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

R.W. HORTON & B.S. MELDRUM

Department of Neurology, Institute of Psychiatry, De Crespigny Park, London SE5 8AF

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

HORTON, R.W. & MELDRUM, B.S. (1973). Seizures induced by allylglycine, 3-mercaptopropionic acid and 4-deoxypyridoxine in mice and photosensitive baboons, and different modes of inhibition of cerebral glutamic acid decarboxylase. *Br. J. Pharmac.* **49**, 52–63.

ORLOWSKI M., REINGOLD, D.F. & STANLEY, M.E. (1977). D- and L-stereoisomers of allylglycine: convulsive action and inhibition of brain L-glutamate decarboxylase. *J. Neurochem.* **28**, 349–353.

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

A.J. KENNEDY & M.J. NEAL

Department of Pharmacology, The School of Pharmacy, University of London, 29/39 Brunswick Square, London WC1N 1AX

(−)-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-

tion of 1 μM to 5 mM. Analysis of the kinetic data obtained provided evidence that DABA entry is mediated by both high and low affinity carriers. Further evidence that DABA entry occurs by more than one transport process was obtained from the Inui constant ratio test (Inui & Christensen, 1966). When cortical slices were incubated in the presence of equimolar [^{3}H]-DABA and [^{14}C]-GABA the ratio of entry of the two radionuclides was found to depend upon the loading concentration. DABA entry was progressively favoured in comparison to GABA entry as the loading concentrations of both amino acids were raised.

The specificity of the uptake of (−)-DABA (1 μM and 1 mM) was examined. GABA and DABA were relatively potent inhibitors of DABA (1 μM) uptake, whereas an equal concentration of histidine did not produce significant inhibition. In contrast, DABA and histidine were markedly more potent as inhibitors of DABA (1 mM) uptake than was GABA.

It is concluded from these experiments that (−)-DABA is transported into cortical slices by a carrier which has high affinities for both DABA and GABA and by a second lower affinity carrier which prefers DABA as a substrate to GABA. On the basis of a comparison of the effects of inhibitors on [^{3}H]-DABA and [^{3}H]-GABA uptake it is estimated that approximately 26% of DABA uptake at 1 μM does not occur by the high affinity carrier whereas at 1 mM DABA this proportion rises to more than 60%.

A.J.K. is an MRC postdoctoral fellow.

References

HARRIS, M., HOPKIN, J.M. & NEAL, M.J. (1973). Effect of centrally acting drugs on the uptake of γ-aminobutyric acid (GABA) by slices of rat cerebral cortex. *Br. J. Pharmac.*, **47**, 229–239.

INUI, Y. & CHRISTENSEN, H.N. (1966). Discrimination of single transport systems. The Na plus sensitive transport of neutral amino acids in the Ehrlich cell. *J. Gen. Physiol.*, **50**, 203–224.

IVERSEN, L.L. & JOHNSTON, G.A.R. (1971). GABA uptake in rat central nervous system. Comparison of uptake in slices and homogenates and the effects of some inhibitors. *J. Neurochem.*, **18**, 1939–1950.

SIMON, J.R. & MARTIN, D.L. (1972). The effects of L-2,4-diaminobutyric acid on the uptake of gamma-aminobutyric acid by a synaptosomal fraction from rat brain. *Arch. Biochem. Biophys.*, **157**, 348–355.

Uptake of 2-amino-6,7-dihydroxy-1,2,3,4- tetrahydronaphthalene (ADTN) into rat brain synaptosomes

A. DAVIS, P.J. ROBERTS & G.N. WOODRUFF

Department of Physiology and Biochemistry, University of Southampton, Southampton SO9 3TU

The 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene (ADTN) molecule contains the dopamine skeleton held in a rigid cyclic conformation. It possesses potent dopamine-like activity and has been found to stimulate locomotor activity (Elkhawad & Woodruff, 1975); to be equipotent with dopamine in its ability to activate dopamine-sensitive adenylate cyclase (Miller, Horn & Iversen, 1974) and to inhibit the firing of cells in the rat nucleus accumbens (Woodruff, McCarthy & Walker, 1976). Recently, we have reported the binding of [^{3}H]-ADTN to receptors on rat brain synaptic membranes (Roberts, Woodruff & Poat, 1977) and in this study we have investigated the possible uptake of ADTN into dopaminergic neurons.

Crude synaptosomes (P_2) were prepared from rat striatum and incubated in Krebs-bicarbonate medium at 37°C containing [^{3}H]-ADTN (0.3 μM). Uptake was linear for at least 4 min and was energy-dependent, as evidenced by the inhibitory effects of lowered temperature and added metabolic inhibitors.

The uptake was mediated by two distinct systems ($K_m = 0.3$ μM; $V_{max} = 0.3$ nmol/mg protein/4 min and, $K_m = 3.1$ μM; $V_{max} = 1.0$ nmol/mg protein/4 min, respectively). Similar systems (K_m's 0.3 and 7.5 μM) were found in the cerebellum, a region devoid of dopaminergic terminals. However, the capacity of these systems were substantially less than in the striatum (V_{max} of 0.028 and 0.125 nmol/mg protein/4 min respectively).

Subcellular distribution studies of striatum demonstrated that of the accumulated [^{3}H]-ADTN, 62.3% was localized in the synaptosomal and, 28.6% in the mitochondrial fractions. TLC of alcoholic tissue extracts indicated that following a 20 min incubation, at least 50% of the radioactivity was attributable to unmetabolized [^{3}H]-ADTN.

To investigate the specificity of the ADTN uptake system, synaptosomes were incubated with [^{3}H]-ADTN (0.3 μM) in the presence of a range of concentrations of drugs known to interact with dopaminergic mechanisms and, IC_{50} values determined.