Repeated Kainic Acid Administration and Hippocampal Neuronal Degeneration

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Abstract: Many animal models have been established to study the mechanisms leading to excitotoxicity. One of the more commonly used models is kainic acid (KA) induced excitotoxicity. Upon administration of KA in rodents, KA produces acute status epilepticus and neuronal damage. The aim of the study was to examine the morphologic alteration in the hippocampus of mature rats, after repeated KA administration. The first group was given KA repeatedly in six doses (10 mg / 1000 g), each second day. The second group was given KA i.p. repeatedly in six smaller doses (5 mg / 1000 g), each second day. The third group (control animals) received corresponding volumes of the normal saline (5 or 10 mg / 1000 g respectively). Animals were transcardially perfused; serial sections were stained with Fluoro-Jade B and DNA-specific dye bis-benzimide (Hoechst). In CA1 region of the first group many degenerating cells were observed. The CA2 region was not as much affected as CA1. In the CA3 region no degenerating cells were observed. In the second group the most prominent was the cell loss both in the CA3 region and in the hilus of the dentate gyrus.

Keywords: Kainic acid – Hippocampus – Neuronal degeneration – Rat

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Introduction

Many animal models have been established to study the mechanisms leading to excitotoxicity. One of the more commonly used models is kainic acid (KA) induced excitotoxicity. KA is a cyclic analogue of glutamate that is known to depolarise both pre- and postsynaptic cells by interaction with the non-NMDA type of glutamate receptor. Upon administration of KA in rodents, KA produces acute status epilepticus and neuronal damage that is restricted to a discrete reproducible spatial pattern that includes lesions in the pyramidal CA1 and CA3 regions of the hippocampus. [1]

In the human brain more than fifty percent of all synapses use glutamate (Glu) as a transmitter. The postsynaptic activity of Glu is mediated by both ionotropic receptors (iGluRs), ligand-gated ion channels, and metabotropic receptors (mGluRs), which are coupled to G-proteins. The iGluRs are constituted by different subunits and classified into the following three heterogeneous types based on the acronym of specific agonists: NMDA (N-methyl-D-aspartic acid), AMPA [(R, S)-2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl) propionic acid] and KA (kainic acid) receptors. [2, 3]

Kainate receptors were originally defined by Watkins and co-workers [4, 5] based on the pharmacology of neuronal responses to excitatory amino acids. The physiological properties of kainate receptors [6, 7] and their roles in synaptic transmission [8, 9, 10], have been discerned only recently. [11, 12, 13]. Although it is well established that kainate receptors constitute an entirely separate group of proteins from AMPA receptors, their physiological functions remain unclear [14].

Succession of nerve cell extinction after a neurotoxic lesion (induced by KA) can reveal some elementary principles of neuroplasticity and can help to understand the relation among components of neuronal circuits.

Material and methods

The experiment was performed using male Wistar albino rats of the body weight 250 to 300 g. Animals were divided into three groups. In the first group KA was administered repeatedly in six doses (10 mg / 1000 g), each second day. Two days after the last application animals were transcardially perfused under the deep pentobarbital anaesthesia with a fixation solution (neutral paraformaldehyde at the room temperature). Brains were removed from the skull and postfixed in the same solution for 24h in a refrigerator. Serial 40 μ cryostat sections were stained with Fluoro-Jade B and DNA-specific dye bis-benzimide (Hoechst), and with Nissl staining. The stained sections were dehydrated, cleared with xylene and mounted in DPX. Material was examined under the epifluorescent microscope Olympus AX-70. The second group of animals was KA administrated i.p. repeatedly in six smaller doses (5 mg / 1000 g), each second day. After last application brains were processed in the same way. The third control group of rodents received corresponding volumes of the normal saline (10 mg, 5 mg / 1000 g KA respectively).

Results

In the first group of animals, which repeatedly received higher doses of KA, the following neuropathological changes have been found: In the CA1 region many degenerating cells were observed and the most affected structure was the layer of pyramidal cells (Colour fig. 7). Numerous glial cells replaced the neuronal population. The CA2 region was not as much affected as CA1. The glial response was less prominent. In the CA3 region no degenerating cells were observed (Colour fig. 8). A massive glial response was observed in this region. The same pathomorphological changes were found in the region of the hilus of dentate gyrus. No obvious pathologic changes were found in the region of the dentate gyrus (both blades, dorsal and ventral) (Colour fig. 9).

In the second group of animals, which repeatedly received lower doses of KA, the most prominent was the destruction of both the CA3 region and the hilus of the dentate gyrus (Colour fig. 10, 11). Almost all pyramidal cells were destroyed and a massive glial response was observed. Also in the CA1 region degenerating cells were found (Colour fig. 12), however the part between CA1 and CA2 regions remained intact. In control animals neuropathological analysis did not detect any changes in the hippocampus.

Discussion

The observed neuropathological changes and their mutual comparison lead us to the conclusion that the most affected structure of the hippocampus is the CA3 region which was almost completely destroyed by the administration of reduced doses.

The CA1 region appears to be more resistant to KA than CA3, yet it remains largely destroyed within a longer period of time. Most resistant are both blades of the dentate gyrus, where no acute morphological changes were observed.

The key question arises whether the observed changes are due to neurotoxic effect of KA, or to what extent they may reflect massive neuronal cells depolarisation, that leads to neuronal death.

The contribution of seizure activity itself to cell damage must be determined on the basis of experiments designed to prevent seizures induced by the i.p. application of KA. It seems very likely that prolonged seizure activity induced by KA contributes to the observed neuropathological changes as was shown in other models in both the adult and immature animals. The indirect support for this view is the fact that morphological changes of similar character could not be detected after the application of sub convulsive (albeit rather high) doses of some excitotoxic acids [15]. The staining procedure and microscopic evaluation used in our experiments do not allow deciding whether the cells died of the necrotic or apoptotic process.

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