Pre- and Postsynaptic Serotonergic Excitation of Globus Pallidus Neurons

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Rav-Acha M, Bergman H, Yarom Y. Pre- and postsynaptic serotonergic excitation of globus pallidus neurons. J Neurophysiol 100: 1053–1066, 2008. First published June 11, 2008; doi:10.1152/jn.00845.2007. The basal ganglia (BG) play a critical role in the pathogenesis and pathophysiology of Parkinson’s disease (PD). Recent studies indicate that serotonergic systems modulate BG activity and may be implicated in the pathophysiology and treatment of PD. The globus pallidus (GP), the rodent homologue of the primate GPe, is the main central nucleus of the basal ganglia, affecting the striatum, the subthalamic nucleus (STN), and BG output structures. We therefore studied the effect of serotonin (5-HT) and specific 5-HT agonists and antagonists on GP neurons from rat brain slices. Using intra- and extracellular recordings of GP neurons we found that serotonin increases the firing rate of GP neurons. Analyzing the effects of specific 5-HT agonists and antagonists on the firing rate of GP neurons showed that the increase in firing rate is due to the activation of 5-HT1A and 5-HT1B receptors. Intra-cellular recordings in both voltage- and current-clamp modes revealed that serotonin mediates its effect via pre- and postsynaptic mechanisms. The presynaptic effect is mediated by attenuation of γ-aminobutyric acid release, probably through activation of 5-HT1B receptors. Postsynaptically, serotonin activates a hyperpolarization-activated cation channel, probably via 5-HT1A receptors. Furthermore, serotonin decreases the fast synaptic depression characteristic of the striatal afferent input. The decreased serotonin concentrations in the BG nuclei in PD may contribute to depressed GP activity and enhance the emergence of BG pathological synchronous oscillations. We therefore suggest that future therapeutics of PD should be directed toward restoration of normal serotonin levels in BG nuclei.

INTRODUCTION

Globus pallidus (GP) activity plays a major role in basal ganglia (BG) function and has been tightly linked to limbic, cognitive, and motor integration leading to action selection and execution (Delong 1971; Kita 2007; Wichmann and Delong 1996). GP function is dominated by GABAergic activity that originates mainly at the striatum and is modulated by dopaminergic, acetylcholine, and serotonin (Chadha et al. 2000; Hikosaka 2007; Mink 1996; Parent et al. 1981; Querejata et al. 2005). Since γ-aminobutyric acid (GABA) is the main carrier of information in the BG network, its modulation by serotonin (5-HT) could indicate serotonin involvement in BG-related clinical disorders such as Parkinson’s disease (PD; Albim et al. 1989; Delong 1971; Obeso et al. 2000; Wichmann and DeLong 1996). Recent studies suggest that nondopaminergic BG modulatory systems, including the serotonergic system, are profoundly involved in PD (Brotchie 2005; Lang and Obeso 2004). This feature has also been crucial in the debate regarding the modulation of serotonin levels in PD therapeutic strategy. There are reports that core parkinsonian motor symptoms have been alleviated using serotonin or specific serotonergic receptor agonists (Bara-Jimenez et al. 2005; Bonuccelli and Del Dotto 2006; Mignon and Wolf 2007) and many studies have shown attenuation of levodopa-induced dyskinesias brought about by serotonin (Bonuccelli and Del Dotto 2006; Brotchie 2005; Nicholson and Brotchie 2002). By contrast, severe extrapyramidal symptoms have been reported in PD patients treated with serotonergic drugs, mainly antidepressants (Caley 1997; Gony et al. 2003). Thus both theory and practice would benefit from a comprehensive exploration of serotonin electrophysiology in the GP nucleus.

Anatomical and immunohistochemical studies have established a direct anatomical connection between the raphe nucleus and multiple basal ganglia nuclei, including the GP (Parent et al. 1981; Steinbusch 1981). The serotonergic effect on pallidal cells is mediated by various 5-HT receptors, including 5-HT1B (Sari et al. 1999, 2004), 5-HT1A (Kia et al. 1996; Pazos and Palacios 1985; Waerber and Moskowski 1995; Wright et al. 1995), and 5-HT4 (Coman et al. 1996; Vilaro et al. 2005). Contradictory reports on 5-HT3C and 5-HT7 receptors preclude a firm conclusion regarding their presence in the BG and particularly in the GP. There are some claims that 5-HT3C receptors exist in the GP (Appel et al. 1990; Kita et al. 2007); however, other studies indicate that these receptors exist in BG nuclei including striatum and substantia nigra pars reticulata but not in the GP (Coman et al. 1998; Eberle-Wang et al. 1997; Mengod et al. 1990). Contrary to previous data (Neumaier et al. 2001; To et al. 1995), recent studies using new tracers for 5-HT3 receptors have not confirmed their presence in rat GP (Bonaventure et al. 2002). However, there is a general consensus that the most prevalent BG serotonin receptors are the 5-HT1B receptors, located at the presynaptic striatopallidal GABAergic terminals (Sari 2004; Sari et al. 1999; Verge and Calas 2000). In fact, the GP contains the highest proportion of this receptor within the telencephalon (Querejata et al. 2005; Verge and Calas 2000). Lesions of the striatum reveal a marked decrease in 5-HT1B immunoreactivity in the ipsilateral GP, thus supporting their striatal origin (Sari et al. 1999; Schwartz and Coyle 1977). 5-HT4 receptors are located on the somata of striatal cells and also on axonal terminals of striatopallidal fibers (Coman et al. 1996; Vilaro et al. 2005). The subcellular distribution of 5-HT1A receptors in rat GP is still not known.

5-HT receptors are known to function as both auto- and heteroreceptors, affecting serotonergic axons and nonserotonergic structures, respectively (Sari et al. 1999, 2004). The
heteroreceptor function of 5-HT receptors is mediated by both presynaptic and postsynaptic effects. Presynaptic modulation of GABA release has been reported in many basal ganglia nuclei (Johnson et al. 1992; Stanford and Lacey 1996). Several lines of evidence suggest that presynaptic activation of the 5-HT1B receptor in the GP decreases GABA release. Direct measurement of H3-GABA release from GP slices shows a decrease after 5-HT1B activation (Chadha et al. 2000). An increase in GP firing rate was measured after intrapallidal injection of serotonin, 5-HT1B agonist (Querejeta et al. 2005), or after intravenous injection of fluoxetine, a serotonin reuptake inhibitor, in anesthetized rats (Bergstrom and Walters 1981). Finally, in vivo intrapallidal injection of 5-HT1B receptor agonist was shown to reverse akinesia caused by striatopallidal GABAergic overactivity in parkinsonian states (Chadha et al. 2000). Thus the presynaptic effect of the 5-HT1B receptor on GP neurons is well established, although it has not been directly demonstrated by biophysical methods.

A postsynaptic serotoninergic impact on neuronal excitability has also been suggested (Bickmeyer et al. 2002; Iwahori et al. 2002; Jiang et al. 2000; Kita et al. 2007; Villalobos et al. 2005). Most studies show regulation of postsynaptic h-current (Ih) by serotonin (Bickmeyer et al. 2002; Cardenas et al. 1999; Iwahori et al. 2002), although others have pointed to postsynaptic regulation of other channels as well (Bickmeyer et al. 2002; Jiang et al. 2000; Villalobos et al. 2005). A direct serotoninergic postsynaptic effect on the GP was suggested only recently (Chen et al. 2008; Kita et al. 2007).

To better circumscribe the effect of serotonin and its possible involvement in PD, we studied the effect of serotonin on GP neurons in vitro. We used whole cell recordings of pallidal activity in GP brain slices that enable bath application of serotonin as well as electrical stimulation of the striatum. Thus the postsynaptic effect could be directly measured, whereas the presynaptic effect could be deduced from the responses to striatal stimulation. Our results indicate that serotonin excites GP cells both pre- and postsynaptically. Presynaptically, serotonin attenuates the release of GABA from presynaptic terminals, whereas postsynaptically it depolarizes the GP cell membrane potential and decreases its input resistance, probably by activation of Ih.

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Slice preparation

Rats (10–16 days old) were anesthetized with ether and then decapitated. The brains were rapidly removed and placed in ice-cold Ringer solution (composition in mM: 124 NaCl, 5 KCl, 1.3 MgSO4, 1.2 KH2PO4, 26 NaHCO3, 10 glucose, 2.4 CaCl2, saturated with 95% O2-5% CO2, pH 7.4 at room temperature). A block containing most of the striatum and GP was isolated and 300-μM parasagittal slices were cut using a microslicer (VT-1000, Leica, Leitz, Germany). The slices were then incubated with oxygenated Ringer solution and maintained at 30°C. After 1–2 h of incubation the slices were transferred to a recording chamber and maintained at 35°C under continuous superfusion with the oxygenated Ringer solution. Housing and surgical procedures were in accordance with the Hebrew University Institutional Animal Care and Use committee guidelines.

Intra- and extracellular recordings

Intracellular whole cell patch-clamp recordings were obtained using glass pipettes (10–12 MΩ) pulled on a Narishige pp-83 puller filled with an intracellular solution (composition in mM: 140 K-glucuronate, 4.0 NaCl, 0.5 CaCl2, 5.0 EGTA, 3.0 Mg-ATP, 10 HEPES, and adjusted to pH 7.3 with KOH). Neurons were visualized using differential interference contrast infrared microscopy (Zeiss, Oberkochen, Germany; Hamamatsu c-2400, Shizuoka, Japan). GP cells were selected on the basis of their location, soma size, and shape (Rav-Acha et al. 2005). Membrane current and voltage were recorded using Axopatch 1D or Axoclamp 2A amplifiers (Axon Instruments, Foster City, CA), in the conventional bridge voltage-clamp or current-clamp mode, respectively.

A bipolar stimulating electrode driven by 0.1-ms pulse (Master 8, AMPI, Jerusalem, Israel; isolation unit, Mk4 Devices, London, UK) was used to stimulate the presynaptic axons. The stimulating electrode was advanced under visual control and placed in the center of the striatum. To facilitate striatal stimulation we used parasagittal slices oriented parallel to the striatopallidal fibers (Rav-Acha et al. 2005).

For extracellular recording, the electrodes were filled with the above-described Ringer solution and the signals were recorded in current-clamp mode. Cells were identified as with the intracellular recordings and the electrodes were placed in close contact with the cell membrane without forming a seal. Stable recordings were characterized by a regular firing frequency and spike pattern for ≥10 min prior to initiating the experiment.

The data were sampled at a rate of 10 kHz with a National Instrument board driven by LabVIEW (National Instruments, Austin, TX) based homemade software that was used for on- and off-line analysis.

Drugs

5-Hydroxytryptamine (5-HT; serotonin creatinine-sulfate complex; Sigma, Poole, UK) was added to the physiological Ringer solution (20–50 μM final bath concentrations). We recorded the effects of serotonin 20 min after its addition to the experimental bath. Complete recovery was obtained only after a prolonged wash period (~80 min). 7-Trimfluoromethyl-4-(4-methyl-1-piperazinyl)pyrrolo-[1,2-a]quinoxaline maleate (CGS-12066A, Sigma), a specific 5-HT1B receptor agonist (10 μM), and N-[2-[(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-(2-pyridyl)cyclhexanecarboxamide trihydrochloride (Way-100635, Sigma), a specific 5-HT1A receptor antagonist (1–5 μM), were used to explore the specific effects of these receptors on GP cells. 4-(N-Ethyl-N-phenylamino-1,2-dimethyl-6-methylamino)pyrimidinium chloride (ZD 7288, 10–100 μM), a specific h-current blocker, was used to study the h-current contribution to GP cell properties. Gabazine (10 μM) was used to block GABA receptors. All drug effects were examined 20 min after their addition to the experimental bath.

Data analysis

The current–voltage (I–V) relations were measured by averaging the voltage response to hyperpolarized and depolarized step currents. Average postsynaptic potentials evoked by striatal stimulation, recorded via current-clamp mode, were measured at a membrane potential of ~80 mV. The spontaneous synaptic currents, recorded via voltage-clamp mode, were detected and segregated from “noise” by an amplitude threshold of 0.02 nA and a half-amplitude duration threshold of 10 ms. The frequency dependence of the synaptic currents was estimated by averaging the synaptic response of GP cells to striatal train stimuli at various intratrain frequencies.

Analysis of extracellular recordings, which included calculating the mean firing frequency, the interspike interval (ISI) histograms, and the autocorrelation functions, was carried out on Matlab programs (Matlab V7.2, MathWorks, Natick, MA). The changes in firing frequency were described by their percentile change relative to the firing frequency in the control condition. The rhythmicity index (RI) was used to quantify the rhythmicity of the firing (Long et al. 2004). The index was calculated from the power spectrum density (using the P Welch
algorithm) of the spike trains. The integral under the peak of the characteristic frequency was calculated using different integration windows (with widths ranging from 0.5 to 5 Hz) and normalized by the total power [from 0 to 1/(minimal ISI Hz)]. Thus a pure sinusoidal process will have a value of RI = 1, whereas a purely random process will have a value of RI very close to zero.

Statistics

Numerical data in the text are expressed as the means ± SD. The statistical significance of data comparing the results of two groups of identical populations of GP cells was done using paired two-tailed t-test. When more than two groups were tested, we used a two-factor ANOVA without replication to test for differences between groups.

RESULTS

Response of GP neurons to serotonin

The effect of serotonin on GP firing pattern was first analyzed by monitoring the extracellular expression of neuronal activity. Single-unit activity was characterized by a biphasic waveform lasting for 3.5 ± 0.8 ms and dominated by the positive phase that reached average amplitude of 3.8 ± 1.8 mV. All extracellular GP recordings (n = 25) revealed regular spontaneous firing at a frequency of 2–20 Hz (6.5 ± 4.5 Hz). The ISI displayed a narrow distribution and the RI was 0.61 ± 0.12 (n = 25) (Fig. 1).

As seen in Fig. 1, serotonin (30 μM) reversibly increased the spontaneous firing frequency of GP neurons. Under control conditions a regular firing pattern was observed (Fig. 1A1). This regularity was expressed as a relatively narrow histogram of the ISI (Fig. 1A2) and pronounced consecutive peaks in the autocorrelogram (Fig. 1A3). The addition of serotonin increased the firing frequency from 19 to 27 Hz (Fig. 1B). Further examination of the ISI histogram (Fig. 1B2) and the autocorrelation (Fig. 1B3) revealed that the regularity of firing was unaffected. The significant recovery after washing (Fig. 1C) indicates that the increase in firing rate induced by serotonin was completely reversible. Despite the large variations in the spontaneous firing rate (2–20 Hz), the average firing frequency after addition of 30 μM serotonin increased significantly by 72 ± 27% (from 6.5 ± 4.5 to 10.8 ± 5.4 Hz, n = 25, P < 0.01, t-test), whereas the firing regularity was unaffected (RI = 0.61 ± 0.12 in the control condition vs. RI = 0.62 ± 0.07 after adding serotonin; n = 25).

The dose–response curve of the serotonin effect was examined in 10 cells. An example is shown in Fig. 2A, where the firing rate gradually increased as a function of the serotonin concentration reaching saturation at a frequency of 10 Hz. The normalized average firing response of 10 cells is shown in Fig. 2B, which shows that 90% saturation was reached at a concentration of 20 μM. Thus the serotonin concentration (30 μM) used in most of our experiments was well above the saturation of the GP cell response to serotonin.

Serotonin extracts its effects through several types of receptors including 5-HT1A and 5-HT1B, which are known to exist in the GP nucleus. We therefore examined the involvement of these two receptor subtypes in the response to serotonin. We used Way-100635, a specific 5-HT1A receptor antagonist, and measured its effect on the response to serotonin. WAY-100635 (5 μM) decreased the firing frequency of GP neurons as well as their response to serotonin (Fig. 3). Way-100635 by itself decreased the firing frequency by 12 ± 5.5% (from 6.2 ± 1.7 to 5.5 ± 1.85 Hz), whereas serotonin (30 μM) in the presence of Way-100635 (5 μM) increased the firing frequency by 19 ± 8.4% (from 6.2 ± 1.7 to 7.7 ± 1.6 Hz; n = 8). Serotonin itself in the absence of WAY-100635 increased the firing frequency by 67 ± 20% (from 6.2 ± 1.7 to 10.5 ± 1.9 Hz; n = 8). The effect of serotonin per se was significantly greater than the effect of serotonin in the presence of Way-100635 (n = 8, P < 0.05; ANOVA).

The dose–response curve of the serotonin effect in the presence of Way-100635 (Fig. 2, C and D) illustrates the noncompetitive blockade of Way-100635. In the example shown in Fig. 2C the firing rate induced by serotonin in the presence of 1 μM WAY-100635 increased from 4.5 to 6 Hz, whereas a much larger increase was measured with serotonin alone (from 5.5 to 10 Hz). Since both effects reached saturation at 30 μM serotonin, we conclude that in this case WAY-100635 is a noncompetitive blocker of serotonin. This conclusion was supported by the average responses measured from four cells (Fig. 2D), demonstrating that the saturated firing rate induced by serotonin decreased by 60% in the presence of 1 μM WAY-100635, independent of serotonin’s concentration. The similarity between the effect of 5 μM WAY-100635 used earlier in eight cells and 1 μM WAY-100635 used in the dose–response experiments indicates that 1 μM is a supersaturated concentration that blocks the 5-HT1A receptor in a noncompetitive manner.

Serotonin 1B receptors (5-HT1B) are densely expressed over the striatopallidal terminals and were shown to regulate presynaptic GABA release (Chadha et al. 2000; Querejeta et al. 2005; Sari et al. 1999). Therefore we examined the effects of CGS-12066A, a specific 5-HT1B agonist, on the spontaneous spike trains of GP neurons. CGS-12066A reversibly increased GP firing rate; however, its effect was smaller than that induced by serotonin (Fig. 3). On average, CGS-12066A (10 μM) increased the firing frequency of GP neurons by 22 ± 11% (from 6.2 ± 3.1 to 8.1 ± 3.7 Hz, n = 5, P < 0.05, t-test), whereas in these cells, the application of serotonin (30 μM) alone increased firing frequency by 77 ± 32% (from 6.2 ± 3.1 to 11 ± 4 Hz; n = 5, P < 0.05, t-test). The firing increase induced by serotonin was significantly larger than that induced by 5-HT1B agonist (n = 5, P < 0.05, ANOVA).

Since 5-HT1B receptors in the GP are located on the GABAergic terminals and are likely to mediate their effect by modulating the release of GABA (Sari 2004; Sari et al. 1999), we examined how blocking GABA_A receptors (by gabazine) would affect the response of GP neurons to serotonin. Recall that the GABAergic receptors on GP cells consist of both GABA_A and GABA_B receptors (Kaneda and Kita 2005; Kita 1992; Kita et al. 2005; Stanford and Cooper 1999). However, the activation of GABA_B receptors was shown to be induced by GP GABAergic collaterals and not by striatopallidal afferents (Kita et al. 2005). Thus 5-HT1B receptors located on striatopallidal terminals (Sari 2004; Verge and Calas 2000) should mainly induce GABA_A-mediated responses. Gabazine (10 μM) increased the firing frequency of GP cells by 21 ± 10% (Fig. 3; from 5.6 ± 2.7 to 7.2 ± 3.1 Hz; n = 7, P < 0.05, t-test), whereas serotonin (30–40 μM) in the presence of gabazine further increased firing frequency by 29 ± 7.6% (from 7.2 ± 3.1 to 9.5 ± 3.6 Hz; n = 7, P < 0.05, ANOVA). It is thus likely that serotonin also has an excitatory postsyn-
aptic effect. The addition of 5-HT$_{1A}$ blocker to serotonin in the presence of gabazine practically restored the original firing rate of the GP cells. The control firing frequency of 5 ± 1.8 Hz increased to 8.2 ± 3.2 Hz after the addition of gabazine + serotonin, and decreased to virtually the control value of 5.3 ± 2.1 Hz in the presence of gabazine + serotonin + 5-HT$_{1A}$.

![FIG. 1. Serotonin (5-HT) excites globus pallidus (GP) cells in rat brain slices. Spike trains (column 1), interspike interval (ISI) histograms (column 2), and autocorrelograms (column 3) of GP firing patterns, respectively. A, B, C: control condition, addition of 30 μM 5-HT, and washout of 5-HT, respectively. GP cell’s activity is characterized by regular repetitive firing, as evidenced by narrow ISI histogram and the periodic autocorrelogram. 5-HT reversibly increases GP cell’s firing rate.](image-url)
antagonist \((n = 3)\). Thus we suggest that 5-HT\(_{1A}\) receptors are involved in serotonin’s postsynaptic effect.

A summary of the effects of serotonin and the various drugs on GP activity, measured via extracellular recording, appears in Fig. 3. The effects of the various drugs are described as their relative effect compared with control condition. The effects of serotonin in the presence of 5-HT\(_{1A}\) blocker, the effect of 5-HT\(_{1B}\) agonist, and the effect of gabazine are comparable. Thus this figure demonstrates that 5-HT\(_{1A}\) and 5-HT\(_{1B}\) activation can account for most of the effects of serotonin on GP cells.

Considering the fact that in our GP slices the subthalamic nucleus (STN)–GP connection was severed—and therefore the main afferent input to GP cells consisted of GABAergic striatopallidal afferents and pallidopallidal axonal collaterals—our extracellular recordings suggest that serotonin has both pre- and postsynaptic excitatory influences on GP cells. In reaching this conclusion, which is in line with previous studies, we assume that the glutamatergic STN input does not exist in our slice preparation. Thus the fact that serotonin excites GP neurons even in the presence of gabazine mandates a postsynaptic effect. The presynaptic effect of serotonin is suggested by the observation that gabazine reduces the effect of serotonin. It is reasonable to assume that this presynaptic effect is mediated by the 5-HT\(_{1B}\) receptors and that the activation of 5-HT\(_{1A}\) receptors is involved in the postsynaptic effect. To unravel the biophysical mechanism that underlies serotonin’s effect and to examine its putative combined pre- and postsynaptic source, we analyzed the changes in the membrane properties of GP cells by using whole cell patch-clamp recordings from pallidal neurons.

**Intracellular recordings from GP neurons**

Stable intracellular recordings were obtained from 50 pallidal neurons. We used the same criteria as those of a previous study.
work (Rav-Acha et al. 2005). Accordingly, pallidal neurons that were included in this study had a resting potential more negative than $-45 \text{ mV}$, a spike amplitude $>50 \text{ mV}$, and presented stable recording for $\geq 40 \text{ min}$. The average quiescent membrane potential and input resistance was $-56 \pm 6.4 \text{ mV}$ and $524 \pm 156 \text{ M} \Omega$, respectively ($n = 50$). The mean threshold was $-46 \pm 5 \text{ mV}$ and spike amplitude $63 \pm 8.7 \text{ mV}$. Similar to the extracellular recordings, most (75%) of these cells exhibited regular spontaneous rhythmic firing at 2–20 Hz ($8.4 \pm 4 \text{ Hz}$). The cells were characterized by time- and voltage-gated anomalous rectification followed by a rebound potential in response to hyperpolarizing step currents.

**Postsynaptic effect of serotonin: reduction in input resistance and increased excitability**

Serotonin at a concentration of $20–50 \mu \text{M} (30 \pm 10 \mu \text{M})$ depolarized the membrane potential and increased the membrane conductance. As shown in Fig. 4, under normal conditions (Fig. 4A) the membrane voltage partially repolarized during negative current injection (Fig. 4A, single arrow). This response, known as “sagging,” reflects the activation of an $h$-like current. This type of current, which is carried by sodium and potassium ions, is activated by hyperpolarization beyond $-50$ to $-70 \text{ mV}$. The activation of this current slowly depolarizes the cell toward an equilibrium potential of approximately $-30 \text{ mV}$ (Pape 1996). In fact, adding Cs ions or ZD 7288 to the extracellular solution completely and reversibly blocked this response (data not shown). As expected from the slow kinetics of the $h$-current, the hyperpolarizing response was followed by rebound depolarization (Fig. 4A, double arrow). The addition of serotonin (30 $\mu \text{M}$) depolarized the membrane potential from $-65$ to $-59 \text{ mV}$ and decreased the input resistance from 700 to 290 $\text{M} \Omega$ (Fig. 4B). An average depolarization of $10.3 \pm 5.1 \text{ mV}$ from $-58.9 \pm 6.4$ to $-48.6 \pm 5.3 \text{ mV}$ and a $43 \pm 15.8\%$ decrease in input resistance from $544 \pm 173$ to $312 \pm 147 \text{ M} \Omega$ were calculated across the population of cells ($n = 35$, $P < 0.01$, $t$-test). Interestingly, serotonin also attenuated the sagging response to hyperpolarized currents. As shown in Fig. 4C these effects were mostly reversible. The decrease in input resistance was further analyzed by comparing the $I$–$V$ curves before and after addition of serotonin (Fig. 4, D and E, respectively). The significant decrease in the slope of the $I$–$V$ curve after addition of serotonin corroborated the reduction in input resistance. It is interesting to note that the two curves intersect at $-35 \text{ mV}$, close to the $h$-current reversal potential (Fig. 4F) (Pape 1996). An average intersection point of $-32 \pm 4.7 \text{ mV}$ was calculated for 30 cells. The attenuated sagging in the response to hyperpolarizing step currents and the comparable reversal potential suggest that serotonin either blocks or continuously activates the $h$-current. Thereafter, the decreased input resistance induced by serotonin suggests continuous $I_h$ activation.

Adding ZD 7288, a specific $h$-current blocker, to the experimental bath in the presence of serotonin restored the input resistance and the membrane potential. As shown in Fig. 5, A and B, adding serotonin (30 $\mu \text{M}$) decreased the input resistance from 900 to 420 $\text{M} \Omega$, whereas adding ZD 7288 (70 $\mu \text{M}$; Fig. 5C) increased the input resistance to 1,000 $\text{M} \Omega$. The resting membrane potential, $-60 \text{ mV}$, was depolarized by serotonin to $-51 \text{ mV}$ and hyperpolarized to $-65 \text{ mV}$ after the addition of ZD 7288. On the population level we analyzed nine cells where the addition of serotonin was followed by the blockade of $I_h$ with ZD 7288 (10–100 $\mu \text{M}$). In these experiments serotonin (30 $\mu \text{M}$) depolarized the cells from $-61 \pm 4.8$ to $-51 \pm 2.9 \text{ mV}$ and decreased the input resistance from 610 $\pm 160$ to 344 $\pm 80 \text{ M} \Omega$. Under these conditions ZD 7288 hyperpolarized the cells to $-65 \pm 6.2 \text{ mV}$ and increased the input resistance to 684 $\pm 182 \text{ M} \Omega$ ($n = 9$, $P < 0.05$, ANOVA). Finally, the effects of serotonin were prevented by previously blocking the $h$-current. This is shown for another GP cell in Fig. 5, D–F where ZD 7288 increased the input resistance from 420 to 570 $\text{M} \Omega$ (Fig. 5, D and E), whereas the addition of serotonin after ZD 7288 had only a slight effect (Fig. 5F). Similar effects were observed in three cells where ZD 7288 (50 $\mu \text{M}$) increased the input resistance from 410 $\pm 32$ to 490 $\pm 45 \text{ M} \Omega$, whereas the addition of serotonin after ZD 7288 decreased the input resistance to 479 $\pm 37 \text{ M} \Omega$ (nonsignificant effect). Thus we suggest that the postsynaptic effect of serotonin on GP cells is mediated...
by activation of $I_h$, which has a major influence on GP activity (Chan et al. 2004).

**Presynaptic effect of serotonin: decrease in evoked inhibitory synaptic potential**

The spontaneous and evoked GABAergic synaptic potentials recorded from GP neurons were used to characterize the presynaptic effect of serotonin. Stimulating the striatal area evoked an inhibitory postsynaptic potential (IPSP) in GP neurons. These synaptic events appeared after a delay of 5.75 ± 1.18 ms, had a mean duration at half-amplitude of 38 ± 13 ms, and their average reversal potential was $-63 \pm 6.3$ mV (Fig. 6, A and C; $n = 12$). The latter is consistent with the expected reversal potential of the GABA$_A$ receptors (Rav-Acha et al. 2005). Serotonin ($30 \mu$M) decreased the amplitude of the PSP by 78% (from 9.5 to 2 mV) and shortened the duration at half-amplitude by 26% (30 to 22 ms) (Fig. 6B). The reversal potential of the evoked PSP was unaffected by serotonin ($-63$ mV; Fig. 6, compare A with B). The average reduction of the PSP amplitude caused by serotonin ($30 \mu$M) was $54 \pm 16\%$ (from $8.6 \pm 2.6$ to $4.1 \pm 1.8$ mV) and the duration at half-amplitude was shortened by $21 \pm 11\%$ (from $38 \pm 13$ to $30 \pm 11$ ms), whereas the average reversal potential was $-63 \pm 6.3$ mV and was unaffected by serotonin ($n = 12$, $P < 0.05$, $t$-test).

The decrease in the amplitude of the PSP without a change in the reversal potential might be due to the decrease in input resistance, rather than a presynaptic effect. In this case, the reduction in PSP amplitude should be equal to the input resistance change induced by serotonin. In the case presented in Fig. 6, we quantified this difference by plotting the PSP amplitude as a function of the membrane potential before (Fig. 6C, black circles) and after (Fig. 6C, red circles) the addition of serotonin. We then calculated the input resistance change induced by serotonin at each of the membrane potentials and estimated the expected change in PSP amplitude, assuming that it was solely due to the change in input resistance. The blue curve in Fig. 6C shows the results of this calculation. The PSP amplitude in the presence of serotonin was smaller than the calculated values of the input resistance change. This difference shows that the postsynaptic effect of serotonin cannot fully account for the reduction in PSP amplitude and points to an additional presynaptic effect. When averaging the result in
serotonin decreased the input resistance by 38 ± 13%, whereas the average decrease in PSP amplitude induced by serotonin was 54 ± 16% (n = 12, P < 0.05, t-test).

We further investigated this possibility by directly measuring the synaptic current, via voltage-clamp mode, which under some circumstances is independent of the membrane resistance. The superimposed traces in Fig. 6D show the synaptic currents before (black trace) and after (red trace) the addition of serotonin (30 μM). Serotonin decreased the synaptic current amplitude by 45%, further supporting direct inhibition of GABA release. An average reduction of the synaptic current amplitude by 49 ± 13% (from 0.11 ± 0.04 to 0.058 ± 0.018 nA) was calculated across a population of GP cells (n = 13, P < 0.05, t-test). Finally, we restored the input resistance by blocking the h-current (see preceding text). This was done to block the main postsynaptic effect of serotonin, leaving the presynaptic mechanism intact. Interestingly, in a few experiments where ZD 7288 (100 μM) was added in the presence of serotonin, a further decrease in the evoked synaptic current was observed (Fig. 6D, green trace). This unexpected result confirms that the decrease in PSP amplitude caused by serotonin is independent of its effect on input resistance and raises the possibility that a presynaptic h-current is involved in GABAergic release.

**Serotonin decreases the spontaneous inhibitory synaptic activity**

The spontaneous synaptic events recorded in vitro in GP neurons are mostly GABAergic, originating either from striatal terminals or GP collaterals. To further examine the presynaptic mechanism of serotonin, we studied its effect on the spontaneous synaptic activity of GP cells. An example of this effect is shown in Fig. 7. The spontaneous synaptic activity in this example had an average amplitude of 0.038 ± 0.013 nA and a frequency of 14.3 ± 5.3 Hz (Fig. 7A), whereas the activity after serotonin application (30 μM) was characterized by an amplitude of 0.029 ± 0.01 nA and a frequency of 8.3 ± 3.2 Hz (Fig. 7B; P < 0.05). The distributions of amplitudes and frequencies of spontaneous synaptic currents in this example are shown in...
Averaging the results of six cells revealed spontaneous synaptic activity with an amplitude and frequency of 0.045 \pm 0.016 nA and 16.5 \pm 6.4 Hz, respectively. Serotonin (30 \mu M) decreased the synaptic amplitude and frequency to 0.032 \pm 0.014 nA and 10.6 \pm 4.4 Hz, respectively (n = 6, P < 0.05, t-test).

Presynaptic effect: decrease in synaptic depression

In our previous work we showed that GP cells are characterized by a frequency-dependent decline of the striatal evoked synaptic response (Rav-Acha et al. 2005). This phenomenon, known as synaptic depression, characterizes many other CNS nuclei (Abbott et al. 1997; O’Donovan and Rinzel 1997). The mechanism underlying depression is a frequency-dependent decrease of transmitter release (Abbott et al. 1997). Since our results indicate that serotonin has a significant presynaptic effect on GABAergic synapses, it could affect the process of synaptic depression. Figure 8 shows the frequency-dependent depression of striatal evoked postsynaptic currents (PSCs). The striatopallidal pathway was stimulated by train stimuli at different frequencies in normal condition (Fig. 8A) and in the presence of 30 \mu M serotonin (Fig. 8B). The PSC amplitude progressively decreased, reaching steady state after 15–20 stimuli (Fig. 8C). This steady-state level decreased as the intratrain frequency increased. Although a similar depression along the train stimuli was observed when serotonin was presented, the relative depression was smaller. Thus in the
control condition during 10-Hz striatal stimulation, the steady-state PSC amplitude decreased to 0.2 of its initial value (Fig. 8C, closed circles), whereas under serotonin it decreased to 0.29 of its initial value (Fig. 8C, open circles). Averaging the results from seven cells revealed that in the control condition during striatal intratrain stimuli of 8 and 20 Hz, the steady-state PSC amplitude decreased to 0.39 ± 0.14 and 0.21 ± 0.06 of its initial value, respectively (Fig. 8D, closed circles). In the presence of serotonin (30 μM), the steady-state PSC amplitude decreased to 0.63 ± 0.17 and 0.47 ± 0.13 of its initial value at the corresponding frequencies of stimulation (Fig. 8D, open circles). These differences induced by serotonin were statistically significant (n = 7, P < 0.05, t-test).

**DISCUSSION**

Although there are various anatomical studies dealing with serotonin in the GP, the functional role of serotonin in this nucleus has never been defined and the physiological data are still inconsistent. Studies of urethane-anesthetized rats (Perkins and Stone 1983) showed that the majority of spontaneously active GP cells were not affected by iontophoretically applied serotonin. Furthermore, 83% of the regularly firing GP cells did not respond to dorsal raphe nucleus stimulation. By contrast, intrapallidal infusion of serotonin (Querejeta et al. 2005) increased the firing of 75% of the pallidal cells of anesthetized rats, decreased the firing in 12.5%, and had no effect in another 12.5%. A different distribution of the responding cells was obtained with intravenous injection of serotonin uptake inhibitor, where 43% of the cells increased their firing rate and another 43% were unaffected (Bergstrom and Walters 1981). A recent extracellular in vivo study of awake monkeys showed an almost equal distribution of increased and decreased firing of GPe (external segment of the globus pallidus) cells induced by local application of serotonin, with no change in mean firing.
rate of the total GPe group (Kita et al. 2007). These results were used to suggest that the suppression of GABAergic release from striatal–GP terminals on one hand and suppression of excitatory glutamatergic release from STN–GP terminals on the other are the source of the inconsistency of serotonin’s effect (Kita et al. 2007). The need for further examination of the precise electrophysiological effects of serotonin on GP cells prompted the current study.

The major findings in this work can be summarized as follows: 1) Serotonin significantly increases the spontaneous firing rate of GP neurons in a slice preparation preserving striatal–GP innervation. 2) The increase in firing rate is due to combined pre- and postsynaptic effects. 3) The postsynaptic effect, which is probably mediated by 5-HT1A receptors, apparently activates the $I_h$ current, thereby depolarizing the neurons and increasing their spontaneous firing rate. 4) The presynaptic effect, which is probably mediated by 5-HT1B receptors, decreases the inhibitory striatopallidal transmission, thereby increasing the spontaneous firing of GP neurons. 5) The reduction of striatopallidal synaptic release leads eventually to a reduction in the process of synaptic depression of this pathway.

These results are basically consistent with a recent publication by Chen et al. (2008), reporting that serotonin has excitatory pre- and postsynaptic effects on GP cells. According to this study the presynaptic effect, which is mediated by 5-HT1B receptors, results from a reduction in the frequency of miniature inhibitory synaptic potentials accompanied by an increased rate of tetrodotoxin (TTX)-sensitive synaptic events. The postsynaptic effect is mediated by 5-HT7 and 5-HT4 receptors. They argue that 5-HT4 receptors activate nonselective cation conductance, whereas the 5-HT7 receptors activate the $I_h$ current. Although we cannot rule out the involvement of these receptors, our results suggest that the 5-HT1A receptors can account for most of the serotonin-driven postsynaptic excitation of GP cells. Notably, some of the above-cited differences could result from the immature rat brain slices used in our study.

![FIG. 8. 5-HT attenuates synaptic depression of GP GABAergic synapse. A and B: voltage-clamp recording of PSC during striatal train stimuli of different frequencies under control condition (A) and in the presence of 5-HT (30 mM; B). C: normalized PSC amplitude (control, closed circle; 5-HT, open circle) as a function of stimulus number within each train of stimuli. Steady-state PSC amplitudes are reached after about 20 stimuli under both control and 5-HT conditions. Note that in the control condition the relative steady-state PSC amplitude was lower than that reached in the presence of 5-HT (dotted lines). D: average ($n = 7$) steady-state PSC amplitude, normalized to the initial PSC amplitude, as a function of intraintrain frequency in control condition (closed circles) and in the presence of 5-HT (open circles).](image-url)
Serotonin was found to have both pre- and postsynaptic effects in various CNS nuclei (Bickmeyer et al. 2002; Iwahori et al. 2002; Jiang et al. 2000; Johnson et al. 1992; Stanford and Lacey 1996; Villalobos et al. 2005). Several studies denoted the prominent role of 5-HT1A receptors that are located on the presynaptic GABAergic terminals and exert their excitatory effect on GP cells by decreasing the release of GABA (Chadha et al. 2000; Querejeta et al. 2005; Sari 2004). Recently, a further suppression of presynaptic release of excitatory glutamatergic input by 5-HT1A receptors was suggested (Kita et al. 2007). However, due to the severing of the STN–GP connection in our slice preparation we could not evaluate the serotonergic influence on the glutamatergic excitatory input.

Contrary to the well-established data regarding presynaptic attenuation of GABAergic release, the possibility of a postsynaptic serotonergic effect on GP cells was suggested only recently from an in vivo study of awake monkeys (Kita et al. 2007). Kita et al. (2007) suggested a postsynaptic effect via 5-HT1A receptors; however, they report an inhibitory effect rather than an excitatory one as found in our study.

A serotonergic effect mediated by the Ih current has been described in other brain structures (Bickmeyer et al. 2002; Iwahori et al. 2002; Liu et al. 2003). For example, activation and inhibition of Ih channels in mouse hippocampal neurons were found to be triggered by the activation of somatic and dendritic serotonin receptors, respectively (Bickmeyer et al. 2002). In GP cells, activation of Ih is of particular importance since it is involved in the high-frequency regular pacemaker-like activity of these neurons. Moreover, Ih has been proposed as the mechanism by which striatal input synchronizes GP firing. In fact, blocking Ih markedly decreased GP firing frequency and disrupted GP cell regularity (Chan et al. 2004). Thus it is likely that serotonin modulates GP firing rate and pattern and can potentially rectify abnormal firing and patterns such as those that occur in PD.

Contrary to past decades, where PD was attributed to a dysfunction of the dopaminergic system per se, work over the last decade has increasingly focused on the role of the serotonergic system in both pathophysiology and treatment of PD (Bonuccelli and Del Dotto 2006; Lang and Obeso 2004; Nicholson and Brodchie 2002). The neurodegenerative process in PD is not limited to the dopaminergic system but rather extends to other neuronal systems as well, including the serotonergic system in both pathophysiology and treatment of PD (Bonuccelli and Del Dotto 2006; Braak and Lacy 1996; Villalobos et al. 2005). Several studies denoted the prominent role of 5-HT1A receptors that are located on the presynaptic GABAergic terminals and exert their excitatory effect on GP cells by decreasing the release of GABA (Chadha et al. 2000; Querejeta et al. 2005; Sari 2004). Recently, a further suppression of presynaptic release of excitatory glutamatergic input by 5-HT1A receptors was suggested (Kita et al. 2007). However, due to the severing of the STN–GP connection in our slice preparation we could not evaluate the serotonergic influence on the glutamatergic excitatory input.

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5-HT EXCITES PALLIDAL NEURONS


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