Effects of Anti-epileptic Drugs on the L-Tryptophan Binding to Human Serum Albumin

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Effects of four anti-epileptic drugs (AED; phenobarbital, phenytoin, carbamazepine and sodium valproate) on the L-tryptophan binding to human serum albumin were studied. Among these drugs examined, only sodium valproate inhibited the binding even within the concentrations of its therapeutic range, and the Klotz plotting analysis revealed that the inhibition was competitive. The results of examinations with sera from epileptic patients medicated with these AED and drug-free normal controls also suggested that the protein binding ratios of L-tryptophan were decreased in the blood plasma of some patients with the high valproate concentrations and the low albumin contents.

Keywords L-tryptophan; human serum albumin; anti-epileptic drug; sodium valproate; binding inhibition

Introduction

L-Tryptophan (Trp) is the sole amino acid present in blood plasma in two forms, a free or ultrafiltrable form and a form bound to albumin.[1] It has also been known that Trp in the central nervous system (CNS) is very important as a precursor of 5-hydroxytryptamin (serotonin) and that only free or ultrafiltrable Trp in the blood plasma can pass through blood-brain barrier into brain tissue. It is therefore significant, from the view-point of studies on the bioavailability of Trp in the central nervous system, to investigate various endogenous and exogenous factors which affect Trp binding to albumin in blood plasma.

The therapeutic ranges of the plasma concentrations of anti-epileptic drugs (AED) are generally high, and some of them, such as phenytoin (PHT) and valproic acid (VPA), have a potent affinity to albumin indicating binding ratios of about 90%.[2]

In order to elucidate the effects of AED on the Trp binding to human serum albumin (HSA), *in vitro* experiments using pooled human serum samples were at first carried out on the basis of the ultrafiltration method. Klotz plotting studies were then performed in order to determine the inhibition mode of VPA. The results of examinations with sera from epileptic patients treated with these AED are also discussed.[3]

Materials and Methods

Materials Phenobarbital (PB), PHT, carbamazepine (CBZ) and VPA (as sodium salt) were purchased from Wako Pure Chemicals (PB and VPA) and Aldrich Chemical company (PHT and CBZ), respectively. The lipid-stripped pooled human serum and purified HSA were obtained from Funakoshi Chemical Co. Sera were also taken from 14 epileptic patients (17—42 years-old, 7 males and 7 females) medicated with at least one of these four AED and from 9 drug-free normal controls (22—41 years-old, 4 males and 5 females). Among 14 epileptic patients, 8 cases were orally given VPA with or without other AED (the VPA group), and 6 others were given only PB, PHT and/or CBZ. All the other chemicals were of analytical grade.

***In Vitro* Examinations for the Detection of Effects of Four AED on the Trp Binding to HSA** The pooled human serum samples with an albumin content of 4.3 g/dl (1.8 ml each) were added to 0.2 ml each of dimethyl sulfoxide (DMSO) solutions of PB, PHT, CBZ and sodium VPA so that their final concentrations were 1×10^{-6}, 1×10^{-5}, 1×10^{-4}, 3×10^{-4}, 1×10^{-3}, 3×10^{-3} and 1×10^{-2} M, respectively (in the case of PHT, higher concentrations than 1×10^{-3} M were excluded due to the low solubility in aqueous media). As a control, only 0.2 ml of DMSO was added. Every mixture (2 ml) thus obtained was allowed to stand at 37 °C for 1 h, and then divided into two 1 ml fractions for measurement of the total and free Trp contents, respectively. The former were added to equal volumes of 10% (w/v) sulfosalicylic acid (SSA) to precipitate the proteins, and the latter were treated with Centricon-10 miniconcentrators (Grace Company) at 740 *g* for 30 min, at room temperature, respectively. Then, 20 μl each of the supernatant after removing the proteins by SSA from the former, as well as 10 μl each of the ultrafiltrates from the latter, was injected into high-performance liquid chromatography (HPLC). HPLC was carried out generally under the conditions used by Martin *et al.*[4] except that detection was performed by monitoring at 280 nm. The Trp contents in samples examined were obtained from a standard curve, and the percent values of Trp concentration ratios of the ultrafiltrates against the original samples (the % free values) were finally calculated for all the samples examined.

Examinations of the Inhibition Mode of VPA on the Trp Binding to HSA Trp was dissolved in a phosphate buffer (0.1 M Na_2HPO_4 adjusted to pH 7.4 by NaH_2PO_4) containing 3.4% (w/v) HSA, at the concentrations of 5×10^{-6} M, 1, 2, 3, 4 and 5×10^{-5} M, respectively. Each mixture was divided into two equal volumes of fractions, and sodium VPA was added to only one of them so that its concentration was 1×10^{-3} M. Changes in the % free values of Trp with and without VPA were measured in the same manner as described above and the obtained data were treated by Klotz plotting analysis.

Examinations with Sera from Epileptic Patients and Normal Controls The total and free (ultrafiltrable) Trp concentrations were measured for 23 (in total) sera from 14 epileptic patients including 8 belonging to the VPA group and from 9 drug-free normal controls (see Materials), in order to elucidate whether the % free values of serum Trp in the VPA group were really elevated. In the VPA group, the serum total VPA concentrations were also determined by capillary isotachophoresis generally under the conditions used by Mikkers *et al.*,[5] employing a Shimadzu IP-2A isotachophoretic analyzer. The serum albumin contents were measured as a routine laboratory test in the hospital.

Results and Discussion

The total and free (ultrafiltrable) Trp concentrations in pooled human serum in the absence of AED was 17.6 and 1.6 μg/ml, respectively, thus indicating that the % free value in the absence of AED was 9.1%. As shown in Fig. 1, additions of VPA with concentrations higher than 1×10^{-4} M gave an increase in the % free values of serum Trp and the value reached more than 70% at a VPA concentration of 1×10^{-2} M. The therapeutic range of the serum total VPA concentration was reported to be 50—100 μg/ml (*ca.* $3—6\times10^{-4}$ M),[6] and the % free values of serum Trp at VPA concentrations of 1×10^{-4} M, 3×10^{-4} M and 1×10^{-3} M were 10.1, 12.8 and 18.5%, respectively. Therefore, these data seemed to suggest that the Trp binding to HSA was inhibited by VPA *in vitro* even within the concentrations of its therapeutic range. On the other hand, PHT at a concentration of 1×10^{-3} M, which is far higher than its therapeutic range (10—20 μg/ml = *ca.* $4—8\times10^{-5}$ M),[7] gave only a slight increase in the % free value of serum Trp from 9.1 in drug-free

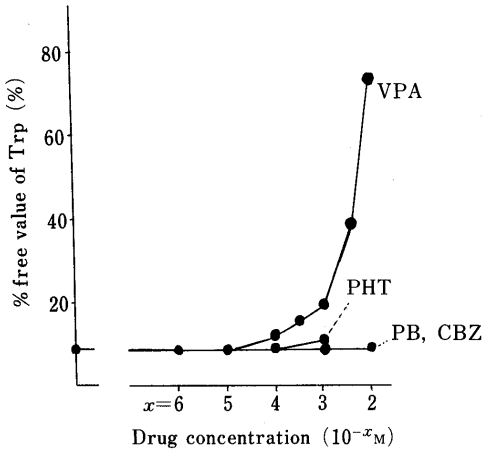

Fig. 1. *In Vitro* Replacement of Serum Trp by AED

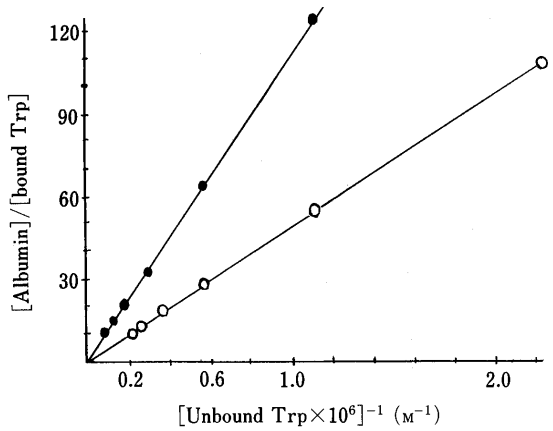

Fig. 2. Inhibitory Effects of Sodium Valproate on the Binding of Trp to HSA

●, with VPA (1 mM); ○, without VPA.

TABLE I. % Free Values of Serum Trp in Epileptic Patients Medicated with AED and Drug-Free Normal Controls

Patients	Range	Mean±S.D.
Medicated with VPA	5.4—11.7%	8.5±2.1%
Medicated with other AED	5.5— 9.4%	7.6±1.5%
Drug-free normal controls	6.1— 9.7%	7.8±1.3%

to 9.7%, although its low solubility in aqueous media did not allow us to study the effects in its higher concentrations. PB and CBZ showed no such effect on the Trp binding to HSA.

As shown in Fig. 2, the Klotz plotting analysis revealed that inhibition by VPA of the Trp binding to HSA is competitive. Concerning the binding positions of Trp to HSA, it has been shown by earlier workers that the binding site of Trp and benzodiazepins is site II of which a major part is located in the fragment C (residues 124—198) and that histidine-146 and lysine-190 have high activities in the binding of this fragment.[8,9] It has also been known that Trp is displaced by benzodiazepins.[10] More recently, competitive inhibition by VPA of the diazepam binding to HSA was demonstrated.[11] Our results also show that Trp, diazepam and VPA have a common binding site on the HSA molecules and that VPA replaces the HSA binding of not only diazepam[12] but also Trp.

Results of measurement of the % serum free values of Trp in 8 cases of the VPA group, 6 other epileptic patients and 9 drug-free controls are summarized in Table I. As shown by the data in Table I, the serum % free value of Trp tended to be higher than those in the other two groups, although statistically no significant difference was observed. On the other hand, the serum total Trp concentrations in these 3 groups were 15.4±4.7 μg/ml ($n=8$), 16.9±5.2 μg/ml ($n=6$) and 16.4±5.5 μg/ml ($n=9$), respectively, showing no difference from each other. The serum % free values of Trp in the VPA group were negatively correlated with the serum albumin contents (3.2—4.7 g/dl; $r=-0.63$), and also positively correlated with the serum VPA concentrations (42—95 μg/ml; $r=0.74$), respectively. Indeed, two elevated % free values of serum Trp (11.0 and 11.7%) in the VPA group were associated with the low albumin contents (3.2 and 3.5 g/dl) and the high VPA concentrations (89 and 95 μg/ml = 5.4 and 5.7×10^{-4} M) in the corresponding sera, respectively. It was considered from these findings that VPA absorbed into the blood plasma can really raise the % free values of Trp slightly under such conditions as the occurrence of the plasma low albumin contents and high VPA concentrations, although the effects are not so clear as in *in vitro*. VPA has been generally used as one of AED, but has occasionally been effective in the treatment of patients with manic-depressive illness[12,13] and schizophrenia,[14] while it was reported that some epileptic child cases exhibited psychotic symptoms as side effects of VPA.[15,16] The authors here point out the possibility that such effects of VPA are not expected only from its actions as AED but can be derived at least partly from the fact that VPA elevated the % free values of Trp in the blood plasma, namely VPA increased the plasma contents of free Trp which can pass through blood-brain barrier into the brain tissues and become a precursor of serotonin in the CNS.

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