Protective Effect of Copper-Rutin Complex in Animals with Experimental Epilepsy

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Copper-rutin complex (2 mg/kg) completely eliminated epileptiform potentials induced by a combination of chlorpromazine and microwave radiation 1-2 min postinjection and suppressed convulsive activity provoked by application of penicillin to the sensorimotor cortex.

Key Words: *epilepsy; epileptiform potentials; active oxygen forms; flavonoid metal complexes*

The term epilepsy includes CNS diseases characterized by repeated seizures caused by synchronous and rhythmic hyperactivity of neuronal populations in the cortex, hippocampus, or thalamus. The pattern of seizures and other external manifestations of epileptic attacks are determined by normal function of the brain structure containing hyperexcitation focus. By the present time, about 2% population of our planet suffer from epilepsy [4]. Hereditary factors play a major role in the etiology of epilepsy. In humans, 12 gene mutations causing different forms of epilepsy were identified. Some genes encode channel-forming proteins [4]. Epilepsy can develop after traumatic or infectious injury to the brain. Free radical processes also play an important role in the pathogenesis of traumatic epilepsy [7]. It was shown that epileptic activity of neurons during seizures is associated with the formation of reactive oxygen forms and NO in the cerebral cortex [1]. That is why antioxidants, including bioflavonoids, were used as the agents suppressing epileptic activity in the CNS [2,6]. We recently showed that complexes of rutin and other flavonoid with metals are more effective antiradical agents and possess a more pronounced cytoprotective effect under conditions of oxidative stress compared to pure flavonoids [3,5]. We investigated the effect of a complex of bivalent copper with rutin (CRC) on bioelectrical activity of the brain under conditions of experimental epilepsy.

MATERIALS AND METHODS

Experiments were carried out on random-bred albino rats of both sexes (150-200 g) narcotized with urethane (1 g/kg intraperitoneally). Electrocorticograms (ECoG) of the right sensorimotor cortex were recorded with an EEG-16S electroencephalograph (Medicor) (standard bipolar lead). Epileptiform activity in the cortex was induced by a combination of chlorpromazine and microwave radiation or by application of penicillin to the cortex. Chlorpromazine in a dose of 5 mg/kg was injected intraperitoneally and animal's head was exposed to 10-min low-intensity microwave radiation (42.2 GHz, 150 μW/cm^2) in a pulse-modulated mode. Benzylpenicillin sodium salt (240 U in 5 μl isotonic phosphate buffer) was injected through a stereotaxically inserted cannula into the subdural space.

Chelate CRC was prepared immediately before each experiment by adding copper bromide to 0.2 mM rutin (aqueous solution) in equimolar quantities under constant stirring.

RESULTS

We previously showed that combination of chlorpromazine with microwave radiation induces epileptiform activity (3-5 Hz spike frequency and amplitude up to

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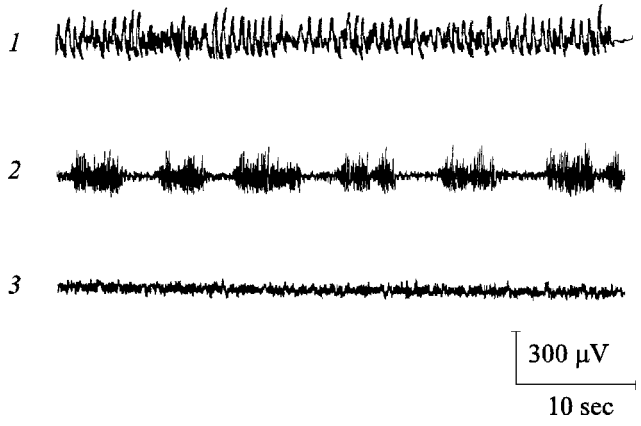

Fig. 1. Bioelectrical activity of rat brain cortex under narcosis (*1*), 10 min after combined treatment with chlorpromazine (5 mg/kg intraperitoneally) and microwave exposure (*2*) and 2 min after intraperitoneal injection of Cu—rutin complex (2 mg/kg) (*3*).

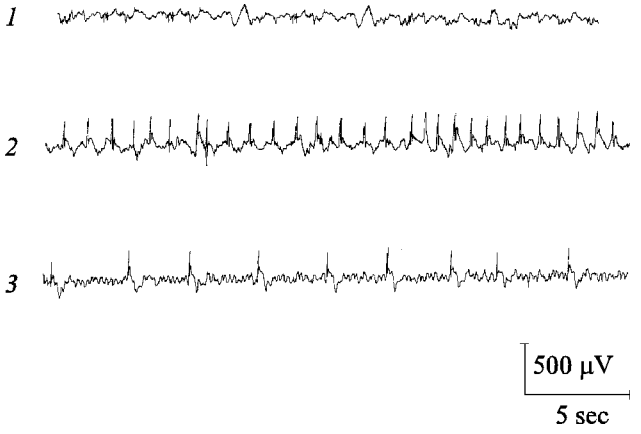

Fig. 2. Bioelectrical activity of rat brain cortex under narcosis (*1*), 5 min after application of penicillin (240 U) (*2*), and 2 min after intraperitoneal injection of Cu—rutin complex (2 mg/kg) (*3*).

300 μV, Fig. 1, *2*) 7-10 min after the start of exposure. Epileptiform activity was not accompanied by muscle convulsions and persisted for 60 min and longer after termination of microwave irradiation. Intraperitoneal injection of CRC (2 mg/kg) completely eliminated epileptiform potentials caused by combination of chlorpromazine and microwave exposure 1-2 min postinjection (Fig. 1, *3*). The effect of CRC persisted for 12±3 min, after which epileptiform activity appeared again. However, repeated injection of CRC completely suppressed epileptiform activity for 10-15 min.

Application of penicillin to the sensorimotor cortex induced spikes with a frequency of 1 Hz and amplitude of up to 500 μV on ECoG 6±2 min after the exposure (Fig. 2, *2*). The seizure lasted for more than 60 min and was paralleled by muscle convulsions coinciding with spike frequency. This type of epileptic activity is called "wet dog shakes" (WDS) [2]. CRC showed a pronounced anticonvulsant effect in penicillin-induced epileptic seizures (Fig. 2, *3*). Three minutes after intraperitoneal injection of CRC in a dose of 2 mg/kg spike frequency significantly decreased ($p<0.05$, Fig. 3). Fifteen minutes postinjection the anticonvulsant effect of CRC decreased, but repeated injection of CRC again significantly reduced spike frequency (Fig. 3).

As CRC is an effective antiradical agent [5,6], pronounced anticonvulsant activity of this drug indirectly confirms the role of active oxygen forms and free-radical processes in electrogenesis of epileptiform activity. Our findings prompt further experimental and technological investigation of flavonoid-metal complexes for creation of new effective anticonvulsant agents.

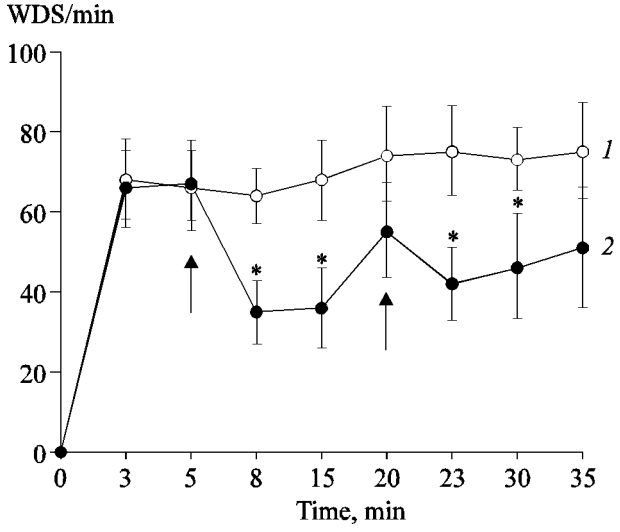

Fig. 3. Frequency of WDS spikes on electrocorticograms after application of penicillin to the motor cortex. *1*) penicillin (control); *2*) penicillin+Cu—rutin (2 mg/kg). *$p<0.05$ compared to the control. Arrows: injections of Cu—rutin complex.

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