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INHIBITION OF γ-AMINOBUTYRIC ACID AMINOTRANSFERASE FROM RAT BRAIN MITOCHONDRIA BY CHLORIDE AND ACETATE

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10–100 mmol/l of acetate inhibits γ-aminobutyric acid aminotransferase from rat brain mitochondria by 35–90% Similarly, 50–100 mmol/l of chloride inhibits this enzyme by 10–30%. In either case, the inhibition is competitive with GABA and noncompetitive with α-ketoglutarate. The effect of sodium and potassium is negligible. γ-Aminobutyric acid aminotransferase from rat liver mitochondria is also inhibited by acetate and chloride. The degree of inhibition of both hepatic and cerebral enzyme is nearly the same.

KEY WORDS: γ-aminobutyric acid aminotransferase, inhibition, chloride, acetate

INTRODUCTION

In the course of our study on the metabolism of γ-aminobutyric acid we obtained evidence that some physiologically occurring anions inhibit the activity of GABA-AT† from rat brain mitochondria. Supposing that this finding may be of some general importance, we studied the inhibition of GABA-AT by acetate and chloride in more detail and report our results here.

MATERIALS AND METHODS

Enzyme Source

Mitochondrial matrix from rat brain and liver was used. The animals were killed by decapitation, the organs removed and homogenized with a ten-fold excess (wt./vol.) of

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† GABA-AT: γ-aminobutyric acid aminotransferase (EC 2.6.1.19); GABA: γ-aminobutyric acid; SSDH: succinic semialdehyde dehydrogenase; PLP: pyridoxal-5-phosphate; AMS: ammonium sulfate.

20 mmol/l phosphate buffer, pH 7.4, containing 0.05 mmol/l PLP. The mitochondria were sedimented by 10 min of centrifugation at 10000 × g. The sediment was washed with the homogenization buffer, centrifuged and resuspended in deionized water. Mitochondrial matrix obtained by the freeze-and-thawing method[1] was dialyzed overnight against two changes of 20-fold sample volume of 0.05 mmol/l PLP buffered to pH 7.4.

Assay of GABA-AT Activity

GABA-AT catalyzes the reaction

$$\text{GABA} + \alpha\text{-ketoglutarate} \rightleftharpoons \text{glutamate} + \text{succinic semialdehyde}. \quad (1)$$

The most common method for the measurement of GABA-AT activity includes the incubation with radioactively labelled GABA and the separation of glutamate with subsequent determination of its radioactivity.[2] Other methods are based on the determination of succinic semialdehyde. This compound yields a fluorescent condensation product with cyclohexane-1,3-dione[3] but the reaction is not very specific. Succinic semialdehyde can also be measured enzymatically by the reaction catalyzed by SSDH,

$$\text{succinic semialdehyde} + \text{NADP} \rightleftharpoons \text{succinate} + \text{NADPH}. \quad (2)$$

Thus, the amount of NADPH formed reflects the activity of GABA-AT. Unfortunately, SSDH is not commercially available. Apart from the tedious isolation of the enzyme from animal tissues,[4,5] SSDH can be prepared from the so called "GABA-ase" which is the lyophilized extract from *Pseudomonas fluorescens* containing SSDH and GABA-AT. The latter activity can be removed by gel filtration[6] or by inactivation followed by dialysis to remove the excess of the inhibitor.[7,8] We obtained a GABA-AT-free SSDH preparation in the following manner:

20 mg of GABA-ase were dissolved in 0.9 ml of tris buffer, pH 7.4 and mixed with 0.1 ml of an aqueous solution (10 mmol/l) of aminooxyacetic acid. After 10 min of incubation at 4°C, the enzyme was precipitated with AMS saturated to 70%, centrifuged for 10 min at 10000 × g, washed with AMS, centrifuged again and dissolved in buffer. At 4°C, the preparation was stable for about one week and at −70°C, the activity remained unchanged for several weeks.

The assay system contained, in 1 ml of total volume, 1 mmol/l NADP, 0.25 units[‡] of SSDH, enzyme, GABA, α-ketoglutarate and 0.05 mol/l buffer, pH 8.2. The concentrations of GABA, α-ketoglutarate, as well as the composition of the buffer are specified in the legends to the tables and figures. The mixture was incubated at 37°C for at least 3 min and the increase in extinction at 340 nm was monitored using the Beckman DU 40 spectral photometer.

[‡] One unit of enzyme activity is defined as the amount of enzyme which catalyzes the formation of 1 μmol of NADPH/min at 37°C.

Other Methods

The concentration of proteins was measured by the method of Smith and coworkers.[9] The amounts of AMS necessary for the required saturation were estimated using the monogram of Dixon.[10] The software package STATGRAPHICS, version 7 of Manugistics, Inc., was used for the statistical evaluation as well as for drawing of figures.

Reagents

GABA, α-ketoglutarate, PLP and NADP were purchased from Serva, Heidelberg; buffer substances and aminooxyacetic acid were obtained form Sigma Chemical Co., St. Louis. GABA-ase was supplied by Boehringer, Mannheim.

RESULTS AND DISCUSSION

It is generally known that any determination of enzyme activity implies the use of an appropriate buffer. Taking account of the possible inhibitory effect of various ions, we purposely examined several buffers to find out which of them did not influence the activity of GABA-AT. Assuming that the degree of inhibition, if any, will depend on the concentration of the inhibitor, we measured the activity of GABA-AT in identical samples using pyrophosphate buffer at concentrations varying between 5 and 50 mmol/l. Under such experimental conditions the activity of GABA-AT remained constant. Furthermore, 50 mmol/l of bicine solution buffered with tris yielded the same results as sodium pyrophosphate. Therefore we concluded that 50 mmol/l of both sodium pyrophosphate and bicine-tris solutions do not interfere with the determination of GABA-AT activity.

Initial screening revealed that acetate and chloride inhibit the activity of GABA-AT. On the other hand, the effect of formate as well as that of the cations (sodium, potassium and ammonium) was negligible (Table 1). A double reciprocal plot with GABA as the variable substrate and acetate as inhibitor show a pattern consistent with the mathematical formula[§] for the competitive inhibition since the intercepts do not differ significantly when tested by ANOVA (Figure 1A). With α-ketoglutarate (Figure 1B) as the variable substrate, the lines intersect at one point to the left of the origin and above the $1/s$ axis which indicates that the inhibition is noncompetitive and that the inhibitor not only diminishes the amount of the enzyme available for the catalysis but also affects the affinity of the free enzyme for the substrate. Similarly, the results shown in Figure 2 allow the conclusion to be drawn that chloride inhibits GABA-AT competitively with GABA and noncompetitively with α-ketoglutarate.

Additional experiments have shown that the matrix of rat liver mitochondria contains a weak GABA-AT activity as well. With respect to the protein concentration, this activity is approximately one tenth of that in the brain. Like the cerebral GABA-AT, the hepatic enzyme is inhibited by chloride and more strongly by acetate (Table 2).

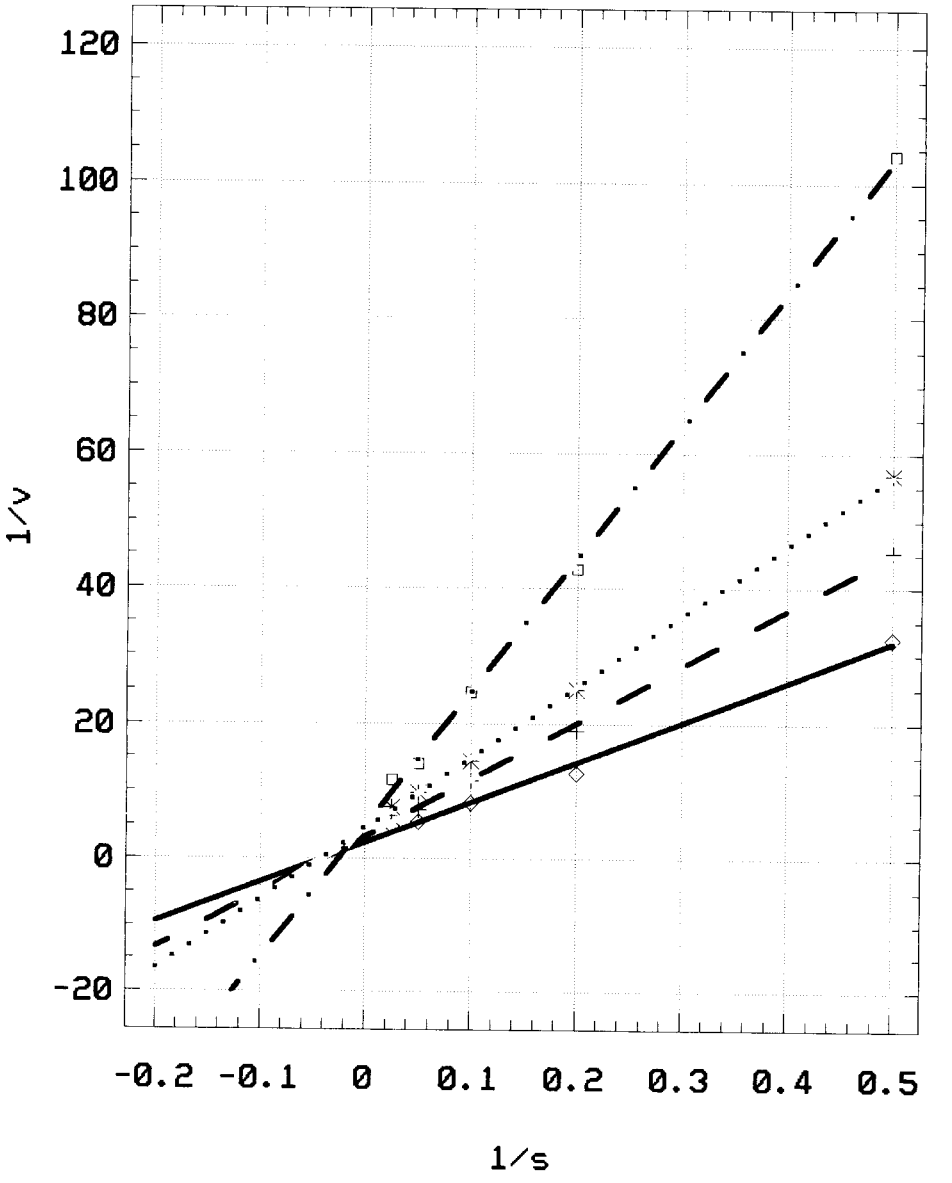
[§] $1/v = k_{ES}/V_{max} . 1/[S] . (1 + [I]/k_{EI}) + 1/V_{max}$.

TABLE 1
Inhibition of GABA-AT from rat brain mitochondria.*

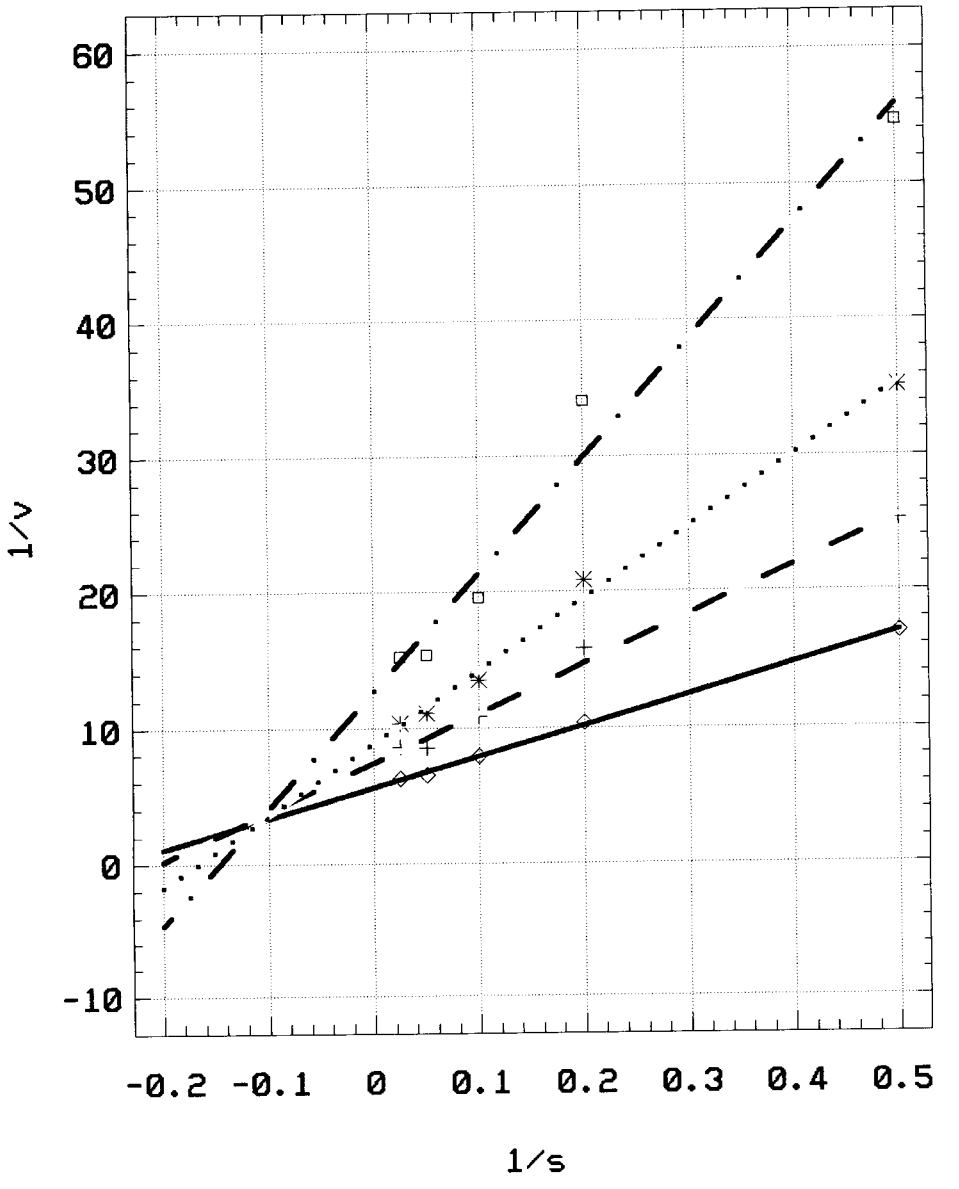
Buffer	Inhibitor	Inhibition
pyrophosphate	$NH_4OOC.CH_3$, 10 mmol/l	35%
	$NH_4OOC.CH_3$, 50 mmol/l	65%
	$NH_4OOC.CH_3$, 100 mmol/l	90%
	NH_4Cl, 50 mmol/l	10%
	NH_4Cl, 100 mmol/l	32%
	NaCl, 100 mmol/l	23%
	KCl, 100 mmol/l	26%
tris-bicine	$NH_4OOC.CH_3$, 50 mmol/l	65%
	Na^+, 50 mmol/l†	≤5%§
	NH_4^+, 50 mmol/l†	≤5%§
	CH_3COO^-, 50 mmol/l‡	68%
	$HCOO^-$, 50 mmol/l‡	7%
	Cl^-, 50 mmol/l‡	19%

* The concentration of GABA in the assay system was 20 mmol/l and that of α-ketoglutarate was 5 mmol/l. The concentration of the buffer was 50 mmol/l. For details, see MATERIALS AND METHODS. † Buffered with bicine. ‡ Buffered with tris. § i.e., less than the experimental error.

The influence of chloride on GABA-AT activity may be of some physiological importance since the inhibitory concentrations of this ion are present in most tissues. It follows that the changes in chloride content may contribute to the regulation of GABA-AT activity and can take effect upon the metabolism of the most important inhibitory neurotransmitter, GABA, thus affecting the signal transduction in central nervous system. In diseases which are accompanied with an extreme hyperchloremia (e.g., nephrotic syndrome) or hypochloremia (e.g., M. Addison or diarrhoea), irregularities in the function of the GABA-ergic system can be expected, but, however, they can be masked by other symptoms caused by the underlying illness. On the other hand, the concentration of acetate in various tissues of the rat[11] are lower by one order of magnitude at least than the concentrations required for the inhibition of GABA-AT and therefore the effect of acetate alone seems to be questionable. However, the results of our preliminary experiments show that some other physiologically occurring anions inhibit GABA-AT as well and that the simultaneous effect of several inhibitors is additive.

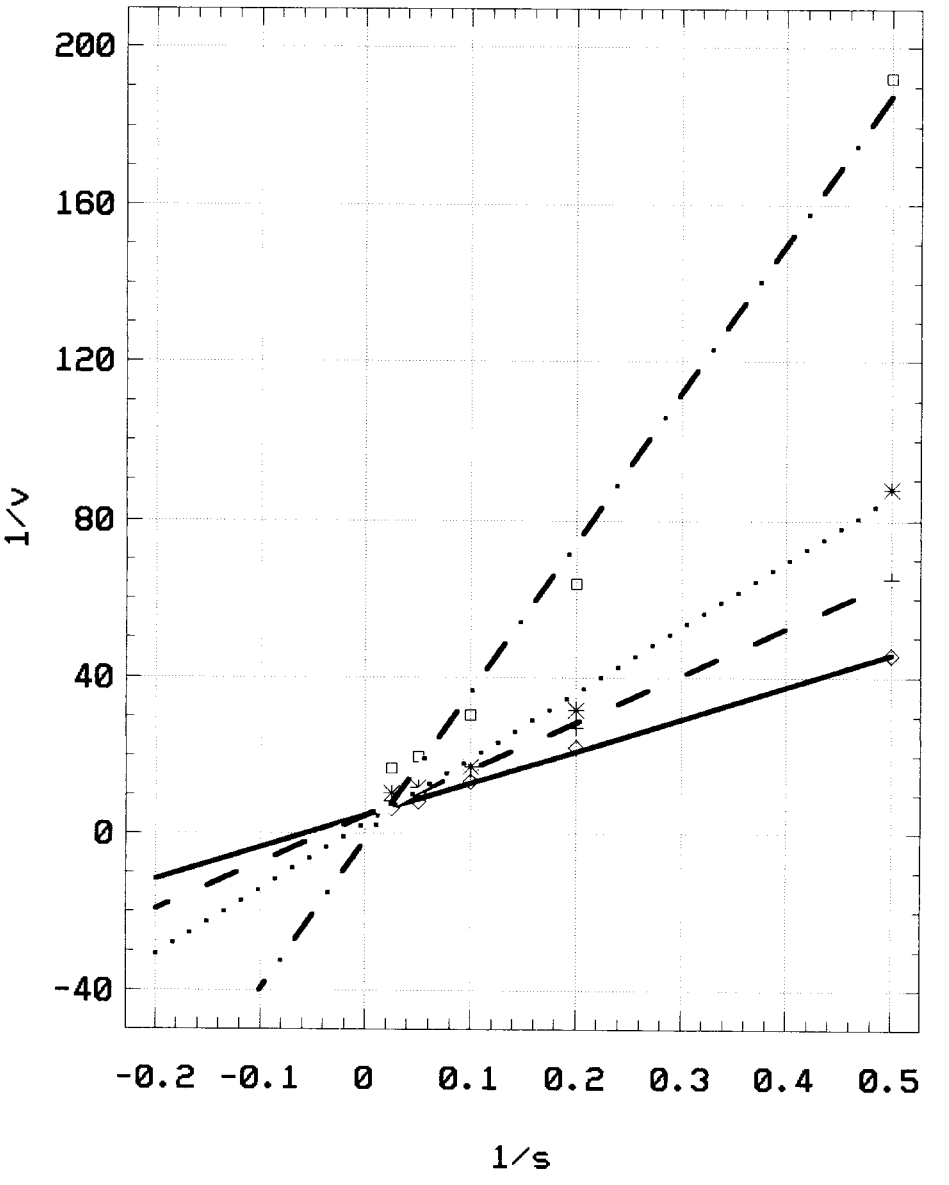


1A



1B

FIGURE 1 The kinetics of GABA-AT inhibition by acetate. The assay system contained GABA, α-ketoglutarate at concentrations indicated below and 50 mmol/l tris-bicine buffer, pH 8.2. (A): GABA was the variable substrate, the concentration of α-ketoglutarate was 5 mmol/l. (B): α-ketoglutarate was the variable substrate, the concentration of GABA was 20 mmol/l. For experimental details, see MATERIALS AND METHODS. S is given in mmol/l, v is given in units of enzyme activity per 1 mg of protein. The concentrations of acetate are denoted as follows: ◇ 0 mmol/l; + 10 mmol/l; * 20 mmol/l; □ 50 mmol/l.



2A

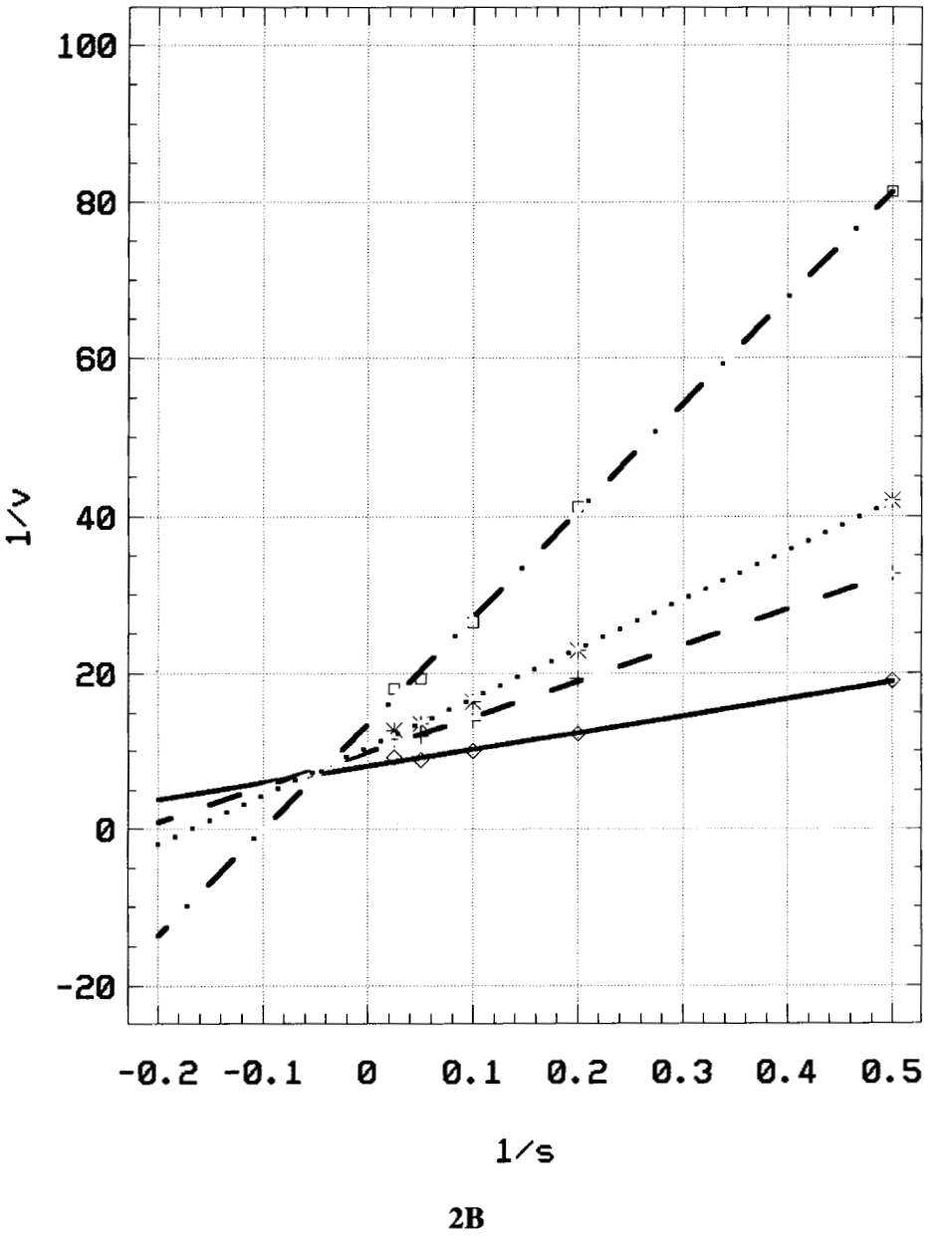


FIGURE 2 The kinetics of GABA-AT inhibition by chloride. The assay system contained GABA, α-ketoglutarate at concentrations indicated below and 50 mmol/l tris-bicine buffer, pH 8.2. (A): GABA was the variable substrate, the concentration of α-ketoglutarate was 5 mmol/l. (B): α-ketoglutarate was the variable substrate, the concentration of GABA was 20 mmol/l. For experimental details, see MATERIALS AND METHODS. The concentrations of chloride are denoted as follows: ◇ 0 mmol/l; + 50 mmol/l; * 100 mmol/l; □ 200 mmol/l.

TABLE 2
Inhibition of hepatic GABA-AT.*

Inhibitor		Inhibition
acetate,†	10 mmol/l	27%
	50 mmol/l	77%
	100 mmol/l	91%
chloride,†	10 mmol/l	38%
	100 mmol/l	58%
	200 mmol/l	88%

* The buffer was 50 mmol/l tris-bicine, pH 8.2. For details, see MATERIALS AND METHODS. † Buffered with tris.

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