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PRELIMINARY COMMUNICATION

INHIBITION OF HUMAN AND MOUSE BRAIN GLUTAMATE DECARBOXYLASE BY THE α-KETO ANALOGS OF CYSTEINE AND HOMOCYSTEINE

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Studies in this laboratory on the mechanism of action of the convulsant allylglycine (2-amino-4-pentenoic acid) have led to the discovery that 2-keto-4-pentenoic acid, formed by the oxidative deamination of allylglycine, is a potent competitive inhibitor of brain glutamate decarboxylase (EC 4.1.1.15) with respect to glutamate [1,2]. The K_i value is 2.4×10^{-6} M [2]. In addition to its convulsive properties [3], allylglycine is a metabolic antagonist of cysteine and has been shown to inhibit the growth of Escherichia coli [4]. Antagonism was postulated to be due to a structural similarity between the sulfhydryl group of cysteine and the vinyl group of allylglycine. Therefore, it was of interest to determine if oxidative deamination of cysteine to its keto analog, β-mercaptopyruvate, would yield an inhibitor of glutamate decarboxylase.

β-Mercaptopyruvate was synthesized by the method of Kun [5] from hydrogen sulfide and bromopyruvate or was generated from L-cysteine in the presence of L-amino acid oxidase. Glutamate decarboxylase activity was determined by measuring the release of $^{14}CO_2$ from [1-^{14}C]-L-glutamate as described previously [1].

The effect of the keto analog of cysteine on the activity of glutamate decarboxylase was studied in preliminary experiments by incubating the enzyme with L-cysteine in the presence of L-amino acid oxidase. The action of L-amino acid oxidase on L-cysteine, under these conditions, is expected to generate β-mercaptopyruvate. The results (Table 1) show that cysteine at the concentration used has no effect on enzyme activity. In the presence of L-amino acid oxidase, however, strong inhibition of the enzyme is observed. Under the same conditions, homocysteine is also converted to an inhibitory derivative, presumably α-keto-γ-mercaptobutyrate. In contrast, the product of oxidative deamination of methionine has no effect on enzyme activity.

The inhibition of glutamate decarboxylase from both human and mouse brain was examined using synthetic β-mercaptopyruvate. The effect of various concentrations of β-mercaptopyruvate

Table 1. Effect of oxidative deamination products of sulfur amino acids on the activity of glutamate decarboxylase*

Reaction mixture	Glutamate decarboxylase activity (μmoles/g/hr)
Full system = A†	33.1
A + L-amino acid oxidase	32.8
A + cysteine (5 mM)	34.3
A + cysteine (5 mM) + L-amino acid oxidase	7.4
A + homocysteine (5 mM)	31.8
A + homocysteine (5 mM) + L-amino acid oxidase	13.8
A + methionine (5 mM)	33.1
A + methionine (5 mM) + L-amino acid oxidase	32.8

*Mouse brain homogenates (20%) were prepared in 0.01 M sodium phosphate buffer (pH 6.8) containing 1.0% Triton X-100. They were centrifuged at 770 g for 10 min and the supernatant solution was used for enzyme determination.

†The full system contained homogenate (50 mg tissue); L-glutamate, 32.5 mM; dithiothreitol, 1.0 mM; pyridoxal-5'-phosphate, 0.5 mM; and sodium phosphate buffer, 70 mM, pH 6.8, in a final volume of 1.0 ml. Incubations were carried out for 60 min at 37°. Data represent the mean value of four determinations.

on enzyme activity is shown in Table 2. The enzyme from both sources is almost completely inhibited at inhibitor concentrations of 1.0 mM. At concentrations of 0.01 mM strong inhibition is observed with both enzymes. Preliminary experiments have shown that the inhibition is competitive with respect to glutamate. The K_i is on the order of 10^{-6} M.

β-Mercaptopyruvate is highly toxic to mice causing convulsions and death following intraperitoneal administration. In preliminary experiments with a dose of 7 μmoles/g, convulsions were accompanied by a significant inhibition of brain glutamate decarboxylase. Detailed studies on the *in vivo* inhibition of the enzyme by β-mercaptopyruvate will be the subject of a separate communication.

The activity of glutamate decarboxylase normally determines the steady-state levels of GABA, a proposed inhibitory neurotransmitter in the mammalian central nervous system [7]. The appearance of convulsions in animals after administration of various compounds which inhibit the enzyme, such as 3-mercaptopropionate [8], thiomalate [9], allylglycine [1,3] and others, has provided the basis for speculation that decreased synthesis of GABA may be one of the factors involved in the etiology of human convulsive disorders [10]. The finding of endogenous metabolites which inhibit glutamate decarboxylase may, therefore, be of considerable interest in the understanding of these disorders.

The present report describes the inhibition of both human and mouse brain glutamate decarboxylase by β-mercaptopyruvate and α-keto-γ-mercaptobutyrate, the α-keto analogs of cysteine and homocysteine respectively. These compounds are strong inhibitors of the enzyme. The K_i of β-mercaptopyruvate is equal to that of the most potent known inhibitors of glutamate decarboxylase [11]. While we did not synthesize α-keto-γ-mercaptobutyrate, it is apparent from our experiments that it is highly inhibitory. Several metabolites, such as α-ketoglutarate, malate and succinic semialdehyde, are known to inhibit glutamate decarboxylase. The inhibition constants of these metabolites are, however, about three orders of magnitude higher than that of β-mercaptopyruvate [11].

Table 2. Inhibition of human and mouse brain glutamate decarboxylase by β-mercaptopyruvate*

Concentration of β-mercaptopyruvate (mM)	Glutamate decarboxylase activity	
	Human brain (μmoles/g/hr)	Mouse brain (nmoles/mg protein/min)
0	3.85	8.82
1.0	0.09 (97.7)	0.30 (96.6)
0.1	0.45 (88.3)	0.75 (91.5)
0.01	1.65 (57.1)	2.50 (71.7)

*Post-mortem human brain was obtained at autopsy and was maintained at -70° until use. Homogenates were prepared as described in Table 1 and the supernatant solution was used to determine activity. The mouse brain enzyme was a partially purified preparation obtained according to Wu et al. [6]. Activity was determined in an incubation mixture containing enzyme (human brain homogenate or mouse brain enzyme); L-glutamate, 10.0 mM; dithiothreitol, 1.0 mM; pyridoxal phosphate, 0.5 mM; and sodium phosphate buffer, 70 mM, pH 6.8, in a final volume of 1.0 ml. Incubations were carried out for 30 min at 37°. Data represent the mean value of three determinations. Values in parentheses represent per cent inhibition relative to control.

β-Mercaptopyruvate is the product of the transamination of cysteine with α-ketoglutarate [12]. Its concentration has not been determined in mammalian tissues. Evidence, however, for its formation in vivo was provided by the detection of β-mercaptolactate-cysteine disulfide as a constituent of human urine [13]. β-Mercaptopyruvate had been shown previously to be a substrate for lactate dehydrogenase [5]. Evidence for the formation of β-mercaptolactate-cysteine disulfide from cysteine was supplied by Crawhall and associates; subjects given a diet supplemented with cysteine showed a marked increase in the excretion of the mixed disulfide in urine [14]. Furthermore, the intravenous administration of [^{14}C]-L-cystine to human subjects led to incorporation of radiolabel into β-mercaptolactate-cysteine disulfide [15].

Evidence was also presented for the accumulation and excretion of significant amounts of the keto analogs of cysteine and homocysteine in patients with homocystinuria. This disorder, characterized by an increased level of homocystine in both plasma and urine [16], is the second most common inborn error of amino acid metabolism [17]. The disulfides β-mercapto-

lactate-homocysteine, α-hydroxy-γ-mercaptobutyrate-homocysteine and α-hydroxy-γ-mercaptobutyrate-cysteine are found in the urine of homocystinuric patients [18]. Therefore, it is of interest that seizures occur in 10-15 per cent of patients with homocystinuria [16]. While seizures in homocystinuric patients are attributed usually to cerebrovascular lesions, the possibility should be considered that the elevated concentrations of β-mercaptopyruvate and α-keto-γ-mercaptobutyrate are a contributory factor.

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