trace amounts of moisture into the system and several conditioning runs must be made each day before an analysis is attempted. Conditioning is completed when the SOF_2 peak, which eluted prior to the SF_4, stays the same on consecutive runs.

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Notes

Paper chromatographic and high-potential paper electrophoretic identification of β-hydroxyglutamic acid in the brain

Recently, β-hydroxy-γ-aminobutyric acid (β-OH-Gaba) was identified in the brain[1]. Regarding the precursor of β-OH-Gaba, it has been reported that Gaba may be converted to β-OH-Gaba by beta oxidation[2]. On the other hand, consideration should be given to the fact that β-hydroxyglutamic acid (β-OH-Glu) may be converted to β-OH-Gaba through decarboxylation[3] in the brain. However, until now, the presence of β-OH-Glu in the brain has not been detected.

In our experiments, fresh brain material was frozen, homogenized and deproteinized with 70% ethanol. The deproteinized liquid was subjected to petroleum ether extraction to remove lipids and was evaporated to dryness at 40° in a vacuum. The residue thus obtained was dissolved in a small amount of distilled water and applied to the bottom half of the starting line on Toyo-Roshi No. 50 filter paper for high-potential paper electrophoresis. A solution of a synthesized sample of *threo*-β-OH-Glu* was then applied to the upper half of the starting line of the filter paper[1]. After high-

* The synthetic racemic samples of *threo*-β-OH-Glu and *erythro*-β-OH-Glu were supplied by Ajinomoto Laboratory and by Dr. SELBY DAVIS, Head, Medical Chemistry, Department of Non-infectious Diseases, Lederle Laboratories, Pearl River, New York. The authors appreciate these generous gifts. *threo*-β-OH-Glu and *erythro*-β-OH-Glu are synonymous with *allo*-β-OH-Glu and β-OH-Glu respectively[4].

potential paper electrophoresis was performed under conditions similar to those described previously[1], a pattern of ninhydrin-positive zones on the filter paper strip was produced as shown in Fig. 1 (top). It can be seen that there are several bands resulting from the brain extract (bottom half) and that one of these bands is continuous with the band formed from the pure *threo*-β-OH-Glu (top half). Fig. 1 (bottom) shows the result obtained in an experiment in which synthetic *threo*-β-OH-Glu was

Fig. 1. High-potential paper electrophoresis of cattle brain extract. Direction of electrophoresis: from left to right. (A) Synthetic sample (*threo*-β-OH-Glu, m.p. 205–206°); (B) prepared extract; (B') prepared extract plus synthetic sample. Arrow (top), the juncture of the area of synthetic sample with the area of prepared extract; arrow (bottom), the layer produced by a synthetic sample superimposed on the area of prepared extract. Conditions of electrophoresis, 125 V/cm, 40 min; electrolyte, formic acid–acetic acid–water (50 ml:150 ml:800 ml), pH 1.9; filter paper, Toyo-Roshi No. 50; stain, 0.5% solution of ninhydrin in acetone.

superimposed on the upper half of the line of application of the brain extract. It is evident that one of the bands due to brain extract was reinforced by the band due to pure *threo*-β-OH-Glu.

The band tentatively identified in Fig. 1 (top and bottom) as *threo*-β-OH-Glu was cut from the filter paper strip before staining and was eluted with water. The eluate was then subjected to two-dimensional paper chromatography.

In Fig. 2 a synthetic sample of *threo*-β-OH-Glu was applied at A and the eluate was applied at B. The first dimension was developed using isopropanol–formic acid–water (8:1:1) as solvent. The resulting R_F for the pure compound was 0.18 (using Whatman No. 1 filter paper). Of the four spots resulting from the eluate, only the encircled spot had an R_F of 0.18. The second dimension was then run using pyridine–water (65:35) as solvent. Again only the encircled spot had an R_F which matched that of the pure compound (0.30). These results would therefore indicate that the encircled spot, which originated in brain extract, was *threo*-β-OH-Glu.

When synthetic *erythro*-β-OH-Glu was substituted for *threo*-β-OH-Glu in the

above described experiments, the former (*erythro*-β-OH-Glu) could not be detected in the brain of humans, cattle, rabbits or rats. Further investigations of *threo*-β-OH-Glu as the precursor of β-OH-Gaba are in progress in this laboratory.

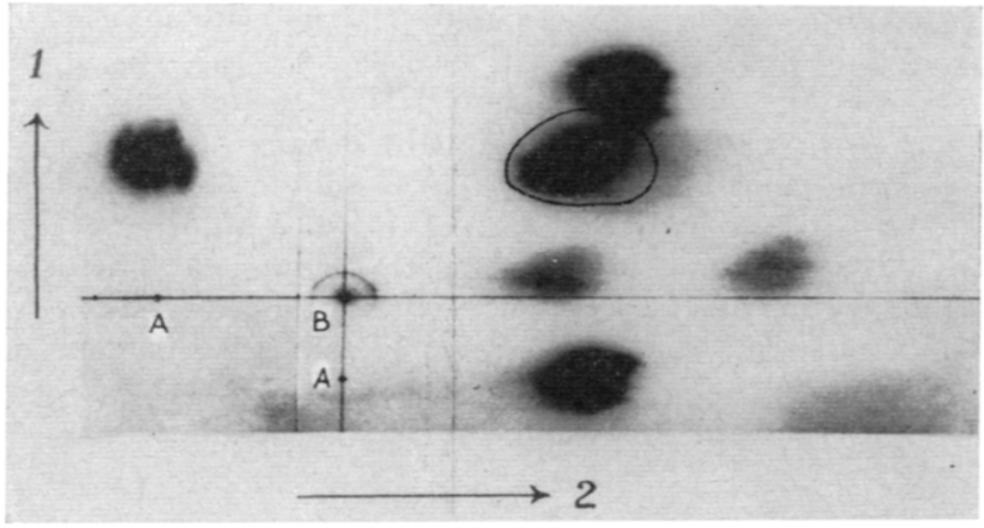

Fig. 2. Two-dimensional paper chromatography of cattle brain extract. (A) Synthetic sample (*threo*-β-OH-Glu); (B) eluted solution (brain extract). Solvent, first dimension: isopropanol–formic acid–water (8:1:1); second dimension: pyridine–water (65:35). Stain, 0.5% solution of ninhydrin in acetone. For A, R_F was 0.18 for first dimension, 0.30 for second dimension. The encircled spot had similar R_F values.

In summary, *threo*-β-hydroxyglutamic acid was identified in the brain of humans and of cattle, using a combination of high-potential paper electrophoresis and two-dimensional paper chromatography. It could not be identified in the brains of mice, rabbits or rats.

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