Glutamate decarboxylase activity in preoptic and hypothalamic nuclei of the rat

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Summary. Freeze-dried samples were used for the determination of glutamate decarboxylase activity in discrete preoptic and hypothalamic nuclei. The activity showed marked regional variation in distribution.

A variety of experiments in mammalian central nervous system have shown that the preoptic area and hypothalamus may play important roles in the regulations of emotional behavior and neuroendocrine mechanisms. Although the hypothalamus contains higher levels of both γ-aminobutyric acid (GABA) and glutamate decarboxylase (GAD)[1], the physiological roles of GABA in the hypothalamus are unclear. On the other hand, there is good evidence that GABA is an inhibitory neurotransmitter in many brain areas and GAD, the synthetic enzyme of GABA, may reflect the function of a GABAergic neuron system[2-4]. It is important, therefore, to determine GAD activities in discrete hypothalamic nuclei. In the present study, using microdissection technique with freeze-dried sections, GAD activities in individual hypothalamic and preoptic nuclei were investigated.

Materials and method. Wistar-king male rats (17-week-old) were used. The animals were killed by decapitation at 16.00 h. The brain was immediately removed and carefully cut with a razor blade on ice. The tissue blocks containing the hypothalamus was frozen in liquid nitrogen and a series of frontal sections, made at 100 µm thickness in a cryostat, were evacuated overnight at -30 °C and 10^{-3} mmHg. The freeze-dried sections were stored in evacuated tubes at -20 °C until use. The individual nuclei were carefully dissected freehand under a stereomicroscope with the guide of the atlas of König and Klippel[5]. The schematic drawings of the dissected nuclei are shown elsewhere[6,7]. Each sample was weighed by an electronic microbalance (Type 4152, Sartorius Co.) with a digital Vm (Type EO-12 Eto Co.). The weight of each sample was 5–10 µg.

GAD activity was measured by a modification of the radiochemical micromethod of Fonnum et al.[8]. Each reaction mixture contained the following components in a final volume of 3 µl: 1.1 mM L-(1-^{14}C) glutamate (50 mCi/mmole, New England Nuclear Co.), 30 mM sodium L-glutamate, 0.15 mM pyridoxal phosphate, 2 mM dithiothreitol, 40 mM potassium phosphate buffer (pH 6.5), 0.2% (v/v) Triton X-100 and 0.05% (w/v) bovine serum albumin. The incubation mixture was added to each microtube containing a dry sample and the microtube was connected by a rubber tubing to another microtube containing 50 µl of hyamine in methanol. After the insides of the microtubes and rubber tubing had been gassed with purified nitrogen, the dual-tube system was incubated at 37 °C for 60 min. The reaction was stopped by placing the system in a boiling water-bath (about 90 °C). A post-incubated period of 60 min at 37 °C was used to allow complete trapping of $^{14}CO_2$ on hyamine solution. The trapping microtube was separated from the connection and transferred into a counting vial containing 10 ml of scintillator toluene and 2 ml of methanol. After shaking the counting vial for 30 min, radioactivity was determined in a Beckman liquid scintillation spectrometer. The counting efficiency was 85%. The preliminary experiments indicated that GAD activity was proportional to the amount of tissue examined under the present experimental conditions.

Results and discussion. The activities of GAD in hypothalamic nuclei and preoptic area are shown in the table. There was approximately 6fold difference between the highest (nucleus preopticus medialis) and the lowest activity (infundibular stem). Low activities were found in median eminence, infundibular stem, nucleus (n.) arcuatus and area retrochiasmaticus. It is of interest that tuberoinfundibular system showed the lowest activity of GAD. In contrast, high activities were found in preoptic area, n. premammillaris, n. periventricularis and n. dorsomedialis. In the hypothalamic nuclei, n. premammillaris ventralis showed the highest GAD activity in the present study, while Tappaz et al.[4] reported that n. dorsomedialis contained the highest GAD activity in the hypothalamus. In order to compare our results with theirs, the activities in the individual nuclei of both results were calculated as ratios to the activity in the median eminence (table). The distribution pattern of GAD activity in the present study was similar to that reported by Tappaz et al.[4]. However, n. premammillaris ventralis and dorsalis in our study showed approximately 2fold higher relative activites than those in their results and also n. posterior showed higher, while n. arcuatus had lower relative activity than in their results. These discrepancies may reflect the differences in the dissection procedures. Tappaz et al. examined by the micropunch method, in which tissue samples were punched out from frozen sections (300 µm) with a small cannula and samples of small nuclei were pooled from 2 rats. Since the nuclei of premammillaris and arcuatus are small and n. posterior is slender, in their study these nuclei might have contamination with surrounding tissues. By contrast, in the

GAD activity in preoptic and hypothalamic nuclei

Nucleus	GAD activity*	Ratio**
Preoptic area		
Nucleus preopticus medialis	452.5±23.3 (4)	5.0
Nucleus preopticus lateralis	445.6±46.5 (5)	4.9
Anterior part of hypothalamus		
Nucleus anterior	327.0±22.0 (5)	3.6
Area lateralis	299.5±16.9 (4)	3.3
Nucleus paraventricularis	262.5±27.9 (5)	2.9
Nucleus periventricularis	307.2±12.6 (4)	3.4
Area retrochiasmaticus	149.8±23.4 (4)	1.6
Medial part of hypothalamus		
Nucleus ventromedialis	310.7±26.3 (5)	3.4
Nucleus dorsomedialis	378.5±16.9 (4)	4.2
Area lateralis	233.3± 7.0 (5)	2.6
Nucleus periventricularis	400.7±21.9 (4)	4.4
Nucleus arcuatus	142.7± 8.9 (5)	1.6
Median eminence	91.3± 5.2 (5)	1.0
Posterior part of hypothalamus		
Nucleus posterior	343.7±19.6 (5)	3.8
Nucleus premammillaris ventralis	426.0±10.8 (4)	4.7
Nucleus premammillaris dorsalis	395.3±29.4 (4)	4.3
Area lateralis	252.9± 5.9 (4)	2.8
Nucleus arcuatus	177.1± 9.9 (5)	1.9
Infundibular stem	78.8± 6.0 (5)	0.9

* The results are expressed as mean values (µmoles $^{14}CO_2$ formed/g dry wt/h) ± SEM; the numbers of animals are in parenthesis.
** The values as ratios to the activity in the median eminence.

present study, each nucleus was dissected from freeze-dried sections (100 µm) and only one dissected sample was used for each assay. In freeze-dried samples, fine structures were visualized clearly as reported by Lowry[9], and the enzyme in the sections stored in evacuated tubes (−20 °C) was stable for at least 12 months.

It is of interest that GAD activity were unevenly distributed in hypothalamus and the distribution is strikingly different from that of other neurotransmitter-related enzymes such as tyrosine hydroxylase[10], choline acetyltransferase[6,7], monoamine oxidase[11,12] and DOPA decarboxylase[13]. In addition, the present method, using microdissection technique with freeze-dried sections, may help in understanding the physiological roles of the neurotransmitter in the hypothalamic nuclei.

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Functional role of arginine residues in glutamic acid decarboxylase from brain and bacteria

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Summary. The arginine-specific reagent phenylglyoxal rapidly inactives glutamic decarboxylase from both mouse brain and *E. coli* when preincubated with the enzyme at concentrations of 3 mM to 40 mM. The rate of inactivation follows pseudo-first-order kinetics and is dependent upon the concentration of phenylglyoxal. These and other data presented support the idea that arginine residues play a key role in the mechanism of action of glutamic decarboxylase.

A number of mono- and dicarboxylic aliphatic acids are pott competitive inhibitors of brain and bacterial glutamic acid decarboxylase (GAD) (4.1.1.15; L-glutamate 1-carboxy-lyase)[1,2]. The requirement of a carboxylic moiety as an integral part of the structure of these inhibitors and the presence of 2 carboxylates in the substrate molecule (glutamate) have let us to speculate the existence of an anionic substrate or inhibitor binding site in these decarboxylases. The most likely amino acid residue involved at the anionic substrate binding site is arginine. Indeed, arginine has been shown to play a general role in the functional binding of anionic cofactors and substrates of a number of enzymes. For example, by using arginine-specific protein modifying agents such as phenylglyoxal and other dicarbonyl compounds, it has been demonstrated that arginine residues are involved in the binding of phosphate-containing cofactors or substrates of several enzymes, notably the enzymes of the glycolytic pathway (see Riordan et al.[3] and references cited therein).

We now report the existence of such functional arginine residues in GAD from mouse brain and *E. coli*. This is the first evidence of the participation of arginine residues in the catalytic action of decarboxylases.

Materials and methods. Swiss white male mice of 9 weeks of age were used as the source of the brain enzyme. The GAD was extracted and an ammonium sulphate fraction prepared as previously described[4].

The mouse enzyme was assayed after the method of Wu et al.[5]. The bacterial enzyme was bought from the Sigma

The inactivation of glutamic acid decarboxylase from mouse brain and *E. coli*

Compound added (10 mM)	Enzyme activity (percent of control) Mouse brain		*E. coli*	
	No borate	50 mM borate	No borate	50 mM borate
None	100	100	100	100
Phenylglyoxal	23	21	15	19
Glyoxal	26	23	21	23
2,4-Pentanedione	82	68	74	39
2,3-Butanedione	88	73	71	33
1,2-Cyclohexanedione	87	80	79	45

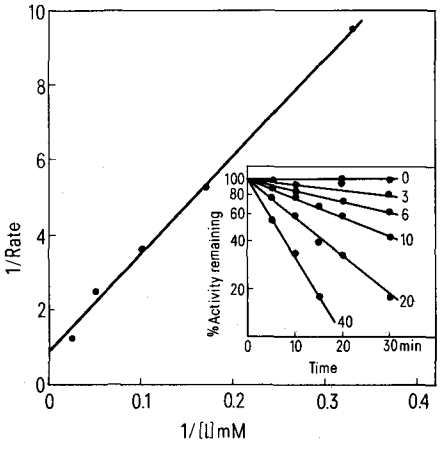

Fig. 1. Inactivation of brain glutamic acid decarboxylase against time by phenylglyoxal (I). Prior to its assay as described in the 'methods' section the enzyme was incubated at 23 °C with varying concentrations (mM) of inactivator as indicated by the numbers 0–40 (see insert). The rate of inactivation (enzyme activity in µmoles/h mg protein lost per min) was calculated and presented as a double reciprocal plot.