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Original Articles

High Frequency Stimulation Extends the Refractory Period and Generates Axonal Block in the Rat Hippocampus

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ABSTRACT

Background: The therapeutic mechanisms of deep brain stimulations (DBS) are not fully understood. Axonal block induced by high frequency stimulation (HFS) has been suggested as one possible underlying mechanism of DBS.
Objective: To investigate the mechanism of the generation of HFS-induced axonal block.
Methods: High frequency pulse trains were applied to the fiber tracts of alveus and Schaffer collaterals in the hippocampal CA1 neurons in anaesthetized rats at 50, 100 and 200 Hz. The amplitude changes of antidromic-evoked population spikes (APS) were measured to determine the degree of axonal block. The amplitude ratio of paired-pulse evoked APS was used to assess the changes of refractory period.
Results: There were two distinct recovery stages of axonal block following the termination of HFS. One frequency-dependent faster phase followed by another frequency-independent slower phase. Experiments with specially designed temporal patterns of stimulation showed that HFS produced an extension of the duration of axonal refractory period thereby causing a fast recovery phase of the axonal block. Thus, prolonged gaps inserted within HFS trains could eliminate the axonal block and induced large population spikes and even epileptiform activity in the upstream or downstream regions.
Conclusions: Extension of refractory period plays an important role on HFS induced axonal block. Stimulation pattern with properly designed pauses could be beneficial for different requirements of excitation or inhibition in DBS therapies.

Introduction

The application of deep brain stimulations (DBS) in the clinic has generated significant excitement as its therapeutic applications have been extended to various brains disorders such as Parkinson's disease, epilepsy, pain, addiction, stroke and depression [1–5]. However, its therapeutic mechanisms are not fully understood. Two important questions remain unanswered: 1) why frequencies greater than 100 Hz are required to produce therapeutic efficacy [6,7]; and 2) which temporal pattern of DBS is more effective: continuous or intermittent stimulation [8,9]?

Recently, strong evidence has shown that the conduction of axons can be blocked by high frequency stimulation (HFS) in the hippocampus and subthalamus both in in-vivo and in-vitro preparation [10–15]. In particular, this axonal block can provide a reversible functional disconnection in both the afferent and efferent axons of hippocampal neurons by HFS with frequencies over 100 Hz [13]. Considering that axonal conduction is essential for the normal function of any complex neuronal networks [6], the effect of HFS on axons could have significant implication on brain function. The goal of the present study is to answer the two questions listed above by focusing on the axonal effects of HFS.

With the application of repeated stimuli of HFS, axons can undergo activity-dependent changes resulting in conduction failures. The changes involve the kinetics alteration of ion channel (e.g., channel inactivation) and the accumulation of ions in intracellular and extracellular areas [16–18] with different time constants. On one hand, the time constant of ion channel kinetics is very short, e.g., ~1 ms activated duration of sodium channels [19], suggesting a fast process. On the other hand, the changes of ionic concentrations,

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such as increase of extracellular potassium concentration ($[K^+]_o$) during intense firings of action potentials and diffusion of the accumulation $[K^+]_o$, suggest a slower process [20]. Therefore, we hypothesized that multiple mechanisms with different time constants could be responsible for both the development and the recovery of axonal failures.

In order to test this hypothesis, we applied HFS with biphasic pulses in the alveus fiber tract of hippocampal CA1 pyramidal cells to induce axonal block in anaesthetized rats. Previous studies indicate that the effective frequency of DBS is in the range of 100–200 Hz, especially 130 Hz. Therefore, we chose 50, 100 and 200 Hz in a wider frequency range to investigate the frequency-dependence of HFS effects. The time courses of the axon failure were examined respectively during and following HFS trains by evaluating the antidromically-evoked population spikes. Two distinct recovery stages of the axon failure with significantly different time constants were observed following HFS with different stimulation frequencies. Furthermore, we studied the mechanism underlying the effect of HFS on axonal block by measuring the changes of refractory period of axons during high frequency stimulation.

Methods and materials

Surgical procedures

All procedures used in this study were carried out in accordance with the Guide for the Care and Use of Laboratory Animals (China Ministry of Health). Adult Sprague Dawley rats (250–350 g) were anesthetized with urethane (1.25 g/kg, i.p.) and placed in a stereotaxic apparatus (Stoelting Co.). Body temperature was maintained at ~37 °C by a wrap with a heating patch and towels. The skull was partially opened to allow the placement of electrodes. A 16 channel recording electrode array (NeuroNexus Technologies) was inserted into the hippocampal CA1 region (AP, −3.5; ML, 2.7; DV, 2.5). Two stimulation electrodes, concentric bipolar stainless-steel electrodes (FHC, Bowdoin, ME 04287, USA), were positioned respectively in two axonal fiber tracts in CA1: the Schaffer collaterals (AP, −2.2; ML, 2.0; DV, 2.8) for orthodromic stimulation and the alveus (AP, −4.8; ML, 2.7; DV, 2.3) for antidromic stimulation. Two separate stainless steel screws were fixed in the nose bone and served as reference electrode and ground electrode. Both multiple unit activity (MUA) and patterns of the evoked potentials at each of the 16 channel recordings were judged to guide the final positions of the recording probe and the stimulation electrodes. Saline was placed over the exposed surface of the dura to keep it moist [13]. At the end of each experiment, the rat was immediately euthanized by an intracardiac injection of saturated potassium chloride.

Recording and stimulation

Potential signals collected by recording probes were amplified by a 16-channel extracellular amplifier (Model 3600, A-M system Inc.) with a filtering frequency range of 0.3–5 kHz. The amplified signals were then sampled by an ML880 Powerlab 16/30 data acquisition system (ADInstruments Inc.) at a sampling rate of 20 kHz/channel with 16-bit precision, and were stored into a hard disk for off-line analysis. The channel with the largest evoked population spikes (PS) and dense unit activity was taken as the location of the pyramidal layer of the CA1 region and the PS amplitudes were calculated.

Custom-made MATLAB codes were used to remove the stimulation artifacts during HFS by linearly interpolating ~1.0 ms signal at each stimulation point. The algorithm was able to remove the artifacts with minimal distortion of the neural signals [13].

Biphasic HFS pulses with constant currents were generated by the Model 2100 isolated pulse stimulator (A-M system Inc.) with a pulse duration of 0.1 ms per phase. According to input–output curves of the orthodromic- or antidromic-stimulations, a current intensity (0.3–0.5 mA) that evoked PSs with an amplitude approximately 3/4 of the maximal PS amplitude was used as HFS stimulation intensity. The inter-pulse intervals (IPI) within HFS trains were set as 20, 10 and 5 ms to obtain the stimulation frequencies of 50, 100 and 200 Hz, respectively. The length of HFS train was set at 1 min. Test stimuli with an identical intensity as HFS were applied at various time points following HFS to show the recovery course of PS activity. Within some of the HFS trains, one prolonged IPI gap of 20 or 100 ms was inserted every 2 s to reveal the change of refractory period of axons. The intervals between sequential HFS trains were >30 min to ensure recovery from previous HFS.

All statistical data were represented as mean ± standard deviation. One-way ANOVA with Post hoc Bonferroni test were used to judge the statistical significance of the differences among data groups.

Results

Two distinct stages in axonal recovery following HFS trains

The axonal block induced by antidromic-HFS (A-HFS) and the time course of its recovery were investigated by stimulation of the alveus, a fiber tract consisting mainly of axons of the hippocampal CA1 pyramidal cells. Under normal condition, paired-pulse stimulation with a 20 ms IPI applied to the alveus induced two antidromically-evoked population spikes (APS) with similar large amplitudes (Fig. 1A). During 1 min long trains of A-HFS with frequencies of 50, 100 or 200 Hz, large APS waveforms were able to follow each stimulation pulse at the beginning of HFS. But, the APS amplitudes decreased rapidly toward the end of HFS trains. The suppression of the APS increased as the frequency of the HFS increased (Fig. 1B). Test pulses applied 3 s and 13 s following each HFS train evoked large and similar APS. Furthermore, the amplitudes of the partially recovered APS at the two time points were similar for all the HFS trains with three different frequencies, in spite of the fact that, at the end of HFS, the APS amplitudes were suppressed to a greater extent at higher frequencies.

As previously reported [13], the suppression of APS could be explained by axon failure since the large orthodromically-evoked PSs (OPS) could still be evoked by orthodromic test stimuli (OTS) during the A-HFS while the APS was markedly suppressed. Since action potentials in CA1 pyramidal cells are thought to be triggered in the axon initial segment, these results indicate that the neuronal cell bodies retained a high level of excitability while the axons were blocked (Fig. 1C). Therefore, in the present study, the amplitudes or areas of APS were used to evaluate the firing and conduction state of the alveus axons during and following HFS.

Although the recovery of the APS immediately following HFS train appeared to be rapid, it was not complete (Fig. 1B). In order to determine the full recovery time course of axon failure induced by A-HFS with different frequencies, we continued the application of test stimuli for 5 min following HFS trains (Fig. 2A). The interval between the test stimuli was set as 10 s for the first 2 min of the test (with the first test stimulus applied at 3 s following the HFS) and then changed to 30 s for the other 3 min. Interestingly, the recovery occurred in two distinct and consecutive phases (fast and slow phases). Within a few seconds following the HFS, the APS amplitude recovered quickly (fast phase). However, the complete recovery of the APS amplitude was much slower and took several minutes (slow phase) (Fig. 2A).