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The Activity of Dopamine β-Hydroxylase of Central Nervous System in Genetically Epilepsy Prone Rats

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Abstract □ Abnormality in the central noradrenergic system may be related to the seizure prone state in the genetically epilepsy prone rats (GEPR). The present work deals with the characterization of the deficit in noradrenergic system if susceptitibility and intensity of seizure are dependent on central noradrenregic activities by comparing the activities of dopamine β-hydroxylase (DBH) which hydroxylates dopamine into noradrenaline. DBH activities were measured in 5 areas of brain of normal rats, naive GEPR, and severe GEPR. The results suggest that lower DBH activities in the midbrain of GEPRs may positively be coupled to the susceptibility to seizure, whereas the same characteristics of the naive or severe GEPR are not necessarily in parallel with the intensity of seizure.

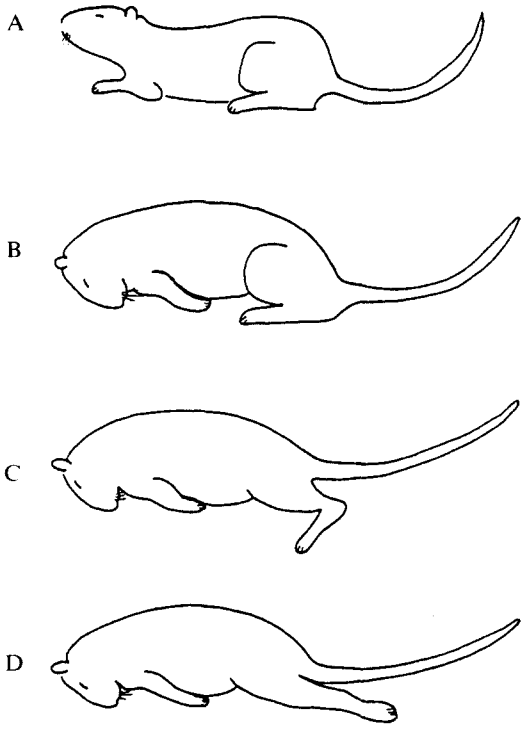
Keywords □ Genetically epilepsy prone rat, dopamine β-hydroxylase

Genetically epilepsy prone rats (GEPR) of which the seizure prone state is characterized by susceptibility to hyperthermia and sound inuced seizures have several characteristics similar to human epilepsy[1]. Both types of epilepsy is under genetically determined. Neurochemical studies indicate that the seizure prone states of GEPR and of the human epileptic may be characterized by similar abnormalities. Serotonergic, acetylcholinergic and catecholaminergic abnormalities have been observed in human epileptics. Similarly abnormalities in free amino acids including GABA, glycine, glutamic acid and taurine may also exist. Innate abnormalities in noradrenergic function in the GEPRs have been reported. Noradrenaline concentrations in most regions of brain[2] and turnover rate of noradrenaline in the midbrain, pons/medulla and spinal cord[3] are lower in the GPPRs. Tyrosine hydroxylase activity in the midbrain but not in other areas of brain is abnormal, and abnormal response to pharmacologically induced alterations in noradrenaline in the brain of GEPR strain has been reported[4]. Because of the potential importance of noradrenergic deficit in this genetic model of epilepsy, it seems important to chanracterize further the nature of this deficit by examining another index of noradrenergic function. Though tyrosine hydroxylase is the rate limiting enzyme in overall noradrenaline biosynthesis, it may be interesting to determine the activity of dopamine β-hydroxylase which converts dopamine into noradrenaline. Thus, the present study is designed to characterize further the noradrenergic deficit in GEPR-3 (naive GEPR) and GEPR-9 (severe GEPR) by comparing activity of dopamine β-hydroxylase in GEPRs and normal rats.

EXPERIMENTAL METHODS

Animals

Male Sprague Dawley rats (125-150 days) from Harlan Sprague Dawley (Madison, WI), GEPR-3 (120-130 days) and GEPR-9 (135-140 days) from the University of Illinois School of medicine, were used throughout the study. Inas much as GEPRs were derived from Sprague Dawley rats, the latter animals were selected as controls. All rats were housed three per cage under environmentally controlled conditions that included a 12 hr light dark cycle (6 A.M.-6 P.M.), 25℃ and free access to water and purina rat chow.



Response to sound stimulation

ARS

0 No response.
1 Running only; no convulsion.
2 Two running phases separated by a refractory period; convulsive endpoint consists of clonus of forelimbs, hindlimbs, head, pinnae, vibrissae and tail. (A)
3 Same as 2 except only one running phase and no refractory period.
4 Two running phases separated by a refractory period; convulsive endpoint consists of tonic flexion of neck, trunk and forelimbs with clonus of hindlimbs. (B)
5 Same as 4 except only one running phase and no refractory period.
6 Two running phases separated by a refractory peroid; convulsive endpont similar to 4 except hind limbs are in partial tonic extension (i. e. tonic extension of thighs and legs with clonus of feet). (C)
7 Same as 6 except only one running phase and no refractory period.
8 Two running phases separated by a refractory period; convulsive endpoint similar to 4 except hindlimbs are in complete tonic extension (i. e. animals in maximal convulsion). (D)
9 Same as 8 except only one running phase and no refractory period.

Fig. 1. Description of audiogenic response score (ARS) system.

Seizure testing

GEPRs used in these studies were screened by subjecting them to three seizure tests. Only rats showing a score of three and nine on the last two tests were used in these studies. The Sprague Dawley rats were subjected to one or two audiogenic seizure test, and none were found to exhibit a seizure. Seizure testing was performed in a cylindrical chamber (41 cm diameter×51 cm high) constructed of galvanized metal. Audiogenic seizures were induced by the standard stimulus of mixed tones generated by two school bells mounted within the chamber. A sound level of ~100 dB relative to 2×10^{-4} dynes/cm^2 was initiated. The audiogenic response score was determined using the system developed by Jobe *et al.*[4] which utilizes a seizure severity score of 0-9 as shown in Fig. 1. Zero represents no response and increasing seizure severity is denoted by increasing numbers with a score of 9 being assigned to a convulsion characterized by full hindlimb extension.

Drugs and chemicals

Standard drugs and chemicals were purchased from Sigma (St. Louis, MO); S-14 C adenosyl methionine (58 mCi /mmol) was purchased from Amersham International Plac.

DBH activity

Rats were decapitated, and the brain was quickly removed and placed on a chilled plate and dissected. Tissue samples were stored until use at −70℃. The assay for DBH was performed using a modification of the method of Molinoff *et al.*[5]. Blanks consisting of tissue homogenates heated to 95℃ for 15 min were run in all experiments as well as internal standard consisting of 40 nmole octopamine added to the reaction mixture. All samples were run in triplicates, and the efficiency of liquid scintillation counting was 85-95%.

Statistical analysis

.The significance of differences among the groups was evaluated by one way analyses of variance (ANOVA) using Newman-Keul test for post-hoc comparison. Differences were considered statistically significant when $p<0.05$ was obtained.

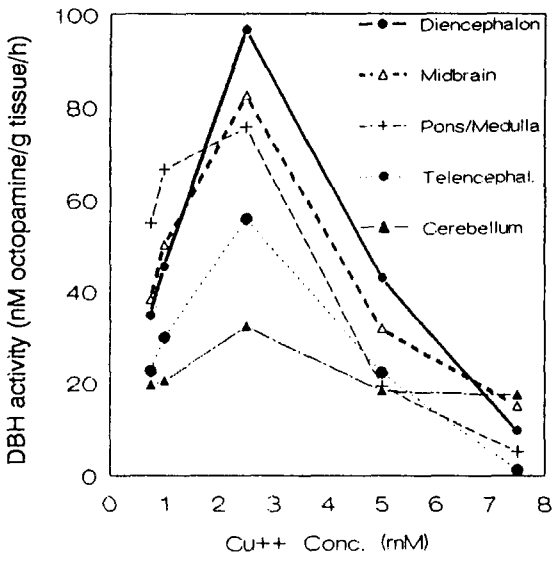


Fig. 2. Effect of Cu^{2+} concentrations on the DBH activities in the various regions of rat brain. Each point represents the mean of the data from 3 replicates.

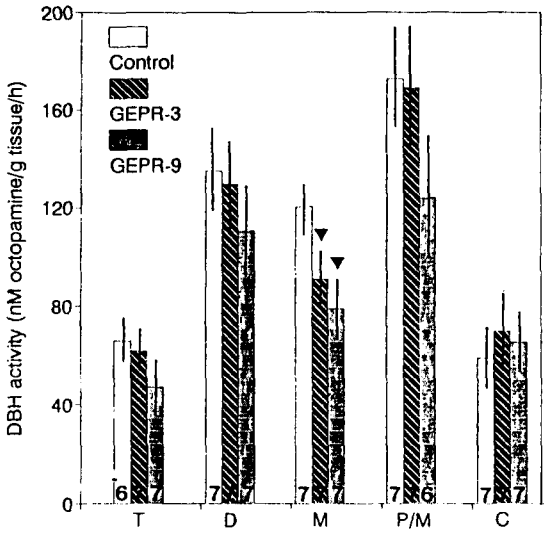


Fig. 3. DBH activities in brain regions of GEPRs and control rats. Each bar represents the mean± S.E. M. of data from at least 6 separate determinations as indicated inside the bars.

▼:$p<.05$, significantly different from control rat, T: Telencephalon, D: Diencephalon, M: Midbrain, P/M: Prons/Medulla and, C: Cerebellum.

RESULTS

Determination of the optimal copper concentration in the DBH assay

DBH is a copper containing protein, whose activity is reduced by endogeneous inhibitors existing in tissue supernatant[5]. To minimize the effect of inhibitors on the DBH assay, $CuSO_4$ is generally added. Since the concentration of copper required for optimum enzyme activity was reported to differ in some brain regions[6], it was necessary to run copper concentration curves for all regions examined. Fig. 2 shows the result of this experiment in the various regions of brain. The optimal copper concentrations were found to be 2.5 mM for all regions; telencephalon, diencephalon, midbrain, pons/medulla and cerebellum.

DBH activity

DBH activities has a tendency to decrease in proportion to the intensity of the seizure in most regions of brain tested except for cerebellum, but the differences were not always statistically significant either between GEPRs and normal rats or GEPR and GEPR-9 as shown in Fig. 3. DBH activities only in midbrain between GEPRs and normal rats were significantly different. DBH activities in cerebellum appeared to be increased in GEPR-3 and GEPR-9, but the differences were not statistically significant.

DISCUSSION

The result shows that DBH activities are lower in GEPR-3 and GEPR-9 than in normal rats in telencephalon, diencephalon, midbrain and pons/medulla. Its deficiency seems to be severer in GEPR-9 than GEPR-3. These differences are, however, not always statistically significant. Only in midbrain, DBH activities in GEPR-3 and GEPR-9 are lower than in normal rats with statistical significance. The deficiency of DBH activity in GEPRs means the possible lack of noradrenergic function.

The finding that the DBH activity of midbrain is deficient in GEPR-3 and GEPR-9 supports the hypothesis of the deficiency of noradrenergic function in GEPRs[4]. The activity of tyrosine hydroxylase which hydroxylates tyrosine into dopa was reported to be decreased in GEPRs' midbrain[7], which might contribute to the noradrenergic deficit. Thus, noradrenergic deficit which may be contributed

from the deficiency of tyrosine hydroxylase activity and dopamine β-hydroxylase activity may be etiologically important as a determinant of susceptibility to seizures in GEPR-3 and GEPR-9.

The difference between GEPR-3 and GEPR-9 lies in the intensity of seizures. According to the result of this experiment, it is impossible to correlate the DBH activity with increasing intensity of seizures because the increasing deficiencies of DBH activity with increasing intensity of seizures are not statistically significant. Recently, Jobe *et al.*[8], reported that DBH activities in cerebral cortex, hypothalamus, hipocampus and inferior colliculus were deficient in GEPR-9. These results are somewhat different from what we have obtained; deficiency only in midbrain of GEPR-3 and GEPR-9. As DBH activities tend to decrease in all regions of brain of GEPR-3 and GEPR-9 examined except cerebellum, it seems important to characterize whether these tendency has statistical meaning with more experimental animals.

DBH activities of cerebellum in GEPR-3 and GEPR-9 appears to be increased though not statistically significant. This result is on the contrary to the findings in other regions such as midbrain, however, it is consistent with the report where noradrenergic contents of GEPR-3 and GEPR-9 have been found to be higher than those of normal rats in cerebellum[9]. This implies that increased noradrenergic function in cerebellum may also have etiological importance in manifestation of seizures in GEPR.

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