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IN VIVO INACTIVATION OF γ-AMINOBUTYRIC ACID-α-KETOGLUTARATE TRANSAMINASE BY 4-AMINO-5-FLUOROPENTANOIC ACID

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SUMMARY

Intraperitoneal injection into mice of varying concentrations of (S)-4-amino-5-fluoropentanoic acid ((S)-AFPA) produces a dose-dependent irreversible decrease in brain γ-aminobutyric acid-α-ketoglutaric acid aminotransferase (E.C. 2.6.1.19) activity. Concomitant with this inactivation is an increase in whole brain γ-aminobutyric acid (GABA) levels. Four hours after a dose of 100 mg/kg body weight of (S)-AFPA to mice, endogenous brain GABA concentrations increase to 16 times that of the untreated animals and the enzyme activity decreases to 20% that of the controls. The binding of (S)-AFPA to GABA receptors was more than three orders of magnitude poorer than for GABA itself.

INTRODUCTION

(S)-4-Amino-5-fluoropentanoic acid was shown to irreversibly inactivate pig brain GABA-T *in vitro* (1) by a mechanism-based mode of action (2). Thus, (S)-AFPA is included in a class of GABA-T inactivators which effect inhibition through a catalytic process of the target enzyme. Other compounds that fit into this category include gabaculine (3), isogabaculine (4), 4-aminohex-5-ynoic acid (5), 4-aminohex-5-enoic acid (6), and ethanolamine-O-sulfate (7).

Since GABA-T is the major catabolic enzyme for the inhibitory neurotransmitter GABA in the mammalian brain (8), it was expected that (S)-AFPA would elevate brain GABA content by the inhibition of GABA-T as has been shown for other mechanism-based inactivators (8-12). In this report we demonstrate that (S)-AFPA is a potent inactivator of GABA-T *in vivo* and produces exaggerated brain GABA levels.

Abbreviations: GABA, γ-aminobutyric acid; GABA-T, γ-aminobutyric acid-α-ketoglutaric acid aminotransferase (EC 2.6.1.19); (S)-AFPA, (S)-4-amino-5-fluoropentanoic acid.

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MATERIALS AND METHODS

(S)-AFPA was prepared as previously reported (13). All substrates and cofactors were purchased from Sigma Chemical Co. Male CD/CR Ham-ICR mice weighing 25-30 g were bought from the Charles River Breeding Labs (Portage, MI).

Groups of three mice were injected intraperitoneally with different concentrations of (S)-AFPA in 0.2 mL of saline; control animals were injected with an equal volume of saline. Brain GABA levels were determined 4 hours after drug treatment by the radioreceptor assay method of Bernasconi et al. (14). Animals were killed by microwave irradiation (General Medical Engineering Corp., Peabody, MA) to prevent postmortem changes in GABA concentrations (14). Residual GABA-T activity, 4 hours after drug treatment, was determined in the brains of animals killed by decapitation. The brains were excised within one minute, frozen on Dry ice, and stored at -80°C until processed for analysis by a modified version of the method of Jung et al. (10). Each brain was hand homogenized at 4°C in 10 volumes (v/m) of a buffer consisting of 0.13% Triton X-100, 0.1 mM glutathione (reduced), 0.1 mM pyridoxal phosphate, 1.0 mM EDTA, 1 mM α-ketoglutarate, 20% (v/v) glycerol and 20 mM potassium phosphate adjusted to pH 6.8 with acetic acid. The homogenates were frozen at -80°C overnight, thawed, and centrifuged at 2500 x g for 20 min. The slightly turbid supernatants were assayed for GABA-T activity at 25°C using the enzyme-coupled, succinic semialdehyde dehydrogenase assay as previously described (2), modified only in that the potassium pyrophosphate concentration was increased to 100 mM. Each brain was assayed in triplicate and the values were averaged. Samples of the supernatants were dialyzed against the homogenization buffer to determine the reversibility of inhibition. GABA receptor binding was carried out the by the method of Bernasconi et al. (14).

RESULTS AND DISCUSSION

Four hours after intraperitoneal injection of different doses of (S)-AFPA into mice, a dose-dependent loss of GABA-T activity concomitant with an increase in whole brain GABA concentration was observed (Figure). Analogous to the *in vitro* results (1), the *in vivo* enzyme activity did not return upon dialysis. This indicates that (S)-AFPA crosses the blood-brain barrier and irreversibly inactivates GABA-T.

(S)-AFPA compares quite favorably to other GABA-T inactivators in its ability to raise whole brain concentrations of GABA. Four hours after drug treatment of mice with 100 mg inactivator/kg body weight, (S)-AFPA caused an increase in the brain GABA levels twice that produced by gabaculine (9) at the same dose; the GABA concentration was 2.5 and 7 times higher than that induced by the same doses of 4-aminohex-5-ynoic acid (10) and 4-aminohex-5-enoic acid (11) respectively after 8 hours. Under these same conditions, brain GABA-T was inactivated 80% by (S)-AFPA, 92% by gabaculine (9), 90% by 4-aminohex-5-ynoic acid (10) and 45% by 4-aminohex-5-enoic acid (11).

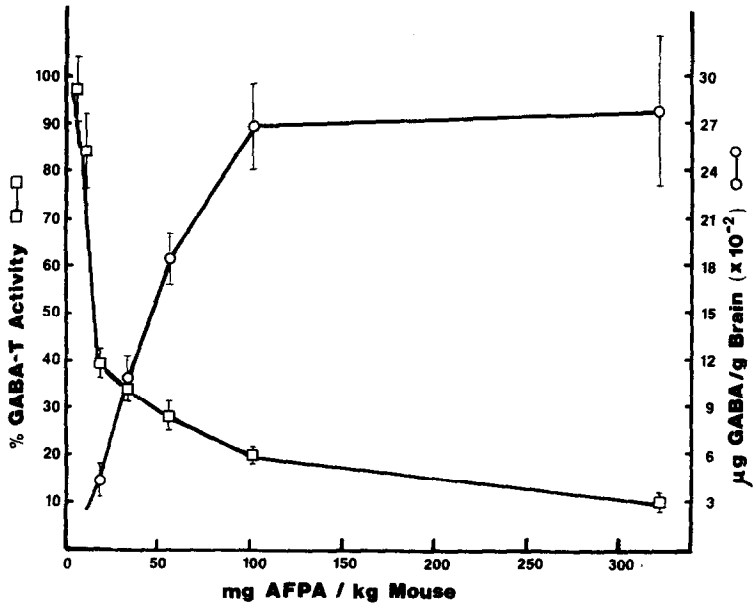

Figure Dose-Dependent Decrease in GABA-T Activity by AFPA Concomitant with an Increase in GABA Concentration.

Mice were injected intraperitoneally with various doses of (S)-AFPA. After 4 hours, the brain GABA levels and GABA-T activity were determined as described in Materials and Methods. The GABA concentration for 6 control mice was 175 ± 10 µg GABA/g brain (wet weight) and the GABA-T activity was 24 ± 2 µmol NADPH / h / g brain. The GABA-T activity is expressed as a % of the controls. Results represent the mean ± SEM.

Binding of (S)-AFPA to the GABA receptor was relatively poor; the IC_{50} values (the drug concentration resulting in 50% inhibition of specific [^{3}H]-muscimol binding (14)) for (S)-AFPA and GABA were 8.6×10^{-5} M and 3.0×10^{-8} M respectively. Therefore, it is unlikely that (S)-AFPA will exhibit any GABA-ergic effects at the receptor sites.

These results indicate that (S)-AFPA is a potent, irreversible inhibitor of GABA-T *in vivo*. Furthermore, preliminary results indicate that (S)-AFPA is a specific GABA-T inactivator; there appears to be little or no *in vitro* or *in vivo* inhibition of brain glutamate decarboxylase, aspartate transaminase, and alanine transaminase. *In vivo* brain GABA-T activity also is lowered by (S)-4-amino-5-chloropentanoic acid (2,13), but to a lesser extent than with (S)-AFPA.

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REFERENCES

1. Silverman, R.B. and Levy, M.A. (1980) Biochem. Biophys. Res. Commun. 95, 250-255.
2. Silverman, R.B. and Levy, M.A. (1981) Biochemistry 20, 1197-1203.
3. Rando, R.R. and Bangerter, F.W. (1976) J. Am. Chem. Soc. 98, 6762-6764.
4. Metcalf, B.W. and Jung, M.J. (1979) Molecular Pharmacol. 16, 539-545.
5. Jung, M.J. and Metcalf, B.W. (1975) Biochem. Biophys. Res. Commun. 67, 301-306.
6. Lippert, B., Metcalf, B.W., Jung, M.J., and Casara, P. (1977) Eur. J. Biochem. 74, 441-445.
7. Fletcher, A. and Fowler, L.J. (1980) Biochem. Pharmacol. 29, 1451-1454.
8. Balazs, R., Machiyama, Y., Hammond, B.J., Julian, T., and Richter, D. (1970) Biochem. J. 116, 445-467.
9. Rando, R.R., and Bangerter, F.W. (1977) Biochem. Biophys. Res. Commun. 76, 1276-1281.
10. Jung, M.J., Lippert, B., Metcalf, B.W., Schechter, P.J., Böhlen, P., and Sjoerdsma, A. (1977) J. Neurochem. 28, 717-723.
11. Jung, M.J., Lippert, B., Metcalf, B.W., Böhlen, P., and Schechter, P.J. (1977) J. Neurochem. 29, 797-802.
12. Leach, M.J. and Walker, J.M.G. (1977) Biochem. Pharmacol. 26, 1569-1572.
13. Silverman, R.B. and Levy, M.A. (1980) J. Org. Chem. 45, 815-818.
14. Bernasconi, R., Bittiger, H., Heid, J., and Martin, P. (1980) J. Neurochem. 34, 614-618.