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# COMPARISON OF THE STRUCTURAL CHARACTERISTICS OF THE 4-AMINOBUTYRATE:2-OXOGLUTARATE TRANSAMINASES FROM RAT AND HUMAN BRAIN, AND OF THEIR AFFINITIES FOR CERTAIN INHIBITORS

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## Summary

4-Aminobutyrate:2-oxoglutarate (4-aminobutyrate:2-oxoglutarate aminotransferase, EC 2.6.1.19) from human brain has been purified 2500-fold with respect to the initial homogenate. The enzyme, which appears to be pure by polyacrylamide gel electrophoresis, N-terminal analysis and immunodiffusion, was compared to rat brain 4-aminobutyrate transaminase, purified to the same extent in an earlier study [15]. The two enzymes, which have approximately the same molecular weight, show large differences in their tryptic fingerprints and in the peptides produced by cyanogen bromide cleavage. The $K_m$ values (limit) for 4-aminobutyrate are different, the human enzyme having four times greater affinity for this substrate. A series of branched-chain fatty acids (including *n*-dipropylacetate), which are structural analogues of 4-aminobutyrate and inhibit rat brain 4-aminobutyrate transaminase, are less powerful inhibitors of the human enzyme.

## Introduction

Study of the effects on neuronal activity of raising cerebral 4-aminobutyrate levels in vivo has often been used as a way of studying this inhibitory mediator. Usually, this increase in 4-aminobutyrate has been effected by the inhibition of its catabolic enzyme, 4-aminobutyrate:2-oxoglutarate aminotransferase (4-aminobutyrate:2-oxoglutarate aminotransferase, EC 2.6.1.19). The inhibitors employed are either molecules which combine with pyridoxal phosphate [1] or are structural analogues of 4-aminobutyrate which inhibit 4-aminobutyrate transaminase, either non-competitive or competitively with respect to 4-aminobutyrate [2—9]. The majority of these inhibitors have been tested on brain

enzymes from rat and mouse both in vivo and in vitro. It was of interest to study the inhibitors which modify the 4-aminobutyrate levels in vivo with respect to the degree of specificity they have for the enzymes of the animals studied. We have compared the activity of certain inhibitors of rat brain 4-aminobutyrate transaminase with their activity in respect to purified human enzyme. These inhibitors are branched-chain fatty acids and also structural analogues of 4-aminobutyrate. Dipropylacetate has anticonvulsive properties in some species of animals and in man [10,11] and its administration to rats and mice causes an increase in cerebral 4-aminobutyrate levels [6] (other inhibitors of the same family have already been described [12,13]). In this study, partially purified human 4-aminobutyrate transaminase [14] was further purified to apparent homogeneity and certain of its structural and kinetic properties are compared to those of the rat enzyme [15]. Certain structural and kinetic properties of the enzyme from brain tissue of other mammals have been reported [16—18].

## Experimental Methods

All procedures were carried out at about 4°C. 330 g of human brain, taken 12 h post-mortem, was used as the starting material. After removal of the corpus callosum the cerebral hemispheres were homogenised with a Waring blendor in 3 vols. of 5 mM phosphate buffer (pH 7.2) containing 10 mg/l pyridoxal phosphate/1 mM aminoethylisothiouronium bromide hydrobromide/0.1 mM EDTA/100 mM sodium succinate. After centrifugation for 15 min at 20 000 × *g*, the pellet was re-extracted and centrifuged under the same conditions, and the supernatants pooled.

*$(NH_4)_2SO_4$ fractionation.* The precipitate which formed between 45 and 70% $(NH_4)_2SO_4$ saturation was redissolved in a minimum quantity of the buffer but without sodium succinate.

*Gel filtration on Sephadex G-200.* This chromatography was performed on a 1 m × 2.5 cm column equilibrated with the solubilisation buffer, 5 ml of the enzyme solution was placed on the column, and the eluate collected in 5 ml fractions.

*DEAE-cellulose chromatography.* The active fractions from Sephadex G-200 chromatography were pooled and adsorbed onto a DEAE-cellulose column (2.5 × 25 cm). The column was extensively rinsed with starting buffer of the following composition: 5 mM phosphate (pH 7.2)/10 mg/l pyridoxal phosphate/1 mM aminoethylisothiouronium bromide hydrobromide/0.1 mM EDTA/10% glycerol. The column was eluted with 500 ml of a linear gradient of 0—130 mM KCl containing the same buffer and the enzyme eluted at about 40 mM KCl.

*DEAE-Sephadex chromatography.* The active fraction from the above chromatography was adsorbed onto a DEAE-Sephadex A-50 column (2.5 × 25 cm), the column rinsed with starting buffer (40 mM KCl + 5 mM phosphate buffer) and eluted with 300 ml of a linear gradient of 40—300 mM KCl. The enzyme was eluted at about 160 mM KCl.

*Hydroxyapatite chromatography.* The active fraction from DEAE-Sephadex was adsorbed onto an hydroxyapatite column (2.5 × 10 cm), rinsed with 30 ml

of 1.5 M KCl plus 5 mM phosphate buffer and eluted with 280 ml of a linear gradient of 5—250 mM phosphate buffer, pH 7.2. The enzyme activity was eluted at about 150 mM phosphate.

*Protein determination.* Protein determination was carried out using either the method of Lowry et al. [19] or the fluorescamine method [20], when the protein concentrations were less than 10 $\mu$g/ml. In each case, it was necessary to remove aminoethylisothiouronium bromide hydrobromide and pyridoxal phosphate (as these compounds interfere with the assays) by precipitation of the proteins with 12% trichloroacetic acid followed by centrifugation. The protein precipitates were dissolved in 1% $Na_2CO_3$ for the Lowry method, and in 0.2 M borate buffer (pH 9) for the fluorescamine method. In each case, a standard curve was obtained using bovine serum albumin treated in the same way.

*Enzyme assays.* Two methods were used: (a) The Salvador-Albers method [21], to assay the enzymatic activity of column eluates. The succinic semialdehyde formed by incubating the enzyme at 38°C in 0.3 M Tris · HCl (pH 8.6) with 10 mM 2-oxoglutarate and 50 mM 4-aminobutyrate is measured as its fluorescent adduct with 3,5-diaminobenzoic acid; (b) Waksman and Roberts method [22], was used to measure the $K_m$ values and the dissociation constants for various inhibitors. This procedure measures the radioactivity of the glutamate formed from U-$^{14}$C-labeled 2-oxoglutarate after incubation with the enzyme as for the method a. The glutamate was separated on a column of Dowex-50 WX8, $H^+$ form.

*Criteria of purity.* Polyacrylamide gel electrophoresis under non-denaturing conditions: 7.5% gels were prepared according to the method of Joivin et al. [23]. Bromophenol Blue was used as a tracking dye, and the protein bands were stained with Coomassie brilliant blue.

N-Terminal analysis: This was performed by the dansylation technique of Zanetta et al. [25] using 100 $\mu$g of protein. The chromatography was carried out both on thin-layer chromatography silica and polyamide plates in the presence of dansylated amino acid markers. The spots corresponding to $\alpha$-dansyl isoleucine and $\epsilon$-dansyl lysine were eluted from silica gel plates and their fluorescence measured.

Preparation of 4-aminobutyrate transaminase antibodies in the rabbit: The pure enzyme was concentrated in an Amicon 8MC cell equipped with a Diaflo PM 10 membrane. Three fractions, each of 0.5 ml (0.5 mg of protein), were conserved in liquid $N_2$. Immediately before use, a fraction was thawed and mixed with 0.5 ml Freunds adjuvant and then injected subcutaneously in three different places in the back of a rabbit (weighing about 1 kg). The injections were repeated twice at 15 days intervals. After 45 days, blood was removed and the resultant serum (after addition of 0.1% $NaN_3$) was conserved at 4°C.

Immunodiffusion: Radial diffusion was used on 1.5% agar gel buffered with 0.2 M phosphate (pH 8) plus 0.1% azide. The antigen consisted of enzyme solution, purified six times, as obtained after $(NH_4)_2SO_4$ precipitation and the diffusion took place for 24 h at 20°C.

*Molecular weight determinations.* Native enzyme: This was determined by gel filtration on Sephadex G-200 in a 75 × 2.5 cm column. The chromatography was carried out in the presence of 1 mg Dextran Blue, 2 mg human serum albumin, 2 mg $\gamma$-globulin, 0.5 mg yeast glucose-6-phosphate dehydrogenase (EC

1.1.1.49) and 2 mg of ovalbumin. The serum albumin, globulin and ovalbumin were detected spectrophotometrically at 280 nm; the glucose-6-phosphate dehydrogenase was measured according to the method of Kornberg and Horecker [26] and the 4-aminobutyrate transaminase activity was measured according to the method of Salvador and Albers [21].

Molecular weight of subunits: The method of Weber and Osborn [24] was used. 10% polyacrylamide gels were prepared containing 0.1% sodium dodecyl sulfate (SDS) with or without 6 M urea. 20 μg of enzyme was preincubated for 15 min at 60°C in the electrophoresis buffer containing 0.1% SDS with or without 10 mM dithiothreitol. This technique was used to determine the molecular weight of subunits of the enzyme compared to standard proteins.

*Amino acid composition.* 100 μg protein were precipitated with 12% trichloroacetic acid and, after centrifugation, the precipitate was washed with 1 ml of 1 M HCl. After centrifugation, the protein was hydrolysed in a sealed tube with 250 μl 6 M HCl (18 h, 100°C). The amino acid composition of the hydrolysate was determined using a Technicon autoanalyser.

*-SH group determination.* 40 μg of enzyme were dialysed at 4°C against 1 l of 0.1 M acetate buffer (pH 5) containing 4 M urea. $^{14}$C-labelled *p*-chloromercuribenzoate (10.4 μCi/nmol) was added to the dialysate to a final concentration of $10^{-4}$ M. After stirring the solution gently for 12 h at room temperature, the mixture was filtered on Diaflo PM 10 membrane, previously equilibrated for 12 h in the dialysis buffer containing $10^{-4}$ M *p*-chloromercuribenzoate. The membrane, which retains the enzyme, was rinsed with the same buffer until the ultrafiltrate contained negligible radioactivity. A control membrane was treated under the same conditions. The radioactivity fixed on the surface of the membranes was determined after combustion on a Packard sample oxidizer.

*Comparison of peptides formed after CNBr cleavage.* About 60 μg each of the rat and human enzyme after precipitation by trichloroacetic acid were redissolved in 250 μl of 79% formic acid. A solution of CNBr was added until the molar ratio CNBr:methionine was about 1 to 500, then the mixture was left at room temperature in a sealed tube for 12 h, and finally lyophilised and redissolved in 0.3 ml of 0.2 M phosphate buffer (pH 8) containing 1% SDS and 1% mercaptoethanol. The solution was incubated for 15 min at 60°C and polyacrylamide gel electrophoresis was performed according to the method of Swank and Munkres [27].

*Tryptic fingerprinting.* About 100 μg of each enzyme, precipitated with 12% trichloroacetic acid, was redissolved in 0.2 ml 0.5 M phosphate buffer (pH 7.8) containing 2 μg trypsin (8000 units/mg, previously treated with diphenylcarbamyl chloride). Proteolysis was carried out for 24 h at 37°C, then the incubation mixture was brought to pH 8.8 with 1 M NaOH. The dansylation of the liberated peptides and chromatography on thin-layer silica gel plates were carried out according to the method of Zanetta et al. [25].

*Determination of $K_m$ and $K_i$ values.* The apparent $K_m$ values of the human enzyme for 4-aminobutyrate were measured at three concentrations of 2-oxoglutarate and those for oxoglutarate at three concentrations of 4-aminobutyrate. The inhibition constants of the human and rat enzymes for various inhibitors were determined at various inhibitor concentrations in the presence of 0.3 mM 2-oxo[U-$^{14}$C]glutarate (0.06 μCi/ml) and 1 or 3 mM 4-aminobutyrate.

The reaction rate was determined by calculating the slope of the regression line, the correlation coefficient showing that the rates determined were always initial rates. The relation $1/V = f(1/S)$ was established by the same method, using the average value of the reaction rate. The inhibition constants are represented graphically by the method of Dixon.

## Results

*Purification of human 4-aminobutyrate transaminase*

About 1 mg of enzyme was obtained from 300 g starting material and had a specific activity of 4.9 μmol/min per mg of protein. It was purified 2450 times with respect to the initial homogenate, with a yield of 8% in activity and 0.003% in protein (Table I). These results are similar to those obtained for the rat except that the specific activity, including that of the homogenate, was usually approx. 2.5 μmol/min per mg of protein. This could be explained by the inevitable delay in obtaining a human brain, causing partial inactivation of some of the enzyme.

*Criteria of purity*

The human enzyme was judged pure by the following criteria: (a) a single protein band (enzymically active) was seen after polyacrylamide gel electrophoresis of 40 μg of enzyme after staining with Coomassie Blue R250; (b) a

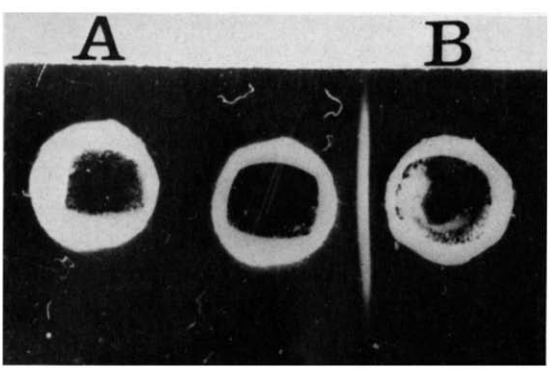



Fig. 1. Electrophoresis of 20 μg of human 4-aminobutyrate transaminase on polyacrylamide gel containing 0.1% SDS.

Fig. 2. Immunodiffusion on gelose of crude human 4-aminobutyrate transaminase (6-fold purified). (A) Serum from normal rabbit. (B) Serum from immunised rabbit.

TABLE I

PURIFICATION OF HUMAN 4-AMINOBUTYRATE TRANSAMINASE

| Stages | Total activity (μmol/min) | Total protein (mg) | Specific activity (μmol/min per mg | Purification | Activity yield (%) | Protein yield |
|---|---|---|---|---|---|---|
| Homogenate | 65 | $32.15 \cdot 10^3$ | 0.0020 | 1 | 100 | 100 |
| Supernatant (20 000 × *g*) | 62 | $10.2 \cdot 10^3$ | 0.0061 | 3 | 96 | 32 |
| Ammonium sulfate fractionation (45—70%) | 30 | $2.5 \cdot 10^3$ | 0.012 | 6 | 47 | 7 |
| Sephadex G-200 | 25 | 416 | 0.060 | 30 | 39 | 1.3 |
| DEAE-cellulose | 14 | 42 | 0.34 | 169 | 22 | 0.13 |
| DEAE-sephadex | 7.5 | 22 | 0.44 | 212 | 12 | 0.068 |
| Hydroxyapatite | 5.4 | 1.1 | 4.91 | 2455 | 8 | 0.003 |

single band was also obtained with SDS-polyacrylamide gel electrophoresis (Fig. 1); (c) N-terminal analysis using the dansylation method gave rise to a single spot corresponding to isoleucine. The ratio of amino acids, N-terminal/lysine = 1/50, is in good accord with the theoretical 1/45 calculated from the amino acid composition of the enzyme (Table III); (d) antiserum to purified human 4-aminobutyrate transaminase was prepared and immunodiffusion in agar gel, with human 4-aminobutyrate transaminase (6-fold purified) as antigen, gave rise to a single precipitation line (Fig. 2). Thus the protein used to make the antiserum contained no precipitating antigen other than 4-aminobutyrate transaminase, under the conditions used.

*Studies of the structure of the human enzyme*

Identical experiments to those described for the rat [15] were performed with the human enzyme: (a) molecular weight of the native enzyme (Sephadex G-200 filtration) is 110 000 ± 5000; (b) the molecular weight of the protein (SDS-polyacrylamide gel electrophoresis) is 57 000 ± 5000. It is suggested from these results that the enzyme may consist of two similar (perhaps identical) polypeptide chains.

Some data obtained for the human and rat brain 4-aminobutyrate transaminases are shown in Table II.

*Amino acid composition*

This was determined as for the rat enzyme and the results are for both enzymes shown in Table III. This analysis confirms the higher molecular weight of the human enzyme. There are differences in the amino acid composition, particularly the levels of aspartic acid plus asparagine, glycine, cysteine, proline, arginine, phenylalanine, tyrosine and leucine. The *p*-chloromercuri[$^{14}$C]benzoate assay suggests the presence of approx. 4 mol sulfhydryl-containing amino acids (presumably cysteine) per mol of enzyme.

*Determination of the apparent $K_m$ values of the general reaction mechanism*

*Apparent $K_m$ values for 4-aminobutyrate.* The apparent $K_m$ values for 4-aminobutyrate (= $K_{m_a}$) were measured at three concentrations of 2-oxoglutarate (0.3, 0.6, 0.9 mM). The graphic representation of $1/V = f(1/S)$ gives a series of parallel lines with apparent $K_{m_a}$ values of 0.22, 0.36 and 0.44 mM (Fig. 3).

TABLE II

COMPARISON OF SOME DATA OBTAINED FOR THE HUMAN AND RAT BRAIN 4-AMINOBUTYRATE TRANSAMINASE

| | Rat enzyme | Human enzyme |
|---|---|---|
| Molecular weight | 105 000 ± 5000 | 109 000 ± 5000 |
| Subunits | Dimer | Dimer |
| Molecular weight of the subunits | 57 000 ± 5000 | 57 000 ± 5000 |
| Ratio N-terminal/lysine | 1/40—1/46 | 1/50 |
| Disulfide bridges | No | No |
| Optimum pH | 8.5—8.6 | 8.5 |
| Thermostability | Rapid denaturation at 55°C | Slow denaturation at 55°C |

TABLE III

COMPARISON OF AMINO ACID COMPOSITION OF RAT AND HUMAN 4-AMINOBUTYRATE TRANSAMINASE

| Amino acid | Rat enzyme | | Human enzyme | |
|---|---|---|---|---|
| | Residues/ molecule | Percentage of total | Residues/ molecule | Percentage of total |
| Glutamic acid | 75 | 9 | 75 | 8.1 |
| Aspartic acid | 81 | 9.7 | 110 | 11.9 |
| Threonine | 30 | 3.6 | 31 | 3.3 |
| Serine | 60 | 7.2 | 69 | 7.5 |
| Glycine | 84 | 10 | 57 | 6.2 |
| Cysteine | 12 | 1.4 | 4 | 0.4 |
| Methionine | 8 | 1 | 8 | 0.9 |
| Valine | 42 | 5 | 46 | 5 |
| Proline | 30 | 3.6 | 50 | 5.4 |
| Arginine | 72 | 8.6 | 37 | 4 |
| Histidine | 39 | 4.7 | 43 | 4.7 |
| Lysine | 90 | 10.8 | 89 | 9.6 |
| Phenylalanine | 27 | 3.2 | 43 | 4.6 |
| Tyrosine | 12 | 1.4 | 30 | 3.2 |
| Leucine | 63 | 7.5 | 108 | 11.7 |
| Isoleucine | 30 | 3.6 | 47 | 5 |
| Alanine | 78 | 9.3 | 75 | 8.2 |
| Tryptophan | — | — | — | — |
| Total | 833 | 100 | 922 | 100 |

The apparent affinity of the enzyme for 4-aminobutyrate extrapolated to infinite 2-oxoglutarate concentration (Fig. 4) gives a $K_m$ limit (= $K_A$) of 0.9 mM.

*Apparent $K_m$ values for 2-oxoglutarate.* These were determined at three concentrations of 4-aminobutyrate (1—3 and 6 mM). Again, a series of parallel

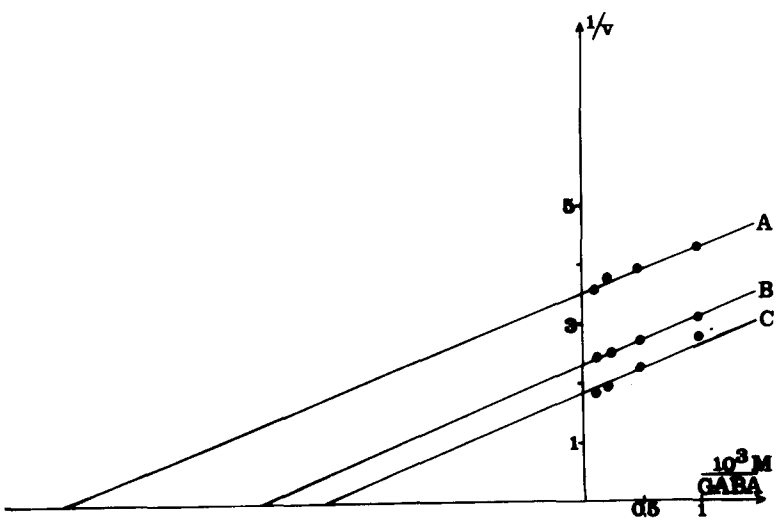



Fig. 3. Determination of apparent $K_m$ values for 4-aminobutyrate (Lineweaver-Burk plot. 2-Oxoglutrate concentration: A, 0.3 mM; B, 0.6 mM; C, 0.9 mM.

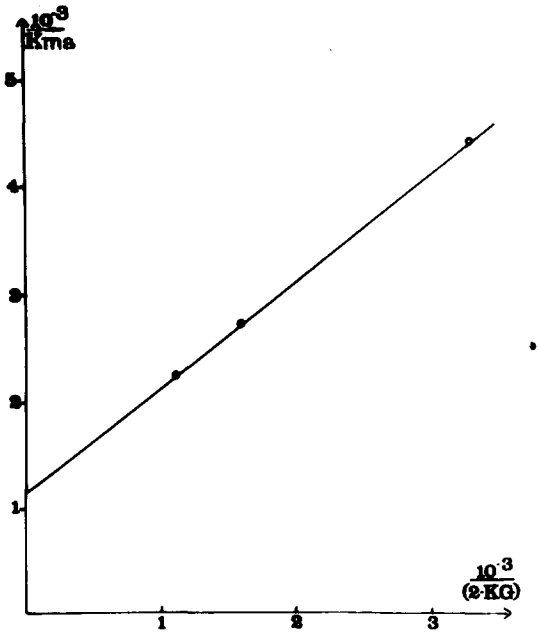



Fig. 4. Apparent $K_m$ values for 4-aminobutyrate as a function of 2-oxoglutarate concentration. Determination of $K_m$ (limit) for 4-aminobutyrate.

lines were obtained (Fig. 5) giving apparent $K_m$ values (= $K_{m_b}$) of 0.32, 0.4 and 0.5 mM. One can also see the inhibition caused by high concentrations of 2-oxoglutarate. Taking account of the $K_A$ previously calculated, the value of $K_B$ = $K$(2-oxoglutarate) = $K_m$ for an infinite concentration of 4-aminobutyrate,

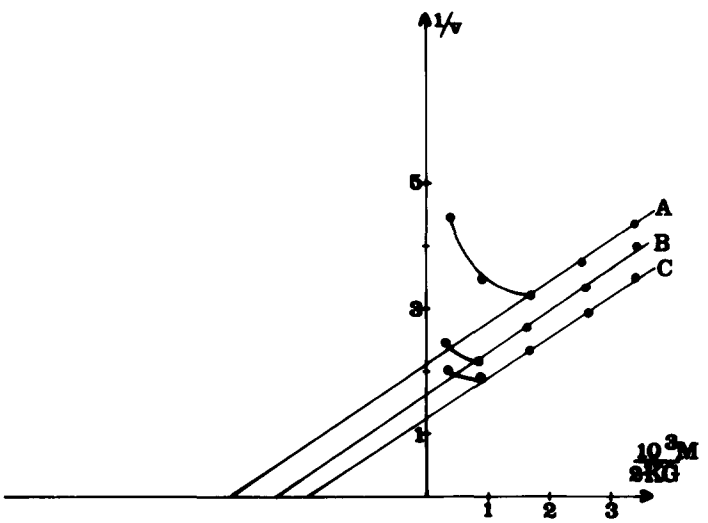



Fig. 5. Determination of apparent $K_m$ values for 2-oxoglutarate. Lineweaver-Burk plot. 4-Aminobutyrate concentration: A, 1 mM; B, 3 mM; C, 6 mM. Inhibition of the enzyme activity at high 2-oxoglutarate concentration.

can be obtained from the equation $1/K_{m_a} = 1/K_A\ (1 + K_B[B])$ which gives $K_B$ = 0.9 mM.

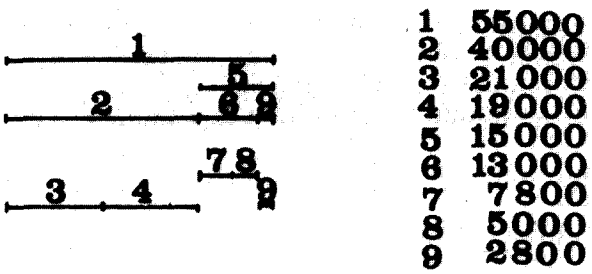
From these results, it should be noted that the affinities of the human enzyme for its substrates are much greater (about four times) than those for the rat enzyme.

*Comparative study of the peptides obtained after CNBr cleavage of the two enzymes*

Nine bands were obtained, from both enzymes, after CNBr treatment and separation of the peptides on polyacrylamide gels. In both cases, the molecular weight of the largest peptide corresponds to various possibilities of rupture of the protein at methionine residues which vary in their susceptibility to CNBr treatment. It can be seen that these peptides are not of the same molecular weight for the two enzymes. In each case, starting from the various peptides, a tentative scheme has been proposed to reconstitute the initial monomer (Figs. 6 and 7).

*Peptide maps of the two enzymes after tryptic digestion*

After tryptic hydrolysis of equal quantities of both enzymes and chromatography separation of the dansylated peptides, the maps shown in Figs. 8 and 9 were obtained and in Fig. 10 the two maps are shown superimposed. There are



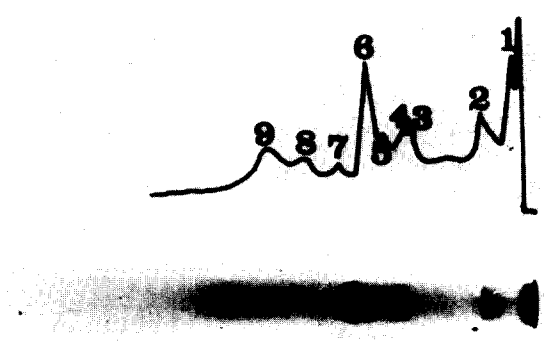




Fig. 6. Molecular weights by polyacrylamide gel electrophoresis of the peptides obtained after cyanogen bromide cleavage of rat 4-aminobutyrate transaminase. The scheme proposed to reconstitute the initial monomer is an hypothesis.

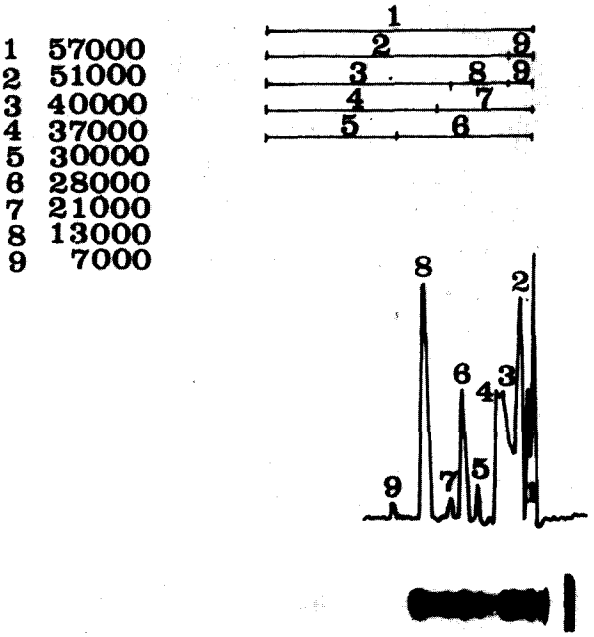
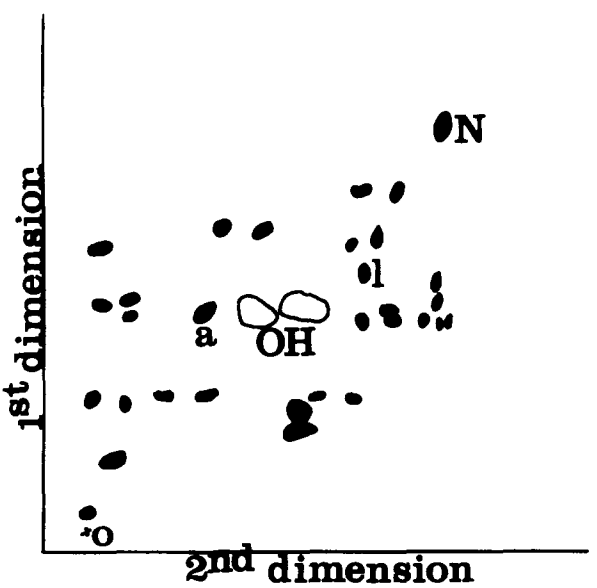



Fig. 7. Molecular weights by polyacrylamide gel electrophoresis of the peptides obtained after cyanogen bromide cleavage of human 4-aminobutyrate transaminase. The scheme proposed to reconstitute the initial monomer is an hypothesis.




Fog. 8. Peptide maps of human 4-aminobutyrate transaminase after tryptic digestion. a, ω-dansyl-arginine; L, ε-dansyl-lysine; OH, dansyl-OH; N, dansyl-$NH_2$; O, origin.

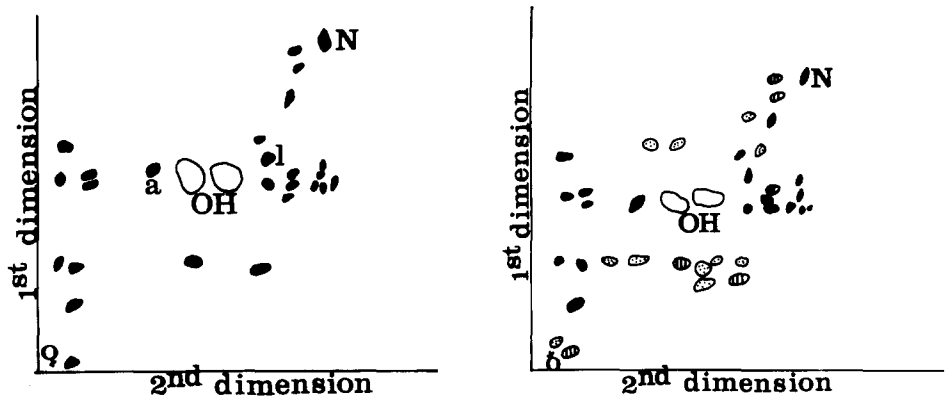



Fig. 9. Peptide maps of rat 4-aminobutyrate transaminase after tryptic digestion. a, ω-dansyl-arginine; L, ε-dansyl-lysine; OH, dansyl-OH; N, dansyl-$NH_2$; ), origin.

Fig. 10. Superimposition of the two peptide maps in order to identify the common spots. •, common spots; ⊙, specific to human 4-aminobutyrate transaminase; ⊘, specific to rat 4-aminobutyrate transaminase.

19 spots common to both enzymes, 11 spots unique to the human enzyme and 6 spots unique to the rat enzyme. These results show that there are similar and different amino acid sequences in these enzymes.

*Determination of the $K_i$ values for some inhibitors*

A certain number of branched-chain fatty acids are inhibitors of rat brain

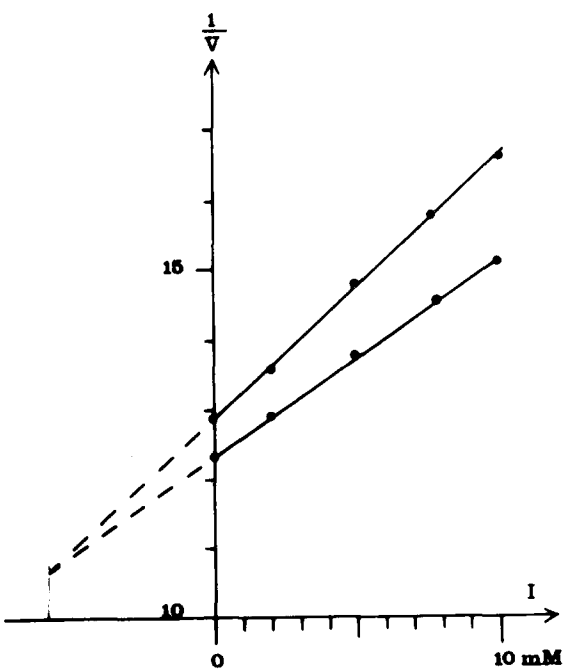



Fig. 11. Graphic determination of the $K_i$ values by Dixon's method. The figure shows an example for 2-propylpenten-2-oic acid.

TABLE IV

$K_i$ VALUES OF THE BRANCHED FATTY ACIDS FOR RAT AND HUMAN 4-AMINOBUTYRATE TRANSAMINASE

| | $K_i$ (mM) rat | $K_i$ (mM) human |
|---|---|---|
| 2-Propylpenten-2-oic acid | 0.5 | 4.5 |
| 2-Methyl-2-ethylhexanoic acid | 1.5 | 6.5 |
| 2,2-Dimethylpentanoic acid | 2 | 9.5 |
| 5,5-Dipropylpentanoic acid | 5 | 20 |
| 2-Propylpentanoic acid (dipropylacetic acid) | 9.5 | 40 |

TABLE V

VALUES OF THE PRODUCT $K_{limit}$ FOR 4-AMINOBUTYRATE $\times$ $K_i$ FOR EACH INHIBITOR

| | Rat | | Human | |
|---|---|---|---|---|
| | $K$(4-amino-butyrate) = 4 mM (mM) | $K$(4-amino-butyrate) $\times$ $K_i$ | $K$(4-amino-butyrate) = 0.9 mM (mM) | $K$(4-amino-butyrate) $\times$ $K_i$ |
| 2-Methyl-2-ethylhexanoic acid | 1.5 | 6 | 6.5 | 5.8 |
| 2,2-Dimethylpentanoic acid | 2 | 8 | 9.5 | 8.5 |
| 2-Propylpenten-2-oic acid | 0.5 | 2 | 4 | 3.6 |
| 5,5-Dipropylpentanoic acid | 6 | 24 | 20 | 18 |
| 2-Propylpentanoic acid (dipropylacetic acid) | 9.5 | 38 | 40 | 36 |

and human brain 4-aminobutyrate transaminase. The graphic determinations of the $K_i$ values according to the method of Dixon (Fig. 11) show that these inhibitors are in every case competitive with 4-aminobutyrate and non-competitive with 2-oxoglutarate.

A comparison of the inhibitor-enzyme dissociation constant, measured under the same conditions, is shown in Table IV. It can be seen that the affinity of the human enzyme for the inhibitors is much lower than that of the rat enzyme.

The value of the product $K$ limit for 4-aminobutyrate $\times K_i$ has been calculated for each inhibitor (Table V). This value is the same for each inhibitor for both enzymes. This suggests that the efficiency of competitive inhibition is directly related to the affinity of the enzyme for 4-aminobutyrate.

## Discussion

This comparative study of certain structural features of rat and human brain 4-aminobutyrate transaminase shows that there are a number of identical sequences, but also number of differences. These results confirm the immunochemical studies of Saito et al. [28], who observed considerable antigenic similarity between mouse and rat 4-aminobutyrate transaminases, but much less between 4-aminobutyrate transaminases from mouse, sheep and human. Complement microfixation tests show that there are significant differences in the 4-aminobutyrate transaminase, for example the rat and the guinea pig [28]. We

have shown that, together with the structural differences between rat and human brain 4-aminobutyrate transaminases, there are also differences in kinetic properties of the two enzymes. Thus, the ratio of affinities for 4-aminobutyrate is about 1 : 4 (human/rat) and $K_m$ (4-aminobutyrate) (limit) for the rat enzyme is higher. Also, the thermostability of the two enzymes under the same experimental conditions is different and the level of inhibition, competitive with 4-aminobutyrate, caused by certain branched-chain fatty acids is different, depending on the source of enzyme. The dissociation constants enzyme-inhibitors are likewise in the ratio 1 : 4 for the two enzymes (rat/human) and the ratio of affinities for 4-aminobutyrate is about 4 : 1 (rat/human), confirming that the inhibition is competitive with the affinity the enzyme has for 4-aminobutyrate. Some of these inhibitors have been used to bring about increases in the cerebral level of 4-aminobutyrate [6,10—12]. These results show that one must be careful when comparing the results of inhibition in vitro of 4-aminobutyrate transaminase from certain animals, e.g. rabbit [29], with the increases of cerebral 4-aminobutyrate after administration of these same inhibitors to other animals, even closely related species, such as rat and mouse. However, the quaternary structure of human 4-aminobutyrate transaminase appears to be similar to that of 4-aminobutyrate transaminase from other sources [17,18] and it is only in the mouse enzyme [16] that two different subunits were found.

Vasiliev and Krylova [30] have reported that the enzyme from rat brain and pig kidney show different affinities toward the inhibitor, ethanolamine-*O*-sulphate. They also found that it was the aldehyde form of the enzyme which had affinity for ethanolamine-*O*-sulphate. Our results also lend support to this view at least with regard to the competitive inhibition of the enzymes produced by the branched-chain fatty acids with respect to 4-aminobutyrate to which they all present structural analogies.

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