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Research Report

Modification of the synaptic glutamate turnover in the hippocampal tissue exposed to low-frequency, pulsed magnetic fields

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Abstract

The influence of pulsed magnetic fields (PMF) on the release and uptake of glutamate was investigated. While the release was examined using hippocampal slices, synaptosomes were chosen to characterize the uptake process. ^{3}H-D-aspartate was used as a marker of glutamergic transmission. The pulsed magnetic fields (9–15 mT) applied according to the pattern which induced epileptic discharges in hippocampus amplified and attenuated the release and uptake of glutamate, respectively. However, the magnetic fields which induced an increase in neuronal excitability without concomitant seizures amplified both processes. These results confirm our previous reports and indicate that the glutamergic synapses are the target of magnetic fields action.

Theme: Excitable membranes and synaptic transmission
Topic: Mechanism of neurotransmitter release

Keywords: Hippocampus; Magnetic field; Glutamate turnover

There are several factors which regulate the efficiency of glutamergic synapses. One of them is the concentration of the glutamate in the synaptic cleft which is the result of the equilibrium between the intensity of release and uptake processes [3]. It has been demonstrated by several laboratories [2,12,16] including ours [8], that any disturbance of that equilibrium can modify excitability of glutamergic neurons. Our previous studies revealed that exposure of hippocampal slices to pulsed magnetic fields [21] increased neuronal excitability in a Ca^{2+}-dependent way [20]. That enhancement of neuronal excitability may reflect changes in synaptic turnover of the neurotransmitter favoring either an increase of the release, or reduced efficiency of the uptake system, or both. The frequency of applied magnetic fields was crucial for the magnitude of the observed effects [21]. Therefore, here we examined the influence of magnetic fields exposure on the release and uptake mechanisms, applying the patterns of magnetic fields, which induced seizures in hippocampal tissue in vitro [1,21] and epileptic episodes in preclinical trials [4,5,9,10]. The preliminary experiments demonstrating the influence of magnetic fields on glutamate release and uptake have been presented by us elsewhere [22].

CD-1 mice of both sexes, 4–6 weeks old were used as a source of the hippocampal tissue, which has been prepared according to the procedures approved by the Committee for Treatment and Use of the Laboratory Animals at CSI. Following decapitation, the brain was removed, both hippocampi were dissected out to cold Ringer's solution and then sliced into 400 μm slices using manual tissue chopper according to the standard procedure used in our laboratory [21,22]. While the release experiments were performed on hippocampal slices exposed to magnetic fields, the uptake was characterized using synaptosomes obtained from hippocampal slices. As a marker of the efficiency of both of these processes, we used radioactive ^{3}H-D-aspartate (^{3}H-D-asp).

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This glutamate analog mimics the action of endogenous glutamate and is widely used to investigate the properties of glutamergic synapses [14,15,19].

1. Release experiments

The slices were preincubated for 1 h at 33 °C in oxygenated Ringer's and then incubated for additional 50 min with ^{3}H-D-asp (0.8 μM). Then 10 slices were placed in the perfusion chamber (0.5 ml, 33 °C) of Brandel Perfusion System and perfused to remove excess of radioactivity from the surface of the slices. The collection of the perfusate (3 min/sample) was initiated 90 min later and magnetic fields were activated usually at the sample #10. The collection of perfusate continued through the whole experiment before, during and after activation of the magnetic fields. Thirty to forty samples were collected in any experiment and the radioactivity was counted in the scintillation counter after addition of scintillation fluid (Beckman and Butler).

2. The uptake experiments

Synaptosomes were prepared from hippocampal slices according to Muzzolini's group [14]. Three tests have been used to evaluate the viability of synaptosomal preparation. In sonicated synaptosomes, the glutamate uptake was reduced to 5.9% of the value observed in intact synaptosomes ($n = 10$; $P < 0.0001$). DL-Threo-β-benzyloxyaspartic acid (TBOA) a specific blocker of glutamate uptake [17] attenuated the efficiency of glutamate to almost 40% (41.4%, $n = 3$, $P < 0.007$). The incubation of the synaptosomes in the medium without Na^{+}, which is essential for glutamate uptake, reduced ^{3}H-D-asp accumulation to 33.5% ($n = 33$, $P < 0.0001$). These three tests demonstrated that synaptosomes prepared from the mouse hippocampal slices retained an active, Na^{+}-dependent glutamate uptake system. The equal volumes of the synaptosomal fraction were pipetted into two separate, constantly oxygenated slice recording chambers and kept at the temperature of 33 °C during the entire experiment [21]. Only the experimental synaptosomes were exposed to magnetic fields applied according to the combination of paradigms depicted in Table 1. Immediately after magnetic field exposure, control and experimental synaptosomal fractions were transferred to two separate manifold filtration chambers containing Ringer's with ^{3}H-D-asp (0.8 μM). After 2 min incubation, the excess of ^{3}H-D-asp which was not accumulated by synaptosomes was filtrated out under vacuum and the filters with ^{3}H-D-asp containing synaptosomes were transferred to the scintillation vials to count the radioactivity. The amount of ^{3}H-D-asp taken up by exposed synaptosomes was expressed as a percent of uptake occurring in control fractions. In some experiments, the synaptosomes were exposed to Paradigm 1 and were taken for uptake analysis 1 h following magnetic field exposure.

Table 1
The pattern of PMF applied according to Paradigms 1, 2 and 3

Paradigm	PMF intensity (mT)	Pattern of application	Number of repetitions
1	9	20 s on/off	4
	12	20 s on/off	4
	15	20 s on/off	8
2	15	5 s on/off	9
		Pause (20 s)	
		5 s on/off	
		Pause (220 s)	6
3	15	2 s on/off	12
		Pause (30 s)	
		2 s on/off	4
		Pause (10 s)	
		2 s on/off	3
		Pause (25 s)	
		2 s on/off	5
		Pause (10 s)	
		2 s on/off	6

While the intensity of PMF varied from 9 to 15 in Paradigm 1, it remained constant (15 mT) in Paradigms 2 and 3. In all Paradigms, the PMF were applied in bursts lasting from 2 to 20 s, and repeated 3 to 12 times. The PMF were applied either according to Paradigm 1 alone, or sequential application of Paradigms 1 and 2, or Paradigms 1, 2 and 3. See the text for further details.

3. The exposure to magnetic fields

Both preparations used in our research (slices and synaptosomes) were exposed to the same type of magnetic fields applied as previously described [21]. A very low-frequency oscillating magnetic fields, which are often called "pulsed" in the literature (pulsed magnetic fields, PMF) were used. PMF can be defined as magnetic fields of different profiles applied in an "on–off" fashion with "on" and "off" periods ranging from seconds to minutes. The shape of PMF used in our experiments was rectangular. To standardize the experimental conditions and to make the description of the experiments uniform, we considered one "on" and "off" period as one cycle. The frequency of PMF application was expressed as the number of these cycles per second. The characteristics of applied magnetic field were patterned according to magnetic fields which induced seizures in epileptic patients [4,5,9,10] and hippocampal slices [21]. The tissue-containing chamber was surrounded by custom made, four magnetic coils (Heavy Armored, Poly-Thermaleze Magnet Wire). Each coil was separately powered by a DC power supply (Dual Power Supply, Model 72-6854 with Instantaneous Current Output Option), and activated/deactivated by automatic timer (XT Series, Chron-Trol) at different frequencies for variable time periods (see Table 1). The power supply was adjusted to maximal current and the settings were not changed during the experiment.