A Model for Hyperphenylalaninaemia due to Tetrahydrobiopterin Deficiency

R. G. H. Cotton  
Birth Defects Research Institute, Royal Children's Hospital Research Foundation, Flemington Road, Parkville, Melbourne, Australia 3052

A model for tetrahydrobiopterin deficiency in mice is described. Elevated levels of phenylalanine produced in the model were shown to be dramatically reduced after injection of tetrahydrobiopterin. A comparison of several reduced pterins for their efficacy in the system is described. The unnatural S isomer of tetrahydrobiopterin was shown to be active in the system.

Tetrahydrobiopterin deficiency leads to hyperphenylalaninaemia (McKusick 26163, 26164 and 26169) and a deficiency of the catecholamine and indole amine neurotransmitters leading to mental retardation and death if untreated (Danks et al., 1978; Dhondt, 1984). Tetrahydrobiopterin (BH₄) was first administered to one of these patients in 1974 (Danks et al., 1975; Danks et al., 1979) and although a dramatic fall in serum phenylalanine followed there was no change in clinical status. However, more recently clinical improvement has been shown in less damaged patients by administration of higher levels of BH₄ (Niederweiser et al., 1982; Kaufman et al., 1982).

Alternative pterins have been used in such patients: these include 6-methylpterin (Kaufman et al., 1982), sepiapterin and 7,8-dihydrobiopterin (Curtius et al., 1979; Niederweiser et al., 1979), tetrahydroneopterin (Curtius et al., 1979) and 1',2'-diacetyl BH₄ (Endres et al., 1982). 6,6-dimethyltetrahydropterin has recently been suggested (Armarego and Waring, 1981; Bailey and Ayling, 1984) but not tried. One great drawback in the search for new pterins is that there is no in vitro model in which to test them. At present new compounds are tested for their kinetic characteristics on purified aromatic amino acid hydroxylases and dihydropteridine reductase. Diaminohydroxyprymidine (DAHP) was shown in 1966 to inhibit biop- terin synthesis in rats as measured by Crithidia assay (Pabst and Rembold, 1966). Much later, pterin synthesis in brain (Gal and Whitacre, 1981) and in a pheochromocytoma (Brautigam et al., 1984) was found to be inhibited.

We have now used the inhibitor DAHP in the diet of mice to create such a BH₄ deficiency that hyperphenylalaninaemia resulted without addition of phenylalanine to the diet. Further, we have shown that BH₄ injection can dramatically reduce this level of hyperphenylalaninaemia as shown in BH₄-deficient patients. The effects of several pterins have been compared using the model.

MS received 15.4.85  Accepted 18.6.85
The information gained from this model of pterin deficiency may also be useful for planning therapy of other disorders such as Parkinson's disease and dystonia, in which BH₄ deficiency in cerebrospinal fluid has been documented (Lovenberg et al., 1979; Williams et al., 1979; Curtius et al., 1982; Le Witt et al., 1983).

MATERIALS AND METHODS

Mice: Balb C mice were sex-matched and used either at weaning (3 weeks) or after 10 weeks of age (see text).

Chemicals: 2,4-Diamino-6-hydroxypyrimidine (DAHP) was from Sigma. 7,8-Dihydrobiopterin (BH₂-7,8) and BH₄ (R,S; R and S) were prepared by Dr W. Arma-rego, Canberra. A second preparation of BH₄ (R,S) was from Roche. Sepiapterin was from Dr Schircks, Jona. L-Phenylalanine and L-tyrosine were from Calbiochem. L-3,4-dihydroxyphenylalanine (L-DOPA) and 5-hydroxytryptophan were from Sigma.

Administration of chemicals: DAHP was mixed (0-7% by weight) with the normal food (ARM rat and mouse pellets, Clarke King), and in some cases L-phenylalanine, L-tyrosine, L-DOPA and 5-hydroxytryptophan were also included at the levels indicated. BH₄ was made up as previously described (Danks et al., 1979) and given by intraperitoneal injection.

Analysis of blood phenylalanine levels: Adult animals were bled from the eye onto Guthrie cards and analyzed using the Guthrie screening method at Mont Park Psychiatric Hospital, Melbourne. Weanling mice were killed and bled and serum phenylalanine was measured by fluorimetry (McCaman and Robins, 1962).

Analysis of tissue BH₄ levels: Mice were killed by decapitation. Livers were snap frozen and stored at -70°C until analysis. Pterins were extracted and analyzed according to the method of Fukushima and Nixon (1980).

Analysis of brain catecholamine metabolites: Brains were collected into liquid nitrogen and stored at -70°C until analysis. Noradrenalin, 3,4-dihydroxyphenylacetic acid (DOPAC) and dopamine were measured by the method of Marley and colleagues (1985).

RESULTS

Preliminary experiments

One of each of the 5 levels (0, 1, 2, 3 and 5%) of DAHP was fed to one of each of 5 pairs of weanling female mice for 7 days. At that time the mice were killed, bled and serum separated for phenylalanine estimation and the liver and brain collected for BH₄ estimation.

The serum phenylalanine level was directly related to the amount of DAHP in the diet and the liver BH₄ content was inversely related to the amount of DAHP.