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EFFECTS OF KAINIC ACID ON GLUTATIONE AND NITRITE IN RAT HIPPOCAMPUS

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ABSTRACT

Epileptiformic activity could result in apoptotic neuronal death, in which oxidative stress could play an important role. In case of decreased antioxidant brain status cellular death could be facilitated. Kainic acid is often used in a model of epilepsy in rats. Up to now there is not enough data evaluating levels of glutathione and nitric oxide in kainic acid-induced epilepsy acutely and several days after the kainic acid exposure. This information will be useful for assessing long term prognosis on a risk of further brain damage.

We studied hippocampal levels of glutathione and nitric oxide at the 3th hour (acute group) and after 7 days of kainic (chronic group) acid exposure.

We found that glutathione level is statistically significantly lower in the hippocampus 7 days after kainic acid exposure, as compared with values measured in the acute group. For both kainic acid treated groups glutathione levels were significantly lower than controls.

Levels of nitric oxide were found to be significantly higher 7 days after kainic acid exposure as compared with acute group. For both kainic acid treated groups nitric oxyde levels were significantly lower than controls.

We conclude that in kainic acid treated rats oxidative stress could be present even after a single treatment. This could be a potentially pathogenic factor for further brain damages.

Key words: kainic acid, glutathione, nitric oxide

Kainic acid (KA) is a potent CNS exitotoxin, producing acute and chronic epilepticform activity [1, 2]. KA induces apoptotic and necrotic cell death in hippocampus and cortical regions of rat brain. It is well documented that reactive oxygen species (ROS) play roles in cell signaling as well as in apoptosis [3] . We also demonstrated that the reactive oxygen species (ROS) and oxidative stress play roles in neuronal apoptosis in hypothalamus [4]. Additionally, ROS have been implicated in pathogenesis of epilepsy [5]. The ability to control ROS is critical in epilepsy, because neuronal damages occur when the endogenous "anti-oxidant-oxidant" balances are disrupted. Several reports suggest that nitric oxide (NO) may increase during epileptic seizures [6, 7]. It was reported earlier that the stimulation of glutamate-KA receptors induces neuronal NO release [8]. There are not enough data documented the effects of KA on the endogenous anti-antioxidant status in rat hippocampus. As the epilepsy is a chronic disease leading to neuronal death, and ROS are implicated in mechanisms of cell death, the studies comparing chronic and acute group treated with KA may provide information on the role of both antioxidant and oxidant status in pathogenesis of epilepsy. The aim of this study was to investigate the effect of kainic acid on the levels of glutathione (GSH) and nitrite in rat hippocampus.

MATERIALS AND METHODS

Animals and treatments

Twenty adult Wistar rats, weighing 180-200 g, were used in the present study. All animals were maintained on a 12 : 12 h light : dark cycle and given continuous access to food and water. Animals received a single i.p. injection of 5 mg/kg bw of KA or vehicle. The acute group animals were killed after 3 h and the chronic group animals were killed after 7 days of single injection of KA, the brains were removed and hippocampus tissues were dissected on ice.

Glutathione (GSH) assay. The cellular levels of glutathione were determined in umol/L/ug of protein by Glutation ASSAY (Calbiochem, USA). Briefly, cells suspensions from hippocampuses were taken for determination of glutathione levels by following the procedure of manufacture.

Nitrite assay. As an indicator of nitric oxide production, the amount of nitrite accumulated in the cells of hippocampus was determined with a calorimetric assay using Griess reagent (1% sulfanilamide, 2.5% H3PO4, 0.1% N-(1-naphthyl) etylenediamine dihydrochloride). Briefly, 50 µl of Gries reagent and 50 µl of the cells suspended supernatant were incubated in the dark at room temperature for 10 min. After incubation, the absorbance at 540 nM was determined with the Spectra Max Plus microplate spectrophotometer. The simple nitrite concentrations were determined from a sodium nitrite standard curve.

Statistical analysis. The data shown in the figures and text are mean ± S.E.M. Data comparisons among multiple groups were made using one-way analysis of

variance. Post hoc tests involved the Student-Newman-Keuls test. A value of $P < 0.05$ was considered significant

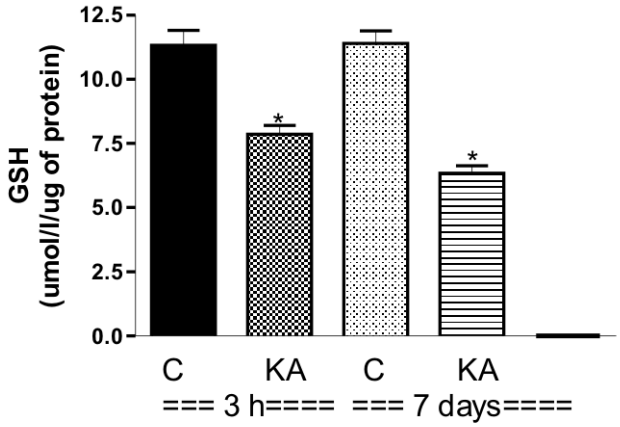

Figure 1. Levels of glutathione in rat hippocampus in all treatment with KA groups. Animals received a single i.p. injection of 5 mg/kg bw of KA or vehicle. The groups of animals (control-C and experimental-KA) were decapitated after 3h (acute experiment) or after 7 days (chronic study). Results are given as umol/l/ug of protein. Data are mean ± SEM. Each group has four animals.

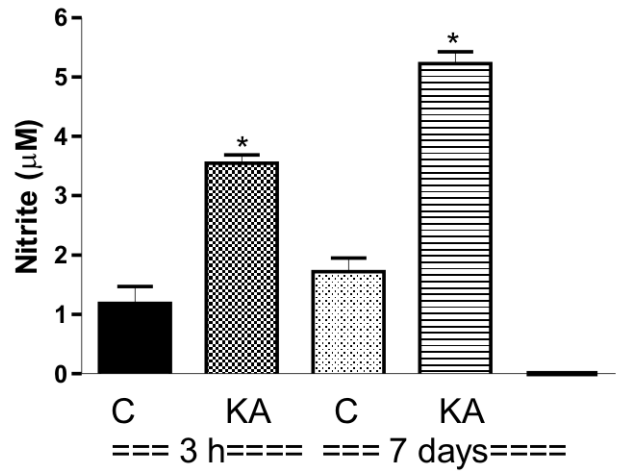

Figure 2. Effect of KA on nitrite in rat hippocampus. Animals received a single i.p. injection of 5 mg/kg bw of KA or vehicle. The groups of animals (control-C and experimental-KA) were decapitated after 3h (acute experiment) or after 7 days (chronic study). Results are present as nitrite (μM). Data are mean+SEM. Each group has 4 animals.

RESULTS AND DISSCUSION

Our results demonstrate that KA decreases the endogenous antioxidant glutathione (fig.1) and increases the levels of NO-nitrites (fig.2) in rat hippocampus. The comparison between acute and chronic effects of KA shows differences. Chronic group of rats have low level of GSH and high concentration of nitrite as compared with the acute group. Kainic acid is a potent glutamate agonist and has been used to induce experimental seizures in animals [1, 2, 9]. It has been shown that KA treatment causes DNA damages in hippocampal neurons and induces cell death [8]. We have been focusing on role of ROS in neuronal cell death [4]. It is well documented that there is balance between antioxidant and oxidant status in neurons. Various substances have capacity to disrupt the physiological free radicals balance in neurons. In our previously studies, we determined that TNF-alpha with or without ethanol disrupted the balance in hypothalamic neurons and caused apoptosis [10]. Here, we demonstrate that KA decreased the levels of endogenous antioxidant GSH. It is well known that GSH is essential for cellular detoxification of reactive oxygen species in brain cells [11, 12, 13]. Increased production of ROS and/or decreased in the antioxidant capacity of cells caused oxidative stress. Compared with other organs as kidney or liver, which cells are able to catalyze the generation of ROS, brain cells contain only low to moderate superoxide dismutase, catalase and glutathion peroxidase activity [14, 15]. This disadvantage of brain cells plays a key role in neurological diseases such as epilepsy. The present study demonstrates the increased concentration of NO and support the involvement of NO in pathogenesis of epilepsy. The experimental findings implicated neuronal NO generation in the pathogenesis of excitotoxic neuronal injuries in vivo [16]. The increase concentrations of NO and decreased levels of GSH support the role of oxidative stress in KA mediated epilepsy.

CONCLUSIONS:

Taken together, the present data demonstrate that KA disrupts the balance between endogenous GSH and nitrite in rat hippocampus. The results suggested that the oxidative status in neurons may play a role in epilepsy.

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