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The effect of vigabatrin on brain and platelet GABA-transaminase activities

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1 The inhibition profiles of GABA-transaminase (GABA-T) in rat brain and platelet have been compared following a single intraperitoneal dose of vigabatrin. The inhibition profiles exhibit similarities. The inhibition produced by the drug is dose-dependent and, in the dose range used in man, the dose-response curves are comparable. The pharmacodynamic effects of the drug (inhibition of central and peripheral GABA-T) remain after the drug has been eliminated from plasma. It is suggested that the measurement of rat platelet GABA-T may be used as a non-invasive assessment of the efficacy of GABA-T inhibitors in the rat CNS.

2 Human blood platelet GABA-T was significantly inhibited by the administration of a single oral dose of vigabatrin. Chronic administration also produces a significant inhibition of platelet GABA-T. As with rats, the pharmacodynamic effects on the platelet enzyme remained after the drug had been eliminated from plasma. If the situation in man is assumed to parallel that found in the rat then measurement of platelet GABA-T inhibition could prove to be a useful indicator of central inhibition.

Keywords vigabatrin GABA-T platelet inhibition

Introduction

Vigabatrin is a selective enzyme-activated irreversible inhibitor of GABA-transaminase (GABA-T) (Jung *et al.*, 1977) which has potent anticonvulsant properties in humans (Rimmer & Richens, 1984; Pederson *et al.*, 1985), presumably resulting from elevation of GABA concentrations in the brain (Jung, 1982). The efficacy of the drug will be affected by at least three factors: its pharmacokinetics; the frequency of dosing and the half-life of the target enzyme. The measurement of the plasma concentration of this drug to assess clinical efficacy is likely to be inadequate because of its mode of action.

Ideally we wish to assess the pharmacodynamic effects of the drug in the CNS (e.g. the extent of inhibition of GABA-T and elevation of GABA concentration) and correlate this with the clinical response (control of seizures).

GABA and its associated enzymes are found in other tissues of the body, including liver, kidney and blood platelet (White & Sato, 1978; White, 1979). GABA-T from platelets resembles that of brain in both its kinetic properties and its response to cofactors and inhibitors (White & Faison, 1980).

This paper describes the experiments performed in order to characterise the relationship between brain and platelet GABA-T inhibition in the mammalian system and to examine the behaviour of human platelet GABA-T during acute and chronic exposure to vigabatrin.

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I. Animal studies

Methods

1) *Animals*

All experiments were carried out in adult, male Wistar rats (250–350 g) which were housed under conditions of constant temperature and day/night cycle and allowed uninhibited to food and water.

Study 1 The time course of inhibition in rat brain and platelet following a single intraperitoneal dose of vigabatrin.

One group of rats was dosed with vigabatrin (1500 mg kg^{-1} i.p. in aqueous solution, 1 ml 100 g^{-1}), another was dosed with saline (1 ml 100 g^{-1}). At various time points after administration (see Figure 2), five rats from each group were anaesthetised with pentobarbitone. Blood (5–10 ml) was obtained via cannulation of the carotid artery and placed in chilled glass tubes containing 0.5 ml of 5% EDTA as anticoagulant. Whole brain was removed and homogenised in chilled EDTA buffer containing glycerol (20% v/v), triton-X-100 (0.13% v/v), reduced glutathione (10^{-4}M), pyridoxal phosphate (10^{-4}M), disodium EDTA (10^{-3}M) and potassium phosphate (10^{-2}M), pH 6.8.

Study 2 The effect of various doses of vigabatrin on rat brain, liver and platelet GABA-T.

Vigabatrin was administered to ten rats in doses ranging from 50 mg kg^{-1} to 3200 mg kg^{-1} (single, i.p. injection in aqueous solution, 1 ml 100 g^{-1}). Six hours later blood was obtained via cannulation of the carotid artery under general anaesthesia (halothane: N_2O/O_2). The rats were sacrificed and the brains and livers removed and homogenised in ice-cold distilled water.

2) *Preparation of platelets*

Blood was centrifuged at 200 *g* (20° C, 10 min). The platelet-rich-plasma was removed and centrifuged at 2450 *g* (4° C, 15 min). The platelet pellet was resuspended in 1 ml EDTA buffer containing EDTA (10^{-3}M), dithiothreitol (0.5×10^{-3}M), pyridoxal phosphate (0.2×10^{-3}M) and potassium phosphate (0.1×10^{-3}M), pH8. Platelets were lysed by three freeze-thaw cycles and then stored at −20° C. Enzyme activity was found to be stable for up to 1 month under these conditions.

3) *Preparation of brain*

Brain homogenate was freeze-thawed once and centrifuged at 2000 *g* (4° C, 30 min). Enzyme activity was determined in the supernatant which was stored at −20° C.

4) *Assays*

GABA-T activity was measured in the samples by the radiometric assay of White (1979). Protein concentration was determined by the method of Bradford (1976). Enzyme activity was calculated in pmol min^{-1} mg^{-1} protein and nmol min^{-1} mg^{-1} protein (or μmol min^{-1} g^{-1} tissue) for platelets and brain respectively. The intra-assay coefficient of variation of the assay was 5% for brain and 14% for platelet respectively. Vigabatrin concentrations were assayed using an h.p.l.c. method involving precolumn derivatisation (dansylation) and fluorimetric detection (Merrell Dow). Phenyl GABA was used as internal standard. The minimum level of detection was 0.1 μg ml^{-1} and the coefficient of variation was < 7%. Statistical analyses were performed by a two-way analysis of variance (two-way ANOVA) and by Student's paired *t*-test.

Study 1

Results

Vigabatrin concentration in plasma and brain fell continuously from 2 h following a single intraperitoneal dose (see Figure 1), being undetectable at 72 h. Peak brain vigabatrin concentrations were about 4% of those in plasma.

The animals were noticeably sedated, hypothermic and diuretic at 24 h after dosing. Some losses of animals occurred in the 168 h and 336 h groups. No other ill effects were noted.

GABA-T activity in whole brain was rapidly inhibited, attaining a maximum inhibition of 65% at 48 h (Figure 2). This residual enzyme activity has been observed previously (Jung *et al.*,1977). The enzyme activity then began to recover slowly, but was still significantly different from control values at 336 h ($P < 0.01$). The inhibition of GABA-T in platelets occurred more rapidly and to a greater extent (Figure 2). A maximum inhibition of almost 100% was reached at 4 h, then the enzyme recovered slowly. At 336 h after dosing the activity was not significantly different from controls. There was a considerable interindividual variation in platelet GABA-T activities which resulted in a large standard error of the mean at each time point.

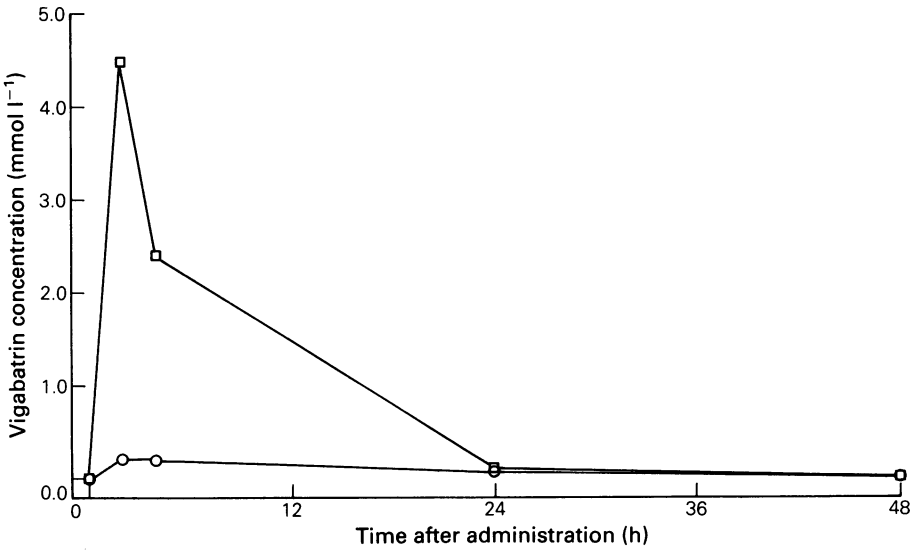

Figure 1 Concentration of vigabatrin in rat plasma (□) and brain (○) following a single intraperitoneal dose (1500 mg kg^{-1}).

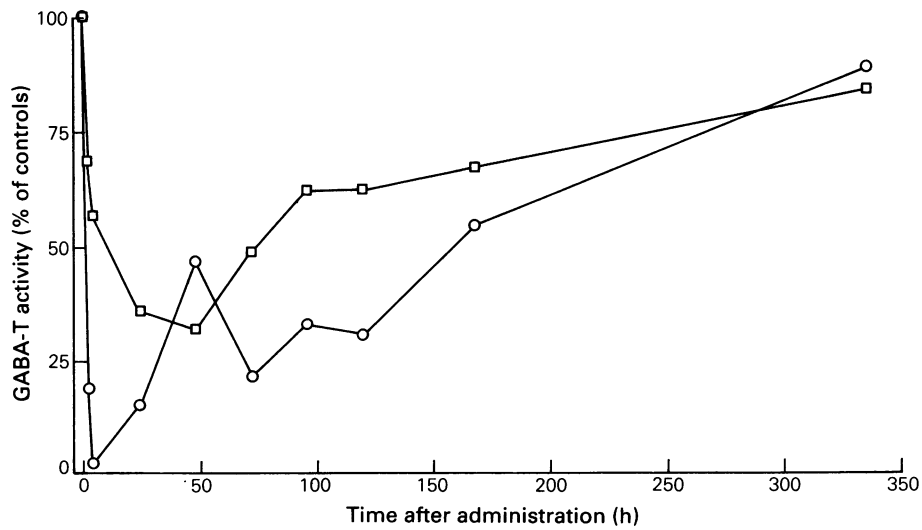

Figure 2 Comparison of the inhibition profiles of rat brain (□) and platelets (○). GABA-T activity is plotted as % activity of controls at each time point.

Study 2

Results

Figure 3 shows the response of rat brain, platelet and liver GABA-T to varying intraperitoneal doses of vigabatrin. The inhibition of brain GABA-T increased almost linearly with increasing doses of vigabatrin, as would be predicted. However, even at the highest dose used a residual activity of approximately 30% remained. The platelet enzyme dose-response relationship was similar to that of brain, except at the highest dose (3200 mg kg^{-1}), where the platelet enzyme was almost completely inhibited. The liver enzyme was 82% inhibited at even the lowest dose of vigabatrin (50 mg kg^{-1}). Increasing the dose after this had only a small effect on enzyme inhibition. At 3200 mg kg^{-1} the liver enzyme was almost completely inhibited (*c.f.* platelet).

Discussion

GABA-T in rat brain and platelets has been shown to be inhibited by a single, intraperitoneal dose of vigabatrin in a dose-dependent manner. The time courses of inhibition of brain GABA-T and platelet GABA-T were similar, although platelet enzyme was inhibited more rapidly and to a greater extent than that of brain. Although platelet GABA-T was almost completely inhibited by this dose of vigabatrin, there remained a residual activity in brain of approximately 30%. (This also occurred in the dose-response relationship study, see Figure 3). This residual enzyme activity has been observed previously (Jung *et al.*, 1977). *In vivo*, as brain GABA-T is inhibited, so the GABA concentration begins to rise. This has two main effects (1) GABA occupies the active site and competes with vigabatrin for that site (direct protection) and (2) the equilibrium between the pyridoxal and pyridoxamine forms of the enzyme is shifted towards the latter (indirect inhibition). The pyridoxamine form has been shown to be refractory to inhibition by vigabatrin (Lippert *et al.*, 1982). No residual enzyme activity occurs in platelets because, presumably, the rise in plasma vigabatrin is so rapid that GABA-T is inhibited before GABA levels begin to rise appreciably. In addition, the synthetic enzyme glutamic acid decarboxylase (GAD) is thought to be absent in platelets and so cannot influence the GABA concentration. The recoveries of each enzyme were remarkably similar given that there is a major difference in the mechanism by which each enzyme recovers its activity. Vigabatrin is an irreversible inhibitor and thus regeneration of brain GABA-T must be due to new protein synthesis. Platelets do not possess a nucleus and, although they do have endoplasmic reticulum and low levels of circulating mRNA, it is unlikely that they could synthesise new protein in

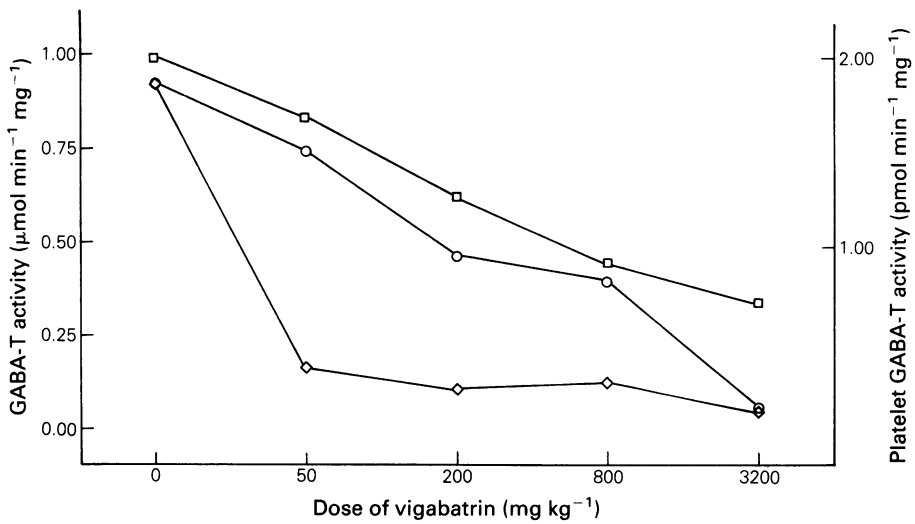

Figure 3 GABA-T activity in rat brain (□), platelet (○) and liver (◇) following a single intraperitoneal dose of vigabatrin (0 to 3200 mg kg^{-1}). Note the different scale for platelets. Each point represents the mean of values obtained from two animals.

sufficient quantities. Recovery of the enzyme must be due to platelet, rather than enzyme protein, regeneration.

Rat brain GABA-T exhibits a similar dose-response relationship to that of mouse brain (Jung *et al.*, 1977). The residual enzyme activity described above occurred again, even at the highest dose of drug used (3200 mg kg^{-1}). The reasons for this have already been discussed. Liver GABA-T possesses similar properties to that of brain GABA-T (White & Sato, 1978) so it is not immediately apparent why the liver enzyme should be inhibited to a greater extent than brain, even at the lowest dose (50 mg kg^{-1}). Possibly the liver receives a larger dose of drug via the portal blood supply. There is also very little glutamate decarboxylase in the liver (Wu, 1982) so that the GABA concentration will not increase so rapidly as that in the brain and exert the protective effect described above. In addition, the existence of isoenzymes of GABA-T has been postulated (Jeremiah & Povey, 1981). Different forms of the enzyme may exhibit differing behaviour when presented with vigabatrin.

At doses up to 200 mg kg^{-1} (the dose range used in patients) there is a good correlation between rat brain and platelet GABA-T responses.

From these results it can be seen that the pharmacodynamic effects of vigabatrin in brain and platelets are present long after the drug has been cleared from plasma. Platelet enzyme inhibition in rats appears to correlate well with inhibition in the brain and may be a useful indicator of the efficacy and central effects of the drug.

II. Human studies

Methods

1) *Design of the study*

(a) *Single dose study in healthy male volunteers* The study design was an open randomised cross-over comparison of three single oral doses of vigabatrin (1, 2 and 4 g) in six healthy male subjects. The age range was 18 to 44 years. Treatment order was allocated according to a randomised latin square design. Each subject received each of the three doses of the drug and consecutive treatments were separated by a minimum interval of 2 weeks. Blood samples (10 ml) were obtained prior to drug administration and at various time intervals after dosing (see Figure 4). A baseline period preceded the three treatments in which normal platelet GABA-T variability was determined, sampling at the same time intervals used during each treatment. Ethical approval was obtained from the Joint Ethics Committee and all subjects gave informed written consent.

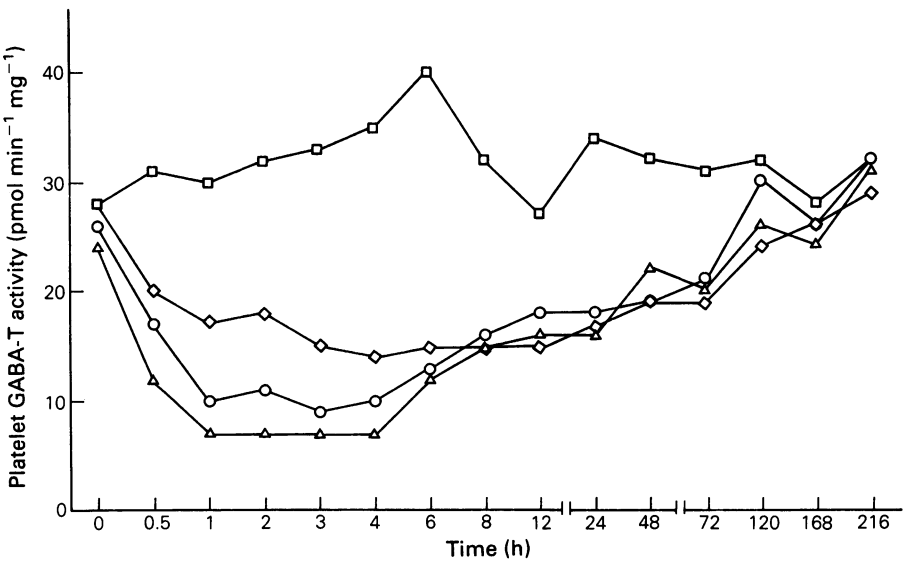

Figure 4 Mean platelet GABA-T in healthy human volunteers following a single oral dose of vigabatrin compared with control values. Each point represents the mean of six results. □ control, ◇ lg, ○ 2 g and △ 4 g vigabatrin.

(b) *Chronic dosing study in patients* Platelet GABA-T activity and plasma vigabatrin levels were studied in eight patients before, during and following a 6 week period of treatment with vigabatrin. The patients, all suffering from chronic refractory epilepsy, were aged between 17 and 51 years. Vigabatrin treatment was provided as an add-on therapy to at least two other antiepileptic drugs (see Table 1). The dosing of these drugs remained constant throughout the study period. Baseline enzyme activity was determined before the study by sampling at least twice. Vigabatrin was added to the usual therapy in doses of 2 g (1 week), 3 g (4 weeks), 2 g (1 week) and then withdrawn completely. Blood samples were obtained at various times during therapy (Figure 5) and also 1 week and 3 weeks after termination of the study. Blood sampling times were standardised for each patient. Sampling occurred at 09.00 h prior to dosing. On one occasion additional blood samples were obtained 1 h and 2 h post administration.

2) *Assays*

Platelets were prepared and GABA-T assays performed exactly as described in the previous section. The vigabatrin assay was performed at Merrell Dow Research Institute, Egham, Surrey, using the h.p.l.c. method described previously. The limit of detection of the assay was 0.1 μg ml^{-1}. The coefficient of variation over the range 1–100 mg ml^{-1} was less than 7% (M. Lush, personal communication).

Results

(a) *Volunteer study*

No adverse effects were reported after the administration of vigabatrin. There was a considerable interindividual variation in baseline platelet GABA-T activity, although the control values for each individual remained constant throughout the baseline period. The mean platelet GABA-T activity during the control period was 31.6 pmol min^{-1} mg^{-1} protein. Figure 4 shows the mean levels of platelet GABA-T activity at the various times following each dose of vigabatrin compared with the baseline period. GABA-T was significantly inhibited at 30 min following all three doses ($P < 0.01$) and inhibition reached a maximum at between 1 and 4 h. Enzyme activity then began to recover and by 120 h was not significantly different from control levels. The minimum enzyme activities after the 1, 2 and 4 g doses of vigabatrin were 43, 30 and 21% respectively compared with the mean control value. Statistical analysis (two-way ANOVA) showed that at 1, 2 and 3 h there were significant differences between the platelet enzyme activities with the three doses (F ratios $5.96\ P < 0.05$, $5.94\ P < 0.05$, $4.48\ P < 0.05$ at 1, 2 and 3 h). The mean platelet GABA-T values of the three doses lay in the expected order and showed a clear dose-response relationship.

Peak plasma vigabatrin concentrations occurred almost always at 1 h post administration and the mean peak concentrations were 34.3,

Table 1 Clinical details of patients

Patient	*Age (years)*	*Sex*	*Seizure type*	*Seizure frequency*	*Medication (daily)*
01	26	F	CPS with 2° generalisation	4 per month	DPH 300 mg CBZ 800 mg
02	38	M	CPS with 2° generalisation	6 per month	DPH 300 mg CBZ 1200 mg
03	31	M	CPS with 2° generalisation	6 per month	DPH 150 mg CBZ 800 mg SV 2 g
04	51	M	CPS with 2° generalisation	8 per month	DPH 400 mg SV 1500 mg
05	40	M	CPS with 2° generalisation	4 per month	DPH 300 mg CBZ 1000 mg Clobazam 20 mg
06	27	F	Tonic-clonic convulsions	3 per month	DPH 180 mg CBZ 800 mg
07	17	M	CPS	4 per month	DPH 350 mg SV 2 g
08	17	M	CPS	8 per month	DPH 300 mg CBZ 800 mg

CPS = Complex partial seizures; DPH = Phenytoin; CBZ = Carbamazepine; SV = Sodium valproate

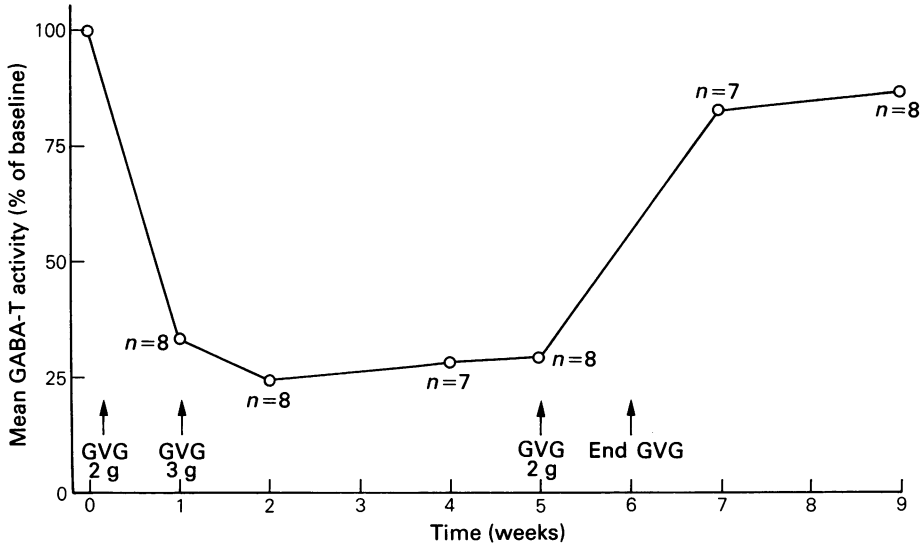

Figure 5 Mean platelet GABA-T activity during vigabatrin therapy as a percentage of each patient's baseline values. Arrows indicate the daily dose of vigabatrin.

75.3 and 143.3 mg l^{-1} for the 1, 2 and 4 g doses were 6.76, 6.87 and 5.52 h respectively. Vigabatrin was undetectable at 48 h. The blood sampling times were not selected primarily to determine the pharmacokinetic parameters, so the figures given should be considered as estimates.

(b) *Patient study*

Again, the interindividual variation in platelet GABA-T baseline activity was considerable, but intrasubject activities were constant.

Vigabatrin, at a dose of 2 g daily, resulted in a marked reduction in enzyme activity in all subjects (Figure 5). The extent of inhibition produced by the higher dose of vigabatrin was not significantly greater than that of the lower (2 g) dose of the drug, even after 4 weeks of treatment. One week after the termination of vigabatrin therapy, enzyme activity was not significantly different from baseline. Mean percentage GABA-T activity compared with baseline is shown in Figure 5.

Table 2 shows the enzyme activity prior to and 1 h and 2 h following the 3 g dose of vigabatrin. There were no significant differences between them. The mean trough levels of vigabatrin (12 h post dose administration) were 5.05 ± 1.97 mg l^{-1} (n = 8, ± s.d.). Vigabatrin concentrations were measured 1 h and 2 h following the 3 g dose and were 44.75 ± 9.39 mg l^{-1} and 36.08 ± 10.87 mg l^{-1} respectively (n = 6). These values after chronic dosing are in fact, lower than the equivalent values after a single 2 g dose in healthy volunteers.

Discussion

Human platelet GABA-T was significantly inhibited in a dose-dependent manner by the administration of single oral doses of vigabatrin. The effect occurred rapidly after drug administration (within 30 min) with marked enzyme inhibition at 8 h with all three doses. The enzyme activity recovered slowly, reaching control levels at 120 h regeneration being due to platelet turnover (as discussed in section 1). A convincing dose-response relationship was demonstrated for all three doses. The maximum degree of

Table 2 Platelet GABA-aminotransferase activity in epileptic patients pre- and post-vigabatrin (3 g)

	Platelet enzyme activity (pmol min^{-1} mg^{-1} protein)		
Patient	*Pre-dose*	*Post vigabatrin*	
		1 h	*2 h*
01	16	10	04
02	02	04	06
03	18	20	32
05	18	08	12
06	12	10	06
08	18	18	22

enzyme inhibition achieved in this study was almost 80%. Thus, after a single dose of the drug, even 4 g failed to inhibit the platelet enzyme completely.

Chronic dosing with vigabatrin produced a significant inhibition in human platelet GABA-T. Unexpectedly, there was no significant difference between the inhibition produced by 1 weeks' treatment with 2 g of drug daily and 4 weeks' treatment with 3 g daily. In addition, there was no significant difference in enzyme activity at the peak and trough plasma vigabatrin concentrations. These two facts suggest that platelet GABA-T is maximally inhibited in each case. Patients on chronic drug therapy exhibited lower peak plasma vigabatrin levels on the 3 g dose than did healthy volunteers on the 2 g dose. It is possible that although vigabatrin is mainly excreted unchanged in urine, some of the ingested dose might be metabolised in the liver and that enzyme induction was present in patients already on chronic antiepileptic drug therapy.

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