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Research article

High frequency stimulation induces LTD of AMPA receptor-mediated postsynaptic responses and LTP of synaptically-evoked firing in the dorsolateral striatum

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ABSTRACT

In the striatum, long term potentiation (LTP) and long-term depression (LTD) of glutamatergic transmission are believed to underlie motor learning and are impaired in animal models of Parkinson's disease. High frequency stimulation (HFS) is often used to induce synaptic plasticity in the brain. In the striatum, the polarity of HFS-induced plasticity is influenced by the recording conditions, which can differ between various studies. Here, we examined the ability of HFS to induce synaptic plasticity in the dorsolateral striatum in the presence of extracellular Mg^{2+} ions, with no $GABA_A$ receptor blocker, and without membrane depolarization during HFS. We found that HFS induced a LTD of excitatory postsynaptic currents (EPSCs) mediated by AMPA receptors (AMPARs) in medium spiny neurons (MSNs) recorded with whole-cell voltage-clamp. However, HFS induced a LTP of field excitatory postsynaptic potentials/population spikes (fEPSP/PSs), which was dependent on the stimulation intensity applied. The rate of synaptically-evoked spiking in MSNs, measured with cell-attached recordings, showed LTP following HFS. LTD and LTP were impaired in the dopamine-depleted striatum of 6-hydroxydopamine (6-OHDA) lesioned mice, a model of Parkinson's disease. This study shows that HFS induces opposing forms of dopamine-dependent synaptic plasticity in the striatum, *i.e.* LTD of AMPAR-EPSCs and LTP of both fEPSP/PS and synaptically-evoked firing in MSNs.

1. Introduction

Long-term changes in the strength of glutamatergic synaptic transmission, such as LTP and LTD, are potential candidates for cellular mechanisms of learning and memory. In the striatum, these changes are involved in motor skill learning and habit formation [8]. Stimulation protocols used to induce synaptic plasticity often consist of HFS applied, in brain slices and *in vivo*, to the cortex or corpus callosum, as well as locally in the striatum. Using whole-cell and intracellular recordings in corticostriatal rodent brain slices, several groups have demonstrated that HFS induces LTD of AMPAR-mediated responses in MSNs of the striatum [1,[8],15]. This LTD is obtained in physiological concentrations of extracellular Mg^{2+} ions, frequently in the presence of antagonists of $GABA_A$ receptors, and in some studies with concomitant postsynaptic membrane depolarization of the recorded neuron during HFS. In contrast, induction of LTP in MSNs following HFS is more difficult to elicit than LTD, and was shown to require removal of Mg^{2+} ions from the perfusion solution to relieve the voltage-dependent block of NMDA receptors [2]. However, several reports have shown that removal of extracellular Mg^{2+} ions is not necessary to induce HFS-LTP, as shown with field potential recordings of the activity of populations of neurons and with whole-cell and intracellular recordings [5,7,11,17]. This discrepancy is likely attributable to distinct effects of HFS on AMPARs and on the excitability of the recorded neurons. Indeed, the population spike measured with extracellular field potential recordings corresponds to a compound response made of AMPAR-mediated postsynaptic depolarizations and firing activity in striatal neurons.

Our aim was to examine the ability of HFS to induce lasting changes in three different synaptically-evoked responses, using three electrophysiological methods: whole-cell voltage-clamp recordings of AMPAR-EPSCs in individual MSNs, extracellular recordings of fEPSP/PSs, and cell-attached recordings of the firing in individual MSNs. Recordings were obtained in slices perfused with a solution which contained a physiological concentration of Mg^{2+} ions and no added pharmacological blockers. In addition, no membrane depolarization was used during voltage-clamp recordings. We found that HFS induces two opposing forms of synaptic plasticity in the striatum, *i.e.* LTD of AMPAR-EPSCs and LTP of synaptically-evoked firing in MSNs as well as of the

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fEPSP/PS. Both LTP and LTD are dependent on dopamine because they are impaired in the dopamine-depleted striatum.

2. Materials and methods

The experimental procedures were approved by our local ethical committee (Stockholms norra djurförsöksetiska nämnd) and were described previously [4,18]. All efforts were made to minimize animal suffering. Male C57BL/6 mice (Envigo, Holland and Janvier, France) aged 5–11 weeks were maintained on a 12:12 h light/dark cycle and had free access to food and water. Fifteen mice underwent unilateral intracerebral stereotaxic injection of 6-OHDA to produce degeneration of dopaminergic neurons and dopamine depletion of the striatum. These mice were anesthetized with intraperitoneal injection of 80 mg/kg ketamine and 5 mg/kg xylazine, placed in a stereotaxic frame, and injected, over 2 min, with 3 µg of 6-OHDA in 0.01% ascorbate in the substantia nigra pars compacta (n = 12 mice; coordinates: AP: −3 mm; ML: −1.1 mm; DV: −4.5 mm relative to bregma and the dural surface), or the medial forebrain bundle (n = 3 mice; AP: −1.2 mm; ML: −1.2 mm; DV: −4.8 mm) of the right hemisphere. Mice were used for electrophysiological experiments 1–2 weeks after the surgery. In the lesioned striatum, the levels of tyrosine hydroxylase, analyzed by Western blotting, were reduced to 10.3 ± 3.8% (n = 15 mice) of the intact striatum levels. Mice underwent cervical dislocation followed by decapitation, and the brains were rapidly removed. Coronal corticostriatal slices (300–400 µm thick) were prepared with a microslicer (VT 1000S, Leica Microsystem, Heppenheim, Germany) in oxygenated (95% O_2 + 5% CO_2) artificial cerebrospinal fluid (aCSF) containing (in mM): NaCl (126), KCl (2.5), NaH_2PO_4 (1.2), $MgCl_2$ (1.3), $CaCl_2$ (2.4), glucose (10) and $NaHCO_3$ (26) pH 7.4 or a sucrose-based aCSF containing NaCl (15.9), KCl (2), NaH_2PO_4 (1), Sucrose (219.7), $MgCl_2$ (5.2), $CaCl_2$ (1.1), glucose (10) and $NaHCO_3$ (26). Slices were incubated for 1 h at 32 °C, and thereafter at 28 °C, in oxygenated aCSF or in a modified oxygenated aCSF containing (in mM): NaCl (126), KCl (2.5), NaH_2PO_4 (1.2), $MgCl_2$ (4.7), $CaCl_2$ (1), glucose (10) and $NaHCO_3$ (23.4). The use of a modified aCSF during slice incubation did not affect the outcome, *i.e.* whether LTP or LTD were induced or not. Slices were transferred to a recording chamber and were continuously perfused with oxygenated aCSF at 28 °C. DNQX (6,7-Dinitroquinoxaline-2,3(1H,4H)-dione, HelloBio, UK) was used at the end of some recordings.

Whole-cell patch-clamp and cell-attached recordings of visually identified MSNs in the dorsolateral part of the striatum were made with patch electrodes (3–5 MΩ) filled with a potassium gluconate-based intracellular solution which contained (in mM): D-gluconic acid potassium salt (120), KCl (20), HEPES (10), EGTA (10), $MgCl_2$ (2), $CaCl_2$ (1), ATP-Mg (2), $GTPNa_3$ (0.3), pH = 7.3. AMPAR-EPSCs were evoked, in voltage-clamped (–80 mV) MSNs, every 15 s by electrical stimulation of the slice through a patch electrode filled with aCSF placed near the recorded neuron. Cell-attached recordings of MSNs (at 0 mV) were performed with patch electrodes (5–8 MΩ). A patch electrode filled with aCSF was placed near the recorded neuron. The position of this stimulation electrode and the stimulation intensity were adjusted to obtain stable synaptically-evoked spiking of a success rate < 40% and a latency > 2.5 ms, evoked every 15 s. A test-pulse was applied after each stimulation to control the quality of the seal.

Extracellular field potentials were recorded using a glass electrode filled with aCSF positioned on the slice surface in the dorsolateral part of the striatum. fEPSP/PSs were evoked by stimulation pulses applied every 15 s to the brain slice through a concentric bipolar stimulating electrode (FHC, Bowdoinham, ME) placed near the recording electrode on the surface of the slice. Single stimuli were applied at an intensity yielding 50 − 60% maximal response as assessed by a stimulus/response curve established, for each slice, at the beginning of the recording session, by measuring the amplitude of the fEPSP/PS evoked by increasing stimulation intensities.

Signals were amplified *via* a GeneClamp 500B, an Axopatch 200B, or an Axopatch 700 B amplifier (Axon Instruments, Foster City, CA), acquired at 10 kHz and filtered at 2 kHz.

Electrical stimulation pulses (0.1 ms duration) were applied through the stimulating electrodes from constant current (Model A365, World Precision Instruments, Hitchin, UK) or constant voltage (Model DS2, Digitimer Ltd., Welwyn Garden City, UK) stimulus isolators. The stimulation intensities were: for field potential recordings, 28–40 µA (10–80 µA for stimulus-response relationships); for voltage-clamp recordings, 10–20 V; and for cell-attached recordings, 35–55 V. These intensities were similar for control and lesioned mice. HFS consisted of 100 Hz trains of one second duration repeated 4 times with a 10 s intertrain interval at the same stimulation intensity as the test pulse. HFS was applied only once to the same slice. A slice or neuron was considered to show plasticity if we observed a change in the response, relative to baseline, which was > 25% (voltage-clamp), > 20% (field), and doubled (cell-attached) 30 min or 1 h after HFS. Two different types of stimulating electrodes were used for field potential and patch-clamp recordings, which could create different stimulation conditions and different neural tissue activation. However, our study compares responses before and after HFS from the same slice and neuron, and not between slices and neurons. In addition, HFS was applied only when the baseline responses were stable for 10–20 min. The fact that synaptic plasticity is not observed in the lesioned striatum and is prevented by various receptor antagonists [12] demonstrates that the changes observed have a biological origin.

Data were acquired and analyzed with the pClamp 9 or pClamp 10 software (Axon Instruments, Foster City CA, USA) and Microsoft Excel (Microsoft Corp., Redmond, Washington, USA). Data are expressed as mean ± s.e.m. The baseline response was measured for each slice or neuron during the 10 min preceding the application of HFS. Statistical analysis was performed using the Student-*t* test for paired and unpaired observations and the Chi square test in GraphPad Prism 5.0 (GraphPad, USA).

3. Results

EPSCs evoked by local stimulation of the slice in whole-cell voltage-clamped (–80 mV) MSNs were mediated by AMPARs as demonstrated by the blockade of the EPSC by the AMPAR antagonist DNQX (10 µM, Fig. 1). In the 6 neurons recorded from 6 control mice, HFS induced a lasting depression of the EPSC amplitude, or LTD (56.2 ± 5.2% of baseline; P = 0.001; Fig. 1). In 5 of the 8 neurons examined in the dopamine-depleted striatum from 8 mice, HFS failed to induce LTD (average from the 8 neurons: 89.1 ± 7.5% of baseline; P = 0.2112; Fig. 1). Two neurons showed LTD and one neuron showed LTP. Thus, HFS induces a dopamine-dependent LTD of AMPAR-EPSCs in MSNs of the dorsolateral striatum.

We measured fEPSP/PSs in the dorsolateral striatum, as described earlier [13]. These field potentials were blocked by DNQX (10 µM) in control and lesioned mice (Fig. 2), demonstrating the contribution of synaptically-released glutamate acting on AMPARs to these responses. HFS induced a lasting increase in the amplitude of the fEPSP/PS in 5/8 slices examined from 7 control mice (average from the 8 slices: 125.0 ± 4.7% of baseline; P = 0.0023; Fig. 2). Three slices did not show a significant increase in the fEPSP/PS amplitude. In the dopamine-depleted striatum, HFS failed to induce LTP in all the 10 slices examined from 7 mice (98.3 ± 2.1% of baseline; P = 0.3745; Fig. 2).

fEPSP/PSs are a compound response composed of AMPAR-mediated depolarizations and firing in a population of neurons in the slice. We thus tested whether the ability of HFS to induce LTP of the fEPSP/PS, while the same protocol induced LTD of the AMPAR-EPSC, was due to a potentiation of the spiking in MSNs during single pulse stimulation. We first determined if HFS affected the fEPSP/PS evoked at low stimulation intensities, which might not induce robust spiking. We then examined the effect of HFS on the firing of individual MSNs. We performed stimulus/response curves by increasing the stimulation intensity in