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PRELIMINARY COMMUNICATIONS

γ-ALLENYL GABA, A NEW INHIBITOR OF 4-AMINO BUTYRATE AMINO TRANSFERASE. COMPARISON WITH OTHER INHIBITORS OF THIS ENZYME

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The recent publication of the synthesis of 4-amino-hepta-dien 5,6-oïc acid (γ-Allenyl GABA) claiming activity of this compound as inhibitor of 4-aminobutyrate-2-ketoglutarate aminotransferase (GABA-T) by two independent groups (1,2) prompted us to release our results concerning the efficiency and enzyme selectivity of this compound. In this preliminary communication we compare the effects of γ-allenyl and γ-vinyl GABA both in vitro and in vivo.

Materials and Methods: (R,S) Allenyl GABA and the R and S enantiomers were synthesized in our Center starting with racemic, R or S-acetylenic GABA as published (2). The following enzymes were prepared from animal tissues and assayed as described in previous publications: GABA-T from pig brain (3), glutamate decarboxylase (GAD) from rat brain (4) and ornithine δ-aminotransferase from rat liver (5). Alanine and aspartate amino-transferase were purchased from SIGMA and assayed following standard methods (6). The time dependent inactivation of the enzymes was followed by measuring remaining enzyme activity after incubation with the potential inhibitors for different time periods and extensive dilution into the respective assay mediums (3).

All animal work was performed on Swiss Albino mice (20-25g). Drugs were given either i.p. or orally as aqueous solutions. The animals were killed by decapitation at appropriate time points. The brains were removed rapidly, split sagitally and frozen until used. One half brain was homogenized in 9 vol. of $HClO_4$ 0.2N and was used for the measurement of GABA levels using an automatic amino-acid analyzer (7). The other half was homogenized in 9 vol. of the following buffer 10 mM KH_2PO_4, pH 6.8 Pyridoxal-phosphate 0.1 mM; EDTA 1 mM; reduced glutathion 0.1 mM; Triton X-100 0.13% (W/v) and 20% glycerol). The 2000g supernatant was used to determine GABA-T and GAD activities (7).

Results and discussion: Upon incubation of partially purified GABA-T with (R,S)-allenyl GABA, there is a time-dependent loss of enzyme activity. As can be seen in fig. 1, the inhibition follows pseudo-first order kinetics for several half-lives and goes essentially to completion. In the same figure, the rates of inhibition of GABA-T by γ-allenyl GABA and γ-vinyl GABA are compared at two concentrations of inhibitors: 0.25 mM of γ-vinyl GABA inhibits the enzyme at the same rate as 0.1 mM of the allenyl derivative. Similar ratios of activity are found at other concentrations, so that one can conclude that γ-allenyl GABA is two to three times more potent than γ-vinyl GABA as a time-dependent inhibitor of GABA-T. As reported for γ-vinyl GABA, the inhibition cannot be reversed by dialysis. Pyridoxal-phosphate, α-ketoglutarate have no effect on the process of inhibition. Mercaptoethanol is routinely added to the incubation medium so that one can rule out that the inhibition of GABA-T by allenyl GABA occurs through the formation of a diffusible alkylating species.

As (R) and (S)-acetylenic GABA were available from previous work (4), the enantiomers of

γ-allenyl GABA were accessible through the same synthetic route as the racemate. It appears from fig. 2 that the (S)-isomer inhibits GABA-T at the same rate as double the concentration of the racemate. On the other hand, the (R)-enantiomer has no significant effect at a millimolar concentration, i.e. at a ten times higher concentration that the (S)-isomer. The mechanism of inhibition of GABA-T by the allenyl substrate analogue, as postulated in (1,2), demands that the hydrogen on C_4 be abstracted as in the normal GABA transamination. For GABA itself, this hydrogen abstraction is stereospecific for the pro-S hydrogen. The observed stereoselectivity of the inhibition is therefore in agreement with the proposed mechanism. It was already known that GABA-T is inhibited preferentially by the (S)-isomers of γ-acetylenic GABA (4) and γ-vinyl GABA (8). Neither the racemate nor any of the enantiomers of allenyl GABA inhibited GAD in vitro even at millimolar concentrations. In this respect, allenyl GABA resembles its vinyl congener, while γ-acetylenic GABA inhibited both bacterial and mammalian GAD although with opposed stereospecificity (4). Similarly (R,S)-allenyl GABA had no time-dependent effect at 1 mM on aspartate and alanine aminotranferases. However, it produced a time-dependent and irreversible inhibition of ornithine amino-transferase: at 0.25 mM of inhibitor, the half-life of this enzyme is 30 min, approximately 10 times longer than for GABA-T. This lack of enzyme selectivity is shared by acetylenic GABA and the gabaculines (5,9). The stereochemistry of ornithine aminotransferase inhibition by γ-allenyl GABA is less clear-cut than the inhibition of GABA-T. Both isomers produce a time-dependent decrease of ornithine transaminase activity. The S-enantiomer is still the most active but the selectivity factor is of the order of 4 only: at 0.5 mM of (S)-γ-allenyl GABA, the half life of enzyme activity is 10 min and 40 min at the same concentration of the R-enantiomer. When given orally or i.p. to mice, (R,S)-allenyl GABA produced a progressive decrease of brain GABA-T activity, accompanied by an increase of brain GABA levels. Fig. 3 compares the dose-dependent changes of brain GABA metabolism in mice which had received either γ-vinyl or γ-allenyl GABA six hours before sacrifice. The dose-dependent inhibition of GABA-T and elevation GABA levels obtained with the two compounds are parallel. As in vitro, γ-allenyl GABA is 2-3 time more potent than the vinyl congener as a means to inhibit GABA-T and to elevate GABA. There are however some differences in the in vivo effects.

γ-Allenyl GABA can inhibit brain GABA-T almost completely, while even at doses of vinyl GABA 3 times higher than those used in fig. 3, there was still 10-15% of residual brain GABA-T activity (7). The decrease of GAD activity in the brains of mice treated with γ-allenyl GABA is more pronounced than for γ-vinyl GABA. Neither compound has a direct effect on GAD in vitro. It had been postulated before that the slow decrease of GAD activity in brains of mice treated with high doses of γ-vinyl GABA is due to a regulation of GAD synthesis by elevated brain GABA levels (7). Given the short-time in the present experiment and the greater decrease of GAD activity with γ-allenyl GABA compared to γ-vinyl GABA for similar brain GABA levels, alternate explanations must be sought. It cannot be ruled out for instance that a metabolite of γ-allenyl GABA has a direct inhibitory effect on GAD in vivo.

In summary, as suggested by the two publications describing its synthesis, (R,S)-allenyl GABA and more precisely the S-enantiomer is indeed a time-dependent irreversible inhibitor of mammalian GABA-T in vitro. The compound is more potent than vinyl GABA but loses in enzyme selectivity as inhibits ornithine aminotransferase at 1/4 the rate of GABA-T. When given to mice, it presumably crosses the blood brain barrier as it produces a decrease of GABA-T activity and an increase of brain GABA levels. Again it appears two to three fold more potent than vinyl GABA. However apar of this moderate increase in potency (R,S)-allenyl GABA offers no advantage over its vinyl congener.

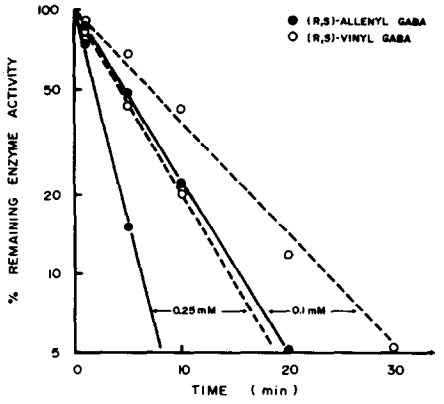

Fig. 1: Time-dependent inactivation of mammalian brain GABA-T by (R-S) allenyl and vinyl GABA.

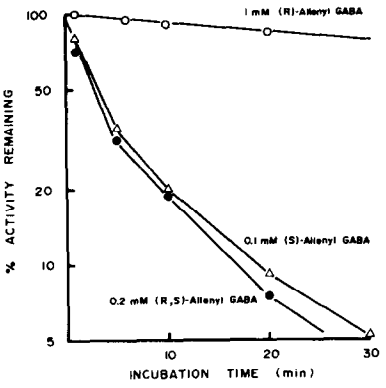

Fig. 2: Stereochemistry of the inhibition of GABA-T by the enantiomers of allenyl-GABA.

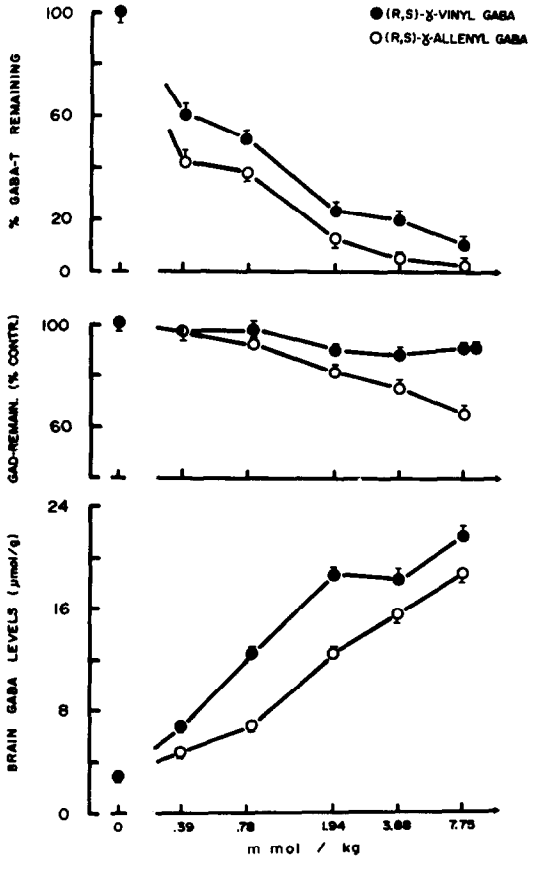

Fig. 3: Dose-dependent changes of brain GABA metabolism after i.p. administration; each value is the mean ± SEM of 5 animals.

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