## A comparison of the effects of allylglycine and 2-keto-4-pentenoic acid on cerebral glutamic acid decarboxylase activity and convulsions in mice

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Intraperitoneal administration of (±)-allylglycine (2-amino 4-pentenoic acid) to mice produces convulsions, with an $ED_{50}$ of 1.0 mmole/kg. Glutamic acid decarboxylase (GAD) activity is inhibited (35%) in mouse brain homogenates following administration of convulsant doses of allylglycine. *In vitro*, allylglycine is a weak inhibitor of GAD activity (Ki about 50 mM) and gives rise to non-linear kinetic plots. This led us to suggest that a metabolite of allyglycine may be responsible for the GAD inhibition (Horton & Meldrum, 1973).

Recently, it has been demonstrated that both (+)- and (−)-isomers of allylglycine are convulsant (Orlowski, Reingold & Stanley, 1977). The (−)-isomer was 3–4 times as effective as the (+)-isomer *in vivo*, but *in vitro* inhibition of GAD activity was similar with both isomers. The inhibitory effects of allylglycine on GAD activity were dramatically increased by the addition of amino acid oxidase to the incubation medium. They suggested that 2-keto-4-pentenoic acid may be a common metabolite formed from (+)- and (−)-allylglycine which is responsible for the inhibitory effects on GAD activity.

We have synthesized 2-keto-4-pentenoic acid enzymically from (−)-allylglycine using purified (−)-amino acid oxidase. The isolated product is a volatile oil, soluble in water with an absorption maximum at 270 nM (pH 6.3) and, gave a yellow crystalline precipitate with 2,4-dinitrophenylhydrazine. The $ED_{50}$ (with 95% confidence limits) for seizure induction after intacerebroventricular injection in Swiss S mice was 14.5 µg (11.3–18.8) compared to 375 µg (264–529) for (−)-allylglycine and 804 µg (561–1151) for (+)-allylglycine.

In contrast to allylglycine, 2-keto-4-pentenoic acid is a very potent inhibitor of cerebral GAD activity. Addition of the GAD extract to a reaction mixture containing a range of substrate and 2-keto-4-pentenoic acid concentrations gave classical competitive inhibition plots with a Ki of $10^{-6}$–$10^{-7}$ M. Preincubation of the enzyme extract with 2-keto-4-pentenoic acid in the absence of substrate reduced the enzyme activity to less than 10% of the control activity within 30 s and abolished the activity completely by 2 minutes. Dialysis of the enzyme inhibitor mixture for 3 h recovered only 10% of the activity. Preincubation (30 min at 37°C) of the enzyme preparation with a range of 2-keto-4-pentenoic acid concentration (in the absence of substrate) gave non-competitive inhibition plots.

These observations can explain the marked cerebral GAD inhibition seen after systemic administration of allylglycine, and the long latency to seizure onset. The lower convulsant potency and different seizure pattern after (+)-allylglycine are probably related to a topographically distinctive (and slower) intracellular accumulation of 2-keto-4-pentenoic acid.

### References

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## Uptake systems for (−)-2,4-diaminobutyric acid in rat cerebral cortical slices

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(−)-2,4-Diaminobutyric acid (DABA) is a relatively strong inhibitor of GABA uptake in the rat cerebral cortex (Harris, Hopkin & Neal, 1973; Iversen & Johnston, 1971). There is, however, evidence to suggest that DABA may be transported by uptake processes other than the high affinity GABA transport system. Simon & Martin (1972) found that GABA inhibited GABA uptake into rat cortical synaptosomes more potently than DABA uptake. We have looked for further evidence for alternative uptake processes mediating DABA entry into cortical slices and have attempted to assess their importance.

The uptake of (−)-[$^3$H]-DABA by rat cerebrocortical slices was studied over a loading concentra-