high CSF levels of carbamazepine[7]. This equates with the potentiation of NMDA-induced depolarization reported in this paper with concentrations of carbamazepine exceeding 50 μM. We have preliminary evidence that perfusion of cortical slices with NMDA releases endogenous glutamate. Thus, carbamazepine, being a tricyclic structure, might inhibit the Na^+-dependent uptake of glutamate at these higher concentrations leading to a potentiated depolarization.

Carbamazepine at all concentrations used in our experiments had no effect on the spontaneous depolarizing shifts which suggests a lack of blocking effect at Na^+ channels especially since tetrodotoxin at 1 μM completely inhibits these spontaneous depolarizing shifts[5]. The site of action of carbamazepine on the NMDA receptor/channel complex cannot be defined from these present experiments. However, it does appear unlikely that it is within the NMDA receptor-operated channel, as compounds such as ketamine and dizocilipine, which block the channel, also inhibit the spontaneous depolarizations[8]. An inhibitory action at either the NMDA recognition site, or the glycine modulatory site, remain as possibilities. Antagonists at the NMDA recognition site have been shown to have potent anticonvulsant activity while antagonists at the glycine site are weakly anticonvulsant[9,10]. The results presented in this paper would favour an antagonistic action at the NMDA receptor site in view of the profound reduction in response seen with low concentrations of carbamazepine.

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0014-4754/92/080751-03$1.50 + 0.20/0

Involvement of D-amino acid oxidase in elimination of D-serine in mouse brain

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Received 12 December 1991; accepted 7 April 1992

Abstract. The physiological role of D-amino acid oxidase (EC 1. 4. 3. 3) in mouse brain is described. The presence of D-enantiomers of neutral common amino acids was surveyed in the brain. D-serine was shown to be present at high concentration only in regions where the enzyme activity was low. In normal mice whose D-amino acid oxidase activity was much higher in the cerebellum than in the cerebrum, free D-serine content was apparently lower in the cerebellum than in the cerebrum. In mice of a mutant strain lacking D-amino acid-oxidase activity, the free D-serine level was remarkably high both in the cerebrum and cerebellum. The results suggest that the enzyme is involved in the elimination of free D-serine in the cerebellum.
Key words. D-amino acid oxidase; D-serine; cerebellum; cerebrum.

D-Amino acid oxidase (DAAO) is a flavoenzyme widespread in many animal tissues that catalyzes the oxidative deamination of free neutral D-amino acids to the corresponding 2-oxo acids[1]. However, the physiological function of the enzyme has remained unknown. One reason is that the substrate D-amino acids have not been believed to be present in animal tissues to any significant extent. Hamilton et al.[2] suggested that the physiological substrates are adducts of amines with glyoxylate. However, substantial amounts of free neutral D-amino acids have recently been found in samples free from the action of DAAO, i.e., human plasma from patients with renal diseases[3], and DAAO-lacking mutant-mouse tissues such as kidney, liver, lung, heart, brain and serum[4]. The presence of D-alanine, D-proline and D-serine has been recently demonstrated by high-performance liquid chromatography (HPLC) analysis in mouse kidney and serum[5]. These studies suggest that DAAO is involved in the catabolism of free neutral D-amino acids.

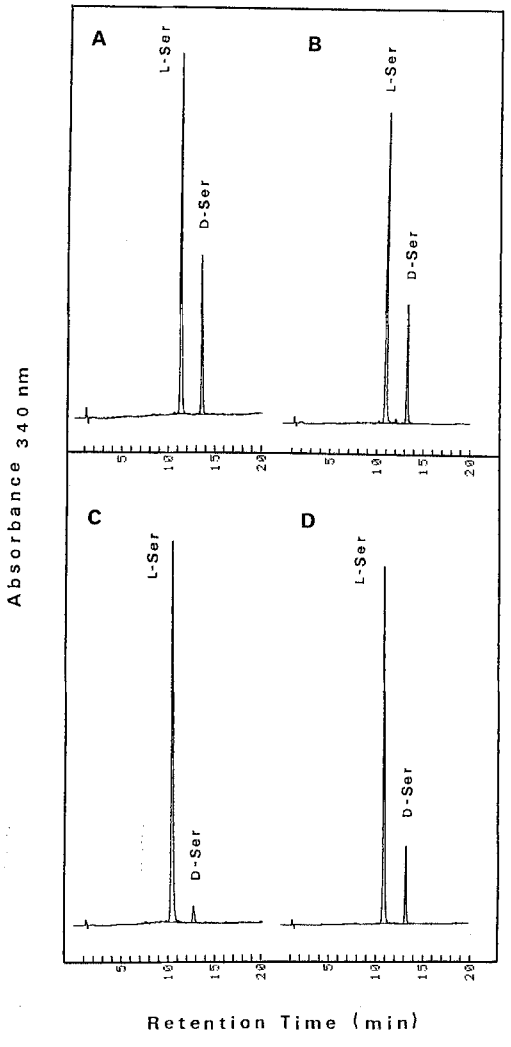
In the present experiment, the relationship between amounts of D-amino acids and DAAO activity was investigated in order to confirm the above hypothesis for the brain, and it has been revealed that the content of free D-serine is high where DAAO activity is low.

Materials and methods

The mutant mouse strain ddY/DAO-, lacking DAAO activity in the kidney and brain, was established[6, 7], and raised together with the normal control ddY/DAO+ mice at Dokkyo University, School of Medicine (Mibu, Tochigi). Male mice (8 weeks old) were chosen for the experiment, since they possess a higher DAAO activity than females[6]. The cerebrum or cerebellum was homogenized with 4 vols of phosphate-buffered saline (150 mM NaCl/10 mM sodium phosphate buffer [pH 7.4]) using a glass homogenizer in an ice bucket at 1100 rpm for 1 min. The homogenate was centrifuged at 16,000 × g for 30 min at 4 °C. Cold trichloroacetic acid (TCA) solution was added to the supernatant extract, to give a final concentration of 5%. The supernatant fraction containing free amino acids was passed through a Dowex 1 × 8 column (acetate form; Muromachi Chemicals, Tokyo) to remove TCA and acidic amino acids, and then, derivatized with 1-fluoro-2,4-dinitropehenyl-5-L-alanine amide (FDAA), according to the method of Marfey[8]. Resolution of enantiomers was performed as described elsewhere[9]. Briefly, HPLC was carried out using a reversed-phase column, Nova-Pak C18 (150 × 3.9 mm ID, Waters, Milford, MA, USA) and an HPLC system (Tosoh, Tokyo). The samples were eluted from the column with a linear gradient of acetonitrile in 50 mM triethylamine-phosphate buffer (pH 3.5) from 10 to 40% over 45 min at 1.0 ml/min and at 22 °C. The eluate was monitored at 340 nm with a D-2500 Chromato-Integrator (Hitachi, Tokyo), and peak areas of the FDAA-amino acids were obtained automatically. Amounts of D- and L-amino acids present were estimated on the basis of the peak areas and the standard curves[9]. DAAO activity was determined using D-alanine as the substrate[10].

Results and discussion

Free serine, threonine, glutamine, asparagine, alanine, proline, methionine, tyrosine, valine, isoleucine, leucine and phenylalanine from the brain were analyzed for the presence of D-enantiomers. Only serine was found to contain a significant amount of D-serine. HPLC profiles of free serine from the cerebrum and cerebellum of the normal and mutant mice are represented in the figure. As seen in the figure, a surprisingly high proportion of serine has the D-configuration in the cerebrum of both the normal (fig. 1 A) and mutant animals (fig. 1 B). In the cerebellum, the D/L ratio of serine seems to be higher in the mutant (fig. 1 D) than in the control mice (fig. 1 C). Table 1 shows the contents of D- and L-serine, the D/L ratio, and DAAO activity in mouse brain. In the mutant mouse, no DAAO activity was observed[7]. In the normal animal, the DAAO activity was much higher in the cerebellum than in the cerebrum ($p < 0.001$). The L-serine content showed no difference between cerebrum and cerebellum, and normal and mutant mice. In contrast, the D-serine content was significantly higher in the cere-



HPLC profiles of free serine from cerebrum of a normal mouse (A) and a DAAO-lacking mutant mouse (B); from cerebellum of the normal mouse (C) and the mutant mouse (D). Aliquots of 5 µl were injected into the HPLC system, and HPLC was performed as described under 'Materials and methods'.

Content of free D- and L-serine, and DAAO activity in mouse cerebrum and cerebellum. Values for normal control and DAAO-lacking mutant mice are shown. The serine content and DAAO activity are expressed as nanomole per g tissues and nanomole D-alanine oxidized per minute per mg protein, respectively. Values are means ± SED for three or four animals.

Mouse	Content of serine enantiomers (nmol/g) D-serine	L-serine	D/L
Cerebrum			
Normal (n = 3)	210.3 ± 12.1	492.7 ± 110.2	0.4428
Mutant (n = 3)	198.0 ± 29.7	459.0 ± 63.6	0.4310
Cerebellum			
Normal (n = 3)	33.0 ± 21.1	473.7 ± 52.2	0.0695
Mutant (n = 3)	137.5 ± 10.6	539.5 ± 72.1	0.2558

	DAAO Activity (nmol/min/mg) Cerebrum	Cerebellum
Normal (n = 4)	0.165 ± 0.087	1.103 ± 0.163

brum than in the cerebellum in normal mice ($p < 0.001$). The D-serine level in the cerebellum was significantly higher in the mutant than in normal mice ($p < 0.01$). The D/L ratio in the cerebrum was high in both the normal and the mutant mice. The high D-serine content in the cerebrum of the normal mice is probably due to the low DAAO activity. The D/L ratio in the cerebellum, was much lower in the normal mice as compared to the mutant animals. As to other amino acids, only the D-enantiomer of alanine was shown to be present. The D/L ratios were 0.0170 and 0.0348, respectively, in the cerebrum and cerebellum of the mutant animal, and 0.0055 and 0.0030, respectively, in the control mouse. Taken together, it appears that DAAO is mostly involved in the catabolism of D-serine.

DAAO is localized in Bergmann glial cells and astrocytes[11]: the greater proportion of the activity is in the glial spaces around the various kinds of synapses. D-serine is known to modulate N-methyl-D-aspartate (NMDA) receptor-mediated responses. D-Serine applied iontophoretically to rat thalamus neurons may have a dual action, a facilitation of NMDA receptor-mediated responses and a non-selective inhibitory action[12]. The effect of a depressant reagent on root potentials of rat spinal cord was reversed by D-serine in vitro[13]. Hence, it seems possible that DAAO plays a role in the mouse nervous system. The physiological meaning of the presence of large amounts of D-serine in the cerebrum is a matter for further study.

Acknowledgment. The author is grateful to Dr. R. Konno of Dokkyo University (Department of Microbiology, School of Medicine, Mibu, Tochigi) for providing the brains of mutant and normal control mice.

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0014-4754/92/080753-03$1.50 + 0.20/0

Methimazole treatment reduces cardiac hypertrophy and mortality without a concomitant reduction in blood pressure in established Goldblatt two-kidney one clip hypertension

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Received 14 October 1991; accepted 14 January 1992

Abstract. The effects of methimazole, an antithyroid drug, on blood pressure and other parameters were evaluated in the established phase of Goldblatt two-kidney one clip (G2K-1C) hypertension. Methimazole was administered via drinking water for five weeks, starting five weeks after hypertension had been induced. After this period of treatment, similarly high blood pressures were observed in methimazole-treated and non-treated G2K-1C rats, despite the fact that a hypothyroid state had been achieved in methimazole-treated rats. Methimazole-treated G2K-1C rats showed reductions in heart rate, ventricular weight, ventricular/body weight ratio and mortality in comparison with rats not treated with methimazole. These results clearly demonstrate that hypothyroidism induced by methimazole: a) does not reverse G2K-1C hypertension, but b) improves the rate of survival and c) reduces relative cardiac hypertrophy, possibly by the reduction in cardiac work observed in Goldblatt hypothyroid rats.
Key words. Goldblatt two-kidney one clip hypertension; methimazole; hypothyroidism; relative cardiac hypertrophy.

Thyroidectomy and antithyroid drugs reduce high blood pressure in experimental hypertension produced in several different ways, despite differences in the underlying pathophysiological mechanisms. This reduction is observed especially in the early phase of hypertension. Thus, when a hypothyroid state is produced in young spontaneously hypertensive rats (SHR), or simultaneously with the induction of hypertension by experimental means, it is able to prevent genetic[2,3], DOCA-salt[4,5], low-renal-mass[6], renal[7], and Goldblatt two-kidney one clip (G2K-1C)[5] hypertension. However, in the established phase of hypertension, discrepancies have been observed depending on the model[2,8], and the duration[3] of hypertension.