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4-Aminobutyrate-2-Ketoglutarate Aminotransferase (GABA-T) in Human Hair Follicle

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GABA-T (4-aminobutyrate-2-ketoglutarate aminotransferase) has been found in human hair follicle. Kinetics experiments with hair follicle homogenate supported a ping-pong type of enzymatic mechanism. Extrapolated K_m values were 1.02 mmol/l for GABA and 0.45 mmol/l for α-ketoglutarate. Hair follicle GABA-T activity was completely inhibited by preincubation of the samples with either 5×10^{-8} mol/l aminooxiacetic acid or 5×10^{-4} mol/l γ-vinyl GABA. The radioenzymatic assay presented is both sensitive enough (only 10 hair follicles are needed for one assay) and economical, making it suitable for clinical practice. Hair follicle GABA-T activity determination could be useful in the study of GABA deficiency diseases (such as epilepsy), congenital GABA-T deficiencies or the control of GABA-T inhibitors treatment.

Key words: GABA-transaminase, Hair follicle.

A deficiency in GABA has been implicated in the etiology of epilepsy, psychosis, movement disorders, pain, hypertension and sleep disturbances (4). GABA-T (4-aminobutyrate-2-ketoglutarate aminotransferase: EC 2.6.1.19) is the enzyme principally responsible for catabolism of GABA. A genetically dysfunctional GABA-T might well produce an abnormality in GABA neurotransmission. In fact, a GABA-T deficiency (detected by means of liver needle biopsy) has been recognized as a new inborn error of the neurotransmitter metabolism (5, 8). On the other hand, it has been demonstrated that GABA-T inhibitors (such as γ-vinyl GABA) are useful for the treatment of GABA related disorders, especially epilepsy (12) both in short (16, 18) and long-term (13) studies. The action of these drugs has been indirectly controlled by measuring cerebrospinal fluid (CSF) GABA increase (6). Both, the study of GABA related diseases and the control of

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GABA-T inhibitors treatment, would be greatly facilitated if readily available GABA enzyme sources could be used.

GABA-T has been identified not only in human brain but also in human kidney, liver, platelets and lymphocytes (3, 19, 21). Platelet GABA-T may be useful in the diagnosis of several illnesses in which aberrations in GABA metabolism have been implicated. In fact, several studies about possible differences between GABA-T activity in platelets of patients with either schizophrenia or affective illnesses against normal controls have been performed (1, 15, 22). Moreover, determination of platelet GABA-T has been proposed to evaluate the action of GABA-T inhibitors treatment (17).

Hair follicles are a readily available enzyme source for the molecular diagnosis of inborn errors of metabolism (2). In fact, the application of hair root catechol-O-methyltransferase activity in the study of psychiatric disorders has been proposed (7). Recently, the determination of a transaminase (1-ornithine ketoacid transaminase: EC 2.6.1.13) in human hair roots has been used for the detection of both homozygote and heterozigote for 1-ornithine transaminase deficiency (9).

The present study was undertaken to demonstrate the presence of GABA-T in human hair follicle, and to describe an assay which may be suitable for clinical practice.

Materials and Methods

Preparation of hair follicle homogenate. — About 40 hairs, collected from several areas of the scalp in healthy adult volunteers, were cut off just above the sheath. The hair roots were put into an all-glass micro UNI-FORM homogenizer and 300 µl of a buffer (pH = 8) containing 0.1 mmol/l EDTA, 0.5 mmol/l dithiothreitol, 0.2 mmol/l pyridoxal phosphate and 0.1 mmol/l potassium phosphate were added. The hair roots were homogenized with 10 strokes by hand; the homogenate was transferred to capped plastic micro test tubes, frozen at -20 °C for at least 24 hr and assayed within one week. No difference in GABA-T activity was found when water was used instead of buffer in the homogenization.

Assay. — GABA-T activity was determined as described for platelet (19, 20) with some modifications: 50 µl of the homogenate (between 0.05 and 0.1 mg of protein) were incubated for 6 h at 37.5 °C and 120 r.p.m. in a total volume containing 0.5 mmol/l α-ketoglutarate, 0.05 mmol/l EDTA, 0.25 mmol/l dithiothreitol, 0.1 mmol/l pyridoxal phosphate, 50 mmol/l phosphate buffer (pH = 8) and 0.67 mmol/l ^{14}C-GABA [4-amino-n-(U-^{14}C) butyric acid, 232 mCi/mmol 50 µCi/ml, Amershan] with a specific activity of 0.37 mCi/mmol. Incubation was performed in open plastic micro test tubes. Blanks were incubated with buffer instead of hair follicle homogenate. When de action of a GABA-T inhibitor was studied, 25 µl of homogenate and 25 µl of the inhibitor solution were preincubated for 30 minutes at 37 °C and used instead of the 50 µl homogenate.

The reaction was stopped by adding 10 µl 2 M HCl and the incubation mixtures were transferred to the surface of resin in 0.5 × 3 cm disposable ion exchange columns (Biorad AG 50W-X8 100-120 mesh, hydrogen form resin, pre-washed with distilled water) in disposable Pasteur pipets, plugged with a small wad of glass wool. Incubation mixtures were eluted directly into scintillation vials using 3 portions of 0.5 ml distilled water. Each portion was placed in the original incubation tube and transferred to the column with the same pipet used to transfer the incubation mixture. Ten ml of scintillation fluid (Pico-fluor 30) were added and activity determined by scintillation techniques. All results were corrected by sub-

tracting cpm obtained in blank assays. GABA-T activity was obtained multiplying sample minus blank cpm by a conversion factor (pmol GABA in an assay divided by cpm obtained by counting directly 50 µl incubation medium) and then dividing it by minutes of incubation and mg of protein in each assay. This activity is expressed as pmol per minute of incubation and per mg of protein.

Protein was measured by the method of LOWRY et al. (11) with human serum albumin as a standard.

Results

Using White's method for platelet, a low GABA-T activity in hair follicle was seen. Therefore, the incubation time was increased from 0.5 to 7 h, obtaining a linear increase in GABA-T activity (fig. 1A). However, an increase in extrapolated intercept of plots of enzyme activity against the amount of the protein in each assay was observed at the same time, both when homogenization and incubation were performed with and without pyridoxal phosphate (fig. 1B). Then, several blanks were tested in order to reduce this intercept to a minimum. The blank in which hair homogenate was changed by the buffer used in homogenization reduced the intercept to an acceptable minimum (fig. 2B).

Plots of reciprocal of GABA-T activity against reciprocal of either α-ketoglutarate (from 0.1 to 0.5 mmol/l) or GABA (from 0.5 to 1.5 mmol/l) concentrations were linear (fig. 3). Beyond 0.5 mmol/l of α-ketoglutarate, a decrease of GABA-T activity was observed (fig. 3B).

With these new analytical conditions (different blank; 6 h of incubation instead of 0.5; 0.5 mmol/l of α-ketoglutarate instead of 0.17, and a ^{14}C-GABA specific activity of 0.37 Ci/mol instead of 10 Ci/mol), a linear relationship between GABA-T activity and either incubation time (fig. 2A) or amount of protein (fig. 2B) in each assay was demonstrated.

GABA-T activity in relation to variable concentrations of GABA and ketoglutarate are shown in table I. Plots of the reciprocal of enzyme activity against reciprocal of substrate concentration were parallel lines, resembling the characteristic ping-pong reaction of other transaminases (20, 21). GABA K_m and α-ketoglutarate K_m, calculated from the secondary plot of

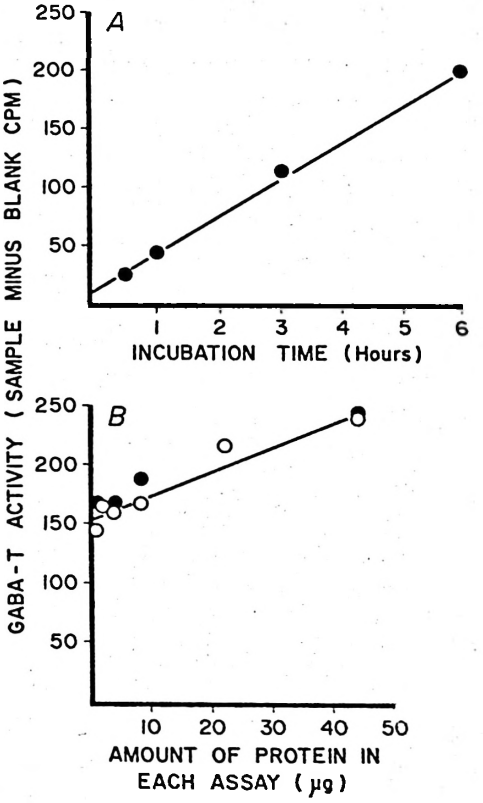

Fig. 1. *Dependence of human hair follicle GABA-aminotransferase (GABA-T) activity on either incubation time (A) or amount of protein in each assay (B).*

In blank assay, ketoglutarate was substituted by distilled water. Samples were incubated with (•) or without (○) 0.1 mmol/l pyridoxal phosphate. In A the amount of protein in each assay was 36 µg; in B incubation time was 6 h.

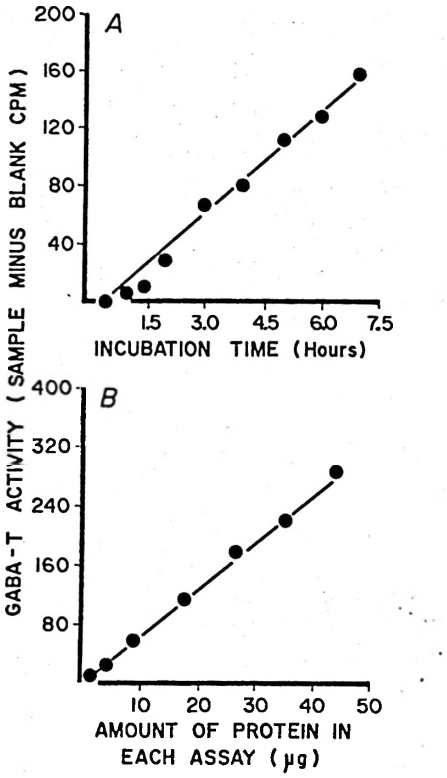

Fig. 2. *Dependence of human hair follicle GABA-aminotransferase (GABA-T) activity on either incubation time (A) or amount of protein in each assay (B).*
In blank assay hair homogenate was substituted by buffer. In A the amount of protein in each assay was 45 µg. In B the incubation time was 6 h.

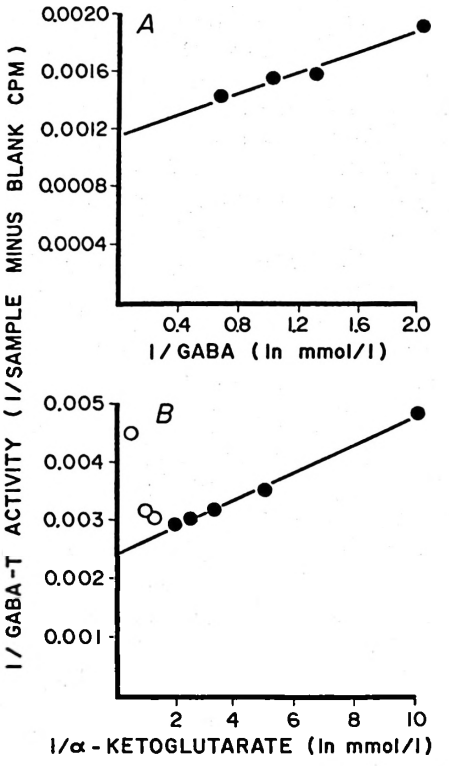

Fig. 3. *Dependence of human hair follicle GABA-aminotransferase (GABA-T) activity on incubation medium concentration of either GABA (A) or α-ketoglutarate (B).*
In A α-ketoglutarate concentration was 0.5 mmol/l, and the amount of protein in each assay was 95 µg. In B GABA concentration was 0.67 mmol/l, and 45 µg of protein was present in each assay. Specific radioactivity was 0.37 Ci/mol and incubation time 6 h.

intercepts against reciprocal of fixed substrate, were 1.02 and 0.45 mmol/l, respectively.

Hair follicle GABA-T activity was reduced to about 50 % of the control when samples were preincubated for 30 min at 37.5 °C along with 5×10^{-9} mmol/l aminooxyacetic acid or 1×10^{-5} mmol/l γ-vinyl GABA. GABA-T activity was completely inhibited (below 5 % of the control) when preincubation was made with 5×10^{-8} mmol/l of aminooxiacetic acid or 5×10^{-4} mmol/l of γ-vinyl GABA (table II).

Discussion

The dependence of the enzyme activity on α-ketoglutarate and GABA-T concentrations, the presence of a ping-pong kinetics and the complete inhibition of this activity not only with aminooxyacetic acid but also with the more specific γ-vinyl GABA, strongly suggest the presence of GABA-aminotransferase in human hair follicle, similar to that described in other

Table I. *Human hair follicle GABA-T activity in relation to concentrations of either GABA or α-ketoglutarate.*
Data are expressed as pmol/min/mg of protein. The amount of protein in each assay was 95 μg and incubation time 6 h.

GABA (nmol/l)	α-Ketoglutarate (nmol/l)			
	0.0625	0.125	0.25	0.5
0.5	8.3	12.6	16.6	20.9
0.75	8.9	12.5	21.5	25.7
1.0	9.1	16.2	22.2	26.2
1.5	9.3	15.3	26.3	28.6

human tissues such as brain, liver and kidney (21) or platelet (19, 20).

Our modification of White's method for platelets (19, 20), increasing incubation time and decreasing ^{14}C-GABA radioactivity, is both sensitive enough (since only 10 hair follicles are required for one determination) and economical, making it suitable for clinical practice.

Hair follicle GABA-T activity determination may greatly improve the study of GABA related illnesses. In the study of GABA deficiency diseases (such as epilepsy), brain (10) or CSF (14) determinations could be substituted for hair follicle studies, allowing, therefore, a wider screening of population. In congenital GABA-T deficiency, liver biopsy (8) may be replaced by hair follicles. In relation to GABA-T inhibitors treatment, GABA-CSF determination (6) may be substituted for the direct measurement of their effects on hair follicle GABA-T activity.

Although at present there is insufficient evidence that hair follicle GABA aminotransferase can be used as a measure of the brain enzyme, the potential value of such a correlation justifies further investigations. Studies about the similarity between hair follicle and brain GABA-T abnormalities in GABA related illnesses deserve attention.

Table II. *Inhibition of the human hair GABA-aminotransferase (GABA-T) activity by aminooxiacetic acid and γ-vinyl-gaba* in vitro.
Samples were incubated along with GABA-T inhibitors for 30 min at 37.5 °C prior the assay. The amount of protein in each assay was 48 μg and the incubation time 6 h.

Concentration (mol/l)	(GABA-T) activity remaining (%)
Aminooxiacetic acid	
0	100
5×10^{-9}	51
1×10^{-8}	18
5×10^{-8}	2
γ-vinyl GABA	
0	100
2.5×10^{-6}	75
5.0×10^{-6}	72
1.0×10^{-5}	56
2.5×10^{-5}	32
1.25×10^{-4}	10
5×10^{-4}	1

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Resumen

Se demuestra la presencia de GABA-T (4-aminobutirato-2-cetoglutarato aminotransferasa) en folículo piloso humano. Estudios cinéticos con homogeneizado de folículo piloso indican un mecanismo enzimático de tipo *ping-pong*. Los valores extrapolados de K_m son de 1,02 mmol/l para el GABA y de 0,45 mmol/l para el α-cetoglutárico. La actividad GABA-T se inhibe totalmente cuando se preincuban las muestras con 5×10^{-8} mol/l de ácido oxoaminoacético ó 5×10^{-4} mol/l de γ-vinil GABA. El método radiométrico que se propone es suficientemente sensible y económico como para aplicarse en la práctica clínica. La determinación de la actividad GABA-T en folículo piloso puede ser útil en el estudio de las enfermedades con déficit de GABA (como la epilepsia), los déficit congénitos de GABA-T o

para el control del tratamiento con inhibidores de la GABA-T.

Palabras clave: GABA-transaminasa, Folículo piloso.

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