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Hippocampal glutamate decarboxylase activity is not altered in gerbils with high seizure susceptibility

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Among different genetic animal models of epilepsy, i.e. genetically predisposed animal species in which seizures either occur spontaneously or in response to sensory stimulation, epilepsy-prone Mongolian gerbils exhibit unique features which make this species particularly useful for epilepsy research (cf. [1]). First, motor seizures can be reliably initiated in seizure-sensitive gerbils by simple external stimuli, such as change in environment [2], handling [3] or exposition of the animals to a blast of compressed air [4]. By the latter technique, seizures can be evoked in more than 98% of randomly bred gerbils, so that no selective breeding is necessary to obtain enough seizure-sensitive animals for experimental studies [5]. Second, the motor seizures are associated with epilepsy-like activity in the electroencephalogram, thus allowing evaluation of electrographic seizure phenomena [6]. Third, there is a progressive age-dependent development of seizure severity in gerbils [2, 5], which represents an interesting parallelity to human absence or myoclonic epilepsies in childhood, which at a later age often proceed to generalized tonic–clonic seizures. Fourth, antiepileptic drug efficacy studies in epileptic gerbils have shown that gerbils of different age can be used for identifying different clinical categories of antiepileptic drugs [5, 7].

The primary mechanisms underlying the seizure-proneness of gerbils are still unknown, although several recent studies have indicated that the inhibitory neurotransmitter γ-aminobutyric acid (GABA) may be involved [8–13]. Of special interest in this respect are recent experiments by Peterson *et al.* [12, 13] using immunocytochemical localization of the GABA-synthesizing enzyme glutamate decarboxylase (GAD), which have shown an increased number of GABAergic neurons in all regions of the hippocampus of seizure-sensitive gerbils compared to seizure-resistant ones. The most pronounced (up to 65%) increases in the number of GAD positive cells were found within the dentate gyrus and the CA 2,3 region of the hippocampal formation. In contrast to the findings in the hippocampal formation, no differences between seizure-sensitive and seizure-resistant gerbils were found in motor cortex, substantia nigra and nucleus reticularis thalami. The increased density of hippocampal GABAergic neurons in seizure-sensitive gerbils was thought to relate, at least in part, to an increased number of basket cells, which provide the main source of feedback and feedforward inhibition to the dentate gyrus and hippocampus proper [12, 13]. If these inhibitory neurons are acting to inhibit each other, the net effect of increased GABAergic activity would be disinhibition of the granule cells and pyramidal cells, which could lead to seizure activity within the hippocampal formation and at distant sites through multisynaptic connections [12, 13]. In fact, morphological studies in seizure-prone gerbils have indicated an increased activity of granule cells in the dentate gyrus, which could be explained by disinhibition [12–14]. The goal of the present paper was (1) to examine if the increase of hippocampal GAD activity found by Peterson *et al.* [12, 13] in seizure-susceptible gerbils by means of immunocytochemical methods can be confirmed when a technique for determination of GAD in synaptosomal fractions of discrete brain regions [15, 16] is used to assess nerve terminal activity of this enzyme, (2) to study if gerbils with different seizure-susceptibility differ in their hippocampal GAD activity, and (3) to determine synaptosomal GAD activities also in various other brain regions of seizure-susceptible and seizure-resistant gerbils. The results obtained do not substantiate the suggestion of Peterson *et al.* [12, 13] that in gerbils the hippocampus is a specific site for seizure-related differences in the GABAergic system.

Materials and methods

From the age of 7–8 weeks, randomly bred gerbils of both sexes were tested once a week for seizure-sensitivity and intensity of seizures. The testing procedure involved

placing the gerbil into an empty plastic bag and exposing the animal to a blast of compressed air (average pressure 5 bars) aimed at the back of the gerbil for 10–15 sec. Details of this technique and classification of seizures stimulated thereby have been described elsewhere [5, 7]. For collection of data shown in Table 1, seizures were differentiated into minor seizures (grade 1–2; localized myoclonic seizures) and major seizures (grade 3–5; generalized myoclonic and tonic–clonic seizures). Whereas most of the randomly bred gerbils of our colony developed seizures upon air blast stimulation, some animals proved to be totally resistant or they showed only some minor seizures after the first stimulations but not after subsequent stimulations for several months (see Table 1). With respect to seizure-sensitivity, we chose three groups for the neurochemical determinations (Table 1): (a) seizure-insensitive gerbils, i.e. animals which did not exhibit seizures in response to weekly air blast stimulation for at least 4 months before GAD determination; (b) seizure-prone gerbils with medium seizure-sensitivity, i.e. animals which showed seizures of inconsistent severity (varying between grade 0 and 5 in the same animal) during the months prior to GAD analysis; and (c) older seizure-prone gerbils with high seizure-sensitivity, i.e. animals which after maximum seizure severity (grade 4–5) was developed displayed consistent grade 5 seizures for several months prior to GAD assay. For the neurochemical determinations, the gerbils of these different groups were killed by decapitation 11 days after the last air blast stimulation. In order to avoid interassay variation error, samples from the 3 groups of gerbils were treated concurrently in all phases of the neurochemical analysis. Brains were rapidly removed and dissected on a cold plate at −18° into 11 brain regions as previously described for rat brain [17]. To obtain enough tissue for the subcellular fractionation, regions were dissected bilaterally. The individual brain regions were then rapidly homogenized in 0.32 M sucrose and synaptosomal fractions were isolated from these homogenates by sucrose density gradient centrifugations as described in detail elsewhere [15, 16]. Synaptosomal pellets obtained by this technique consist primarily of synaptosomes, whereas contamination with free mitochondria and glia cells is very low [16]. For analysis of GAD activity, each synaptosomal pellet was suspended in 1 ml of chilled distilled water containing Triton X-100 (0.5% v/v), 1 mM 2-mercaptoethanol, and 0.1 mM pyridoxal phosphate. The activity of GAD was measured according to the fluorimetric method of Lowe *et al.* [18] as described previously [19]. In a final volume of 1 ml, the incubation medium contained 5 mM L-glutamic acid, 0.24 mM pyridoxal phosphate, 100 mM phosphate buffer (pH 6.4) and 0.5 ml of the pellet suspension.

Results and discussion

GAD activities determined in synaptosomal fractions of discrete brain areas of seizure-insensitive and seizure-prone gerbils are shown in Fig. 1. In seizure-insensitive animals, the highest GAD activities were found in hypothalamus, tectum (inferior and superior colliculus) and thalamus, whereas in the substantia nigra, synaptosomal GAD activity was conspicuously low compared to other rodent species [16, 20] (see below). In gerbils with medium seizure-sensitivity, the only significant difference to seizure-insensitive gerbils was a 40% increase in hippocampal GAD activity. In contrast, in older epileptic animals with high seizure-sensitivity hippocampal GAD was not different from insensitive animals. In the seizure-insensitive group, which contained both younger and older animals (see number of stimulations in Table 1), no age-related differences in GAD activity were found for any region indicating that differences in age between animals was not a bias for the present GAD determinations. In this respect, it should be noted that by using the air blast technique for inducing seizures in gerbils, only very few animals (one or two among 100) are seizure-resistant. Thus, it is very difficult and time-consuming to get enough "control" animals for biochemical studies in seizure-sensitive gerbils. This explains the variation of age in the present seizure-insensitive group, because more than one year of selection was necessary to find six seizure-insensitive animals.

Table 1. Details of seizure-sensitive and seizure-resistant gerbils used for the neurochemical experiments

Group	No. of stimulations	No. of seizures			Mean seizure severity at the last 8 stimulations
		Grade 0	Grade 1–2	Grade 3–5	
Seizure-insensitive					
Gerbil No. 1	82	82	0	0	0
Gerbil No. 2	81	79	2	0	0
Gerbil No. 3	29	23	6	0	0
Gerbil No. 4	29	28	1	0	0
Gerbil No. 5	18	18	0	0	0
Gerbil No. 6	18	18	0	0	0
Seizure-sensitive (medium)					
Gerbil No. 1	17	9	1	7	2.3
Gerbil No. 2	28	8	8	12	2.4
Gerbil No. 3	18	3	7	8	2.8
Gerbil No. 4	29	12	6	11	1.9
Gerbil No. 5	27	8	7	12	2.5
Seizure-sensitive (high)					
Gerbil No. 1	102	12	6	84	5
Gerbil No. 2	92	6	5	81	5
Gerbil No. 3	79	11	9	59	5
Gerbil No. 4	73	10	7	57	5
Gerbil No. 5	80	2	8	70	5
Gerbil No. 6	64	4	8	52	5

From an age of 7–8 weeks, all gerbils were stimulated once weekly by a blast of compressed air.

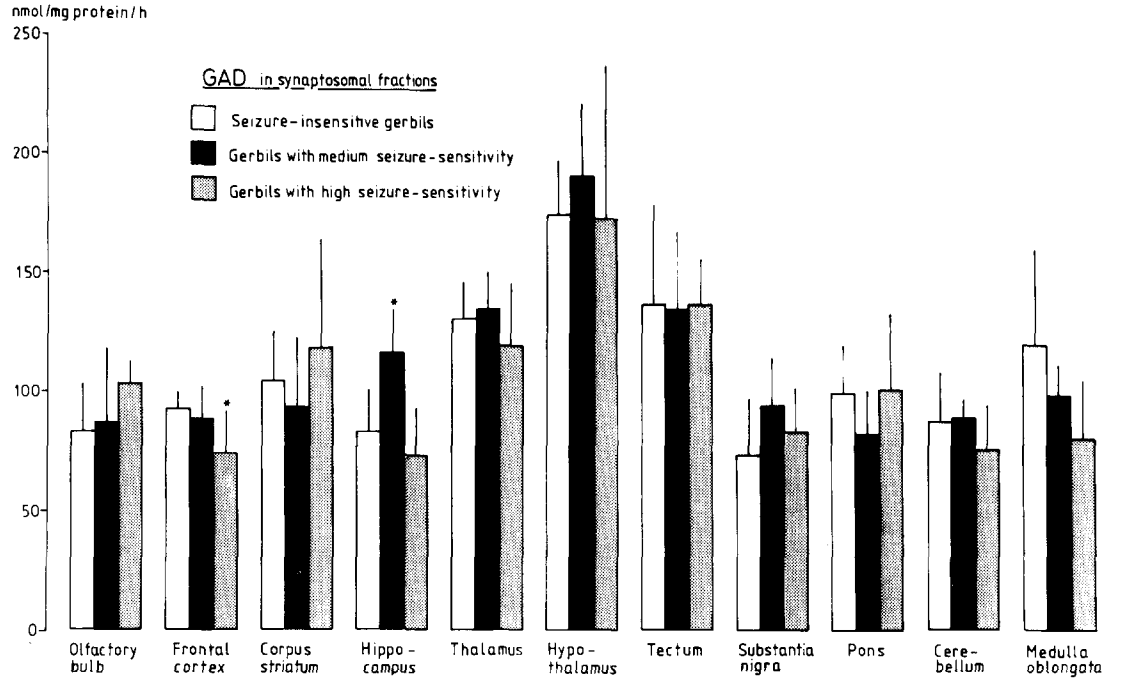

Fig. 1. Activities of GAD in synaptosomal fractions of 11 brain regions of seizure-insensitive gerbils and gerbils with medium and high seizure-sensitivity (see Table 1). Data are means ± SD of 5–6 animals per determination. Significance of differences between seizure-insensitive and seizure-sensitive gerbils was calculated by Student's two-sided *t*-test and is indicated by asterisks ($P < 0.05$).

Whereas the present data on gerbils with medium seizure-sensitivity seem to confirm the recent immunocytochemical studies of Peterson *et al.* [12, 13] in that hippocampal GAD activity is approximately 40% higher in seizure-sensitive animals compared to seizure-resistant ones, the lack of hippocampal GAD alterations in older seizure-sensitive gerbils with maximum seizure severity cast doubt on the possibility that GABAergic transmission in the hippocampus is critically involved in the seizure-prone state in gerbils. In fact, in these animals with maximum stimulus-responsiveness, the only difference in GAD activity to seizure-insensitive animals was a moderate decrease of enzyme activity in the frontal cortex, thus substantiating previous data on low cortical GABA levels in seizure-sensitive gerbils [8]. It should be noted, however, that the present and previous studies on hippocampal GAD differ in some aspects. First, Peterson *et al.* [12, 13] counted GAD positive cells in different regions of the hippocampal formation, whereas we determined GAD activity in whole hippocampus. Second, Peterson *et al.* [12, 13], used selectively bred seizure-sensitive and seizure-resistant gerbils in which seizure-sensitivity was tested by change in environment, whereas we used randomly bred gerbils in which seizure testing was performed by air blast stimulation, which is a much stronger stimulus for seizure induction in this species [5]. Third, in the studies of Peterson *et al.* [12, 13], seizure severity and age of the animals used for GAD determination were not specified, although it was stated that in five selected animals with high seizure ratings the number of GAD-positive cells in the dentate gyrus was positively correlated with seizure intensity. Interestingly, an increased number of GAD positive hippocampal cells was also found in the progeny of seizure-sensitive animals prior to the age at which seizure activity begins, which indicates that the increase in GABAergic neurons found by Peterson and co-workers was not solely a compensatory response to seizure activity in the brain.

Irrespective of the differences in hippocampal GAD activities, in all three groups of gerbils studied in the present paper conspicuously low activities of GAD were present in synaptosomal fractions of the substantia nigra, a region which in other rodent species contains very high amounts of synaptosomal GAD [16, 20]. In fact, average synaptosomal GAD activities determined in synaptosomes of the nigra by the present technique in different strains of rats ranges between 400 and 700 nmol/mg protein/hr [16, 20] compared to the 70–100 nmol/mg protein/hr determined in the different groups of gerbils. Since GABA synapses in the nigra are though to be critically involved in the control of seizure propagation [15, 21], a low GABA synthesis in this brain region could favor the generalization of seizure activity emanating from more rostral loci. In this respect, the recent binding studies of Olsen *et al.* [11] are of interest which indicated that the functional state of the benzodiazepine/GABA receptor complex is impaired in the substantia nigra of seizure-prone gerbils. In order to study further the possible role of regional alterations of the GABA system in epileptic gerbils, we plan to investigate the effect of microinjections of GABAmimetic drugs into different brain regions, including the substantia nigra, on seizure severity in gerbils.

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