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Differential effects of vigabatrin and zonisamide on the neuropeptide Y system in the hippocampus of seizure prone gerbil

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Abstract

Changed neuropeptide Y (NPY) system in the hippocampus has been reported in various experimental epileptic models. However, there have been little data concerning the alteration in the NPY system in the epileptic hippocampus following treatment of anti-epileptic drugs (AEDs). In the present study, therefore, we performed analyses of effects of vigabatrin (VGB) and zonisamide (ZNS) treatment on the NPY system in the hippocampus of the seizure sensitive (SS) gerbils. In SS gerbil, NPY immunoreactivity in the hippocampus was lower than that in seizure resistant gerbil. Following VGB treatment, the number of NPY immunoreactive neurons and NPY mRNA expression were increased in the hilus and the hippocampus proper. In contrast, ZNS treatment markedly elevated only the density of NPY immunoreactive fibers in the dentate gyrus, not in the hippocampus proper, as compared with saline-treated animals. These patterns were observed in the dose-dependent manners. These findings suggest that AEDs treatments may distinctly affect the NPY system in the SS gerbil hippocampus.

Keywords: Neuropeptide Y; Hippocampus; Gerbil; Epilepsy; Zonisamide; Vigabatrin

1. Introduction

Neuropeptide Y (NPY) is supposed as one of the endogenous anticonvulsive substances, since NPY coexists with γ-aminobutyric acid (GABA) in neurons of the dentate gyrus and it inhibits the excitatory neuronal transmission (Colmers and Bahh, 2003). This suggestion is supported by a previous report demonstrating increase in seizure susceptibility in NPY-deficient mice (DePrato Primeaux et al., 2000). In addition, status epilepticus induced by electrical stimulus has been reported to cause a long-lasting elevation of NPY immunoreactivity, especially noted in the hippocampus (Husum et al., 2002). It is thus suggested that the biosynthesis of NPY in the hippocampus may contribute to the seizure susceptibility in the animal models of epilepsy.

On the other hand, the hypothesis of seizure activity is based on the impaired inhibitory transmission in the brain. Thus, the strategy of developing of anti-epileptic drug (AED) and the studies on epileptogenesis are focused on the metabolism of γ-aminobutyric acid (GABA), the major inhibitory neurotransmitter in the central nervous system. Although anti-epileptic drugs (AEDs) affect various neurotransmitter systems in the

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hippocampus (Kang et al., 2003a,b), little evidence supports the relationship between AEDs on the NPY system. Thus, the issue remains to be clarified as to whether AEDs may affect the NPY system in the hippocampus. In the present study, therefore, comparative analysis of the NPY system following two distinct AEDs, vigabatrin (VGB) and zonisamide (ZNS, 1,2-benzisoxazole-3-methanesulfonamide), was conducted in Mongolian gerbil (one of genetic epilepsy models), since the deficiency of NPY in the hippocampus may be one of the factors in seizure activity in this animal (Kang et al., 2000a).

2. Materials and methods

2.1. Experimental animals and drug treatments

This study utilized the progeny of Mongolian gerbils (*Meriones unguiculatus*) obtained from Experimental Animal Center, Hallym University, Chunchon, South Korea. Animals were housed at constant temperature (23 °C) and relative humidity (60%) with a fixed 12 h light/dark cycle and free access to food and water. Procedures involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with international laws and policies (NIH Guide for the Care and Use of Laboratory Animals, NIH Publication No. 85-23, 1985). Each animal was tested a minimum of three times as described by Paul et al. (1981). Only animals with a consistent stage 4 or 5 seizure score, according to the seizure severity rating scale of Loskota et al. (1974), were included in the present study as SS gerbils. Forty SS gerbils (about three-month old) were used in the present experiment. Animals were divided into five groups ($n = 8$, respectively), and each group was given below drug once a day for 1 week: (1) vigabatrin (VGB, γ-vinyl-GABA, Sigma USA, 30 mg/kg, IP); (2) VGB (15 mg/kg, IP); (3) zonisamide (ZNS, Eisai Korea Inc., Korea, 30 mg/kg, IP); (4) ZNS (15 mg/kg, IP); (5) saline. Eight SR gerbils were used as normal control. Prior to tissue processing, each animal was tested three times using the methods described above for checking behavioral effects (Kang et al., 2003d).

2.2. Tissue processing and immunohistochemistry

One hours after the last injection, animals were anesthetized with ketamine, and perfused via the ascending aorta with 200 ml of 4% paraformaldehyde in phosphate buffer (pH 7.4). The brains were removed, postfixed in the same fixative for 4 h and rinsed in PB containing 30% sucrose at 4 °C for 2 days. Thereafter the tissues were frozen and sectioned with a cryostat at 30 μm and consecutive sections were collected in six-well plates containing phosphate buffered saline (PBS). These free-floating sections were first incubated with 10% normal goat serum for 30 min at room temperature. They were then incubated in the rabbit anti-NPY antiserum (diluted 1:1, 500, Peninsula, USA) in PBS containing 0.3% Triton X-100 and 2% normal goat serum overnight at room temperature. After washing three times for 10 min with PBS, sections were incubated sequentially, in goat anti-rabbit IgG (Vector, USA) and streptavidin (Vector, USA), diluted 1:200 in the same solution as the primary antiserum. Between the incubations, the tissues were washed with PBS three times for 10 min each. The sections were visualized with DAB in 0.1 M Tris buffer and mounted on the gelatin-coated slides. The immunoreactions were observed under the Axioscope microscope (Carl Zeiss, Germany). In order to establish the specificity of the immunostaining, a negative control test was carried out with pre-immune serum instead of primary antibody. The negative control resulted in the absence of immunoreactivity in any structures.

2.3. In situ hybridization

For in situ hybridization study, the 28-mer antisense oligodeoxynucleotide probe (Sigma Genosys Australia Pty. Ltd) of the following sequence, complementary to nucleotides 195–222 of NPY cDNA (BC043012) was used to label NPY mRNA: 5′-GAG TAG TAT CTG GCC ATG TCC TCT GCT G-3′ (Hwang et al., 2004). The antisense oligodexoynucleotide probe was labeled with biotin using the Fast-Tag oligonucleotide labeling kit (Vector, USA) by the manufacturer's protocol (http://www.vecterlabs.com). In situ hybridization was carried out by previous protocols (Kang et al., 2003c). In order to establish the specificity of the in situ hybridization, pre-treatment with RNase A was performed, which showed the absence of reactivity in any structure.

2.4. Quantitation of data and statistical analysis

Cell counts were carried out with a computerized image analysis system (Leica image scale). Sections (15 sections per each animal) were viewed through a microscope connected via a CCD camera to a PC monitor. At a magnification of 25–50×, the hippocampal regions were outlined and their surface areas measured. NPY positive neurons were counted by clicking on the monitor, at a magnification of 100×. All NPY immunoreactive cells were counted regardless the intensity of labeling. Cell counts were performed by two different investigators who were blind to the classification of tissues. The estimated cell number (n) was the average of values from three adjacent sections. Since the nucleus size measurement was used to correct the potential sampling bias, the area measurement for each nucleus in the sample population was also measured at a magnification