The over-expression of somatostatin in the gerbil entorhinal cortex induced by seizure

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Abstract

In present study, we investigated the immunohistochemical distribution of somatostatin (SRIF) in the hippocampal complex of the Mongolian gerbil and its association with different sequelae of spontaneous seizures, in an effort to identify the roles of SRIF in the self-recovery mechanisms in these animals. In the dentate gyrus and subiculum, SRIF immunoreactive (SRIF\textsuperscript{+}) cells were similar in both the seizure resistant and the pre-seizure group of seizure sensitive gerbils. Interestingly, SRIF immunoreactivity was markedly decreased until 12 h postictal. Twenty-four hours after the on-set of seizure, the distribution of SRIF immunoreactivity in these regions had slightly increased. In contrast, in the entorhinal cortex the population of SRIF\textsuperscript{+} cells and their density were significantly elevated compared to pre-seizure group 30 min postictal. Twelve hours after the on-set of seizure, however, the population of SRIF\textsuperscript{+} cells and their density declined, approximately 70–80\% compared to the situation at 30 min postictal. These findings suggest that the enhancement of SRIF expression in gerbil entorhinal cortex may affect tissue excitability and have a role in modulating recurrent excitation following seizures.

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1. Introduction

The Mongolian gerbil provides an opportunity for studying the mechanisms of epileptogenesis, because naturally epileptic and non-epileptic animals can be compared directly to detect differences in brain anatomy and electrophysiology that correlate with seizure behavior. In particular, these animals also provide an opportunity to investigate self-recovery mechanisms after seizure on-set [3,13,17].

Recent studies [8,12,29] have demonstrated that seizure activity in experimental models leads to dramatic changes in the phenotypic capacities of different classes of neurons, particularly in the hippocampus. It is noteworthy that the expression of various neuropeptides in distinct forebrain areas and their release from neurons, and the density and/or affinity of their receptor subtypes are profoundly changed by seizures. Many previous studies have indicate that somatostatin (SRIF) containing neurons in the hippocampus play an important role in hippocampal excitability in epilepsy, because: (i) SRIF is preferentially released from neurons during seizures [1,9,28]; (ii) Marked changes in the expression of SRIF mRNA, the levels of the peptide and its receptors occur after experimentally induced seizures and in human epileptic tissue; and (iii) intracerebral injections of SRIF, its analogues or SRIF-specific antibodies affect seizures and epileptogenesis in rats (reviews [21,29]).

In the hilus of the dentate gyrus, distinct populations of peptidergic neurons are affected by seizures and seizure-induced cell damage. A subset of GABAergic interneurons

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containing SRIF are highly vulnerable to damage induced by sustained stimulation of the perforant path or by kainate-induced seizures in rats [24–26] and in human temporal lobe epilepsy specimens [19]. The same neurons, however, show increased SRIF after repeated electroconvulsive shocks and enhanced mRNA expression in kindled rat [2,16,23]. Furthermore, in the Mongolian gerbil, altered SRIF expression has not been definitively determined; Wolf-Dieter et al. [31] reported that SRIF levels elevated in a seizure sensitive group, though Bucikmaster et al. [4] observed the number of SRIF immunoreactive (SRIF\(^+\)) neurons in the dentate gyrus was not significantly different in seizure resistant and seizure sensitive groups. Therefore, in the present study, we investigated the immunohistochemical distribution of SRIF in the hippocampal complex of the Mongolian gerbil and its association with different sequelae of spontaneous seizure to identify the roles of SRIF in the recovery mechanisms in these animals.

2. Materials and methods

2.1. Experimental animals

This study utilized the progeny of Mongolian gerbils (Meriones unguiculatus) obtained from Experimental Animal Center, Hallym University, Chuncheon, South Korea. The animals were housed at constant temperature (23\(^\circ\)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. [17]. Only animals with a consistent stage four or five seizure score, according to the seizure severity rating scale of Loskota et al. [15], were included in the present study as SS gerbils. SR gerbils never demonstrated the seizure activity, thus they were assigned seizure severity scores of zero.

2.2. Tissue processing and immunohistochemistry

Fifty seizure sensitive (SS) and ten seizure resistant (SR) gerbils (about 8-months-old) were used in the present experiment. To examine the temporal changes of SRIF expression following seizure, SS gerbils were divided into five groups; pre-seizure group (n=10), post-seizure group I, II, III and IV (n=10, respectively) that recovered normally at 30 min, 12, 24 or 36 h after the on-set of tonic–clonic generalized seizure, respectively [12]. The gerbils were anesthetized with pentobarbital sodium, and perfused via the ascending aorta with 200 ml of 4% paraformaldehyde in phosphate buffer. The brains were removed, post-fixed in the same fixative for 4 h and rinsed in PB containing 30% sucrose at 4\(^\circ\)C for 2 days. Thereafter the tissues were frozen and sectioned with a cryostat at 30 \(\mu\)m 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-SRIF antiserum (diluted 1:2000, Peninsula, USA), which not cross-react with prosRIF, 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 Axioskop 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. Quantitation of data and statistical analysis

Cell counting was performed on the sections incubated for immunohistochemistry to determine the relative number of SRIF\(^+\) neurons per section. SRIF\(^+\) soma were counted in each group of the hippocampal complex. Cell counts were carried out with a computerized image analysis system (Leica image scale). Sections were viewed through a microscope connected via CCD camera to a PC monitor. At a magnification of 25–50\(\times\), the region was outlined on the monitor and measured their area. Neurons per-mm\(^2\) were counted by clicking on their image on the monitor, at a magnification of 100\(\times\). Images of SRIF immunoreactivity in hippocampal complex were also captured with an Applescanner. The brightness and contrast of each image file were uniformly enhanced by Adobe Photoshop version 2.4.1, followed by analysis using NIH Image 1.59 software. Values of background staining were obtained and subtracted from the SRIF immunoreactive intensities.

All data obtained from the quantitative data were analyzed using one-way ANOVA test to determine statistical significance. Bonferroni’s test was used for post-hoc comparisons. P-value below 0.01 or 0.05 was considered statistically significant.

3. Results

3.1. Hippocampus proper

SRIF\(^+\) neurons were scattered in the hippocampal
proper. Its immunoreactivity was highly localized within the cell bodies and extended throughout their short processes. On the basis of their localization and morphology these cells were identified as interneurons. Their morphologies, population and immunodensities were similar in all groups (data not shown).

3.2. The dentate gyrus

In the present study, SRIF$^+$ cells in the dentate hilus were usually observed with multiple processes. In the pre-seizure group of SS gerbils, neurons in the dentate hilus, were strongly stained for SRIF, than was the case in SR group (Figs. 1A, B and 5). The populations of SRIF$^+$ neurons were similar in these two groups, 17.9±6.08 and 18.2±4.07 per-mm$^2$, respectively (Fig. 4). The distribution pattern of SRIF$^+$ neurons was similar in pre-seizure and post-seizure groups, however, SRIF immunoreactivity was weaker in post-seizure group I ($P<0.01$). In post-seizure group II, the number of SRIF$^+$ cells (4.3±2.11 per-mm$^2$) had significantly declined due to loss of SRIF immunoreactivity in the neurons (Figs. 1C, 4 and 5). The density of SRIF immunoreactivity and the number of SRIF$^+$ cells (7.4±3.23 per-mm$^2$) in post-seizure group III were slightly elevated compared to post-seizure group II (Fig. 1D).

Thirty-six hours after seizure on-set, the SRIF density recovered to the SS level (Figs. 4 and 5).

3.3. The subiculum

In the subiculum, SRIF$^+$ cells were similar pattern to hippocampus proper, usually with short multiple processes. There were no differences in the SRIF immunoreactivity of the SR and pre-seizure group of SS gerbils (Fig. 2A). The

Fig. 1. Showing coronal sections of the dentate hilus of SR and SS gerbils. Compared to the case of SR (A), neurons in the dentate hilus, are strongly stained for SRIF in the pre-seizure group of SS gerbils (B, arrows). At 12 h postictal, the number of SRIF$^+$ cells was significantly declined due to the loss of SRIF immunoreactivity in the neurons (C). The density of SRIF immunoreactivity 24 h after the on-set of seizure had slightly elevated (D, arrows). GL: granular layer. Bar=30 μm.
The population of SRIF$^+$ cells per-mm$^2$ were $11.1 \pm 4.01$ and $12.2 \pm 4.91$, respectively (Fig. 4). Interestingly, the SRIF$^+$ immunoreactivities were markedly decreased in post-seizure groups I and II (Fig. 2B and 5). The population of SRIF$^+$ cells was also declined, $9.4 \pm 3.21$ and $1.3 \pm 2.19$ per-mm$^2$, respectively (Fig. 4). In these groups of SS gerbils, neurons in the subiculum were nearly devoid of SRIF immunoreactivity. In post-seizure group III, the distribution of SRIF immunoreactivity in the subiculum was slightly increased, thus the pattern of SRIF immunoreactivity observed in the subiculum was similar to that of the dentate hilus (Figs. 2C, 4 and 5).

### 3.4. The entorhinal cortex

In the entorhinal cortex, SRIF$^+$ neurons were densely localized in layers II–V with multiple processes. The numbers of SRIF$^+$ cells ($15.2 \pm 3.99$ and $14.2 \pm 4.24$ per-mm$^2$, respectively) and their density in SR and pre-seizure groups were similar (Fig. 3A and B). In the post-seizure group I, the population ($36.2 \pm 7.87$ per-mm$^2$) and density of SRIF$^+$ cells were significantly elevated compared to pre-seizure group (Figs. 3C, 4 and 5). In post-seizure groups II and III of SS gerbils, however, the population of SRIF$^+$ cells ($14.5 \pm 5.23$ and $9.3 \pm 4.31$ per-mm$^2$, respectively) in the EC had significantly decreased. Particularly, in post-seizure group III the population of SRIF$^+$ cells decreased by approximately 70–80% of post-seizure group I (Figs. 3D, 4 and 5). Thirty-six hours after seizure on-set, the level of SRIF density has recovered to the SS level.

### 4. Discussion

Many previous studies [18,20–22,29] in the hippocampal complex have indeed shown that SRIF has an inhibitory action on the spontaneous activity of pyramidal cells, and that long-duration pressure ejection or bath application of the peptide induces dendritic hyperpolarization. The stimulation of SRIF receptors in the hippocampus suppressed chronic susceptibility to metrazole-induced convulsions in kainic acid-treated rats. Furthermore, seizure-induced damage to SRIF neurons in the dentate hilus has been demonstrated in several experimental models of epilepsy [5,10,22,25–27,32].

In the present study, the similar distribution pattern of SRIF$^+$ neurons was observed, with the exception of the intensity of SRIF immunoreactivity in the pre-seizure group, and in the dentate gyrus and the subiculum of both SR and pre-seizure groups. These results are consistent with previous studies, which reported population similarities in these regions in both SR and SS gerbils [4] and the enhancement of its expression in the surviving SRIF neurons [26,27].

In our study a decline of SRIF immunoreactivity in the dentate gyrus and subiculum were observed over the first
Fig. 3. Showing coronal sections of the entorhinal cortex of SR and SS gerbils. The numbers of SRIF \(^+\) cells and their density in SR and pre-seizure groups are similar to each other (A and B). In the post-seizure group I, the population of SRIF \(^+\) cells is significantly elevated compared to pre-seizure group (C). In the post-seizure groups III of SS gerbils, however, the population of SRIF \(^+\) cells in the entorhinal cortex is significantly decreased (D). IV: layer IV in the entorhinal cortex, respectively. Bar=30 \(\mu\)m.

On the other hand, we are able to observe an increase of SRIF expression in the entorhinal cortex 30 min postictal, and a decline in SRIF immunoreactivity until 12 h postictal. This chronological data is very interesting when compared to the kainate-induced seizure model [30], which does not involve a change in SRIF expression in the entorhinal cortex. This suggests, that unlike the kainate induced seizure model, this peptide play an important role in regulating neuronal activity into and out of the hippocampal complex during the early seizure stage in the gerbil. Because hippocampal output fibers from CA1 and the subiculum, strongly activate deep layers of the entorhinal cortex, which can then drive synchronous discharges to the superficial layers (II and III). Layer II, which sends the major excitatory input to the granular cell layer in the

12 postictal. These results indicate that the release of SRIF may attenuate the seizure activity; hippocampal SRIF neurons form symmetric synapses consistent with an inhibitory function [6,14], and SRIF is released at a higher rate than other peptides during epileptogenesis, not allowing SRIF accumulation in the neurons [30].

The recovery of SRIF immunoreactivities in these regions was detected 36 h postictal. This finding suggests that SRIF may be stored in the neuron perikarya as a consequence of enhanced synthesis, which is supported by previous reports [26,27] described that SRIF immunoreactivity was strongly decreased in the hippocampus 3 days after the injection of kainate, but recovered after 10 days as a result of the enhancement of SRIF expression in the interneurons of the dentate gyrus.
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