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Effects of increasing doses of vigabatrin on platelet γ-aminobutyric acid-transaminase and brain γ-aminobutyric acid in rats

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Abstract

We report the relationship of GABA-transaminase inhibition in platelets and brain with the increase in brain γ-aminobutyric acid (GABA), as percents of the control, at 24 h after single and after 3 and 8 days of treatment with increasing doses (1, 3, 10, 30, 100 and 300 mg kg^{-1} day^{-1}) of vigabatrin in rats. The inhibition of GABA-transaminase in platelets correlated at least as well as that in brain with the increase in brain GABA after 3 days ($r = -0.87$ vs. $r = -0.78$), and 8 days of treatment ($r = -0.77$ vs. $r = -0.74$), and when the data of single and multiple doses were pooled ($r = -0.77$ vs. $r = -0.75$). The correlation between platelet GABA-transaminase and brain GABA fitted to a power curve, the increase in brain GABA being significant only when platelet GABA-transaminase was inhibited to less than 50% of the control. Our results suggest that platelet GABA-transaminase could be a peripheral marker of the effect of vigabatrin on brain GABA in rats.

Keywords: Blood platelet; Epilepsy; GABA (γ-aminobutyric acid); GABA-(γ-aminobutyric acid-) transaminase; Vigabatrin

1. Introduction

Vigabatrin (γ-vinyl GABA) is a selective and *suicide* dose-dependent inhibitor of brain 4-aminobutyrate-2-ketoglutarate aminotransferase (EC 2.6.1.19, GABA-transaminase) both in vitro and in vivo (Jung et al., 1977; Metcalf, 1979). Vigabatrin increases the γ-aminobutyric acid (GABA) concentration in whole brain (Schechter et al., 1977) and synaptosomal fraction (Löscher, 1981) in rodents and in cerebrospinal fluid (CSF) (Ben-Menachem et al., 1989; Grove et al., 1981) and plasma (Löscher et al., 1993) in epileptic patients. In rodents, the increase in brain GABA concentration paralleled that in synaptosomal fraction (Löscher, 1981) and has been related to the anticonvulsant effects of vigabatrin in some experimental models of convulsion (Bernasconi et al., 1988; Gale, 1989; Schechter et al., 1977).

Vigabatrin plasma concentrations do not properly reflect the clinical effects of this drug (Gram et al., 1983, 1985; Browne et al., 1987; Sivenius et al., 1987; Mumford and Dam, 1989). Therefore, it seems advisable to look for another parameter that reflects them better.

GABA-transaminase has been identified in platelets (White, 1979), and has enzymatic characteristics similar to those of brain GABA-transaminase (White, 1979; White and Faison, 1980). These enzymes are inhibited by vigabatrin in rats (Bolton et al., 1989; Valdizán and Armijo, 1991) and epileptic patients (Rimmer et al., 1988; Arteaga et al., 1992). A correlation between the inhibition of GABA-transaminase in brain and platelets has been described in rats after single doses but not after multiple doses of vigabatrin (Valdizán and Armijo, 1991).

Although the determination of platelet GABA-transaminase activity has been proposed as a way of controlling treatment with GABA-transaminase inhibitors such as vigabatrin (White, 1979; Rimmer et al., 1988), the relationship between the effects of vigabatrin on platelet GABA-transaminase and brain GABA has not yet been investigated.

We have studied the effects of single and multiple increasing doses of vigabatrin on brain GABA and the relationship with GABA-transaminase inhibition in brain and platelets in rats.

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2. Materials and methods

2.1. Animals and groups

The study was carried out in 21 groups of 10 adult male Wistar rats of similar age weighing between 450 and 500 g, housed at a temperature of 22 ± 2°C under natural light/dark conditions, and allowed food and water ad lib. Seven groups were treated with a single i.p. dose (9 a.m.) of saline (control group), or 1, 3, 10, 30, 100 and 300 mg kg^{-1} of vigabatrin in aqueous solution. Another seven groups were treated for 3 days and the remaining seven groups for 8 days with the same doses of vigabatrin once a day.

2.2. Samples

Twenty four hours after the last vigabatrin dose, rats were anaesthetised with ether, 10 ml of blood being collected from each rat by heart puncture and placed into glass tubes containing 0.5 ml of 5% ethylenediaminetetraacetic acid (EDTA) to measure platelet GABA-transaminase activity. The whole brain was immediately removed, divided sagittally and weighed. One-half was homogenised in 5 ml of ice-cold water. Homogenates were then centrifuged at $2500 \times g$ for 10 min at 4°C to measure brain GABA-transaminase activity in the supernatant. The other half was homogenised in 5 ml of ice-cold methanol and then centrifuged as described to measure GABA concentration in the supernatant. Preparation of platelet extracts was performed according to the method of White (1979). Supernatant and platelet lysate samples were stored at −20°C until assay.

2.3. Assays

Platelet and brain GABA-transaminase activity was measured by the radiometric method described by White and Faison (1980) and modified as described by Valdizán and Armijo (1991). Mean GABA-transaminase activity in control groups was 912 pmol min^{-1} mg^{-1} of protein in brain, and 9.4 pmol min^{-1} mg^{-1} of protein in platelets. Quality control was assessed in five control samples assayed for 9 days in triplicate. Within-assay and between-assay coefficients of variation were 6.0 ± 0.9 and 12.4 ± 1.8%, respectively, for brain and 9.1 ± 2.2 and 13.8 ± 5.0%, respectively, for platelets.

Brain GABA concentration was assayed by the liquid chromatographic method of Turnell and Cooper (1982) modified as described by Valdizán and Armijo (1992). Mean brain GABA concentration in control groups was 2.1 μmol g^{-1} of tissue. Within-assay and between-assay coefficients of variation in the five control samples were 2.3 ± 0.9 and 6.8 ± 3.4%, respectively.

Platelet extract and brain homogenate upper-phase concentrations of protein were determined by the method of Lowry et al. (1951), using human albumin as a standard.

2.4. Data analysis

Statistical analysis was carried out using SPSS/PC + software. Differences between platelet and brain GABA-transaminase activity in the same samples were analysed by paired Student's *t*-test. Comparison among the effects

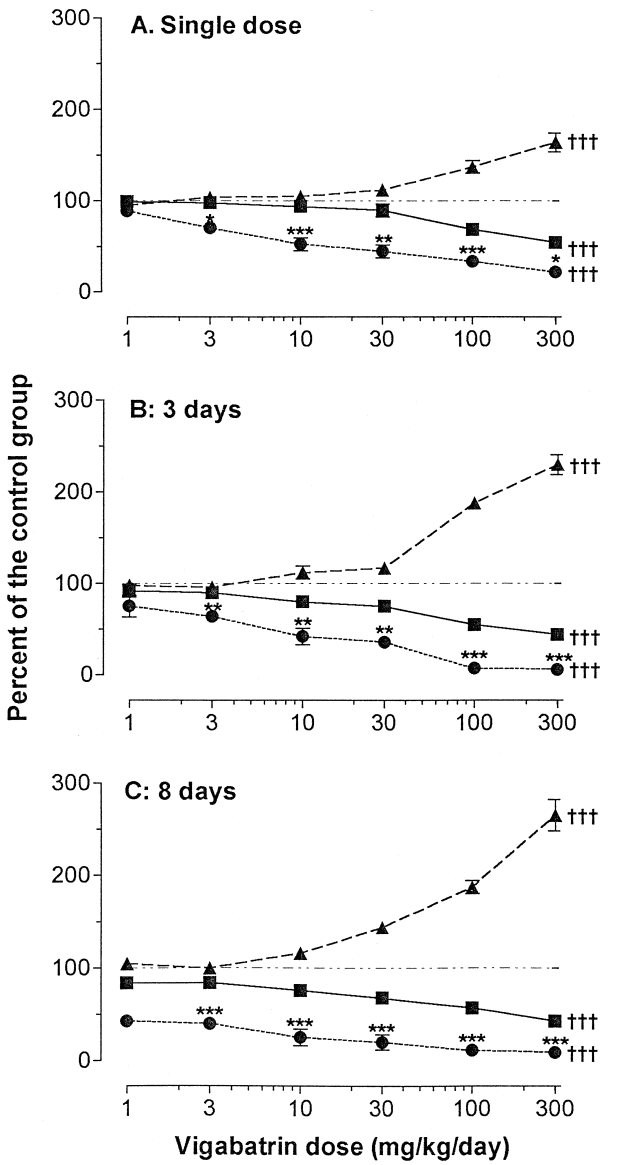

Fig. 1. Platelet GABA-transaminase activity (●), brain GABA-transaminase activity (■) and brain GABA concentration (▲) observed in rats after single doses (A), after 3 days (B) and after 8 days (C) of treatment with 1, 3, 10, 30, 100 and 300 mg kg^{-1} of i.p. vigabatrin. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ vs. brain GABA-transaminase by paired Student's *t*-test. Differences between platelet and brain GABA-transaminase were significant after single dose ($F = 14$, $P < 0.01$), 3 days ($F = 15$, $P < 0.01$) and 8 days of treatment ($F = 13$, $P < 0.01$; by analysis of covariance). Data are means ± S.E.M. Samples were collected 24 h after vigabatrin administration. $^{†††}P < 0.001$ between the effects of increasing doses of vigabatrin by analysis of variance.

of a single dose, 3 days and 8 days of treatment for each individual vigabatrin dose was performed by one-way analysis of variance. Differences between series, for instance, between the effects of single and multiple doses were assessed by analysis of covariance. Correlation was assessed in the samples in which all the GABAergic parameters were available. It was estimated by linear, exponential, logarithmic and power regression methods by using the least-squares method. Because the coefficients of correlation were overall higher by power regression than by linear, logarithmic or exponential regression, only power coefficients of correlation are shown in the table. Data are expressed as mean ± S.D. in the text and as mean ± S.E.M. in the figures. A significance of $P < 0.05$, two-sided test, was accepted throughout.

3. Results

3.1. Single vs. multiple doses of vigabatrin

Platelet GABA-transaminase activity was reduced in a dose-dependent manner by increasing single doses of vigabatrin. Brain GABA-transaminase was progressively inhibited by single doses of vigabatrin higher than 30 mg kg^{-1}, this being accompanied by a progressive increase in brain GABA concentration. The effects of vigabatrin on both enzymes and GABA after 3 days and 8 days of treatment were greater than after single doses (Fig. 1): the effects of 3 days of treatment were significantly greater than those of single doses only for brain GABA-transaminase ($F = 7$, $P < 0.05$ by analysis of covariance whereas the effects of 8 days of treatment were significantly greater than those of single doses for brain GABA-transaminase ($F = 6$, $P < 0.05$), platelet GABA-transaminase ($F = 8$, $P < 0.05$) and brain GABA ($F = 6$, $P < 0.05$).

3.2. Platelet vs. brain GABA-transaminase inhibition

The inhibition of GABA-transaminase activity by increasing doses of vigabatrin in platelets was greater than in brain by analysis of covariance, and paralleled that of brain after single doses higher than 30 mg kg^{-1}, and after 3 and 8 days of treatment (Fig. 1).

The correlation between the inhibition of GABA-transaminase activity in platelets and brain in individual rats, as percent of the control values, was significant after single doses ($r = 0.45$), 3 days ($r = 0.83$) and 8 days ($r = 0.61$) of treatment, and also when the three series were analysed together (Table 1). As shown in Fig. 2 (top), the data fitted better to a power curve than to a straight line. The GABA-transaminase activity was almost totally inhibited by vigabatrin in platelets, whereas a residual GABA-transaminase activity of about 40% of the control remains in brain.

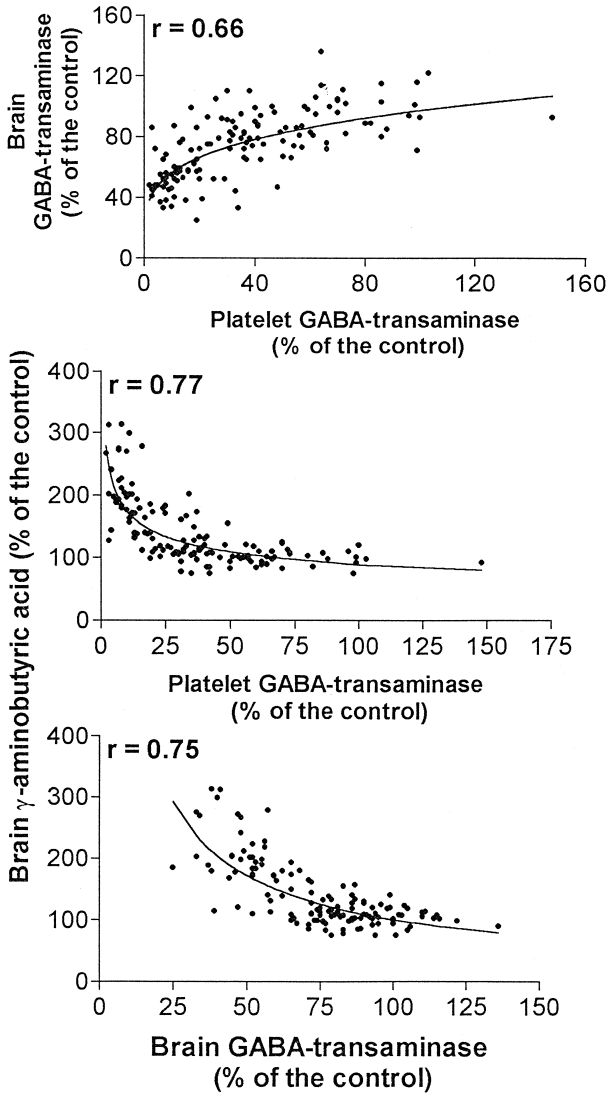

Fig. 2. Correlation between platelet and brain GABA-transaminase activity (top), and between brain GABA and both platelet GABA-transaminase (middle) and brain GABA-transaminase (bottom) in individual rats after single doses, 3 days and 8 days of treatment with 1, 3, 10, 30, 100 and 300 mg/kg of i.p. vigabatrin once a day. r = Coefficient of power correlation. Samples were collected 24 h after vigabatrin administration.

Table 1
Correlation between the effects of single and multiple increasing doses of vigabatrin on brain and platelet GABA-transaminase (GABA-T) and brain GABA in individual rats

Dosing	N	Platelet GABA-T/ brain GABA-T	Platelet GABA-T/ brain GABA	Platelet GABA-T/ brain GABA
Single	44	0.45[a]	−0.57[b]	−0.72[b]
3 days	38	0.83[b]	−0.87[b]	−0.78[b]
8 days	49	0.61[b]	−0.77[b]	−0.74[b]
All	131	0.66[b]	−0.77[b]	−0.75[b]

Data are the coefficients of power correlation between the GABAergic effects of 1, 3, 10, 30, 100 and 300 mg kg^{-1} of vigabatrin after single doses and after 3 days and 8 days of treatment with the same dose once a day expressed as percent of each control group. N = number of rats. [a]$P < 0.01$. [b]$P < 0.001$.

3.3. Relationship between brain GABA increase and GABA-transaminase inhibition

Overall, the progressive inhibition of GABA-transaminase in brain and platelets achieved by increasing doses of vigabatrin was accompanied by a progressive increase in brain GABA levels. After single doses, the mirror image with brain GABA was better for brain GABA-transaminase than for the platelet enzyme (Fig. 1, top), and this was accompanied by a better power correlation in individual rats ($r = -0.72$ and $r = -0.57$, respectively) (Table 1). After 3 days and 8 days of treatment, the mirror images with brain GABA were similar for the two enzymes (Fig. 1, middle and bottom), which were accompanied by a better correlation with the platelet enzyme ($r = -0.78$ for the brain and $r = -0.87$ for the platelet enzyme after 3 days, and $r = -0.74$ vs. $r = -0.77$ after 8 days). The correlation between the increase in brain GABA and the inhibition of GABA-transaminase in individual rats was also slightly better for the platelet enzyme when data from single, 3 days and 8 days of treatment were pooled (Table 1). The data fitted better to a power curve than to a straight line, but there were differences between the two enzymes (Fig. 2). For platelet GABA-transaminase, the increase in brain GABA was significant only when platelet GABA-transaminase activity was lower than 50% of the control. Therefore, the difference between the linear ($r = -0.63$) and power ($r = -0.77$) correlations was large. A significant increase in brain GABA was observed only when platelet GABA-transaminase activity was inhibited from 50% to 0% of the control (Fig. 2, middle). In contrast, the increase in brain GABA correlated almost linearly with the inhibition of brain GABA-transaminase (Fig. 2, bottom), the difference between the linear ($r = -0.71$) and power ($r = -0.75$) correlations being smaller.

4. Discussion

The most interesting result in this study was the significant correlation between the increase in brain GABA and the inhibition of platelet GABA-transaminase after multiple doses of vigabatrin. Furthermore, we have found a good correlation between the inhibition of GABA-transaminase in brain and in platelets after multiple doses of vigabatrin.

The correlation between platelet and brain GABA-transaminase after single doses of vigabatrin has been described previously (Valdizán and Armijo, 1991). However, a correlation between the effects of vigabatrin on the two enzymes after multiple doses could not be demonstrated in that study because platelet GABA-transaminase was totally inhibited. This correlation has been demonstrated in the present study in which lower doses of vigabatrin were used.

The power correlation between platelet GABA-transaminase and brain GABA in individual rats was worse than that observed with the brain enzyme after single doses but it was slightly better after multiple doses. After single doses, there was no increase in brain GABA or inhibition of brain GABA-transaminase after 3, 10 or 30 mg kg^{-1} of vigabatrin whereas platelet GABA-transaminase was progressively inhibited. However, after multiple days of treatment, the mirror image between the increase in brain GABA and the inhibition of GABA-transaminase was similar for the two enzymes, giving similar coefficients of correlation with brain GABA in individual rats.

When data obtained after single doses, 3 days and 8 days of treatment were analysed together, similar coefficients of correlation were also found. It should be pointed out that the relationship between the inhibition of platelet GABA-transaminase activity and the increase in brain GABA-concentration fitted to a power curve, and that a significant increase in brain GABA was observed only when platelet GABA-transaminase activity was inhibited to less than 50% of the control group.

Platelet GABA-transaminase activity could be used as a peripheral marker of the effects of vigabatrin on GABA concentrations in whole brain in rats. The increase in whole brain GABA has been related to the anticonvulsant effects of vigabatrin in some models of epilepsy in rodents (Bernasconi et al., 1988; Gale, 1989; Schechter et al., 1977). Therefore, it is possible that the inhibition of platelet GABA-transaminase could also reflect the anticonvulsant effects of vigabatrin. We did not analyse plasma concentrations of vigabatrin because they are almost undetectable at 24 h, nor was the anticonvulsant effect of vigabatrin assessed in this study. More specific studies are needed to determine if platelet GABA-transaminase reflects the anticonvulsant effects of vigabatrin better or worse than brain GABA, brain GABA-transaminase, vigabatrin plasma concentrations or other peripheral markers.

Data on the effects of vigabatrin on platelet GABA-transaminase in epileptic patients are scarce (Bolton et al., 1989; Arteaga et al., 1992). In the study of Arteaga et al. (1992), a relationship between the degree of inhibition of platelet GABA-transaminase and seizure reduction was observed in some vigabatrin respondent children in whom the vigabatrin dose was gradually increased, but a correlation between the two parameters could not be demonstrated (Arteaga et al., 1992). This lack of correlation may be attributed to the fact that platelet GABA-transaminase activity was maximally inhibited by the vigabatrin dose used in the study. In fact, platelet GABA-transaminase activity, after a mean dose of 57 mg kg^{-1} of vigabatrin, did not decrease when the dose was increased to 84 mg kg^{-1} (Arteaga et al., 1992). However, the possibility of a relationship between the inhibition of platelet GABA-transaminase and the antiepileptic effects of vigabatrin at doses that do not totally inhibit platelet GABA-transaminase was not discarded.

Other peripheral markers such as GABA or glutamate in CSF and GABA in plasma have also been proposed for monitoring the effects of vigabatrin. In fact, vigabatrin respondent patients had a higher CSF increase in total GABA (Riekkinen et al., 1989), a higher baseline CSF glutamate (Kälviäinen et al., 1993), and a higher increase in plasma GABA (Löscher et al., 1993) as compared with both controls and non-responders. However, the increase in CSF GABA during vigabatrin add-on treatment in mentally retarded patients was similar in patients with both good and poor responses (Pitkänen et al., 1988).

In summary, our results demonstrate a significant power correlation between the inhibition of GABA-transaminase in platelets and the increase in GABA concentration in brain at 24 h after single and multiple increasing doses of vigabatrin in rats, the correlation with platelet GABA-transaminase being at least as good as that with the brain enzyme after multiple doses. These results suggest that platelet GABA-transaminase could be used as a peripheral marker of the effects of this antiepileptic drug on brain GABA in rats especially at doses which inhibit platelet GABA-transaminase activity to less than 50% of the control group but do not totally inhibit the activity of this enzyme.

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