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SHORT COMMUNICATION

Kainic Acid Increases Activity Only of Some Cortical Neurons in the Rat

M. KONRÁD*, V. PAVLÍK, P. MAREŠ

Institute of Physiology, Academy of Sciences of the Czech Republic, Prague
**Present address: Faculty of Sciences, Charles University, Prague*

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Summary

The influence of a subconvulsant dose of kainic acid (KA) on the activity of neurons was studied in the sensorimotor cortical area of urethane-anesthetized rats. A total of 41 neurons was recorded, 38 of these in layer V (probably pyramidal cells). The activity of 18 neurons was recorded before as well as more than 30 min after KA administration (6 mg/kg i.p.). Nine out of these 18 neurons increased their firing rate significantly even 20 min after KA injection, whereas the remaining neurons did not change their activity. Altogether, the increase in the firing rate was significant. KA was found to enhance markedly the firing rate of a part of cortical neurons at very early stages of its action.

Key words

Cerebral cortex • Kainic acid • Unit activity • Rat

Kainic acid is an agonist acting at one type of ionotropic excitatory amino acid receptors (Barnard and Henley 1990). If administered systemically to rodents, it induces epileptic seizures qualified as a model of complex partial (temporal) seizures with secondary generalization (Ben-Ari 1985). The phenomena elicited by kainic acid exhibit a clear-cut dependence on the dose administered. The first phenomena induced are epileptic automatisms (the most conspicuous being represented by wet dog shakes). When a sufficiently high dose of kainic acid is injected, automatisms are followed by motor seizures lasting for several hours (Sperk *et al.* 1985). This represents a model of convulsive status epilepticus. Numerous data have been published on kainic acid-induced motor status epilepticus (Sperk 1994, Babb *et al.* 1995), but there are only exceptional reports on nonconvulsive kainic acid-induced phenomena (Sperk *et al.* 1985) and their possible consequences (Berger *et al.* 1989). We therefore started to study the acute as well as delayed effects of a subconvulsant dose of kainic acid in rats and we described acute changes in behavior (Mikulecká *et al.* 1999) and in phenomena induced by electrical stimulation of the cerebral cortex (Koryntová *et al.* 1997, Koryntová and Mareš 1998). We demonstrated that the threshold for spreading of epileptic afterdischarges from the cerebral cortex into the limbic system was decreased immediately after kainic acid administration. Surprisingly, an increase was found in the

Fax +420 2 293 894
E-mail physres@biomed.cas.cz http://www.lf2.cuni.cz/physiolres/physiolres.htm

thresholds for movements and spike-and-wave epileptic afterdischarges elicited by stimulation of the sensorimotor cortex (Koryntová and Mareš 1998). This indicates that a subconvulsant dose of kainic acid not only influences limbic structures but also the cerebral cortex. Therefore, we decided to study the acute effects of kainic acid in the same dose as in our previous papers (6 mg/kg i.p.) on the activity of neurons in the sensorimotor cortical area of adult rats.

The experiments were performed on 27 adult male albino rats of the Wistar strain. The animals were anesthetized by an intraperitoneal injection of urethane in a dose of 1.5 g/kg. A trephine opening (3 mm in diameter) was made over the sensorimotor cortical area (center of the opening on coordinates AP=0, L=3.5 mm in relation to the bregma). The dura mater was removed from the central part of the trephine opening.

The animals were fixed into a stereotaxic apparatus on a pad heated electrically to 37° C. Glass microelectrodes filled with 1 M NaCl (with a resistance between 5 and 20 MΩ) were introduced into the cerebral cortex by means of a microdrive with a stepping motor (with steps of 2 μm). An indifferent electrode was placed on the nasal bone. Recording was started when stable unit activity was found. All recorded cells were classified as randomly firing neurons. Electrical activity was amplified and action potential transformed into rectangular pulses of 1 ms duration. Original unit firing as well as the transformed pulses were followed on the screen of an oscilloscope. These pulses were stored in an 8-bit microcomputer and their frequency was counted. Then the animals received an injection of kainic acid (Sigma, St.Louis, Mo) at a dose of 6 mg/kg i.p. (freshly dissolved in physiological saline in a concentration of 6 mg/ml). Recording was repeated between 20-22 min and between 30-32 min after kainic acid administration. If a unit was lost, the activity of another neuron was recorded. The depth of recording electrodes from the cortical surface was measured using the moment of electrical contact as zero. Statistical evaluation of data was performed by means of Repeated Measure ANOVA with multiple posthoc comparison using Dunnett's test (SigmaStat® Jandel). The level of significance was set at 5 %.

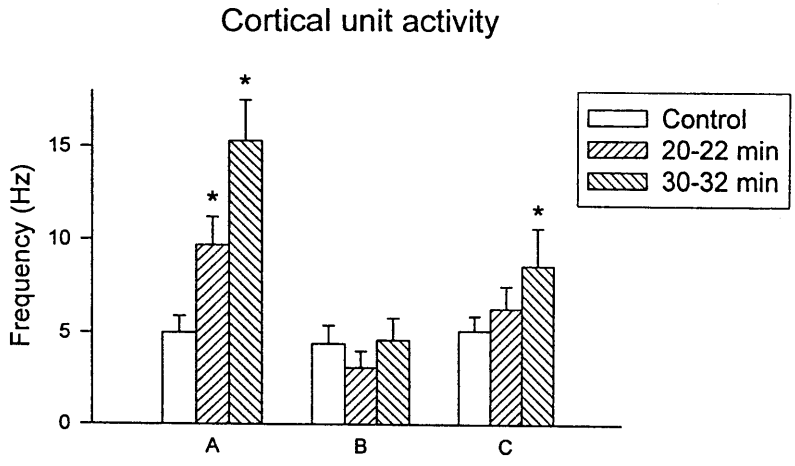

Fig. 1. *Frequency of firing of cortical neurons (mean ± S.E.M.) before, 20-22 and 30-32 min after kainic acid administration (see inset). Abscissa: A – activity of nine neurons increasing activity; B – activity of nine neurons which did not change their firing frequency; C – activity of all neurons, including those recorded only at one time period. Ordinate: frequency in Hz. Asterisks denote significant differences in comparison with the corresponding predrug period.*

The activity of 18 neurons was recorded in all three time periods, that of other 23 cells were recorded before kainic acid administration and the activity of 8 more neurons was registered between 20-22 min after kainic acid injection. Most of the cells recorded were localized in layer V (at a depth from 800 to 1200 μm), only three neurons were localized more superficially, around 500 μm below the cortical surface, i.e. in layer II/III. Nine out of the 18 neurons recorded throughout the experiment increased their firing rate significantly in the first and second interval after kainic acid administration from 5.0±0.9 Hz to 9.7±1.5 Hz and 15.3±2.2 Hz, respectively (Fig. 1A). In spite of the marked change of the firing rate, the pattern of firing did not change, i.e. action potentials were generated in a random manner. The other half of neurons did not exhibit significant changes in the firing rate, the tendency to decrease between 20-22 min (27 % on the average) did not reach the level of statistical significance. The mean frequency in the second post-drug interval was exactly the same as before kainic acid

injection (Fig. 1B). These neurons behaved similarly to the control cells (16 neurons in layer V recorded in another experiment) and exhibited only moderate variations of their firing frequency during the same time period (unpublished data). There was no marked difference in the depth where affected and unaffected neurons were localized. Taking all neurons together, the average frequency before kainic acid administration was 5.1±0.8 Hz (n=41) and after administration 6.3±1.2 Hz (n=26) and 8.6±2.0 Hz (n=18), respectively (Fig. 1C). The behavior of three neurons recorded in layer II/III did not differ from that of layer V cells.

In spite of the relatively low number of neurons recorded at all three chosen intervals, our results demonstrate clearly that a part of neurons in cortical layer V (with a high probability pyramidal cells) increased markedly the firing rate under the influence of kainic acid even 20 min after administration. This finding proves an early involvement of cerebral cortex in the epileptic activity induced by kainic acid, at the time when behavioral automatisms (probably of limbic origin) can be observed in freely moving animals (Ben-Ari 1985, Sperk *et al.* 1985). An early involvement of the cerebral cortex was demonstrated especially in immature rats (Cavalheiro *et al.* 1983). The difference between immature and adult animals might be due to an uneven development of kainate type of receptors for excitatory amino acids in the cortex and hippocampus (Miller *et al.* 1990). Direct activation of pyramidal neurons may explain this effect. Kainate receptors were demonstrated especially in layers V and VI of the rat cortex (Unnerstall and Wamsley 1983) but other possibilities have to be taken into account. The most probable mechanism concerns the spread of seizure activity from the hippocampus which is the first structure activated by kainate (Ben-Ari 1985, Sperk 1994).

Only randomly firing neurons were recorded and they maintained their original firing pattern, i.e. the transition into the "bursting" type of activity characteristic for epileptic neurons (Prince 1978) was never seen. Such a transition might be expected in connection with EEG recordings in freely moving rats with implanted electrodes, demonstrating isolated (not very frequent) spikes in the sensorimotor cortical area at the time after injection of the 6 mg/kg dose of kainic acid (Koryntová *et al.* 1997). This failure of neurons to generate bursts of action potentials might be due to the general anesthesia used in the present study but not in EEG experiments.

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Reprint requests

P. Mareš, M.D., D.Sc. Institute of Physiology, Academy of Sciences of the Czech Republic, Vídeňská 1083, 142 20 Prague 4, Czech Republic. e-mail: maresp@biomed.cas.cz