NOTE *Anatomy*

Reduced Immunoreactivity of Tyrosine Hydroxylase in the Hypothalamic Paraventricular Nucleus of the Seizure Sensitive Gerbil

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ABSTRACT. We compared the immunoreactivity and numbers of tyrosine hydroxylase (TH) immunoreactive neurons and neuropil in the paraventricular nucleus (PVN) of the hypothalamus between the seizure sensitive (SS) and seizure resistant (SR) gerbils. The distributional pattern of TH immunoreactivity was similar in both groups: TH immunoreactivity was seen mainly in magnocellular neurons of the PVN. However, total TH immunoreactivity in the neurons and neuropil in the SS gerbils was significantly lower than that in the SR gerbils. In addition, the number of TH immunoreactive neurons in the SS gerbils was also much lower than those in the SR gerbils. These results indicate that SS gerbils have a low TH immunoreactivity in the hypothalamic PVN compared with that in SR gerbils.
KEY WORDS: Mongolian gerbil, seizure, Tyrosine hydroxylase.

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The hypothalamic paraventricular nucleus (PVN) is known to be a major integrative center of the autonomic, physiologic and endocrine regulations to maintain homeostasis [32]. The PVN consists of magnocellular and parvocellular neuroendocrine neurons and autonomic-related neurons [31, 33]. Tyrosine hydroxylase (TH), the first and rate-limiting enzyme in catecholamine synthesis converting L-tyrosine to L-DOPA [20], is primarily found in the magnocellular neurons of the PVN in humans, rats and rabbits, and so on [12, 18, 29]. Li *et al.* [12] demonstrated that TH immunoreactive cells accounted for about 40% of paraventricular magnocellular neurons in the human. In addition, catecholaminergic system including TH may influence vasopressin release via synaptic mechanisms [6].

Seizure is a transient, synchronous and rhythmic firing of populations of central nervous system neurons [17]. It has been known that seizure produces a number of biochemical changes in the brain such as releases of many neurotransmitters, inductions of selected enzymes and the up or down regulation of receptors [30].

Some researchers have reviewed the several genetic models of epilepsy; photosensitive baboon (*Papio papio*), audiogenic seizure-prone mice, genetically epilepsy-prone rats (GEPRs), Mongolian gerbils (*Meriones unguiculatus*), photosensitive fowl, tottering mice, and epileptic dogs [10, 14]. Among these epilepsy models, the Mongolian gerbil is a good animal model of inherited epilepsy and exhibits spontaneous tonic-clonic motor seizures in response to a variety of stimuli [3, 15]. Based on the neuroanatomy and electrophysiology, Mongolian gerbils are hereditary seizure prone or seizure resistant to be directly compared [15].

Few studies have evaluated the morphological characteristics of the TH containing neurons in the PVN of the Mongolian gerbil. Therefore, we examined the difference of TH expression in the PVN between the seizure sensitive (SS) and seizure resistant (SR) gerbils.

This study used the Mongolian gerbils (*Meriones unguiculatus*) obtained from the Experimental Animal Center, Hallym University, Chuncheon, South Korea. They were housed individually in a light- and temperature-controlled room (12:12-hr light-dark cycle, 24 ± 1°C). The animals were provided with food and water *ad libitu*m. All animal care and experimental procedures were approved by the Animal Care and Use Committee at Seoul National University and conformed to the NIH guidelines for the Care and Use of Laboratory Animals. All experiment was conducted to minimize the number of animals used and suffering used in this study.

Ten SS and SR male gerbils (about 3 months old; 70 ± 5 g body weight) were used in the present experiment. Each animal was stimulated by vigorous stroking on the back with a pencil, as described by Paul *et al.* [24] and tested a minimum of 5 times. The motor and behavioral characteristics, as well as the severity of the seizures, were graded from stage 0 (no seizure) to stage 5 (tonic-clonic convulsions with automatisms, opisthotonos and rollover) [15]. According to the seizure severity rating scale, only animals with a consistent stage 4 or 5 seizure score were included in the present study as SS gerbils. SR gerbils did not demonstrate the seizure activity, namely stage 0. SS gerbil (pre-seizure state) did not show seizure activities at least 72 hr prior to the per-

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fusion.

The animals were anesthetized with a mixture of ketamine (50 mg/kg) and xylazine (10 mg/kg) and perfused via ascending aorta with 0.1 M phosphate-buffered saline (PBS, pH 7.4) followed by 4% paraformaldehyde in 0.1 M PBS (pH 7.4). The brains were removed, post-fixed in the same fixative for 4 hr and cryoprotected by infiltration with 30% sucrose overnight. The brain tissues were sectioned with a cryostat at 30-μm consecutive coronal sections: Coronal sections from 0.7 mm to 1.1 mm posterior to bregma were used [16].

The sections were sequentially treated with 0.3% hydrogen peroxide for 20 min and 10% normal goat serum for 30 min. The sections were then incubated in rabbit anti-TH antiserum (1:400. Chemicon International, Temecula, U.S.A.) for 48 hr at 4°C and subsequently exposed to biotinylated goat anti-rabbit IgG and streptavidin peroxidase complex (1:200, Vector, Burlingame, CA). And they were visualized with 3,3'-diaminobenzidine tetrachloride (Sigma, St Louis, MO) in 0.1 M Tris-HCl buffer and mounted on the gelatin-coated slides. After dehydration the sections were mounted with Canada balsam (Kanto, Tokyo, Japan). A negative control test was carried out using preimmune serum instead of primary antibody in order to establish the specificity of the immunostaining. The negative control resulted in the absence of immunoreactivity in any structures.

Fifteen sections per animal were selected in order to quantitatively analyze TH immunoreactivity in the PVN. The corresponding areas in the PVN were measured on the monitor at a magnification of 25–50 ×. Images of all TH immunoreactive structures taken from the PVN were obtained through a BX51 light microscope (Olympus, Tokyo, Japan) equipped with a digital camera (DP71, Olympus) connected to a PC monitor. The numbers of TH immunopositive neurons were counted in the whole PVN of each section. The images were processed into an array of 512 × 512 pixels corresponding to a tissue area of 140 × 140 μm (40 × primary magnification). Each pixel resolution was 256 gray levels. The total staining intensity of all TH immunoreactive structures was evaluated on the basis of a optical density (OD), which was obtained after the transformation of the mean gray level using the formula: OD = log (256/mean gray level). The OD of background was taken from areas adjacent to the measured area. After the background density was subtracted, a ratio of the OD of image file was calibrated as % (relative optical density, ROD) using Adobe Photoshop version 8.0 and then analyzed using NIH Image 1.59 software.

The data shown here represent the means ± SEM of experiments performed for each PVN. Differences among the means were statistically analyzed by student *t*-test analysis of variance to elucidate differences between SR and SS groups. Statistical significance was considered at $P<0.01$.

TH immunoreactive neurons and neuropil were found in the PVN of the SS and SR gerbils: The distributional pattern of TH immunoreactive neurons and neuropil were similar in both groups (Fig. 1). The TH immunoreactive neurons were multipolar with long processes: TH immunoreactivity was seen mainly in magnocellular neurons of the PVN. Total TH immunoreactivity (ROD) in the PVN was strikingly different between SR and SS gerbils (Figs. 1 and 2). The ROD in the neurons and neuropil in the PVN of the SS gerbils was significantly lower than that in the SR gerbils through rostral/caudal level (Figs. 1 and 2).

Also, the number of TH immunoreactive neurons in the PVN of the SS gerbils was lower than those in the SR gerbils (Table 1). In the PVN of the SR gerbils, the number of TH immunoreactive neurons was about 50~70 at the level from 0.7 mm to 1.1 mm posterior to the bregma. On the other hand, the number of TH immunopositive neurons in the PVN of the SS gerbils was about 10~30 at the corresponding level ($p<0.01$).

TH activities in the central and peripheral nervous systems are subject to changes in stress, aging, osmotic stimuli and other physiological or endocrinological manipulations [2, 9, 22, 25, 34].

It has been known that TH is expressed mainly in the magnocellular neurons of PVN: This ectopic expression of TH is described in various species [12, 18, 29]. In the present study, TH immunoreactive neurons and neuropil were found in the PVN of the SR and SS gerbils: TH immu-

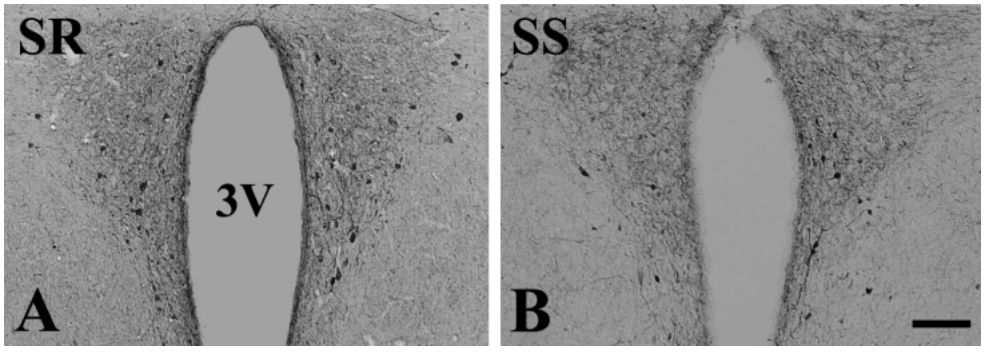

Fig. 1. TH immunoreactivity in the PVN of the SR (A) and SS gerbils (B). The number of TH immunoreactive neurons in the SS gerbils was much lesser than that in the SR gerbils. The photographs are shown at the level of 1.0 mm posterior to the bregma. 3V: 3rd ventricle. Bar = 100 μm.

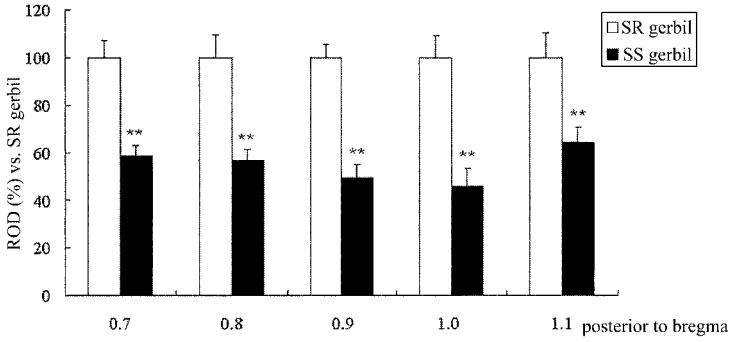

Fig. 2. The relative optical density (ROD) of total TH immunoreactivity from rostral to caudal region of the PVN in SR and SS gerbils (** $P<0.01$, significantly different from the SR group at the same level). The bars indicate the means ± SEM.

Table 1. The number of TH immunoreactive neurons in the PVN of the SR and SS gerbils according to the rostral/caudal level

	SR gerbil	SS gerbil
–0.70 mm bregma	55.7 ± 3.8	23.2 ± 4.8**
–0.80 mm bregma	56.2 ± 7.1	18.1 ± 4.9**
–0.90 mm bregma	66.1 ± 10.7	18.7 ± 2.3**
–0.10 mm bregma	50.0 ± 3.9	22.3 ± 2.2**
–0.11 mm bregma	66.7 ± 6.4	31.5 ± 4.1**

The numbers of TH immunopositive neurons were counted in the whole PVN of each section.
Values indicate means ± SEM.
** $P<0.01$, significantly different from the SR group at the same level.

noreactivity was seen mainly in magnocellular neurons of the PVN. Many investigators reported that the magnocellular neurons of the PVN produced a number of chemical messengers, neuropeptides and peptide hormones such as oxytocin and vasopressin [18, 26]. Panayotacopoulou *et al.* [23] urged that TH immunoreactive neurons synthesized vasopressin, and TH colocalized with vasopressin. In addition, it has been reported that vasopressin has an anticonvulsant effect although there is much room for discussion [1, 8, 11].

It was reported that TH immunoreactivity in some brain regions was decreased in GEPRs as compared with control Sprague Dawley (SD) rats, and that repeated electroconvulsive shock treatment and repeated audiogenic seizures in GEPRs showed further decreased TH immunoreactivity [21, 27]. It was also investigated that a seizure episode resulted in norepinephrine release from synaptic terminals, and the repeated increase of norepinephrine in synaptic levels via repeated seizure might activate inhibitory α_2-adrenergic autoreceptors and then decrease neuronal activities [27].

Seizure activities induced by various convulsant agents are reduced when noradrenergic activity is increased due to the stimulation of the locus coeruleus [13, 19, 28]. It was reported that significant deficits of norepinephrine concentration were found in the hypothalamus/thalamus of GEPRs, and norepinephrine deficits were innate characteristics of GEPRs rather than consequences of seizure episodes [5, 7, 27]. In addition, it was reported that, in the gerbil, the transection of the pineal stalk produced seizure activity and depressions of norepinephrine level in the hypothalamus [4]. In the present study, although TH immunoreactivity was found in both SR and SS gerbils, the number and total immunoreactivity of TH immunoreactive neurons in the SS gerbils were significantly lower than those in the SR gerbils.

Based on the above mentioned researches, we postulate that levels of TH expression and noradrenergic activities in the PVN are lower in the SS gerbil than in the SR gerbil because of repeated seizure experiences. We presume that reduced noradrenergic activity in the PVN may be one of the causes of epilepsy in the SS gerbils, based on the levels of TH expression which could be used as indices of the noradrenergic functions. In conclusion, our finding provides that TH immunoreactivity in the PVN of the epileptic gerbil is much lower than that in the non-epileptic gerbil.

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