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Hippocampal quinolinic acid concentrations in epileptogenesis in rats

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AIM: To study the changes of hippocampal quinolinic acid (QA) concentrations during acute and chronic seizures induced by ip injection of kainic acid (KA, 12 mg kg^{-1}) in rats. **METHODS**: The extraction and measurement of QA in the hippocampus were performed using a gas chromatography-mass spectrometry method. **RESULTS**: When acute seizures were fully established 3 h after KA injection, no significant changes of hippocampal QA were found. During chronic seizures observed on d 30 after KA injection, there was even a 55±8 % significant decrease. When neither acute nor chronic seizures were detectable but astroglial proliferation in the hippocampus and secondary neuronal degeneration in extrahippocampal regions became gradually prominent 2 d and 7 d after KA injection, there were 56±13 % and 156±13 % dramatic increases of hippocampal QA concentrations, respectively. **CONCLUSION**: The increase of hippocampal QA hardly plays any key role in the initiation of KA-induced seizures but may contribute to astroglial proliferation and neuronal degeneration by activation of *N*-methyl-*D*-aspartate receptors.

KEY WORDS epilepsy; hippocampus; quinolinic acid; kainic acid; mass fragmentography

Since quinolinic acid (QA) in the CNS is the most potent member of kynurenine metabolic pathway in causing convulsions and cell death, QA is worthy of serious consideration as a causative factor in convulsive disorders[1-3]. Our preliminary work found no increase, but a decrease, of QA in cerebrospinal fluid (CSF) of epileptic patients compared with controls[4]. This decrease was neither the effect of antiepileptic medication nor the effect of the causes of seizures[4]. The result did not exclude the possibility that early increases in brain QA were involved in pathogenesis of epilepsy, since all these patients had had recurrent seizures for 6±8 a[4]. In the present study, kainic acid (KA) model of temporal lobe epilepsy[5, 6] was used to analyze the hippocampal QA concentrations at different time points after the establishment of acute and chronic seizures in rats.

MATERIALS AND METHODS

Wistar rats (♂, 50 for KA-treated and 12 for control group) weighing 196±*s* 16 g were decapited at different time points after ip injection of KA (12 mg kg^{-1})[5, 6]: h 3 (n=12), d 2 (n=10), d 7 (n=15), and d 30 (n=13). Control rats (n=12, 3 for each time points) were injected with the solvent phosphate buffer solution (PBS, pH 7.4) alone. After the injection, rat behavior was monitored for 30 d.

The extraction and measurement of QA in the hippocampus were performed using a gas chromatography-mass spectrometry method previously described[7] with some modifications. In brief, the hippocampus was homogenized in 2 mL 80 % ethanol containing 100 μL NaOH 0.5 mol L^{-1} and 2,4-pyridine dicarboxylic acid (2,4-PDA) 2 nmol L^{-1} as internal standard. The hippocampal QA and 2,4-PDA were derivatized to hexafluoroisopropanol (HFIP) ester, which was then dissolved in 10 μL acetone. Two μL of this solution was injected into the gas chromatography-mass spec-

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trometer (GC-MS 2000). The following GC were used: sylanized glass column (2.5 m × 0.2 mm) containing 5 % OV-17 on Chromosorb, 100—200 mesh; helium flow rate: 15 mL min^{-1}; oven temperature 145 ℃; flash heater temperature 190 C. MS conditions were: separator temperature 240 C; electron energy 60 eV; acceleration voltage 3.5 kV. Under such ionization conditions, the predominant ion had mass/charge (m/z) of 272, therefore the content of QA was obtained by comparing the height of its peak with that of its internal standard at m/z 272. Data were expressed as $\bar{x} \pm s$ and statistically analyzed using t test.

RESULTS

The behavioral seizures within 1 d after KA injection were called acute seizures, characterized by "wet dog shakes", forepaws tremor, loss of posture control, and generalized seizures. Three hours after KA injection, the rats exhibited a fully developed status epilepticus. We therefore selected this time point to assess the possible changes of hippocampal QA concentrations which may occur in conjunction with acute seizures. However, no such early changes were detected (Tab 1).

Tab 1. Hippocampal quinolinic acid (QA) concentrations (nmol/g wet tissue) at different time points after ip kainic acid 12 mg kg^{-1}. Number of rats in parentheses. $^bP<0.05$, $^cP<0.01$ *vs* control.

Time after KA	Control	KA
3 h	0.91±0.20 (3)	0.91±0.32 (12)
2 d	0.82±0.26 (3)	1.28±0.31 (10)^b
7 d	0.80±0.13 (3)	2.05±0.43 (15)^c
30 d	0.93±0.25 (3)	0.42±0.15 (13)^b

All the rats showed a relatively "silent" period with no seizure behavior 2—7 d after KA. But compared with control, QA concentrations in the hippocampus increased 56±13 % and 156±13 % on d 2 and d 7, respectively.

The recurrent seizures occurred 8—30 d after KA treatment were called chronic seizures, characterized by generalized seizures. A 55±8 % decrease in QA concentrations in the hippocampus was detected 30 d after KA treatment.

DISCUSSION

The time course of changes of hippocampal QA concentrations was determined in the present study to examine if there was any increase of brain QA which might be involved in the initiation of KA-induced acute and/or chronic behavioral seizures. When acute seizures were well established 3 h after KA treatment, no significant change in hippocampal QA concentrations was detected. Hippocampal QA was even decreased during chronic seizures 30 d after KA injection. The increases of hippocampal QA concentrations were observed 2—7 d after KA administration, which corresponded temporally with neither acute nor chronic seizures, but with a relatively "silent period" for behavioral responses. These results indicated that the activation of NMDA receptors by QA hardly played a key role in the initiation of KA-induced acute and chronic seizures. This conclusion is consistent with the observation that NMDA antagonists failed to block the initiation of KA-induced epileptic burst discharges in hippocampal CA3 neurons[8]. Besides, the decrease in hippocampal QA concentrations during KA-induced chronic seizures is consistent with our previous clinical observations showing that CSF QA concentrations in chronic epileptic patients also decreased markedly[4].

KA causes neuron loss and astroglial proliferation in both hippocampal and some distinct extrahippocampal (distant) regions[5]. These pathological changes were particularly pronounced between 2—7 d after KA injec-

tion, the time which corresponded to the increase of QA concentrations in the hippocampus observed in the present study. Since both QA synthetic enzyme 3-hydroxyanthranilate 3, 4-dioxygenase (3-HAD) and QA degradation enzyme QA phosphoribosyltransferase (QPRT) are localized predominantly in glial cells[1-3,9-11], we propose that the increase of QA concentrations in the hippocampus 2 d and 7 d after KA treatment be related to reactive astrogliotic events. Besides, "distant" extrahippocampal damage was frequently observed 3 — 8 d following KA treatment, which was mediated by secondary release of endogenous excitotoxic NMDA agonist[5], we propose that QA may be such a candidate and may play a causative role in the genesis of secondary neuronal degeneration.

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癫痫形成时大鼠海马喹啉酸浓度的变化

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目的：观察大鼠海马内喹啉酸(QA)浓度在卡英酸(KA)致癫过程的不同时期的动态变化特点. **方法**：采用气相色谱—质谱联用法分离并测定海马 QA 浓度. **结果**：腹腔注射 KA 后3 h 的急性惊厥期海马内 QA 的浓度较对照组相应值无显著变化；注入 KA 后30 d 的慢性惊厥期海马内 QA 的浓度反而较对照组相应值显著减低55±8 %；但在注入 KA 后2及7 d，急性惊厥已基本消失而慢性惊厥尚未出现，但同时海马及海马外某些脑区神经元变性及胶质增生反应显著的时候，海马内 QA 的浓度却分别较对照组相应值显著增高56±13 %及156±13 % ($P<0.01$). **结论**：海马内 QA 浓度的增高对 KA 所致惊厥行为的产生可能不起关键作用，但可能通过激活 NMDA 受体参与神经元变性及胶质增生反应.

关键词 癫痫；海马；喹啉酸类；卡英酸；质量碎片质谱法