Press et al. 1986) was used to determine the statistical significance of the amplitude distributions of spontaneous IPSCs.

RESULTS

DYN13 hyperpolarizes some GP neurons

Bath application of DYN13 (1 μM) hyperpolarized 15 of 61 GP neurons that were recorded using the current-clamp method under three different recording conditions (Table 1). Fourteen and 38 neurons were recorded with the low- and high-Cl electrolyte containing pipettes, respectively. Nine others were recorded with the high-Cl electrolyte containing pipettes in the presence of TTX (1 μM). DYN13 (1 μM) hyperpolarized approximately one-quarter of neurons in every condition (Table 1). The DYN13-induced hyperpolarization was accompanied by a decrease (16.2 ± 3.6%, n = 15) in the input resistance at the resting membrane potential (Fig. 1). The hyperpolarization was reversed by washing (n = 7), by the nonselective opioid antagonist naloxone (5 μM, n = 4), or by the κ-opioid receptor selective antagonist nor-BNI (0.2 μM, n = 4; Fig. 1A), but not by the δ-antagonist naltrindole (1 μM, n = 3) or the μ-antagonist CTOP (1 μM, n = 3). DYN13 depolarized one of the neurons recorded with a high-Cl electrolyte containing pipette (Table 1). Washing reversed the depolarization, but no further tests were possible because of a clogged recording pipette.

All of the DYN13-sensitive neurons generated sustained firing without strong accommodation on injection of suprathreshold current pulses (Fig. 1B). Stimulation of DYN13-insensitive neurons generated either sustained firing or a short train of firing with strong accommodation. Intracellular staining with Neurobiotin revealed that the neurons with sustained firing were of medium size (12.3 ± 0.2 × 27.8 ± 0.5 μm, mean ± SE, n = 15) and had two to four thick, slowly tapering, smooth dendrites (an example shown in Fig. 2). Distal dendrites had occasional small spines. The neurons with strong accommodation had smaller (9.8 ± 0.2 × 24.2 ± 1.0 μm, n = 9) somata and had two to five thin dendrites with spines (data not shown) (see Kita and Kitai 1994; Nambu and Llinas 1997).

The current-voltage relationship curves obtained from the DYN13-sensitive neurons before and after DYN13 application crossed at 85.2 ± 1.7 mV (n = 15), which is near the potassium-equilibrium potential of ~83 mV estimated by using the Nernst equation. This suggested the possibility that DYN13 activated inwardly rectifying potassium channels in these neurons. To test this possibility, barium chloride (100 μM) was applied to the slice before DYN13. Barium caused a depolarization (5.7 ± 0.6 mV, n = 22) and an increase (147–208%) in the input resistance of all 22 GP neurons tested. None of these barium chloride–treated neurons were hyperpolarized by DYN13.

DYN13 diminishes inhibitory postsynaptic response

Stimulation of the Str evoked GABAergic responses in GP neurons of slices perfused with ACSF containing DNQX (50 μM) and CPP (10 μM). Recent anatomical studies have revealed that a large number of GP neurons project to the Str and that all of these neurons have local collateral axons (Bevan et al. 1998; Kita and Kitai 1994; Kita et al. 1999; Nambu and Llinas 1997). Thus it can be expected that Str stimulation activates both striato-pallidal fibers and local collaterals of pallido-striatal fibers. Str stimulation induced responses were recorded in the initial part of the study with a current-clamp amplifier (n = 24) and later with a voltage-clamp amplifier (n = 73). Stimulation at threshold intensity evoked very small (<3 mV or 10 pA) IPSPs (n = 22) and IPSCs (n = 64) with a latency of 7–10 ms in 86 of 97 GP neurons tested. When the stimulus intensity was gradually increased, the response amplitude gradually increased, and the latency gradually shortened to about 6 ms (example in Fig. 4A). When the stimulus intensity was increased to some value, the response amplitude steeply increased, and the latency steeply decreased to about 4 ms. In the majority (81 of 86) of the neurons, the long-latency component blended into the preceding short-latency component and could not be isolated. In five neurons, however, the late component was large enough so that the amplitude could be measured from the crest of the short-latency response. Data obtained from these five neurons are presented below. In 11 of 97 neurons, Str stimulation at threshold intensity evoked short-latency (approximately 4 ms) large IPSPs (n = 2) and IPSCs (n = 9) without the long-latency IPSCs seen with other neu-

<table>
<thead>
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<th>Number of Cells Tested</th>
<th>Hyperpolarized</th>
<th>Depolarized</th>
<th>No Effect</th>
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<tr>
<td>Low-Cl</td>
<td>14</td>
<td>3 (21.4%, 5.3 ± 2.3 mV)</td>
<td>0</td>
</tr>
<tr>
<td>High-Cl</td>
<td>38</td>
<td>10 (26.3%, 4.4 ± 0.7 mV)</td>
<td>1 (2.6%)</td>
</tr>
<tr>
<td>High-Cl with TTX</td>
<td>9</td>
<td>2 (22.2%, 5.5 ± 1.5 mV)</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>61</td>
<td>15 (24.6%, 4.7 ± 0.6 mV)</td>
<td>1</td>
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Neurons were recorded with the low-Cl (5 mM) electrolyte-containing pipettes, the high-Cl (50 mM) electrolyte-containing pipettes, and with the high-Cl electrolyte-containing pipettes in the presence of tetrodotoxin (TTX, 1 μM). DYN13 (1 μM) hyperpolarized approximately one-quarter of neurons in every recording condition. DYN13 depolarized only one of the neurons recorded with the high-Cl electrolyte containing pipettes.
The results indicate that the DYN13-induced hyperpolarization was accompanied by a decrease in the input resistance. The two current-voltage curves cross approximately 7 mV. A decrease in the input resistance. The noisy appearance of the trace is due to a large number of spontaneous depolarizing inhibitory postsynaptic potentials (IPSPs). Bath application of DYN13 (1 μM) hyperpolarized this GP neuron approximately 7 mV. A decrease in the input resistance and a decrease in the amplitude of spontaneous IPSPs accompanied the hyperpolarization. B: responses to depolarizing and hyperpolarizing current pulses were recorded from another GP neuron with a high-Cl electrolyte containing pipette before and after application of DYN13 (1 μM). Suprathreshold current pulses triggered repetitive spikes without strong accommodation. DYN13 (1 μM) hyperpolarized this neuron from −70 to −73 mV. C: current-voltage relationship curves obtained from the neuron are shown. The membrane potentials at the terminations of current pulses were measured on 4–5 traces with no observable IPSPs at that time and the averages were plotted. The results indicate that the DYN13-induced hyperpolarization was accompanied by a decrease in the input resistance. The two current-voltage curves cross at approximately −85 mV.

The effect of DYN13 on GABA receptors at the postsynaptic membrane was studied using the iontophoretic GABA application method. Two-barreled pipettes, one barrel containing 0.1 M GABA and the other containing saline, were placed approximately 30 μm from the somata of the recording neurons. Iontophoresis of GABA by constant current pulses (5–30 nA in amplitude and 30–100 ms in duration) between the GABA and the saline containing pipette induced 13- to 25-mV depolarizations in the neurons when recordings were made with high-Cl electrolyte containing pipettes (n = 10). The response was sensitive to bicuculline methiodide (50 μM) and thus was considered GABA_A receptor mediated (Fig. 6). Of 10 neurons tested, 8 were DYN insensitive and two DYN sensitive. In the eight DYN-insensitive neurons, bath application of DYN13 (2 μM) did not change the response to exogenous GABA (Fig. 6). In the two DYN-sensitive neurons, DYN13 (2 μM) decreased the response to GABA by approximately 10% (data not shown). The reduction was much smaller than that observed in the synaptically induced GABAergic responses. This small reduction might be due to an increase of the membrane conductance at distal dendrites. These results indicate that DYN13 does not change the GABA sensitivity of the postsynaptic membrane.
DYN13 decreases the frequency of spontaneous IPSCs

All the GP neurons examined exhibited spontaneous GABAergic IPSCs. The amplitudes of spontaneous IPSCs were as high as 220 pA when the membrane potential was clamped at the resting membrane potential of −65 to −75 mV. We analyzed spontaneous IPSCs that were obtained from DYN-insensitive neurons and that had the amplitudes exceeding two times the noise level (i.e., typically 1–3 pA). The rise time of these spontaneous IPSCs was 1.7 ± 0.05 ms (n = 20). DYN13 (1 μM) diminished the frequency of the spontaneous IPSCs (Fig. 7C) without changing their mean amplitude (Fig. 7D) or the amplitude distribution (Fig. 7E). The decrease in frequency was fully reversible with washing (Fig. 7C) and was also completely blocked by nor-BNI (0.2 μM; n = 3, data not shown). These results and the iontophoretic GABA application experiment described above suggest that DYN13 suppresses presynaptic GABA release but not postsynaptic GABA sensitivity. To investigate this further, the following experiments were performed.

**FIG. 2.** Example of a DYN-sensitive GP neuron intracellularly stained with Neurobiotin. A: photomicrograph shows that the neuron has a medium-sized soma and thick, smooth dendrites without spines. The light staining of the blood vessels in the background was due to an efflux of Neurobiotin from the recording pipette tip prior to patching the cell. B: camera-lucida drawing of the neuron shows slowly tapered spine free dendrites extending 300–400 μm from the soma. C: the star in the drawing of the slice marks the location of the neuron in the GP. For all figures, top is dorsal and left is rostral.

**FIG. 3.** Effects of DYN13 on neostriatum (Str) stimulation induced IPSPs and inhibitory postsynaptic currents (IPSCs) in GP neurons. Recordings were made with high-Cl electrolyte containing pipettes. Electrical stimulation was applied through a bipolar electrode. To block glutamatergic responses, 6,7-dinitroquinoxaline-2,3-dione (DNQX; 50 μM) and 3-(2-carboxypiperizin-4-yl)-propyl-1-phosphonic acid (CPP; 10 μM) were added to artificial cerebrospinal fluid (ACSF). A: IPSPs recorded from a GP neuron that had a resting membrane potential of −72 mV. DYN13 reduced the amplitude of IPSPs. The effect was reversible on washing. DYN13 did not alter the resting membrane potential or the input resistance of this neuron. Bicuculline methiodide (50 μM) completely blocked the IPSPs indicating that the IPSPs were GABAA receptor mediated. B: IPSCs recorded from another GP neuron. This neuron was voltage clamped at −68 mV. Like the IPSPs, DYN13 reversibly reduced the amplitude of the IPSCs. C: a change of the IPSP amplitude occurred during the course of the experiment. DYN13 was applied for 10 min and the IPSPs were evoked at 1-min intervals during the experiment. Each point in the graph represents a normalized average amplitude ± SE (percentage of controls) of the IPSPs recorded from four DYN-insensitive neurons (i.e., DYN13 did not hyperpolarize these neurons). D: a dose-response curve of the DYN13 effect on evoked-IPSCs. Each point represents the mean ± SE of 3–15 experiments, and the value was expressed as a percentage of control amplitude. The effect is nearly maximal (i.e., about 85% decrease in IPSC amplitudes) with 5 μM DYN13. The dose-response curve was fitted with a Langmuir isotherm (see text for details). EC50 for the best fit of the curve is 0.68 μM.
Action potential–independent mIPSCs were recorded from GP neurons using the high-Cl electrolyte containing pipettes in the presence of TTX (1 μM), CPP (10 μM), and DNQX (50 μM). Under these conditions, the amplitudes of mIPSCs were as high as 150 pA, and the frequency was <1 Hz (Fig. 8, A and D). In five neurons, application of DYN13 (1 μM) resulted in an insignificant decrease (9%, P = 0.07, paired t-test) in the frequency with no change in the amplitude. Application of cadmium (200 μM) reduced the frequency of mIPSCs by approximately 60% without altering the amplitude. Thus the insignificant reduction of mIPSCs frequency by DYN13 might be explained if only the Ca-dependent portion of mIPSCs was modulated by DYN13. To depolarize synaptic terminals and increase the occurrence of Ca-dependent mIPSCs, the extracellular potassium concentration was raised to 20 mM by substituting a portion of NaCl with KCl in ACSF (Doze et al. 1995). The somatic membrane of the recording neurons was clamped at its resting membrane potential in control ACSF. Switching to the high-potassium ACSF did not change the amplitude but did increase the frequency of mIPSCs in all seven neurons tested (Fig. 8, A, B, and E). DYN13 (1 μM) decreased the frequency of mIPSCs in all these neurons (Fig. 8, A and F), without changing their mean amplitude (Fig. 8B) or amplitude distributions (Fig. 8C). Washing the slices with the high-potassium ACSF reversed the DYN13 effect (Fig. 8, A and B). The application of cadmium (200 μM) to the high-potassium ACSF diminished the frequency, but not the amplitude, of mIPSCs by approximately two-thirds below that measured in control ACSF (Fig. 8, A and B). DYN13 had no effect on the cadmium-insensitive mIPSPs (Fig. 8, A and B). These results suggest that DYN13 inhibits Ca-dependent, but not quantal, GABA release from terminals.

**DYN13 decreases the success probability of IPSCs evoked by Str stimulation**

To gain additional information about the presynaptic action of DYN13, the effect of DYN13 on the IPSCs with paired Str stimulation induced IPSCs with 2 clearly separable latencies in this neuron (see inset). Amplitudes of the IPSCs changed with the change in stimulus intensity. Plots indicate that Str stimulation induced IPSCs consist of multiple components. C: IPSCs recorded from another GP neuron. Str stimulation induced IPSCs with 2 clearly separable latencies in this neuron (see inset). Amplitudes of the IPSCs were measured as shown in inset. DYN13 (1 μM) diminished the amplitudes of both the short- and long-latency IPSCs. DYN13 was applied for 4.5 min, and IPSCs were evoked at 20-s intervals during the experiment. Each point in the graph represents a normalized (percentage of control) average amplitude ± SE of three IPSCs.

**FIG. 4.** Effects of DYN13 on Str stimulation induced short- and long-latency IPSCs. A: sample traces show IPSCs to Str stimulation with various intensities. Amplitudes and latencies of IPSCs changed with the change in stimulus intensity. Plots indicate that Str stimulation induced IPSCs consist of multiple components. C: IPSCs recorded from another GP neuron. Str stimulation induced IPSCs with 2 clearly separable latencies in this neuron (see inset). Amplitudes of the IPSCs were measured as shown in inset. DYN13 (1 μM) diminished the amplitudes of both the short- and long-latency IPSCs. DYN13 was applied for 4.5 min, and IPSCs were evoked at 20-s intervals during the experiment. Each point in the graph represents a normalized (percentage of control) average amplitude ± SE of three IPSCs.

**FIG. 5.** Kappa-receptor antagonists block the DYN13 effect on evoked-IPSCs. DYN13 (1 μM) reduced the amplitude of IPSCs to 39.7 ± 3.7% (n = 15) of the control. A nonselective opioid receptor antagonist naloxone (5 μM; n = 3, 101.3 ± 1.8% of the control) and the κ-selective antagonist nor-binaltorphimine dihydrochloride (nor-BNI; 0.2 μM; n = 6, 99.3 ± 7.3% of the control) completely antagonized the DYN13 effect. The δ-antagonist naltrindole (1 μM; n = 5, 34.2 ± 6.8% of the control) had no effect (P > 0.05, ANOVA). Data are expressed as means ± SE. Asterisks indicate P < 0.0005 using ANOVA.

**FIG. 6.** DYN13 failed to diminish the response to exogenous GABA. Two-barreled pipettes, one barrel contained GABA (0.1 M, pH 4.8) and the other saline, were placed about 30 μm from the recorded neuron. Currents with 6 nA in amplitude and 60 ms in duration were passed between the two barrels to ejection GABA. Iontophoretically applied GABA induced a large response in the GP neuron recorded with pipettes containing the high-Cl electrolyte. The response was sensitive to bicuculline methiodide (50 μM) but was unaltered by bath application of DYN13 (1 μM).
stimulation was examined. As mentioned above, Str stimulation with threshold intensity induced large IPSCs with a latency of approximately 4 ms in eight neurons. In five of the eight neurons, IPSCs with a constant latency and fairly constant amplitude could be evoked (Fig. 9A). The amplitude and the latency were unchanged over a wide range of stimulus intensities (e.g., 35–90 μA for the neuron shown in Fig. 9). In control ACSF, the success probability and the amplitude of the successfully evoked IPSCs to the first and second stimulus with an interstimulus interval more than 10 ms were very similar (Fig. 9, A, B, and D). Thus the probability ratio (i.e., success probability to the 2nd/1st stimulus) was approximately one (Fig. 9C). In the presence of DYN13 (1 μM), the success probability of evoking IPSCs to the first stimulus decreased significantly (Fig. 9B). The success probability to the second stimulus was higher than that to the first stimulus with an interstimulus interval of 10–60 ms. We chose the inter-stimulus interval of 25 ms in this study because it yielded the highest success probability to the second stimulus in three neurons tested with various inter-stimulus intervals. Thus the probability ratio was significantly higher in the presence of DYN13 than in control ACSF (Fig. 9B). DYN13 did not reduce the amplitude of successfully evoked IPSCs (Fig. 9D).

**DYN13 does not modulate presynaptic glutamatergic release or postsynaptic glutamate sensitivity**

Stimulation of the internal capsule evoked glutamatergic responses in GP neurons. DYN13 (1–2 μM) did not affect the EPSPs of 11 DYN-insensitive GP neurons. Three others that were DYN sensitive were hyperpolarized (5.3 ± 2.3 mV), and their input resistance was decreased (16.4 ± 6.4%). In these DYN-sensitive neurons, DYN13 decreased the amplitude of EPSPs by 39.9 ± 12.3%.

The effect of DYN13 on the postsynaptic glutamate receptors was studied using iontophoretic glutamate application with appropriate receptor blockers. The method of iontophoretic application of glutamate was similar to that used for GABA application. Both CPP-sensitive NMDA responses and DNQX-sensitive α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)/kainate responses evoked in DYN-insensitive neurons (n = 8) were unaffected by DYN13 (1 μM). These results suggest that DYN13 does not change the glutamate sensitivity of the postsynaptic membrane.

**DISCUSSION**

This is the first report of DYN13 effects on single GP neurons in slice preparations using the whole cell recording technique. The results indicate that DYN13 exerts both postsynaptic effects on GP neurons.

**Postsynaptic effects of DYN13**

DYN13 caused a hyperpolarization and a decrease in the input resistance in approximately one-quarter of the GP neurons. The hyperpolarization was thought to be due to an activation of κ-receptors because only the κ-receptor–selective antagonist, not δ- or μ-antagonists, blocked the response. The hyperpolarization was insensitive to TTX and was independent of the intracellular chloride concentration. The current-voltage relationship curves before and after DYN13 application crossed at approximately −85 mV, very close to the predicted potassium equilibrium potential of these neurons. These results suggested that the postsynaptic effect of DYN13 involved a potassium conductance. Direct pharmacological examination of the response was difficult because only a quarter of GP neurons were DYN sensitive and because it was difficult to maintain long recordings for repeated application of DYN13 to single neurons. However, we tested the effect of barium on neurons that were not tested for DYN sensitivity. The result that DYN13 hyperpolarized none of 22 barium-treated neurons suggested that the hyperpolarization was due to the activation...
of a barium-sensitive inwardly rectifying potassium conductance. Activation of the potassium conductance by \( \kappa \)-agonists has been reported in rat bulbothalamic neurons (Hayar and Guyenet 1998) and in guinea pig substantia gelatinsa neurons (Grudt and Williams 1993). It has been also shown that \( \kappa \)-receptors and inwardly rectifying potassium channels coexpressed by Xenopus oocytes formed functional couplings (Henry et al. 1995; Ikeda at al. 1995). The hyperpolarization of GP neurons by DYN13 was consistent with the results of a unit study in anesthetized rats showing that iontophoretically applied glutamate in some GP neurons (Huffman and Frey 1989).

All DYN13-sensitive GP neurons were medium-sized aspiny neurons, and all were capable of firing continuously without strong spike accommodation. These neurons are known to project to the subthalamic nucleus and the substantia nigra. Some of these neurons project to the Str as well (Bevan et al. 1998; Kita and Kitai 1994; Nambu and Linus 1997). It should be noted that DYN13 failed to hyperpolarize some aspiny neurons that shared very similar morphological and physiological properties with the DYN13-sensitive neurons.

**DYN13 effects on GABAergic synaptic transmission**

DYN13 greatly reduced the amplitude of postsynaptic GABAergic responses in all of the GP neurons tested. This effect was most likely mediated by \( \kappa \)-receptors because the \( \delta \)-or \( \mu \)-agonists did not. A recent report indicated that \( \mu \)-agonists had a similar effect in the GP (Stanford and Cooper 1999). We have confirmed Stanford and Cooper’s results that the \( \mu \)-agonist Tyr-d-Ala-Gly-(NMe) Phe-Gly-ol (DAMGO) reduced the amplitude of IPSCs and that the effect was antagonized by the \( \mu \)-agonist CTOP (1 \( \mu \)M) but not by the \( \kappa \)-antagonist nor-BNI (Ogura and Kita 1999). Thus it was unlikely in the present study that DYN13 and nor-BNI acted as a partial \( \mu \)-agonist and antagonist, respectively.

DYN13 failed to decrease the response to iontophoretically applied GABA. DYN13 decreased the frequency of both spontaneous IPSCs and high-potassium–induced IPSCs without changing the amplitude of either. In the paired-stimulation test, DYN13 decreased the success probability of evoking IPSCs but increased the probability ratio (i.e., probability of the 2nd/the 1st stimulation). These results suggest that DYN13 presynaptically modulates GABAergic transmission. The reduction of neurotransmitter release by \( \kappa \)-agonists has been reported in several brain areas including the glutamatergic mossy fibers in the hippocampus (Gannon and Terrian 1991; Wagner et al. 1993; Weisskopf et al. 1993), glutamatergic inputs to bulbothalamic neurons (Hayar and Guyenet 1998), dopaminergic fibers in the Str (Mulder et al. 1984), glutamate release in Str synaptosomes (Hill and Brotchie 1995), and the dorsal root ganglion in culture (Macdonald and Nelson 1978).

Multiple mechanisms may account for the DYN13 modulation of presynaptic GABA release in the GP. One mechanism may be to decrease spiking at the somata of DYN-sensitive GP neurons, hence reducing the number of the spikes arriving to the local-collateral terminals. Another mechanism may be due to the direct action of DYN13 on the synaptic terminals. DYN13 may hyperpolarize the synaptic terminals of DYN-sensitive GP neurons by activating an inwardly rectifying potassium current as it did in their somata. The hyperpolarization would indirectly reduce Ca-inflow to the terminals by enhancing the rate of spike repolarization, thereby reducing GABA release. Our preliminary results indicate that barium partially blocked the DYN13 effect on the evoked IPSCs (M. Ogura and H. Kita, unpublished data). These preliminary data

![Image](http://jn.physiology.org/)

*[FIG. 8. Effects of DYN13 on miniature IPSCs (mIPSCs). mIPSCs were recorded from GP neurons using pipettes containing the high-Cl electrolyte in the presence of TTX (1 \( \mu \)M), CPP (10 \( \mu \)M), and DNQX (50 \( \mu \)M). A and B: the frequency and the amplitude of mIPSCs was determined from 3 min of continuous recording from 7 neurons under each condition. An elevation of extracellular potassium from 6.24 to 20 mM increased the frequency of mIPSCs from 0.75 \( \pm \) 0.11 to 1.31 \( \pm \) 0.20 Hz. DYN13 (1 \( \mu \)M) significantly decreased the frequency to 0.67 \( \pm \) 0.09 Hz (\( P < 0.01 \), paired \( t \)-test). The effect was reversed by washing, and the frequency increased to 1.21 \( \pm \) 0.2 Hz. Application of 200 \( \mu \)M cadmium greatly decreased the frequency of mIPSCs to 0.37 \( \pm \) 0.07 Hz (\( P < 0.01 \), paired \( t \)-test). DYN13 failed to decrease the frequency (0.34 \( \pm \) 0.14 Hz) of the cadmium-independent mIPSCs. The amplitude of mIPSCs was not altered significantly during these treatments. The amplitude was as follows: 16.3 \( \pm \) 3.9 pA in the control; 16.8 \( \pm \) 2.9 pA in 20 mM potassium; 13.8 \( \pm \) 4.2 pA in DYN13; 14.8 \( \pm \) 2.2 pA in the wash; 12.1 \( \pm \) 3.6 pA in cadmium; and 13.5 \( \pm \) 2.9 pA in cadmium and DYN13. Error bars in A and B are SE. C: cumulative amplitude distributions of mIPSCs show no amplitude shift between the control, in 20 mM potassium, and during application of DYN13 (\( P > 0.05 \), Kolmogorov-Smirnov test). D–F: sample traces show mIPSCs in the control (D), in 20 mM potassium (E), and in DYN13 (F).]*
also suggested that multiple mechanisms may be involved in the presynaptic action of DYN13. Other possible mechanisms include an activation of a voltage-gated potassium current ( Muller et al. 1999; Simmons and Chavkin 1996; Vaughan et al. 1997) and an inhibition of the high-threshold voltage-gated calcium channels ( Kanemasa et al. 1995; Moises et al. 1994; Rusin et al. 1997; Simmons and Chavkin 1996; Vaughan et al. 1997). The result that DYN13 reduced the frequency but not the amplitude of the high-potassium–induced mIPSCs is consistent with the possibility that κ-agonists might directly reduce voltage-gated Ca-currents.

**Origins of GABAergic responses**

It has become increasingly clear that at least one-third of GP neurons project to the Str ( Bevan et al. 1998; Kita and Kitai 1994; Kita et al. 1999). Thus electrical stimulation of the Str might activate both striato-pallidal and pallido-striatal GABAergic fibers. The response observed in most GP neurons to low-intensity Str stimulation was a long-latency small IPSC or IPSC. A gradual increase in the stimulus intensity resulted in a large jump in amplitude and a shortening of the latency to approximately 4 ms. We consider that the long- and the short-latency responses were mediated by striato-pallidal axons and the local collateral axons of pallido-striatal neurons, respectively, for the following reasons: 1) the threshold current needed to stimulate the somata of Str projection neurons should be lower than that for the pallido-striatal axons, 2) the striato-pallidal axons have much slower conduction velocity than the pallido-striatal axons ( Kita 1994a; Nambu and Llinas 1994), 3) the striato-pallidal axons form synapses mainly on the dendrites of GP neurons ( Falls et al. 1983; Okoyama et al. 1987; Smith et al. 1998), 4) all the pallido-striatal neurons have intrinsic collateral axons that form synapses on the soma and proximal dendrites of GP neurons ( Bevan et al. 1998; Kita and Kitai 1991, 1994; Smith et al. 1998), and 5) the latency of the antidromic spikes recorded in the GP after Str stimulation ranges from 1–3 ms ( Kita and Kitai 1991; Nambu and Llinas 1994; Walker et al. 1989). This latency is consistent with the short-latency response obtained in this study. It is also likely that the spontaneous and TTX-independent mIPSCs originated from the local collateral axons of GP neurons. The spontaneous IPSCs that had a large amplitude and a short rise time might have been evoked by the synapses on the soma and proximal dendrites of the recorded neurons.

We have examined the effects of DYN13 mainly on the short-latency IPSCs because they were large in amplitude and very stable for long periods. The long-latency IPSCs could be examined with DYN13 in five neurons. The results of the present study suggest that DYN13 reduced GABA release from the terminals of both the striato-pallidal and local-collateral axons of GP projection neurons.

**Modulation of glutamatergic input by κ-agonist**

The GP receives the major glutamatergic input from the subthalamic nucleus ( Kita 1994a; Kita and Kitai 1991; Smith et al. 1998). The subthalamic input plays a significant role in maintaining the activity and in shaping the firing patterns of GP neurons. The possibility that the opioids released from Str terminals heterosynaptically modulate the subthalamic input was suggested by an observation of Huffman and Frey ( 1989). They showed that iontophoretically applied κ-agonists, DYN13 and benzomorphan, depressed spontaneous firing and discharges to iontophoretically applied glutamate in some GP neurons in anesthetized rats. The present results show that DYN13 does not modulate EPSPs or the response to the iontophoretically applied glutamate in DYN-insensitive neurons. DYN13 decreased the amplitude of EPSPs only in DYN-sensitive neurons. Thus it is likely that DYN13 reduces glutamatergic responses by decreasing the input resistance of neurons but not by presynaptic modulation.
Functional significance

Possible functional roles of the opioids contained in the Str spiny projection neurons have begun to be described. Jiang and North (1992) have reported a presynaptic inhibition of GABAergic transmission by the opioids in rat Str. They observed that the local stimulation-induced GABAergic response was reduced by δ-agonists but not by κ- and μ-agonists, and speculated that δ-agonists affected local collateral axons of the Str projection neurons. More recently, Stanford and Cooper (1999) showed that μ- and δ-agonists presynaptically inhibit GABAergic transmission in the GP. These authors considered 1) that μ-receptors are located on the terminals of striato-pallidal axons and local-collateral axons of spontaneously active GP neurons and 2) that δ-receptors are located on the local axon terminals of quiescent GP neurons. We report here that the κ-agonist DYN13 affects the GP in two different manners. One is a direct inhibitory action on approximately one-quarter of GP neurons by activating the inwardly rectifying potassium conductance, and the other is a presynaptic inhibition of the GABAergic terminals of the local collateral axons of GP projection neurons and the striato-pallidal axons.

In the GP, DYN is contained in the collaterals of striato-entopeduncular and striato-nigral axons. It can be speculated from the results obtained in the present study that DYN released in the GP by activation of the Str efferent pathways will have multiple effects on GP neurons. DYN may directly inhibit a small subpopulation of GP projection neurons by activating postsynaptic μ-receptors. In a larger subpopulation, DYN and another striatal opioid, enkephalin, may act to disinhibit GP neurons by presynaptically suppressing GABA release from both the striato-pallidal and the local collateral terminals. The results of Stanford and Cooper (1999) and the present experiment indicate that the suppression of GABA release by the striatal opioids is accompanied by a development of the paired pulse facilitation. Thus it can be further suggested that the striatal opioids might alter response characteristics of GP neurons to high-frequency burst GABAergic inputs.

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