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AN ANALYSIS OF THE KINETICS OF THE INHIBITION OF RABBIT BRAIN γ-AMINOBUTYRATE AMINOTRANSFERASE BY SODIUM *n*-DIPROPYLACETATE AND SOME OTHER SIMPLE CARBOXYLIC ACIDS

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Abstract—The kinetics of inhibition of GABA-transaminase by the antiepileptic drug sodium *n*-dipropylacetate have been studied using a preparation of the enzyme partially purified from rabbit brain and an assay method which determines one of the immediate products, i.e. succinic semialdehyde. It has been shown that the compound is a very weak inhibitor of the binding to the enzyme of both GABA (K_1 = 42 mM) and α-ketoglutarate (K_2 = 92 mM). Other simple carboxylic acids *n*-butyric, *n*-valeric and propionic acid have been shown to be more potent inhibitors of the enzyme. The significance of these results with regard to the mechanism of inhibition of GABA-transaminase by carboxylic acids has been discussed.

It has been reported that sodium *n*-dipropylacetate (*n*-DPA) has anticonvulsive properties which are related to its ability to inhibit γ-aminobutyrate aminotransferase (GABA-T EC 2.6.1.19) [1]. Large doses of the compound cause a small increase in γ-aminobutyric acid (GABA) levels *in vivo* in mice and give protection against audiogenic seizures [1]. It has also been claimed that such doses gave protection against convulsions induced by electroshock or pentalenetetrazol in mice rats and rabbits [2, 3]. In man, the drug is effective in the treatment of petit mal epilepsy [4]. It is believed that *n*-DPA is a reversible inhibitor of GABA-T and it has not been possible to measure decreased enzyme activity *in vivo* caused by its administration. Therefore, the discussion of possible modes of action of the drug *in vivo* is based on extrapolations from experiments *in vitro* on the inhibition of the enzyme [1, 5].

n-DPA is a carboxylic acid which is an analogue of the substrates of GABA-T. It is well known that aspartate aminotransferase is inhibited by carboxylic acids which are substrate analogues [6–9] and some studies on pig kidney GABA-T have shown inhibition of the enzyme *in vitro* by several simple carboxylic acids [10]. Therefore we considered it of interest to investigate further the mechanism of inhibition of GABA-T by *n*-DPA and to compare the effect with that found with some other acids, i.e. propionic, *n*-butyric and *n*-valeric acids.

MATERIALS AND METHODS

Rabbit brain was obtained from the Buxted-Olac Rabbit Co., Great Totes Farm, Buxted, Sussex, U.K. The brains were removed as soon as possible after slaughter and stored frozen. A partially purified preparation of GABA-T activity was obtained by the method previously described for rat brain [11]. The extracts obtained contained 0·05–0·10 units/mg protein (1 unit of enzyme activity being defined as 1 μ mole of succinic semialdehyde produced per min at 37°) and were 20–30-fold purified over the original homogenate. They could be stored at 4° for at least several weeks without loss of activity, and because of this stability and the ease of repetition this GABA-T preparation was considered ideal for these experiments.

GABA-T activity was measured by the method of Salvador and Albers [12]. Incubation mixtures (total vol. 0·1 ml) were prepared in 0·1 M sodium borate buffer pH 8·2 and incubated at 37° for 20 min. Approximately 5×10^{-3} units of enzyme activity were used per incubation to ensure sufficient activity which was also a true measure of initial velocity. During all the studies on the effects of inhibitors on the enzyme, control experiments without inhibitor were performed at the same time and with the same concentration of enzyme.

Protein concentration was measured by the method of Lowry *et al* [13].

n-DPA was a gift from the Laboratoire Berthier, Grenoble, France. GABA, α-ketoglutaric acid, propionic acid, *n*-butyric acid, *n*-valeric acid and 3,5 diaminobenzoic acid were supplied by Sigma Chemical Co., Surrey, U.K. All other chemicals used were 'Analar' grade supplied by British Drug Houses Ltd, Poole, Dorset, U.K.

RESULTS AND DISCUSSION

The effect of several concentrations of *n*-DPA (10, 20 and 40 mM) on GABA-T was studied at a series of GABA concentrations with α-ketoglutarate concentration fixed at 10 mM and also at a series of α-ketoglutarate concentrations with the GABA concentration fixed at 10 mM. Figures 1a and 2a show that results

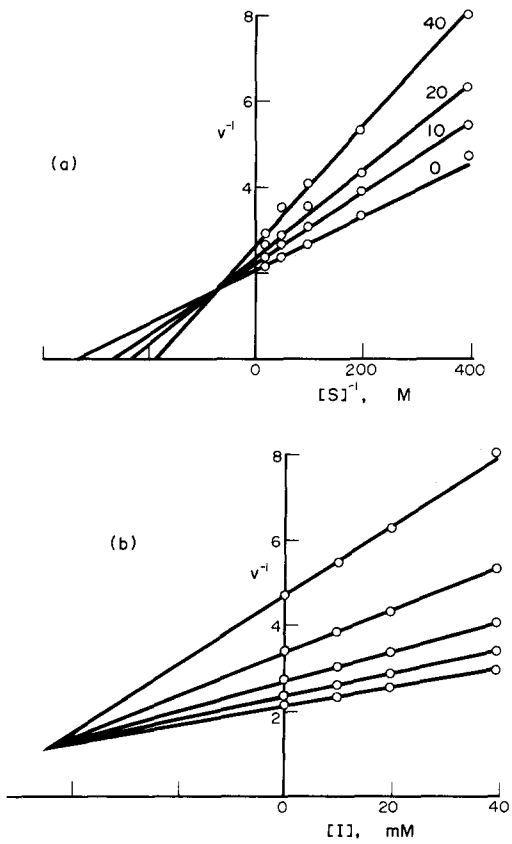

Fig. 1. The effect of *n*-DPA on GABA-T activity. (a) Reciprocal plot v^{-1} against $[GABA]^{-1}$, the numbers above the lines show the concentration of inhibitor used. (b) The same data plotted as v^{-1} against inhibitor concentration to obtain the dissociation constant. The initial velocity of reaction is expressed in arbitrary fluorimetric units.

of these experiments displayed as double reciprocal plots. The term given to the type of inhibition indicated by these plots depends on the source used to obtain definitions of types of reversible inhibition. Reference to Dixon and Webb [14] for example would lead to the conclusion that the inhibitions were 'mixed' and 'non-competitive' respectively whereas reference to Cleland would describe them both as 'non-competitive'. The inhibition patterns shown in Fig. 3a, which will be fully described below would be described as 'competitive' by both sources. These terms are only helpful in understanding the mechanism of the inhibition if it is realised that they may simply represent particular cases of the same binding process. The results can all be explained in terms of a simple mechanism in which the inhibitor binds to the same substrate-binding site on both aldimine and amine forms of the enzyme. The reciprocal rate equation for such inhibition has been derived by Velick and Vavra [6] for the inhibition of aspartate aminotransferase by maleate.

$$\frac{1}{v} = \frac{[I]}{V}\left(\frac{K_A}{[A]K_1} + \frac{K_B}{[B]K_2}\right) + \frac{1}{V}\left(\frac{K_A}{[A]} + \frac{K_B}{[B]} + 1\right) \quad (1)$$

A and B are amino and keto acid substrates respectively, I is the inhibitor, K_A and K_B are complex Michaelis constants and K_1 and K_2 are dissociation constants for the inhibitor with aldimine and amine forms of the enzyme respectively. Velick and Vavra pointed out that reciprocal plots of $1/v$ against $1/[A]$ or $1/[B]$ at several values of [I] show both slope and intercept on $1/v$ axis to be affected by [I]. We wish to consider the point at which the lines intersect since when this happens on the $1/v$ axis the inhibition would be defined as 'competitive'. Examination of equation (1) shows that the coordinates of the intersection point when $1/v$ is plotted against $1/[A]$ are

$$\frac{1}{v'} = \frac{1}{V}\left[1 + \frac{K_B}{[B]}\left(1 - \frac{K_1}{K_2}\right)\right] \quad (2)$$

and

$$\frac{1}{[A]'} = \frac{K_1 K_B}{K_2 K_A [B]} \quad (3)$$

When $1/v$ is plotted against $1/[B]$ the coordinates of the intersection point are given by

$$\frac{1}{v'} = \frac{1}{V}\left[1 + \frac{K_A}{[A]}\left(1 - \frac{K_2}{K_1}\right)\right] \quad (4)$$

and

$$\frac{1}{[B]'} = \frac{K_2 K_A}{K_1 K_B [A]} \quad (5)$$

Thus when $K_2K_A[B] \gg K_1K_B$ inhibition will appear competitive when $1/v$ is plotted against $1/[A]$. Consideration of the mechanistic situation existing when $K_2K_A[B] \gg K_1K_B$ shows that the predominant enzyme species will be the free aldimine enzyme (E) and its complexes with amino acid substrate A. Thus the inhibition is due largely to competition between I and A for the substrate binding site. If, under these conditions, $1/v$ is plotted against $1/[B]$ the intersection point will be far from the $1/[B]$ axis. In this case therefore, although the inhibitor may well be binding to exactly the same site on E there is not, strictly speaking, competition between I and B because B does not combine productively to this form of the enzyme. There is still however, competition between A and I for the same binding site on the predominant form of the enzyme.

The dissociation constants of *n*-DPA for both forms of the enzyme were determined by replotting the data of Figs. 1a and 2a as $1/v$ against [I] (Figs. 1b and 2b). As pointed out by Velick and Vavra, the intersection point occurs at $[I] = -K_1$ when $1/v$ is plotted against I at a series of fixed A concentrations and $[I] = -K_2$ when the plot is made at a series of fixed B concentrations K_1 and K_2 were found to be 42 and 92 mM respectively. This difference may reflect small changes in active site geometry in the aldimine and amine forms of the enzyme. The values of K_A and K_B were determined from Fig. 1. The intercept on the $1/[A]$ axis for the line obtained without inhibitor is $(1/K_A)[(K_B/[B]) + 1]$ and the corresponding intercept on the $1/[B]$ axis is $(1/K_B)[(K_A/[A]) + 1]$. Since [B] and [A] were both 10 mM K_A and K_B were calculated to be 4·1 and 3·6 mM. Using the determined values of K_1, K_2, K_A and K_B and equations (3) and (5), coordinates for the intersection

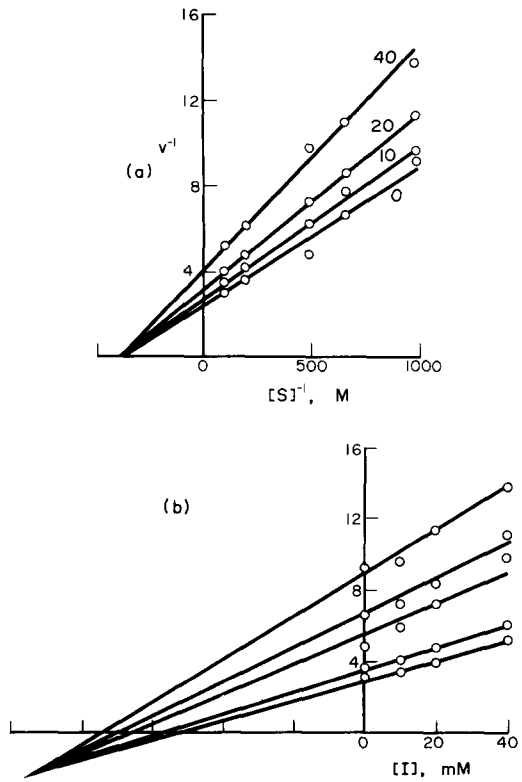

Fig. 2. The effect of *n*-DPA on GABA-T activity. (a) Reciprocal plot v^{-1} against [α-ketoglutarate]$^{-1}$, the numbers above the lines show the concentration of inhibitor used. (b) The same data plotted as v^{-1} against inhibitor concentration to obtain the dissociation constant. The initial velocity of reaction is expressed in arbitrary fluorimetric units.

points were determined to be $1/[A]' = 40\,M^{-1}$ and $1/[B]' = 250\,M^{-1}$ in good agreement with Fig. 1. It is clear therefore that depending on which way the data are plotted fairly small differences in K_1 and K_2 and K_A and K_B lead to considerable differences in the intersection point for a system in which the same basic inhibition mechanism operating in both directions. The way in which inhibition patterns vary is illustrated in Fig. 3 which shows reciprocal plots constructed according to equation (1). Different values have been assigned to K_1 and K_2 in order to produce the required effect. In all three plots K_A and K_B were given values of 5 and the fixed substrate was given a value of 10 and V was set at 1. The same basic mechanism therefore provides examples of (a) competitive, (b) uncompetitive and (c) non-competitive or mixed inhibitions.

Inhibition of GABA-T *by carboxylic acids*

The effects of certain simple monocarboxylic acids, propionic, *n*-butyric and *n*-valeric acid, on GABA-T activity are shown in Figs. 4a and b. The results are plotted in reciprocal manner and are shown (a) with varying GABA concentration (2·5–50 mM), fixed α-ketoglutarate (10 mM) and inhibitor (20 mM) and (b) with varying α-ketoglutarate concentration (1–10 mM), fixed GABA (10 mM) and inhibitor (10 mM). These graphs appear to show that the acids are 'competitive' inhibitors for GABA binding to the enzyme but 'noncompetitive' inhibitors of α-ketoglutarate binding. The observations are most simply explained by the proposal that these monocarboxylic acids bind to a single substrate binding site present in both forms of the enzyme, but that they bind much more strongly to the aldimine than to the amine form of the enzyme.

Thus the inhibition of GABA-T by *n*-DPA and by the simple monocarboxylic acids would appear to be by the same mechanism. This is not surprising since they are analogues of the substrates of the reaction and the inhibition of aminotransferases by carboxylic acids has long been known [6–9]. In fact, the simple acids are closer analogues of the substrates and would appear to be stronger inhibitors of the enzyme than *n*-DPA.

With regard to the possible mode of action of *n*-DPA *in vivo* as an anticonvulsant it is debatable whether such a weak inhibitor would have a very marked effect on GABA-T *in vivo*. Since it has been shown that *in vivo* there is a spare capacity of enzyme of approximately 50 per cent [16], very high concentrations of inhibitor would be needed to raise the

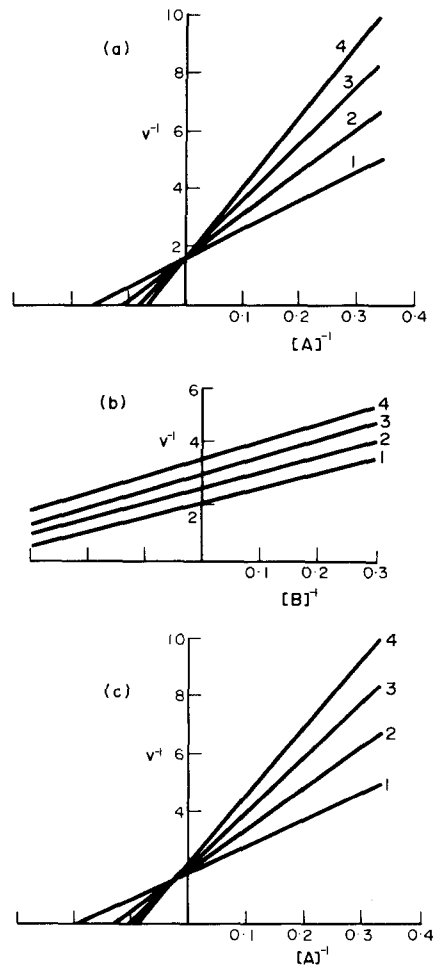

Fig. 3. Calculated plots derived from the rate equation of Velick and Vavra [6]. The numbers above the lines show the concentration of inhibitor. (a) v^{-1} against $[A]^{-1}$ with $V_{max} = 1$; $K_A = 5$, $K_B = 5$, $K_1 = 1$, $K_2 = 20$ and B = 10. (b) v^{-1} against $[B]^{-1}$ with $V_{max} = 1$; $K_A = 5$, $K_B = 5$, $K_1 = 1$, $K_2 = 20$ and A = 10. (c) v^{-1} against $[A]^{-1}$ with $V_{max} = 1$; $K_A = 5$, $K_B = 5$, $K_1 = 1$, $K_2 = 5$ and B = 10.

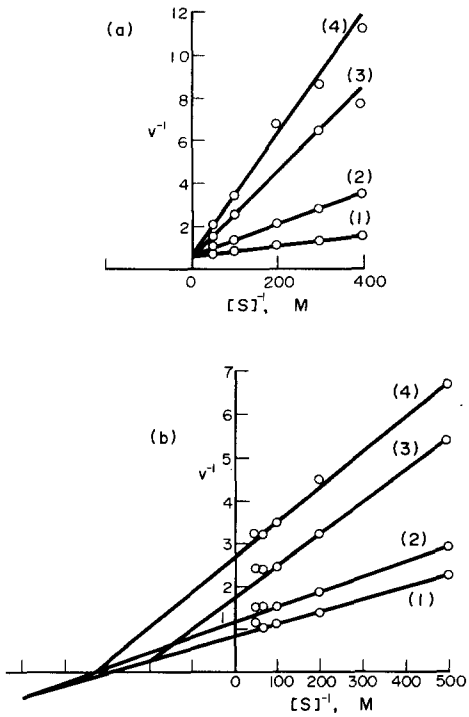

Fig. 4. The effect of carboxylic acids on GABA-T activity: (1) no addition (2) *n*-valeric acid (3) propionic acid (4) *n*-butyric acid. (a) With variation of GABA concentration and excess fixed α-ketoglutarate. (b) With variation of α-ketoglutarate concentration and excess fixed GABA. Initial velocity is expressed in arbitrary fluorimetric units.

GABA level by this mechanism. However, if GABA-T inhibition is the primary mode of action of this anticonvulsant then with correct interpretation of kinetic data it should be possible to design and evaluate compounds which are stronger inhibitors of the enzyme than *n*-DPA.

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