Acetylcholine Precursor Choline Evokes NMDA-Dependent Epileptoid Activity in Rat Hippocampal CA1 Area

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Application of choline (5 and 10 mM) to electrically stimulated (1 Hz) rat hippocampal slices evoked epileptoid activity manifested by generation of extra population spikes. Application of methyllycaconitine (10 nM), a specific agonist for α7-subunit of nicotinic acetylcholine receptors, did not prevent generation of extra population spikes. In contrast, pretreatment of slices with Mg^{2+} (5 mM) or blockade of NMDA-type glutamate receptors with MK-801 (100 μM) prevented generation of the extra population spikes. It was hypothesized that elevation of choline concentration during cerebral pathology can promote activation of NMDA-receptors and provoke epileptoid activity not related to activation of α7-subunit of nicotinic acetylcholine receptor.

Key Words: *hippocampus; CA1; choline; epileptoid activity*

Choline, a precursor of neurotransmitter acetylcholine, is produced in the synapses during depolarization after hydrolysis of acetylcholine by acetylcholine esterase and during some pathological states such as hypoxia [4,6], ischemia [3], and epilepsy [5]. Under these conditions, the extracellular concentration of choline increases by 3-4 times and attains the level of 9 to 20 μM. As an absolute agonist and desensitizing agent, choline interacts with nicotinic α7-acetylcholine receptors (α7)nAChR, while as a partial (weak) agonist it interacts with (α3β4)nAChR [1].

In the hippocampus, the glutamatergic inputs of the pyramidal neurons are modulated by (α7)nAChR and (α3β4)nAChR located in the pyramidal and intercalary neurons. Moreover, nAChR, AMPA, and NMDA receptors control neurotransmission in such neurons within CA1 area of hippocampal slices [2]. Generation of epileptoid activity in surviving hippocampal slices *in vitro* is related to disturbances in the balance between the excitatory and inhibitory processes. This fact is used to develop a model of epileptoid activity by removing Mg^{2+} from the perfusion solution of the cerebral slices. In this case, the balance between inhibition and excitation is shifted towards excitation due to activation of NMDA-receptors normally blocked with Mg^{2+} [9]. It is a common knowledge that activation of these receptors *in vivo* modulates choline metabolism [10]. Hypothetically, elevation of choline concentration during various pathological states could induce epileptoid activity in rat hippocampal CA1 pyramidal neurons.

Our aim was to test this hypothesis and to examine the effect of choline on (α7)nAChR and NMDA receptors.

MATERIALS AND METHODS

The experiments were carried out on hippocampal slices of Wistar rats (*n*=28) weighing 150-200 g. Isolation and incubation of 350-μ slices are described elsewhere [8]. The slices were perfused (2 ml/min) with ACSF solution containing (in mM): 126 NaCl, 3.0 KCl, 1.2 $MgSO_4$, 1.25 NaH_2PO_4, 2.0 $CaCl_2$, 26 $NaHCO_3$, 10 glucose (pH 7.4) saturated with 95% O_2 and 5% CO_2 at 34.0±0.5°C. The slices were adapted to this medium for 1 h and then the initial parameters were recorded. The total electrical activity was recorded in CA1 re-

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gion via single-channel microelectrodes filled with NaCl (0.15 M). Population spikes (PS) were recorded with electrodes places into *stratum pyramidale* region of CA1 area. Orthodromic electrical stimulation was performed with rectangular voltage pulses (amplitude 3-8 V, duration 0.1 msec) applied via bipolar platinum electrodes onto *stratum radiatum* region with Schaffer collaterals. The signals were amplified within a frequency band of 30 kHz and fed to PC via a digitizer. Epileptization was induced by removal of Mg^{2+} from the perfusion solution or application of choline. To assess the degree of epileptization, we stimulated Schaffer collaterals at a rate of 1 Hz (30 pulses over 30 sec) while recording all PS [7]. The degree of epileptization was evaluated according to appearance of extra PS measured with peak-to-peak amplitude as in the case of basic PS.

The data were processed statistically by Student's *t* test.

MK-801 and methyllycaconitine (MLA) were used as antagonists of NMDA receptor-channel complex and (α7)nAChR, respectively.

RESULTS

Removal of Mg^{2+} from ACSF unblocked NMDA-receptors and triggered generation of extra PS. In the control, the amplitude and shape of the 1st PS and 30th PS did not change during electrical stimulation of CA1 field with 30 pulses at a repetition rate of 1 Hz. On minute 40 after removal of magnesium (series I, n=5), epileptization increased by 57.14±0.72%, which was manifested by persistent increase in the number of PS depending on the stimulus number from the first to the 30th (Fig. 1, *a*).

In series II, we examined the effect of choline in concentrations of 1, 5, or 10 mM on generation of extra PS in *stratum pyramidale* of CA1 area of rat hippocampus. Addition of 1 mM choline (n=3) to ACSF produced no effect on the magnitude and shape of the 30th PS in comparison with control (Fig. 1, *b*). In contrast, 5 mM choline (n=5) enhanced epileptization by 19.63±0.52% observed as persistent increase in the number of PS during presentation of 30 pulses (Fig. 1, *c*; Fig. 3). Similar enhancement of epileptoid activ-

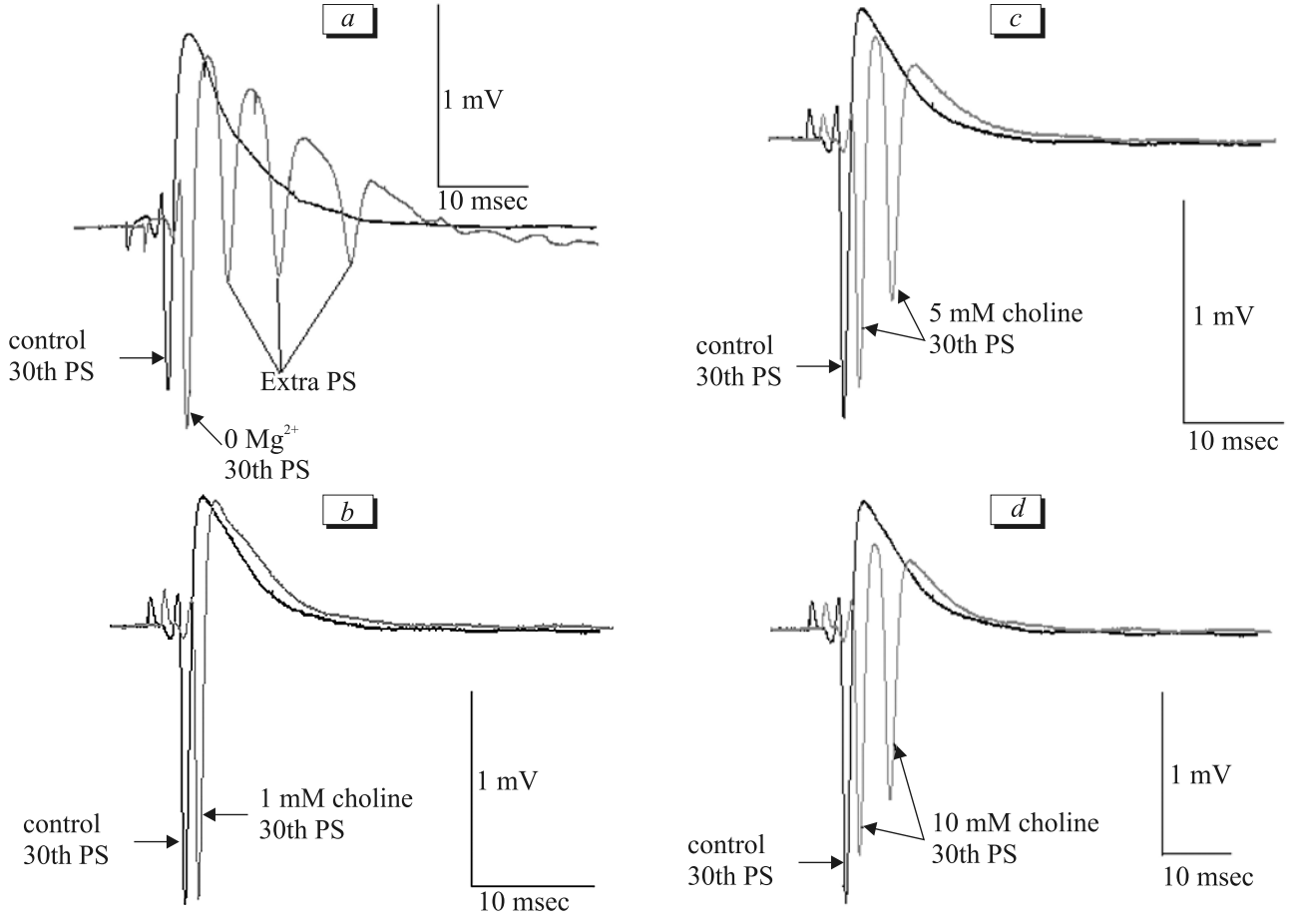

Fig. 1. Effect of magnesium removal and choline application on epileptoid activity. *a*) generation of epileptoid activity manifested by extra PS in response to the 30th stimulus applied on minute 40 after removal of Mg^{2+} from ACSF; *b*) application of 1 mM choline to ACSF did not induced extra PS; 5 mM (*c*) and 10 mM (*d*) choline in ACSF induced extra PS.

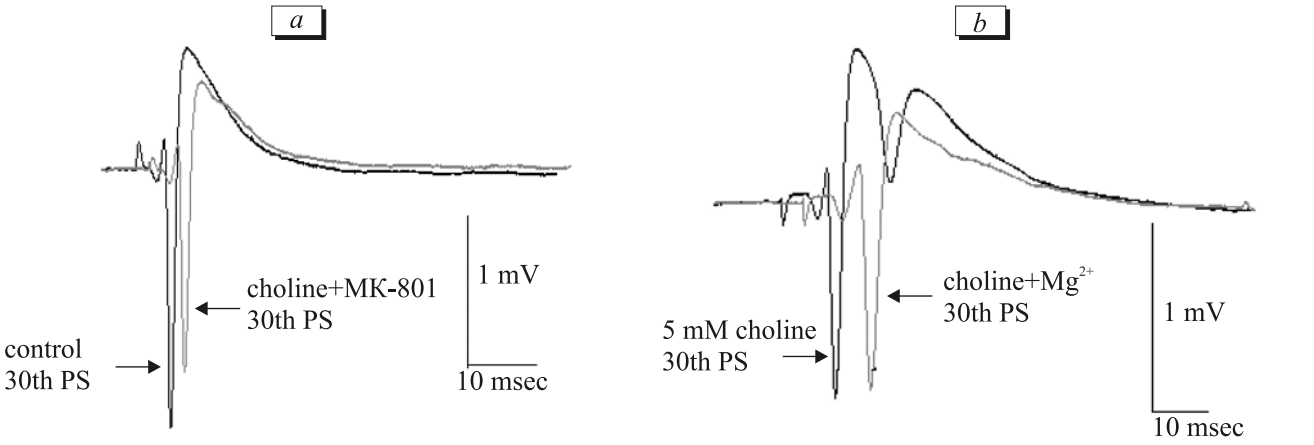

Fig. 2. Effect of blockade of NMDA-receptors with MK-801 and Mg^{2+} on the action of choline in various concentrations. *a*) preliminary addition of MK-801 (100 μM) to ACSF prevented generation of extra PS evoked by 10 mM choline; *b*) application of 5 mM choline to ACSF generated extra PS, while addition of Mg^{2+} prevented generation of this extra PS.

ity by 15.46±0.88% was produced by 10 mM choline (*n*=5; Fig. 1, *d*; Fig. 3).

In series III (*n*=6), we examined the effect of 10 mM choline on generation of extra PS in *stratum pyramidale* of CA1 area of rat hippocampus under conditions of blockade of NMDA receptors with MK-801. Preliminary application of MK-801 (100 μM) prevented generation of extra PS by 10 mM choline (Fig. 2, *a*). Similar effect was observed after addition of Mg^{2+} (5 mM) to ACSF (Fig. 2, *b*; Fig. 3).

In series IV (*n*=5), the effect of 10 mM choline on generation of extra PS in *stratum pyramidale* of CA1 area of rat hippocampus was examined under conditions of blockade of (α7)nAChR with MLA. Here, the preliminary addition of 10 nM MLA to ACSF did not prevent generation of extra PS evoked by 10 mM choline.

The data obtained showed that choline could induce epileptoid activity in the neural network of CA1 area in rat hippocampal slices, which can be revealed only by the method of frequency stimulation at 1 Hz [7]. However, it was not clear which receptors mediate this effect of choline, because MLA (10 nM), a specific blocker of (α7)nAChR, did not prevent generation of epileptoid activity [1], while MK-801 (100 μM), a specific blocker of NMDA receptors, completely prevented generation of this activity. Specificity of MK-801 effect is corroborated by the fact that addition of Mg^{2+} (5 mM) to ACSF prevented generation of extra PS in response to 5 mM choline.

It is hypothesized that elevation of choline concentration characteristic of some pathological conditions of the brain can promote activation of NMDA receptors resulting in epileptoid activity unrelated to activation of (α7)nAChR. However, further studies are needed to comprehensively reveal the mechanism of choline action.

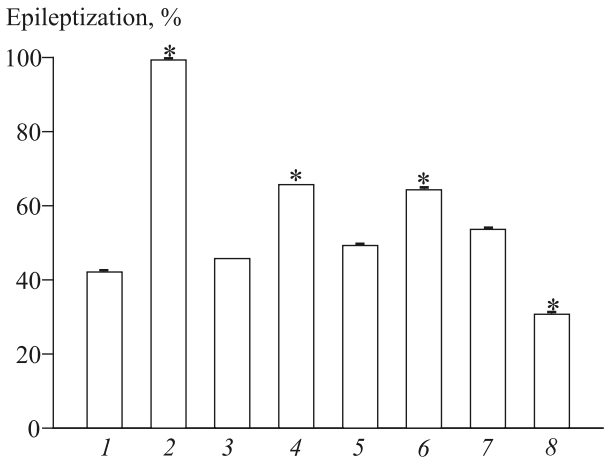

Fig. 3. General indices of the effects of NMDA blockers on choline-evoked epileptoid activity. *1*) control (30th PS); *2*) 30th PS at 0 Mg^{2+} and minute 40; *3*) control (30th PS); *4*) choline 5 mM; *5*) control (30th PS); *6*) choline 10 mM; *7*) control (30th PS) after addition of 10 mM choline; *8*) 30th PS after addition of 10 mM choline+5 mM Mg^{2+}. *p<0.001 in comparison with the corresponding control.

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