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Alterations of corticostriatal plasticity by ammonium and rescue by green tea polyphenols

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ABSTRACT

Hyperammonemia is a major pathophysiological factor in encephalopathies associated with acute and chronic liver failure. On mouse brain slice preparations we analyzed the effects of ammonium on the characteristics of corticostriatal long-term potentiation (LTP) induced by high-frequency electrical stimulation (HFS) of cortical input and the long-lasting effects of pharmacological NMDA receptor (NMDAR) activation. Ammonium chloride exposure enhanced the expression of HFS-induced LTP at the expense of LTD and promoted the generation of NMDA-induced LTD. This treatment did not affect two NMDAR-independent forms of plasticity: taurine-induced LTP and histamine-induced LTD. Alterations in NMDA-induced plasticity were prevented by treatment with green tea polyphenols suggesting the contribution of oxidative stress to the expression of abnormal corticostriatal plasticity.

Introduction

The basal ganglia, a key part of the extrapyramidal motor system, are involved in motor planning, procedural learning, habit formation and execution of motor skills [1–3]. In humans, dysfunction of basal ganglia circuits has been associated with numerous debilitating conditions including Parkinson's disease, Huntington's disease, Tourette's syndrome, schizophrenia, attention-deficit disorder, obsessive–compulsive disorder, and addictions. Hepatic encephalopathy (HE)[1] as a major complication of liver dysfunction is characterized by motor and cognitive deficits as well as changes in the level of alertness. In minimal HE, characteristics of liver cirrhosis, or even in liver cirrhosis without encephalopathy, extrapyramidal symptoms manifest themselves in psychomotor slowing, impairment of fine motor skills, tremor and gait impairment [4–7]. Psychological tests revealed also deficits in learning and memory capacities [8] which may persist after resolution of overt HE [9]. The impairment of locomotor and cognitive functions is also consistently observed in animal models of HE as well as during hyperammonemia without liver dysfunction [10–13].

The striatum is the primary input nucleus of the basal ganglia receiving information from all regions of the cerebral cortex through topographically-organized glutamatergic projections. Information from all regions of the cerebral cortex is integrated by striatal principal neurons, medium size spiny neurons (MSNs) under control of striatal interneurons and dopaminergic input from the substantia nigra. Activity-dependent alterations of synaptic efficacy in the corticostriatal pathway form the basis for motor learning [14–16]. Two major forms of synaptic plasticity, long-term depression (LTD) and long-term potentiation (LTP) of post-synaptic responses in neostriatal principal neurons can be triggered by repetitive stimulation of the corticostriatal input, both critically depending on cell depolarization, calcium entry and the integrity of dopaminergic innervation of the neostriatum [17,18]. Corticostriatal LTP is induced by calcium entry via NMDA receptors (NMDAR) and requires co-activation of D1 dopamine receptors and M1 cholinoceptors, while LTD is initiated by calcium entry via L-type calcium channels and requires co-activation of D2 dopamine receptors, group I metabotropic glutamate receptors (mGluR I) [14,19] and CB1 receptors for endocannabinoids [20]. Changes in the expression and direction of synaptic plasticity in a corticostriatal pathway were reported for pathological conditions modelling Parkinson's disease at different stages and L-DOPA-induced dyskinesia [21–23], Huntington's disease [24,25], and dystonia [26]. Impairment of corticostriatal synaptic plasticity was found to accompany alcoholism [27,28] and brain aging [29]. Although changes in the efficiency of corticostriatal input must have a great impact on the function of basal ganglia circuitry modulating motor activity, only a few studies analysed changes in striatal synaptic plasticity in an animal model of HE [13] and hyperammonemia [30].

Liver dysfunction and hyperammonemia, the key factor in the pathogenesis of HE [31,32], affect signalling associated with activation of NMDA and mGlu I glutamate receptors [33,34] which are

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[1] *Abbreviations used:* HE, Hepatic encephalopathy; LTD, long-term depression; LTP, long-term potentiation; mGluR I, group I metabotropic glutamate receptors; NMDAR, NMDA receptors; ACSF, artificial cerebral spinal fluid; HFS, high-frequency stimulation; NMDA, N-methyl-D-aspartate; NH4Cl, Ammonium chloride; NOS, NO synthase; PP60, polyphenon 60

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involved in the generation of corticostriatal LTP and LTD, respectively. Studies of NMDAR-dependent LTP in the hippocampus showed that the major features of LTP deficits observed in animal models of chronic hyperammonemia and HE [35,36] can be reproduced by long *in vitro* exposure of hippocampal slices to ammonium ions [34,36–38]. In our previous study on mouse brain slice preparations we used a similar *in vitro* model to analyze the effects of hyperammonemia on the characteristics of corticostriatal long-term depression (LTD) induced by electrical stimulation of cortical input or pharmacological activation of mGluR I. Long exposure of neostriatal slices to ammonium chloride impaired the induction and/or expression of all studied forms of LTD. This impairment was reversed by the phosphodiesterase inhibitor zaprinast implying lowered cGMP signalling in LTD suppression [30]. Using a similar *in vitro* model of hyperammonemia, we analyzed now its effects on NMDAR-dependent LTP and NMDAR-independent synaptic plasticity induced by taurine and histamine. We also report that some of these changes can be prevented by treatment with green tea polyphenols.

Materials and methods

Slice preparation and field potential recording

Corticostriatal slices were prepared from the brain of male C57Bl/6 mice aged 6–10 weeks. All procedures were conducted according to German law and the local guidelines and were in accordance with the European Communities Council directive regarding care and use of animals for experimental procedures. After decapitation the brain was rapidly removed from the skull and immersed in an ice-cold artificial cerebral spinal fluid (ACSF) containing (in mM) 125 NaCl, 1.8 KCl, 1.2 KH_2PO_4, 2.4 $CaCl_2$, 1.2 $MgCl_2$, 26 $NaHCO_3$ and 10 D-glucose and saturated with 95% O_2: 5% CO_2 gas mixture (pH = 7.4). Horizontal slices containing the dorsal striatum (neostriatum) and neocortex were cut, 400 μm thick, on a Vibroslicer (Campden Instruments) and stored in submersion chambers filled with a standard solution at room temperature until recording. After a 1 h adaptation period, ammonium chloride (NH_4Cl) was added to one of the chambers at 1 or 5 mM and the slices were incubated for at least 4 h. For recording a single slice was transferred to a chamber of submersion type and perfused at 1.5–2 ml/min with warm (32 °C) and oxygenated ACSF. The slices preincubated with 1 mM NH_4Cl were also perfused with the NH_4Cl-containing medium during the recording, while the slices treated for 4 h with 5 mM NH_4Cl (alone or in combination with polyphenon 60) were first washed in a store chamber for at least 30 min and then perfused with a standard solution during the recording. This was necessitated by the strongly reduced responsiveness of slices continuously exposed to 5 mM NH_4Cl.

Corticostriatal field potentials were recorded with low-resistance (2–4 mOhm) ACSF-filled micropipettes positioned within the striatum at a distance of up to 0.5 mm from the stimulation point in the subcortical white matter at the border between cortex and striatum, where a bipolar nickel-chrome electrode was positioned. The corticostriatal field response includes a negative peak (N2 peak) representing a synchronous spike discharge of striatal medium spiny neurons after activation of excitatory cortical input [39,40] which is completely suppressed by AMPA receptor anagonists [40,41]. After initial testing of stimulus–response relationships, the stimulus intensity was adjusted to induce a postsynaptic response of about 50–60% of its maximal value, and stimulation frequency was set to 0.033 Hz. Stimulus intensity remained the same for the entire experiment. Maximal amplitudes of the N2 peak varied from 0.4 to 1.9 mV, slices showing a maximal response less than 0.4 mV or a trend to decrease the response amplitude with time were discarded. After 20–30 min baseline recording, an electrical or chemical conditioning stimulus was applied to induce long-term changes in corticostriatal postsynaptic responses. The corticostriatal responses were then recorded for 40–60 min to determine the presence of long-term changes in neurotransmission. The following conditioning stimuli were used: (i) high-frequency stimulation (HFS) consisting of three 3 s trains of pulses at 100 Hz separated by 30 s intervals; (ii) 10 min perfusion with the NMDAR co-agonist glycine at 10 mM; (iii) 10 min perfusion with NMDA at 20 μM; (iv) 10 min perfusion with histamine at 10 μM; (v) 20 min perfusion with taurine at 10 mM.

Drugs

Ammonium chloride (NH_4Cl), glycine, taurine, histamine dihydrochloride, and polyphenon 60 (PP60), were purchased from Sigma–Aldrich (Germany), N-methyl-D-aspartate (NMDA) from Ascent. NMDA was prepared as a stock solution, stored in aliquots at −20°, and was defrozen and diluted to the required concentration by ACSF before application. All other drugs were prepared freshly before use.

Experimental setup and data analysis

Two similar experimental setups were used for field response recordings. Signals were amplified with Axoclamp-2A and NPI amplifiers, digitized at 10 kHz using a Digidata 1200 interface, and recorded on hard disk using Clampex8 (pClamp8 software, Axon Instruments). A Master-8 pulse generator and isolated stimulators with voltage output were used for delivering electrical stimuli. The recordings were analyzed off-line using Clampfit8 (pClamp8 software, Axon Instruments). The N2 peak amplitude was measured as the average of the amplitude from the peak of the early positivity to the peak negativity, and the amplitude from peak negativity to peak late positivity. All values were normalized to baseline, the mean of a 15–20 min period before the conditioning stimulation. The presence of LTP or LTD was determined by a persistent increase or decrease, respectively, in N2 peak amplitude by $\geqslant$15% by the end of recording. The magnitude of LTP or LTD was measured as an average of relative values of N2 amplitudes within 30–40 min after electrical and 40–50 min after chemical conditioning. Data were expressed as the mean ± SEM with *n* indicating the number of slices per group (each group included slices from at least four animals). Statistical analysis was performed by GraphPad Prism software.

Results

Long exposure to 1 mM ammonia does not affect basal corticostriatal neurotransmission

Analysis of stimulus–response relationships (input–output curves) showed no difference between control corticostriatal slices and the preparations treated with 1 mM NH_4Cl when recording in either standard (Fig. 1B and D) or magnesium-free ACSF (Fig. 1C and E). Thus, long exposure to ammonium chloride did not induce any changes in basal neurotransmission.

High-frequency stimulation of the cortical input

NMDAR-dependent plasticity of corticostriatal neurotransmission was first studied using high-frequency electrical stimulation (HFS) of the cortical input. When recording in standard ACSF, HFS induced predominantly LTD or only short-term changes of field responses in the control corticostriatal slices. Treatment with