High-Frequency Stimulation Produces a Transient Blockade of Voltage-Gated Currents in Subthalamic Neurons

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INTRODUCTION

The observation that deep brain stimulation applied at a high-frequency (HFS) in the subthalamic nucleus (STN) and its surgical destruction, both greatly ameliorate motor signs of Parkinson’s disease in patients, led to the hypothesis that HFS blocks, partly or completely, the activity of STN neurons. In keeping with this, HFS in the STN has been shown to significantly decrease the frequency of extracellularly recorded STN neurons in rats in vivo (Benazzouz et al. 1997). As STN neurons are glutamatergic excitatory output neurons (Hammond et al. 1978; Robledo and Féger 1990; Smith and Parent 1988), the immediate consequence of their reduction of activity is the persistent Na+-current (I\(_{Na,p}\)) was totally blocked (by 99%), the Ca\(^{2+}\)-sensitive, hyperpolarization-activated cationic current (I\(_{NaP}\)) was little affected. Thus a high-frequency tetanus produces a blockade of the spontaneous activities of STN neurons as a result of a strong depression of intrinsic voltage-gated currents underlying single-spike and bursting modes of discharge. These effects of HFS, which are completely independent of synaptic transmission, provide a mechanism for interrupting ongoing activities of STN neurons.

METHODS

Slice preparation

Experiments were performed on STN neurons in slices obtained from 20- to 28-day-old male Wistar rats. Rats were anesthetized with ether and decapitated. The brain was quickly removed, and a block of pallidum internal part (EP/GPi) was as observed in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated monkeys and naive rats (Benazzouz et al. 1995; Burbaud et al. 1994; Hayase et al. 1996). It has also been suggested that the consequence of clinical HFS will be to somehow counteract the abnormal bursting pattern recorded in the STN in animal models of Parkinson disease (Bergman et al. 1994; Hassani et al. 1996; Hollerman and Grace 1992; Vila et al. 2000).

To understand the contribution of HFS in pathological conditions, it is clearly essential to determine whether a HFS of the STN could modify or block the intrinsic activities of STN neurons and to analyze the underlying mechanisms. This is best achieved in vitro, as slice preparations enable to better isolate the various effects of a tetanus on neuronal properties. In the present study, using patch-clamp recordings of rat STN neurons in slices, we report that HFS of the STN suppresses the spontaneous activity of both single-spike and bursting STN neurons. The effects of HFS are synaptic-independent and are mediated by a blockade of the voltage-gated currents and particularly the persistent Na\(^{+}\)-current (I\(_{Na,p}\)) and the L- and T-type Ca\(^{2+}\)-currents (I\(_{Ca,L}\) and I\(_{Ca,T}\)) that are known to generate the intrinsic spontaneous discharge modes of STN neurons (Beurrier et al. 1999, 2000; Bevan and Wilson 1999).

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recording chamber, maintained at 30 ± 2°C (mean ± SD) and continuously superfused (1–1.5 ml/min) with the oxygenated Krebs solution.

STN stimulation

The stimulating electrode was positioned in the middle of the STN identified as an ovoid structure just lying at the border of the basal part of the cerebral peduncle. Two types of stimulating electrodes were tested: The bipolar concentric electrode measuring 0.5 mm in diameter (NEX-100, Rhodes Medical Instruments) used by Burbaud (Burbaud et al. 1994) and Benazzouz (Benazzouz et al. 1995) for the in vivo stimulation of the rat STN and a much thinner electrode (0.01 mm in diameter) that we designed to avoid any mechanical lesion of the STN.

Electrophysiological recordings

Slices were visualized using a dissecting microscope and the recording electrode was precisely positioned in the STN. Electrophysiological recordings of STN neurons were performed in the current- or voltage-clamp mode using the blind patch-clamp technique in the whole cell configuration. Patch electrodes were pulled from filamented borosilicate thin-wall glass capillaries (GC150F-15, Clark Electromedical Instruments, Pangbourne, UK) with a vertical puller (PP-830, Narishige, Japan) and had a resistance of 10–12 MΩ when filled with the following (in mM): 120 Kgluconate, 10 KCl, 10 NaCl, 10 ethylene glycol-bis(b-aminoethyl ether)-N,N′,N′,N′-tetracetic acid (EGTA), 10 HEPES, 1 CaCl₂, 2 MgATP, and 0.5 NaGTP, pH 7.25.

Reagents

Drugs were applied by bath. Reagents were procured from Sigma (St. Louis, MO), except 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), d-(-)-2-amino-5-phosphophentanoic acid (d-APV), and bicuculline, which were purchased from Tocris (Bristol, UK).

Data analysis

Membrane potential was recorded using Axoclamp 2A or Axopatch 1D amplifier (Axon Instruments, Foster City, CA), displayed simultaneously on a storage oscilloscope and a four-channel chart recorder (Gould Instruments, Longjumeau, France), digitized (DR-890, NeuroData Instruments, New York), and stored on a videotape for subsequent off-line analysis. During voltage-clamp recordings, membrane currents were fed into an A/D converter (Digidata 1200, Axon Instruments), stored, and analyzed on a PC using pCLAMP software (version 6.0.3, Axon Instruments). Corrections for the liquid junction potential were performed according to Neher (1992): −6 mV for the K-glutamate-based pipette solution as estimated with a 3 M KCl ground electrode.

RESULTS

HFS-induced arrest of single-spike or bursting activity

STN activity was recorded in current-clamp mode (whole cell configuration) for at least 1 min before the HFS was applied. Using a bipolar concentric stimulating electrode similar to that used in rat in vivo (see METHODS), a brief (1 min) HFS consisting of 100 µs stimuli of 5–8 V amplitude, produced a blockade of ongoing activity whether it was in single-spike (Fig. 1) or bursting (Fig. 2) mode. This effect was frequency dependent (Figs. 1A and 2) with an optimal frequency of 166 up to 250 Hz that produced a full blockade of the activity (n = 17). The latency of the HFS-induced silence could not be determined in detail as during the 1-min stimulation period, artifacts prevented analysis of the activity. Nevertheless as shown in Figs. 1B and 2, above a certain frequency, the onset of the blockade was immediately obvious by the end of the train. Interestingly, HFS blocked both single spike (Fig. 1) and burst firing (Fig. 2) modes, suggesting that its mechanisms do not involve a current(s) that is expressed only in one type of discharge.

The suppression of STN spontaneous activity was observed for 5.8 ± 0.7 min (range: 1.1–18.0, n = 31) after HFS. At the end of the silence period, spontaneous activity slowly recovered in the same mode as before stimulation (Figs. 1B to 6). During cell silence, membrane potential remained stable at −52.2 ± 0.8 mV (range: −40 to −68, n = 45) for tonic cells and at −56.2 ± 1.4 mV (range: −48 to −61 mV, n = 8) for bursting cells. These membrane potentials were significantly more depolarized than the potentials at which cells were silent in control conditions: before HFS, cells tested in the tonic mode were silent at −60.2 ± 0.6 mV (range: −49 to −68 mV, n = 45, P < 0.001, paired t-test) and cells tested in the bursting mode were silent at −63.5 ± 1.3 mV (range: −56 to −68 mV, n = 8, P = 0.015, paired t-test). This suggested that HFS did not stop STN cell activity simply by transiently hyperpolarizing the membrane.

Spikes could still be evoked during the silence period in all tested neurons (n = 60). However, in half of the cells, spike threshold was significantly higher during the silence period.
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HFS-induced suppression of activity is independent of synaptic activity

An important issue was to determine whether effects of the train were mediated by synaptic transmission. Bath application of ionotropic glutamate and GABA<sub>A</sub> receptor antagonists, CNQX (20 μM), d-APV (40 μM), and bicuculline (10 μM) failed to prevent the effects of HFS (n = 6, Fig. 4). Furthermore HFS still suppressed single-spike activity when synaptic transmission was blocked by 2 mM Co<sup>2+</sup> (n = 16, Fig. 5A, top). Since the silencing effect of HFS did not require Ca<sup>2+</sup>-dependent transmitter release, we tested whether it was possible to mimic this effect with intracellular stimulation of the recorded cell. When comparing the two types of HFS (extracellular and intracellular) in the same tonically active STN neurons (n = 8), it appeared that both HFS resulted in a silence of the cell. However, intracellular HFS had a different effect on membrane potential: there was a strong hyperpolarization of the membrane at the break of the intracellular pulses (to −63.2 ± 3.1 mV) that declined in about 20 s to −48.1 ± 4.1 mV, a potential at which tonic activity recovered (n = 8, data not shown). Such an after hyperpolarization and slow membrane repolarization were never observed after extracellular HFS where membrane potential remained stable during cell silence (Figs. 1–6).

HFS-induced decrease of voltage-gated currents

We hypothesized that HFS induced a modification of voltage-sensitive currents essential for the expression of tonic and burst-firing modes (Burrer et al. 2000; Bevan and Wilson 1999). In the tonic mode, the silencing effect of HFS did not require Ca<sup>2+</sup> influx since it was still observed in the presence of 2 mM Co<sup>2+</sup> nor increase of intracellular Ca<sup>2+</sup> concentration since it was present in BAPTA-loaded cells (n = 4, data not shown). We therefore tested the effect of HFS on spontaneous tonic activity and I<sub>NaP</sub> recorded from the same STN neurons by shifting from current- to voltage-clamp mode before, during, and after HFS-induced silence. In voltage-clamp mode, in response to a voltage ramp and in the continuous presence of Co<sup>2+</sup>, a TTX-sensitive inward current that had characteristics of a persistent Na<sup>+</sup> current was recorded. It was strongly reduced during HFS-induced silence (Fig. 5). I-V relationships before and during HFS-induced silence showed that peak amplitude of I<sub>NaP</sub> was reduced by 99% during cell silence as compared with the control (from −122.2 ± 13.1 to −1.1 ± 1.1 pA, n = 9; Fig. 5, B and C). This effect reversed to 78% of control (to −92.5 ± 9.9 pA, n = 8) once cell activity recov-
Co$_2^+$ synaptic transmission.

A

When applied at the end of the experiment, TTX (1 μM) totally abolished this current, confirming that it was $I_{\text{NaP}}$ (Fig. 5A).

Spontaneous bursting mode and $I_{\text{Ca}}$ were then analyzed. However, since the recording of Ca$_{2}^{2+}$ currents requires the presence of K$^+$ channel blockers, a procedure incompatible with the recording of burst firing in current-clamp mode, the amplitude of Ca$_{2}^{2+}$ currents was therefore evaluated from the evoked potentials they underlie: the rebound depolarization, also called low-threshold Ca$^{2+}$ current and the Ca$_{2}^{2+}$-activated inward current (Beurrier et al. 1999). Following HFS, during minutes of silence, plateau duration was reduced by 62% (from 1119.4 ± 150.6 to 425.6 ± 111.4 ms, n = 32) sometimes with a total suppression of the after spike depolarization (Fig. 6, A, top and middle, and B, left). Concomitantly, the amplitude of the rebound potential was reduced by 75.9% (from 8.8 ± 0.4 to 2.1 ± 0.5 mV, n = 23; Fig. 6, A, top and bottom, and B, right). Once cell activity recovered, the effects on plateau potential duration and on the amplitude of rebound potential reversed to 66% of control (to 739.4 ± 217.9 ms, n = 18) and to 39% of control (to 3.4 ± 0.8 mV, n = 14), respectively.

In contrast, the Cs$^+$-sensitive, hyperpolarization-activated cation current ($I_{\text{h}}$) was not affected by HFS at potentials normally traversed by the membrane during tonic firing. It was...
Effect of HFS on the hyperpolarization-activated cation current $I_h$. Left: from a holding potential of $-50$ mV, a family of currents was evoked in response to 1.500-msec hyperpolarizing steps from $-60$ to $-110$ mV (10-mV increment) before HFS (control) and during HFS-induced silence (HFS). Right: $I-V$ relationship of $I_h$ before (control), during HFS-induced silence (HFS), and in the presence of 1–3 mM cesium in the bath ($V_H = -50$ mV). Values of $I$ were obtained by subtracting the value of the current at the beginning of the hyperpolarizing pulse from that at the end of the pulse. Currents were normalized ($I/I_{\text{max}}$) to the maximal current ($I_{\text{max}}$) recorded at $-110$ mV.

Reduced between $-80$ and $-110$ mV (by 26.5% at $-90$ mV, $n = 5$, Fig. 7). Consistent with these findings on $I_h$, the amplitude of the depolarizing sag observed during a hyperpolarizing current pulse was not significantly affected (it was reduced by 4.8%, from $5.21 \pm 0.82$ to $4.99 \pm 0.80$ mV, $P = 0.69$, $n = 12$; Fig 6A, bottom).

**Discussion**

Our results show that HFS blocks the spontaneous activity of tonic and bursting STN neurons with a mechanism that does not require $Ca^{2+}$-dependent transmitter release. The silencing effect of HFS has a short latency, is brief, reversible, can be repeated several times with little change, and is frequency dependent. It is mediated by a dramatic reduction of $Na^+$ and $Ca^{2+}$ voltage-gated currents leading to an interruption of the spontaneous activities of the neurons. In fact, in single-spike activity, a TTX-sensitive, persistent $Na^+$ current ($I_{\text{NaP}}$), underlies the slow pacemaker depolarization that spontaneously depolarizes the membrane from the peak of the after spike hyperpolarization to the threshold potential for spike initiation (Beurrier et al. 2000; Bevan and Wilson 1999). In contrast, in burst-firing mode, the interplay between a T-type $Ca^{2+}$ current ($I_{\text{CaT}}$), an L-type $Ca^{2+}$ current ($I_{\text{CaL}}$), and a $Ca^{2+}$-activated inward current, all insensitive to TTX, underlie recurrent membrane oscillations (Beurrier et al. 1999). The blockade of these subliminal currents can also explain the increase of membrane resistance observed during HFS-induced silence.

The silencing effect of HFS does not result from the activation of a local network and is not mediated by the stimulation of afferents to the STN, since it was still observed in the presence of blockers of glutamatergic and GABAergic ionic synaptic transmission and in the presence of cobalt at a concentration that totally blocked synaptic transmission in the STN. It was in fact reproduced by direct stimulation of the recorded STN cell as previously tested by Borde et al. (2000) in hippocampal CA1 pyramidal neurons. In this preparation, a low-frequency intracellular stimulation induced a depression of activity that developed rapidly, was reversible, persisted up to 3 min and was still observed when synaptic transmission was strongly reduced by the P-type $Ca^{2+}$ channel blocker $\omega$-agatoxin IVA or enhanced by 4-aminopyridine. The insensitivity of depression to synaptic blockade indicates little if any involvement of synaptic mechanisms and implies that postsynaptic mechanisms are key factors as observed in the present study with extracellular HFS. However, mechanisms underlying intracellular stimulation may be different from those underlying extracellular HFS. The silencing effect of intracellular stimulation is $Ca^{2+}$-dependent since it requires $Ca^{2+}$ influx and intracellular $Ca^{2+}$ increase in the stimulated cell (Borde et al. 2000), whereas that of extracellular HFS is $Ca^{2+}$-independent (the present study).

As the pattern of discharge of STN neurons may play an important role in the physio-pathology of Parkinsonism (Bergman et al. 1994; Hollerman and Grace 1992), it is tempting to correlate the present effects of in vitro HFS on the spontaneous STN activity, to the beneficial effects of high-frequency deep brain stimulation in the STN of MPTP-treated monkeys (Benazzouz et al. 1992; Hayase et al. 1996) or parkinsonian patients (Benabid et al. 1994; Limousin et al. 1998). However, such a direct correlation needs further experiments. First, clinical HFS is performed in vivo where it could affect the whole basal ganglia network, at least at the onset of stimulation. Second, clinical HFS is efficient at lower frequencies (125–185 Hz) than sometimes in vitro HFS does. This could be explained by the differences in the characteristics of the stimulating electrode. Finally, beneficial clinical effects are observed during the continuous application of the stimulation and only for a short while after the stimulation, whereas in the present study, only events that followed the stimulation have been studied. Nevertheless, the present results give some insights in the way intrinsic activity of STN neurons can be depressed.

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**References**


