various amine transmitters are regulated differently. The existence of distinct substrate-specific aromatic amino acid decarboxylases could explain our results.


Inhibition of Kynurenine Hydrolase by Benserazide, Carbidopa and other Aromatic Hydrazine Derivatives: Evidence for Sub-Clinical Iatrogenic Nicotin Deficiency

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It has been shown previously that kynurenine hydrolase (L-kynurenine hydrolase, EC 3.7.1.3) is inhibited by the hydrazide Benserazide (R_{1,4-4602}, DL-serine 2-[(2,3,4-trihydroxyphenyl)methyl] hydrazide), a compound that is used clinically in the treatment of Parkinson's disease (Bender et al., 1977). Benserazide is a potent inhibitor of aromatic amino acid decarboxylase (L-aromatic amino acid carboxy-lyase, EC 4.1.1.28) and is used in conjunction with dopa (3,4-dihydroxyphenylalanine) therapy. Since Benserazide does not cross the blood–brain barrier, it will inhibit only extra-cerebral aromatic amino acid decarboxylase, and hence allow a greater proportion of the administered dose of dopa to enter the brain for cerebral dopamine (3,4-dihydroxyphenethylamine) synthesis. This allows use of smaller doses of dopa, and hence decreases the incidence of dopa-induced side effects in Parkinsonian patients.

Since Benserazide is a hydrazide, it is not surprising that it inhibits kynurenine hydrolase, which is a pyridoxal phosphate-dependent enzyme. It is well established that many hydrazine derivatives inhibit pyridoxal phosphate-dependent enzymes, by formation of biologically inactive hydrazones of the cofactor (Vilter, 1964). In the present study the pattern of inhibition of kynurenine hydrolase by Benserazide has been studied. At the same time another hydrazine inhibitor of aromatic amino acid decarboxylase that is used in treatment of Parkinsonism together with dopa, Carbidopa (MK-486, S-α-hydrazino-3,4-dihydroxy-α-methylbenzenepropionic acid monohydrate) has also been studied. For comparison, four other aromatic hydrazine derivatives have also been used: isonicotinic acid hydrazide, an anti-tuberculosis agent known to inhibit kynurenine hydrolase in vivo under clinical conditions (Roc, 1971), phenylhydrazine, Girard's reagent T (2-hydrazino-NNN-trimethyl-2-oxoethanaminium chloride) and Girard's reagent P (2-hydrazino-2-oxoethane-N-pyrindinium chloride).

Liver from freshly killed rats was homogenized in 2 ml of 0.15 M-NaCl/g of tissue; 0.5 ml of this homogenate and 0.5 ml of 0.2 M-sodium phosphate buffer, pH 7.0, were incubated together for 5 min at 30°C. For inhibition studies the inhibitor was dissolled in the buffer, so that it was present with the enzyme throughout this preincubation period. The reaction was initiated by addition of 0.2 ml of a solution of L- kynurenine; five concentrations of substrate were used, over the range 0.5–4.0 mm. After incubation for a further 30 min the reaction was stopped by addition of 1 ml of 1 M-trichloroacetic acid. Denatured protein was removed by centrifugation and the supernatant was made approximately neutral by the addition of 0.5 ml of 1 M-NaOH. The volume of the supernatant was then adjusted to 5 ml by the addition of 1 M-sodium phosphate buffer, pH 5.5, and the fluorescence due to anthranilic acid measured (activation 310 nm, 1978
Fig. 1. Inhibition of kynurenine hydrolase by aromatic hydrazine derivatives

For clarity, individual points are not shown. Lines are from unweighted least-squares fitting to triplicate determinations at each of five concentrations of substrate (given beside each line in μM). (a) Benserazide; (b) Carbidopa; (c) isoniazid; (d) phenylhydrazine; (e) Girard reagent T; (f) Girard reagent P.

emission 420 nm). $K_m$ and $V_{max}$ values were calculated by unweighted least-squares fitting to the double-reciprocal (Lineweaver-Burk) plot, with triplicate incubations at each substrate concentration. $K_i$ values were calculated by plotting $1/\text{(rate of reaction)}$ against inhibitor concentration, in the same way.

For the reaction in the absence of inhibitor the mean observed $K_m$ for D,L-kynurenine was $590 \pm 110 \mu M$, a value in agreement with previous results from mouse liver preparations (McDermot et al., 1973; Bender et al., 1977). The mean observed $V_{max}$ was $2.93 \pm 0.32 \text{nmol of anthranilic acid formed/min}$, equivalent to $17.5 \pm 1.9 \text{nmol/min per g of liver}$.

As shown in Fig. 1, Benserazide, isoniazid and the two Girard reagents all gave
non-competitive-inhibition patterns, as might be expected with compounds that interact directly with the cofactor. With Benserazide there was some evidence of an uncompetitive character to the inhibition at low concentrations of the inhibitor. Carbidopa was mainly uncompetitive throughout the inhibitor range used. Phenylhydrazine was mainly competitive, but with a clear non-competitive element at lower concentrations of inhibitor. It is possible that the mixed character of the inhibition patterns observed may reflect re-activation of the enzyme by free pyridoxal phosphate present in the crude tissue extract used; at higher concentrations of inhibitor this would be less important than at low concentrations.

\[ K_i \] values were calculated as follows: Carbidopa \(4.7 \pm 1.3 \mu M\); Benserazide \(26.4 \pm 6.2 \mu M\); phenylhydrazine \(55.8 \pm 9.8 \mu M\); Girard reagent T \(237 \pm 24 \mu M\); isoniazid \(480 \pm 30 \mu M\).

It is well established that administration of isoniazid to tuberculosis patients without vitamin B₆ supplementation can lead to signs of pellagra (niacin deficiency) because of kynurenine hydrolase inhibition, and hence decreased formation of NAD from tryptophan (Roe, 1971). The usual daily dose of isoniazid is about 300mg (approx. 2.2 mmol/day). The doses of Benserazide and Carbidopa used in treatment of Parkinsonism are between 0.4 and 0.8 mmol/day; since these two compounds are very much more potent inhibitors of kynurenine hydrolase \textit{in vitro} than is isoniazid, it might be expected that pellagra would be observed in patients treated with them. To date there has been no report of clinical pellagra after Benserazide or Carbidopa therapy. However, preliminary studies indicate that such patients are marginally niacin deficient, excreting significantly less \(N^1\)-methylnicotinamide than do control subjects. In 15 patients treated with Sinemet (Merck, Sharp and Dohme; Carbidopa+dopa) the mean urinary excretion of \(N^1\)-methylnicotinamide was 0.57±0.25 mmol/mol of creatinine, and in three patients treated with Madopar (Roche; Benserazide+dopa) the mean excretion was 0.69±0.32 mmol/mol of creatinine. In a group of nine age-matched control subjects (Parkinsonian patients treated with anti-cholinergic agents or bromocriptine, and post-menopausal women attending the menopause clinic at the Hospital for Women, Soho, London, U.K.) mean excretion was 1.20±0.44 mmol/mol of creatinine. This difference is statistically highly significant. Hence there is evidence of at least biochemical niacin deficiency in patients treated with Benserazide or Carbidopa.

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