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Increased levels of amino acid neurotransmitters in the inferior colliculus of the genetically epilepsy-prone rat

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Previous studies have shown an increase in the number of GABAergic and total neurons in the inferior colliculus (IC) of the genetically epilepsy-prone rat (GEPR). Amino acid analysis of the central nucleus of the IC, as well as cerebellum, sensorimotor, temporal, and occipital cerebral cortices of GEPRs with high pressure liquid chromatography showed significant increases in the levels of GABA, taurine and glutamate. The IC of GEPR displayed a 2.3-fold increase in GABA as compared to that of non-epileptic rats, a 2.4-fold increase of taurine, and a 1.9-fold increase of glutamate. In addition, taurine and glutamate were increased in the sensorimotor and temporal cortex, respectively. These results are consistent with previous anatomical data on the GABAergic system in the IC and provide additional information. The increase in taurine and glutamate in the IC indicates that other neurotransmitters could be involved in the mechanism of seizure activity.

INTRODUCTION

The genetically epilepsy-prone rat (GEPR) exhibits severe audiogenic seizure activity in response to loud auditory stimuli. The results of lesion studies indicate that bilateral lesions of the inferior colliculus (IC) prevent seizure activity. Also, the results of pharmacological studies have shown that neurons in the IC of the GEPR may be less sensitive to the iontophoresis of GABA and benzodiazepine as compared to the neurons in the IC of non-epileptic rats.

Previous morphological studies have shown an increase in the number of GABAergic and total neurons observed in the IC of the GEPR as compared to non-epileptic Sprague–Dawley (SD) rats. Other studies from this laboratory have also shown an increase in the number of total neurons in the IC of young GEPR offspring that have not yet developed seizure activity. This latter study indicated that the increase in the GABAergic and total number of neurons is not caused by seizures. Moreover, biochemical studies have shown that a small (10–35%), insignificant increase in GABA occurs in the IC of GEPRs. Since the largest increase in the number of GABA neurons occurred in the central nucleus of the IC with much smaller increases in the other nuclei of the IC, it is possi-
ble that the change in the central nucleus was masked by the inclusion of these other areas of the IC in the previous biochemical studies. To determine whether the increased numbers of GABAergic neurons are associated with significant biochemical changes in the IC of the GEPR as compared to non-epileptic SD rats, the central nucleus of the IC was punched out of fresh midbrain sections for amino acid analysis. In addition, the amino acid neurotransmitters in the cerebellum and sensorimotor, temporal, and occipital cerebral cortices of GEPRs and SD rats were analyzed. If an increase in the level of GABA is present in the IC of the GEPRs as compared to SD rats, it would confirm our previous results that showed an increase in the number of GABAergic neurons in this brain region of the GEPR.

METHODS

The rats used in this experiment were tested in a sound chamber that is equipped with two doorbells. Their seizure activities were evaluated and given an audiogenic response score (ARS). The GEPR always displayed maximal tonic seizures (ARS = 9) whereas SD rats showed no seizing activity (ARS = 0). Seven GEPRs and 8 SD rats were analyzed.

The animals were decapitated with a guillotine to obtain fresh brain tissue for chemical analysis. Five different brain regions as described above were dissected over ice as rapidly as possible and frozen in liquid isopentane. The removal of tissue from the central nucleus of the IC required a special procedure. The caudal midbrain containing the IC was dissected in the coronal plane as one large slab of tissue. Then, a large-bore, blunt-tipped syringe containing a small volume of normal saline was used to remove a core of tissue from the IC that contained mainly the central nucleus. The temperature of isopentane was kept at -20 °C, and brain specimens were dropped into the solution for no more than 30 sec to avoid fracture of the tissue. The vials that contained frozen brain tissues were stored at -70 °C to -90 °C and were given codes so the biochemical analysis would be performed without any bias.

Each sample was weighed in its vial, then homogenized in 1 ml of ice-cold 0.1 N HCl. The vial was then weighed again to get the net weight of tissue. The suspension was then heated for 5 min in a 100 °C water bath, and centrifuged to get rid of most cell debris and precipitated proteins. Usually, 0.8 ml of supernatant was removed and diluted to between 3.2 and 4.8 ml with water (5–8-fold dilution) and filtered through a 0.2 μm filter. Two or three 0.5 ml samples were then frozen (-80 °C) to await analysis. The high pressure liquid chromatography (HPLC) method was a pre-column derivatization using o-phthalaldialdehyde and β-mercaptoethanol. The column was a C18 reverse phase (Spherisorb). The mobile phase solvents were 0.5 M lithium acetate and methanol with a gradient running from 5% methanol to 95% methanol. A 10–40 μl sample of saved supernatant was then mixed with 20 μl of 'internal standard' (IS) and water to make 200 μl total volume. Of this, 50 μl were actually analyzed. The IS contained cysteic acid (CYS) and norvaline so that the final concentration was 100 pmoles/50 μl of assayed fluid. The norvaline was the reference standard to account for variations of sensitivity from run to run. The CYS came off very early in the run and was used as a quick monitor that everything was working properly. It also gave an approximation of the precision of the method because each sample should have given 100 pmoles. The 'external standard' for the HPLC analysis contained GABA, taurine, glutamate, glutamine, glycine, aspartate, serine and alanine so that 50 μl contained 100 pmoles.

RESULTS

The central nucleus of the IC in GEPRs had a 2.3-fold increase in the level of GABA as compared to that of non-seizing SD rats (Fig. 1). The values were 0.72 ± 0.07 (mean ± S.E.) μmol/g for the GEPRs and 0.31 ± 0.06 μmol/g for the SD rats. The significance of this difference was P < 0.001 (Student’s t test). Significant increases in the levels of taurine and glutamate (Fig. 1) were also observed in the IC of GEPRs as compared to SD rats: a 2.4-fold increase of taurine (0.54 ± 0.05 vs. 0.22 ± 0.05 μmol/g, P < 0.001), and a 1.9-fold increase of glutamate (1.44 ± 0.21 vs. 0.76 ± 0.14
Increased levels of taurine and glutamate were also observed in the cerebral cortex of GEPRs as compared to that of SD rats (Fig. 1). The sensorimotor cortex had a 1.5-fold increase of taurine (4.24 ± 0.57 vs. 2.89 ± 0.30 μmol/g, P < 0.05), whereas the temporal cortex had a 1.9-fold increase of glutamate (5.98 ± 1.08 vs. 3.16 ± 0.44 μmol/g, P < 0.05). The occipital cortex and cerebellum displayed no significant differences in the levels of amino acids between GEPR and SD rats. No other differences were found.

DISCUSSION

The major finding of this study is the significant increase in the level of GABA, glutamate and taurine in the central nucleus of the inferior colliculus (IC). These increases were probably found as a result of the selection of only the central nucleus for analysis because previous studies that analyzed the entire inferior colliculus failed to show any significant changes in these 3 amino acids. In addition, the present study displayed differences in the levels of glutamate and taurine in 2 other brain regions.

GABA

The 2.3-fold increase in the level of GABA in

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**TABLE I**

|                | GABA   | Taurine | Glutamate | Aspartate | Glycine | Glutamine | Alanine | Serine |
|----------------|--------|---------|-----------|-----------|---------|-----------|---------|--------|
| Inferior colliculus | 0.721±0.073 | 0.542±0.046 | 1.437±0.208 | 0.987±0.305 | 1.054±0.270 | 0.242±0.053 | 0.330±0.110 | 0.698±0.352 |
| Cerebellum       | 1.536±0.346 | 3.013±0.622 | 3.939±0.522 | 0.928±0.120 | 0.878±0.130 | 0.796±0.086 | 0.697±0.210 | 0.418±0.084 |
| Occipital cortex  | 1.751±0.151 | 4.013±0.673 | 4.209±0.389 | 0.963±0.405 | 0.912±0.031 | 0.567±0.064 | 0.412±0.066 | 0.467±0.046 |
| Temporal cortex  | 2.078±0.319 | 5.346±1.421 | 5.976±1.081 | 1.521±0.414 | 1.095±0.161 | 0.895±0.174 | 0.690±0.184 | 0.667±0.137 |
| Sensorimotor cortex | 2.009±0.421 | 4.240±0.567 | 4.066±0.710 | 1.096±0.134 | 0.845±0.097 | 0.550±0.061 | 0.458±0.080 | 0.375±0.079 |
| Inferior colliculus | 0.314±0.061 | 0.224±0.045 | 0.756±0.138 | 0.860±0.192 | 1.755±0.442 | 0.301±0.070 | 0.575±0.143 | 1.843±0.528 |
| Cerebellum       | 2.104±0.290 | 2.566±0.272 | 3.223±0.285 | 1.302±0.138 | 0.876±0.112 | 0.762±0.088 | 0.919±0.144 | 0.503±0.086 |
| Occipital cortex  | 2.702±0.403 | 2.791±0.317 | 3.505±0.413 | 1.599±0.227 | 0.942±0.136 | 0.586±0.112 | 0.655±0.095 | 0.567±0.120 |
| Temporal cortex  | 2.127±0.297 | 3.055±0.204 | 3.163±0.439 | 1.364±0.288 | 0.884±0.814 | 0.485±0.107 | 0.669±0.091 | 0.513±0.079 |
| Sensorimotor cortex | 2.132±0.221 | 2.891±0.296 | 2.547±0.278 | 0.988±0.094 | 0.763±0.083 | 0.382±0.051 | 0.546±0.058 | 0.302±0.048 |
the central nucleus of the IC supplements and is in conformity with previous anatomical data from this laboratory. The magnitude of the increase in the level of GABA in the central nucleus of GEPR as compared to that of non-epileptic SD rats is consistent with the 3.0-fold increase in the number of GABAergic neurons observed in this region.

The functional significance of this increase in GABA in the central nucleus of the IC remains unclear. The inferior colliculus is considered the most important sensory brain nucleus implicated in eliciting audiogenic seizures in the GEPR. Studies have shown that the manipulation of the level of GABA in the IC has an effect on seizure behavior. For example, the iontophoretic dose (current) of GABA required to suppress neuronal firing in the GEPR was significantly greater than that required in neurons of non-epileptic rats. Another study showed that this effect involves GABA because suppression of neuronal firing in the IC is blocked by the GABA<sub>α</sub> receptor antagonist, bicuculline, and is magnified by a GABA uptake inhibitor. Furthermore, microinjection of bicuculline directly into the IC of normal rats causes these animals to be susceptible to audiogenic seizures. Together these data support the notion that an abnormal (dysfunctional) GABAergic system exists in the IC of GEPRs. This abnormality could involve increased disinhibition of excitatory projection neurons during intense auditory stimuli as suggested by Roberts et al. or it could involve a problem with the GABA<sub>α</sub> receptor. Further studies are required to resolve why high GABA and low efficacy occur in the IC.

**Glutamate**

A 1.9-fold increase of glutamate in the central nucleus of the IC and a 1.9-fold increase of glutamate in temporal cortex of GEPR were observed in this study. The increased levels of glutamate in the IC may reflect increased disinhibition of excitatory neurons as originally proposed by Roberts et al. If the excitatory neurons are more active, they may contain higher levels of an excitatory neurotransmitter, such as glutamate. The increase in glutamate in auditory cortex is interesting because other regions of the cortex lacked differences (see Table I) and a previous study has shown that the entire cortex displays no changes in the level of glutamate<sup>1</sup>. This increase in auditory cortex may reflect an increased level of excitation in the auditory pathways of the GEPR as a result of the increased activity of excitatory neurons in the IC.

**Taurine**

Taurine, an inhibitory substance, is one of the most abundant amino acids in the brain and is a powerful anticonvulsant in genetic seizures. It is not known if taurine is a neuromodulator or a neurotransmitter. However, it seems to have more of a modulatory role than a transmitter function<sup>7</sup>. A 2.4-fold increase of taurine in the central nucleus of the IC and a 1.5-fold increase of taurine in sensorimotor cortex of GEPR as compared to SD rat in this study suggest some participation of taurine in the mechanism of the seizure activity. At this time, it is difficult to explain the increase in the level of taurine in the central nucleus of the IC. The activity of glutamate decarboxylase (the synthesizing enzyme for GABA) in the brains of seizure-resistant rats was not affected by the addition of taurine. However, in the brains of seizure susceptible rats, the addition of the same concentration of taurine (10 mM) results in a 20% increase in the rate of decarboxylation of glutamate. The selective effect of taurine on the decarboxylation of glutamate could be a basis for the anticonvulsive response of taurine. A similar type of study indicates that taurine directly influences the metabolism of glutamic acid; when a loss of endogenous taurine from epileptic cortex occurs, it is similarly associated with a decrease in glutamic acid<sup>13</sup>.

Other amino acids that were analyzed did not display significant differences between GEPR and non-epileptic SD rats. Except for glutamine which was reported to decrease in the cerebellum and the whole IC, these results are in general agreement with those of Chapman et al.<sup>1</sup>

In conclusion, the 2.3-fold increase in the level of GABA in the central nucleus of the IC of GEPRs confirms the previous anatomical data that described an increase in GABA neurons in this region<sup>12</sup>. The increase in the levels of taurine and glutamate in this structure as well as the increase of taurine in sensorimotor cortex and glutamate in temporal cortex indicate that other amino acid neurotransmitters are involved in the mecha-
nism or consequences of the seizure activity.

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