THE IN VIVO INHIBITION OF GABA-TRANSAMINASE BY GABACULINE

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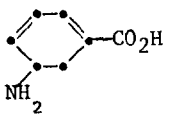
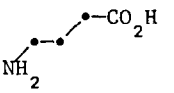
Summary

The naturally occurring amino acid gabaculine ((-)-5-amino-1,3 cyclohexadiene carboxylic acid) is a potent irreversible inhibitor of mouse brain γ-aminobutyric acid (GABA)-α-ketoglutaric acid transaminase. When administered I.P. gabaculine, irreversibly inhibits the mouse brain enzyme in a time dependent fashion. Concomitant with this inhibition is a rise in endogenous brain GABA levels. Administration of gabaculine at a concentration of 100 mg/kg mouse leads to the complete inhibition of the enzyme after 4 hrs. Brain levels of GABA continually rise after the administration of the drug. After 20 hrs they are 15-20 times higher than levels in the untreated animals.

Gabaculine, (-)-5-amino-1,3-cyclohexadiene carboxylic acid, is a naturally occurring neurotoxin isolated from Strep. toyocaenis (1). It is an



Gabaculine



GABA

irreversible inhibitor of pyridoxal phosphate linked γ-aminobutyric acid (GABA)-α-ketoglutaric acid transaminase isolated from mammalian and bacterial sources (2, 3). In mammals, this enzyme terminates the action of the inhibitory neurotransmitter GABA. Gabaculine is of a class of irreversible enzyme inhibitors which are substrates for their target enzyme (4). As a consequence of the catalytic turnover of these molecules the enzyme is irreversibly inactivated. In the case of gabaculine, the mechanism of the inactivation process, at least with the bacterial enzyme, involves transamination of the inhibitor followed by spontaneous aromatization which generates meta-carboxyphenylpyridoxamine phosphate (CPPp) (3, 5). The tight

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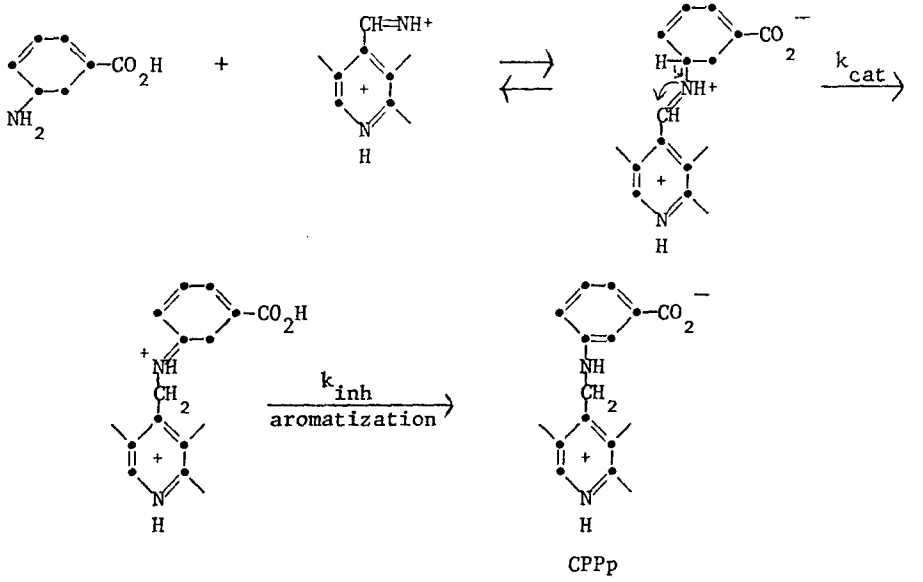
binding of this stable compound to the active-site of the enzyme results in its irreversible inhibition.



CPPp

In this report we show that gabaculine leads to the irreversible inhibition of mouse brain GABA transaminase when administered to mice I.P. As a result of this inhibition, there is a dramatic increase in the brain levels of the inhibitory neurotransmitter GABA. Thus, gabaculine promises to be of great utility as a pharmacological tool in studies on gabanergic neurotransmission.

Materials and Methods

Racemic gabaculine was synthesized by the published methods (1). GABA, α-ketoglutaric acid, L-glutamate, pyridoxal phosphate, nicotinamide adenine dinucleotide, and GABA-transaminase/succinic semialdehyde dehydrogenase (GABAase) were products of the Sigma Chemical Co. Male albino mice (CD_1) were purchased from the Charles River Co. The mice weighed between 20-25 g. The mice were housed 6 to a cage and allowed food and water ad libitum.

GABA transaminase activity was determined both by the method of Wu (6) and by coupling it to succinic semialdehyde dehydrogenase (7). Both assays gave equivalent results. GABA levels were determined with GABAase (Sigma Chemical Co.). In this coupled assay system the GABA is enzymatically converted into succinic acid semialdehyde which is quantitatively oxidized to succinic acid by succinic semialdehyde dehydrogenase and nicotinamide adenine dinucleotide. The optical density increase at 340 nm (reduced nicotinamide adenine dinucleotide) is directly related to the endogeneous GABA levels. Standards containing known concentrations of GABA were determinations of endogenous GABA levels. Protein concentrations were measured by the Lowry method (8).

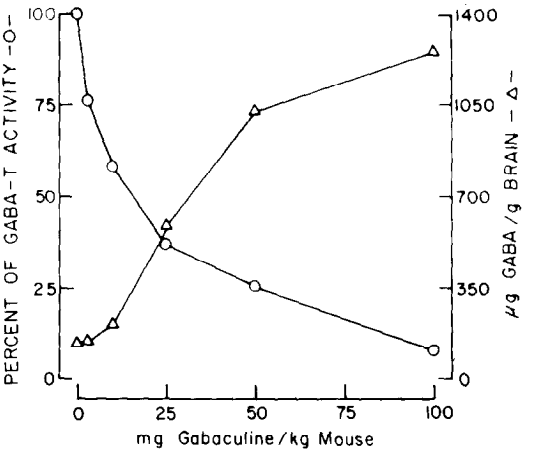


Fig. 1. The dose dependent inhibition of GABA-transaminase activity by gabaculine and the concomitant rise in endogeneous GABA levels. Mice were injected I.P. with gabaculine at the inducted concentrations. After 4 hrs the animals were sacrificed and the remaining GABA-transaminase activities and GABA levels were determined. GABA-transaminase activities were normalized to the same protein concentrations.

D,L gabaculine was dissolved in saline and injected into the animals stomach. Control animals were injected with the same volume of saline. When a determination of GABA-transaminase or whole brain GABA was to be made, the animal was killed by decapitation and the brain was immediately removed (within 60 sec) and frozen in liquid nitrogen. For the determination of GABA the frozen brains were weighed and homogenized in 1 mM EDTA and 0.5 M $HClO_4$ at 15 volumes $HClO_4$/1 volume tissue. The material was then centrifuged at 2,000 x g for 20". The supernatent was neutralized with $KHCO_3$ and recentrifuged. The resulting supernatent was stored overnight before assaying for GABA levels by the procedures described above. Brain GABA levels determined in this fashion did not differ from those determined by freezing the skull in liquid nitrogen prior to removal of the brain. The GABA-transaminase levels were determined by homogenizing the brains in 5 volumes of a cold buffer of the following composition: (1) glycerol 20% (v/v) (2) 10 mM KPO_4 buffer, pH = 7.5 (3) 1 mM Na_2 EDTA (4) 0.1 mM glutathione and (5) 1.3% 1/10 buffer diluted Triton X-100. The final pH of the buffer was 7.2. The homogenates were frozen and thawed once before centrifugation at 2,000 x G at 4° for 30". The enzymatic activity was found in the supernatent fraction. The protein concentration of this fraction was determined. 50-λ aliquots of this material were assayed for transaminase activity.

In the Figures shown, each point is an average of three determinations, each on three mice. Activity of the gabaculine inactivated enzymes could not be reactivated by dialyzing the inhibited enzyme overnight against pH = 8.0 KPO_4 buffer containing 0.1 mM pyridoxal phosphate. The endogeneous brain levels of gabaculine were never high enough to interfere with either the determinations of the remaining transaminase activities or GABA levels.

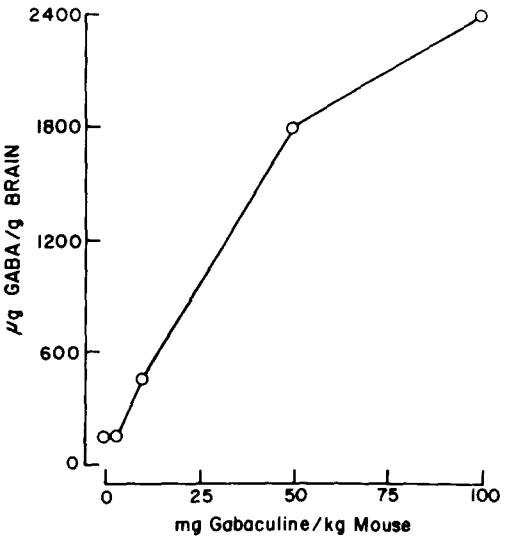


Fig. 2. The dose dependent increase in endogenous GABA levels after 20 hrs. Mice treated as in Fig. 1 were sacrificed after 20 hrs and the brain levels of GABA were determined.

Results and Discussion

In Vivo Inhibition of GABA Transaminase and the Rise in GABA Levels

Mice were injected I.P. with varying concentrations of gabaculine. After four hours the animals were decapitated and endogenous levels of GABA-transaminase and GABA were determined (Fig. 1). As can be seen in this figure the rate of irreversible inhibition of the enzyme is dose dependent as are the increases in GABA levels. Gabaculine is an exceedingly potent irreversible inhibitor of mouse brain GABA transaminase *in vitro* (2). The measured K_I for the drug is 5.86×10^{-7}M and the half-life of the enzyme at this concentration of gabaculine is ca. 5" (2). The relatively high concentrations of gabaculine required for the *in vivo* inhibition suggests that gabaculine does not transverse the blood-brain barrier very effectively This conclusion seems warranted in light of the fact that GABA has been reported not to get across (9). Preliminary experiments with 2-[^{3}H]-gabaculine show that less than 0.001% of gabaculine injected into the stomach eventually

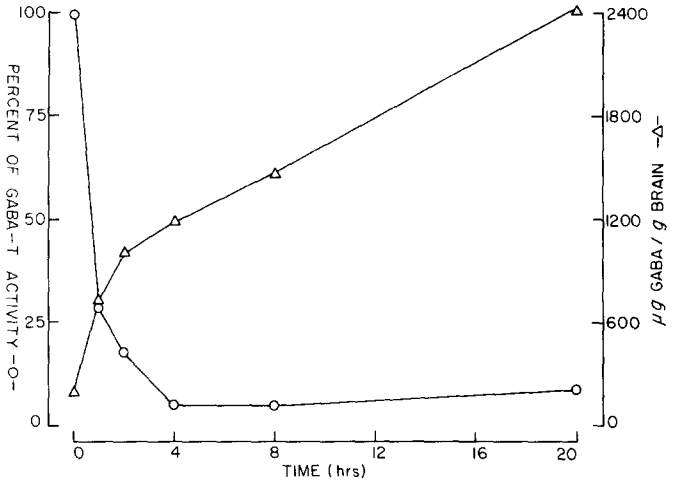


Fig. 3. The time dependent increase in GABA levels and decrease in GABA-transaminase at a single dose of gabaculine (100 mg/kg). Mice were injected I.P. with gabaculine at 100 mg/kg. At the indicated times, GABA levels were determined as well as GABA-transaminase activities.

reaches the brain (unpublished ezperiments, F.W. Bangerter).

Mice were injected I.P. with various levels of gabaculine as in Fig. 1. At the end of 20 hrs the animals were decapitated and the endogenous levels of GABA were determined (Fig. 2). As can be seen from this figure, there is a large time dependent increase in GABA levels with time. From these experiments it is clear that GABA is not a feed back inhibitor of glutamate decarboxylase in vivo. In Fig. 3, the time dependent inhibition of GABA transaminase and the time dependent increase in GABA levels are determined at a 100 mg/kg dose of gabaculine. At this concentration of gabaculine, GABA levels are increased by 15-20 fold after 20 hrs. It should be stressed that these values represent whole brain levels of GABA. We have not yet determined the localization of the transmitter.

In summary, the results reported here show that gabaculine is an irreversible inhibitor of GABA transaminase in vivo. Since gabaculine is effective when administered peripherally it is likely to be a useful agent in studying the pharmacology of GABA. Insofar as we can tell, gabaculine is a specific

inhibitor of GABA transaminase. When tested in the millimolar range no irreversible inhibition of the following pyridoxal phosphate linked enzymes was observed: glutamate decarboxylase, ornithine decarboxylase, aspartate aminotransferase, and alanine aminotransferase.

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