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COMMENTARY

THE ROLES OF FOLATE AND PTERIDINE DERIVATIVES IN NEUROTRANSMITTER METABOLISM

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The biologically active folate coenzymes are the derivatives of tetrahydrofolic acid (FH_4) that function in the transfer of 1-carbon groups in a variety of reactions in purine, pyrimidine and amino acid metabolism [1]. The pteridine moiety, in addition to forming an integral part of the folic acid molecule, is also required as a donor of reducing equivalents in several amino acid hydroxylation reactions. A number of defects in both folate and pteridine metabolism are known to affect neurological function, suggesting an essential role for these compounds in the maturation of the nervous system and in synaptic dynamics [2–4]. This review will concentrate on aspects of the metabolism of these compounds of current relevance to neurochemistry and, in particular, to the metabolism of amino acid and amine neurotransmitters. Such studies have been limited in the past because of the low concentrations of folate derivatives in tissues, their instability and problems associated with their analysis, although radioimmunoassay procedures are now available for folate measurements. The structures of folates and pteridines and the biochemistry of one-carbon transfer reactions have been described in detail elsewhere [1], although pathways relevant to this essay are shown in Fig. 1. Throughout this article the term 'pterin' will be used to refer to derivatives of the naturally occurring 2-amino-4-hydroxypteridine, as suggested elsewhere [1]. The role of folates in nucleic acid metabolism in brain will not be discussed here.

Folate metabolism in brain. Although the synthesis of the pteridine ring system has been extensively studied in bacterial and insect systems, there is little information available concerning the biosynthesis of pterins in mammalian systems. However, it has been established that various mammalian tissues, including brain, possess the capacity to synthesize biopterin from GTP [5], and neuroblastoma cell lines have been shown to effect a similar synthesis [6]. The pathway of mammalian pterin synthesis is presumed to be similar to that found in microbial systems. In contrast mammalian tissues do not have the capacity to synthesize folate derivatives and for human beings there is a daily dietary requirement of about 50 μg folic acid [2].

The folic acid molecule is composed of a pterin linked through a *p*-aminobenzoyl moiety to one or more glutamate residues in γ-carboxyl linkage (folate mono- and oligo-glutamates). 5-Methyltetrahydrofolate (CH_3FH_4) is the major form of folate present in serum and peripheral tissues as well as in the developing brain. However this is apparently not the case in the mature brain where almost 50 per cent of the total folate is present as FH_4 oligoglutamates [4, 7]. Folate levels in brain and cerebrospinal fluid are 2–3 times higher than those found in serum even in states of folate deficiency [8]. This indicates the importance of these compounds to the brain and suggests the existence of a regulatory mechanism for maintaining brain folate levels. CH_3FH_4 exists predominantly in a protein bound form in blood [9] and is concentrated into brain by a saturable uptake mechanism specific for folates [10]. This requirement for the transport of reduced folates from blood is necessitated by the absence in brain of significant quantities of dihydrofolate reductase [11]. Formiminotransferase, an enzyme which can contribute to the one-carbon pool in liver is also apparently absent from brain [12], suggesting that serine is the major endogenous source of one-carbon units in this tissue through the action of serine hydroxymethyltransferase. All the enzymes required for the *de novo* synthesis of *S*-adenosylmethionine from serine are known to occur in brain (see Fig. 1).

A number of studies [12–16] have examined the subcellular distributions of folate-metabolizing enzymes in nerve tissue and although there is general agreement on the cytosolic location of methylene FH_4 reductase, methionine synthetase and methionine adenosyl transferase, discrepancies are apparent in the reported distribution of serine hydroxymethyltransferase. Davies and Johnston [13] and McClain *et al.* [12] have reported significant proportions (20–30%) of serine hydroxymethyltransferase in the

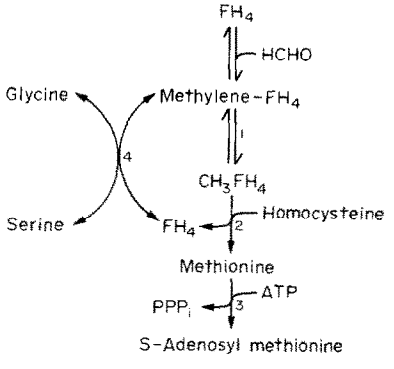

Fig. 1. Interconversion of FH_4 derivatives in relation to amino acid and amine metabolism. The principal enzymes involved are: (1) methylene-FH_4 reductase (EC 1.1.1.68); (2) CH_3FH_4 homocysteine methyltransferase (EC 2.1.1.13); (3) methionine adenosyltransferase (EC 2.5.1.6); (4) serine hydroxymethyltransferase (EC 2.1.2.1). The association of FH_4 with formaldehyde is presumed to be non-enzymic.

cytosol, with the majority of the remaining activity occurring in the mitochondrial fraction as in liver. In contrast, other workers have concluded that this enzyme occurs almost exclusively in mitochondria with negligible activity being present in the cytosol [14–16]. The discrepancies may reflect the differences in homogenisation and fractionation conditions employed in these studies. A careful re-investigation of the localisation of serine hydroxymethyltransferase in brain is important because of its possible involvement in a one-carbon shuttle system across the inner mitochondrial membrane [17], which would require both cytosolic and mitochondrial forms of the enzyme.

Although a considerable proportion of cellular folates are present as their oligoglutamate derivatives, most studies on folate-metabolizing enzymes have tended to use the monoglutamates as substrates because of their ready availability. However the oligoglutamate forms of the folates have been shown to be better substrates than the monoglutamates for a number of folate-interconverting enzymes [18–20]. Thus the oligoglutamate derivatives may not only represent storage forms of the folates as previously suggested [1], but may function both as natural substrates and as regulators of intracellular folate-dependent reactions. Such possibilities should be borne in mind in studies on the enzymology of folate metabolism.

The role of pterins in the synthesis of biogenic amines. The primary and rate-limiting step in the synthesis of the catecholamines involves the conversion of L-tyrosine to L-3,4-dihydroxyphenylalanine (DOPA) by the enzyme tyrosine hydroxylase [21]. This enzyme catalyses a 3-substrate reaction involving tyrosine, oxygen and a reduced pterin cofactor (probably tetrahydrobiopterin, BH_4) as shown in reaction (1):

$$\text{L-tyrosine} + BH_4 + O_2 \rightarrow \text{DOPA} + BH_2 + H_2O \quad (1)$$

$$BH_2 + \text{NADH} + H^+ \rightarrow BH_4 + \text{NAD} \quad . \quad (2)$$

Regeneration of the reduced pterin is carried out by a pyridine nucleotide-dependent enzyme, dihydropteridine reductase [22] (reaction 2). Phenylalanine [23] and tryptophan hydroxylases [24] also use pterin cofactors for the donation of reducing equivalents and similarly require pteridine reductase for regenerating the reduced cofactor. The related enzyme dihydrofolate reductase probably plays no role in the supply of BH_4 [25]. The regulation of catecholamine synthesis occurs mainly at the tyrosine hydroxylase step and may involve long-term modifications of the amount of tyrosine hydroxylase protein (trans-synaptic induction) and short-term regulatory processes independent of changes in enzyme levels [26]. This review will consider only the latter mechanisms.

It is now well established that dopamine and noradrenaline inhibit tyrosine hydroxylase competitively with respect to the pterin cofactor [27]. Such observations suggest that release of catecholamines from the nerve ending may result in an increase in the rate of amine synthesis due to the relief of end-product inhibition of tyrosine hydroxylase. Indeed, several studies have demonstrated an increase in the rate of catecholamine biosynthesis following amine release due to electrical stimulation or K^+-induced depolarization [28, 29]. In addition cyclic nucleotides have been reported to regulate amine synthesis [30], and cyclic AMP has been shown to mediate activation of tyrosine hydroxylase *in vitro* by reducing the K_m of the enzyme for the pterin cofactor [31, 32]. It is at present uncertain whether or not the activation by cyclic AMP involves a direct phosphorylation of tyrosine hydroxylase by a cyclic AMP-dependent protein kinase [32, 33].

The synthesis of a number of neurotransmitters including the catecholamines may be regulated by precursor availability (for discussion see ref. 34). For example, elevation of tyrosine levels can bring about a transient increase in catecholamine synthesis [35] and it has also been proposed that the levels of reduced pterin cofactor may regulate tyrosine hydroxylation and amine synthesis [36]. This suggestion is sustained by the demonstration that intraventricular injection of biopterin [37] or its addition to sympathetic nerve tissue in culture [38] or synaptosomal preparations [39] results in an increased conversion of tyrosine to catecholamines. Furthermore the use of an inhibitor of pteridine reductase has been shown to abolish noradrenaline fluorescence in neuronal processes [38]. Glucagon administration increases both pteridine reductase levels and the conversion of phenylalanine to tyrosine in rat liver [40]. This observation further supports the hypothesis that availability of reduced pterin is a regulatory factor in such hydroxylation reactions.

Chemical sympathectomy with 6-hydroxydopamine has been reported to have no effect on the levels of rat brain pteridine reductase [41] which would suggest that only a small proportion (probably less than 10 per cent) of the total pteridine reductase in brain is located in catecholaminergic neurons. Some will of course be present in serotonergic neurons, but it would appear that the majority of pteridine reductase activity is extraneuronal and may serve in, as yet, unidentified hydroxylation reactions.

Less is known about the control of serotonin synthesis in brain, although tryptophan hydroxylation is thought to be the most likely site for regulation to occur. Brain serotonin levels are also controlled by precursor availability and are increased by treatments that raise brain tryptophan levels [34]. Studies on the effects of administered biopterin on serotonin synthesis have not been reported, although it is likely that pterin levels are a regulatory factor in the biosynthesis of the indoleamines, by analogy with the regulation of catecholamine biosynthesis.

Folates and transmethylation reactions. Several methyl transferases are of importance in the metabolism of biogenic amines [42]. For example, both the biosynthesis of adrenaline and the inactivation of histamine involve *N*-methylation reactions, whereas the formation of melatonin and the inactivation of catecholamines involve *O*-methylation processes. A number of methylated derivatives of the biogenic amines (e.g. mescaline and *N,N*-dimethyltryptamine) produce psychotomimetic effects in human beings and hence abnormal or excessive methylation of amine neurotransmitters has been implicated in the aetiology of psychotic illnesses [43]. This transmethylation

hypothesis of schizophrenia [44], originally proposed in 1952, is supported by the ability of the methyl donor methionine to intensify psychotic symptoms in a significant proportion of schizophrenic patients. However, at present there is no convincing evidence for significant differences in the levels of psychotomimetic, methylated amines between schizophrenic patients and controls. Recent reviews have discussed the varying popularity of this transmethylation hypothesis [43, 45].

A critical factor in the concept of an "endogenous hallucinogen" would be the ability of mammalian tissues to synthesize psychotomimetic compounds such as dimethyltryptamine. A significant advance was provided in 1961 by Axelrod [46] who demonstrated the presence, in rabbit lung, of an enzyme capable of forming *N*-methylated tryptamine derivatives from the parent amine. This enzymic activity was also shown to be present in brain [47], and *N*-methylated tryptamines have been reported to occur in various mammalian tissues [48], although the specificity of identification of these compounds using thin layer chromatographic studies has been challenged [49].

In all the above work it had been tacitly assumed that the methyl donor in such methylation reactions was *S*-adenosyl methionine (SAM). This assumption was challenged by Laduron [50] who proposed that CH_3FH_4 may function directly as methyl donor to biogenic amines. Previously CH_3FH_4 had merely been regarded as the donor of a methyl group to homocysteine in the biosynthesis of methionine [42]. Further reports from Laduron [51] and others [52, 53] described an enzyme from brain that could apparently donate the methyl group of CH_3FH_4 to a variety of catecholamine derivatives to form both *O*- and *N*-methylated products. Characterization of this enzyme from brain showed it to be more active than SAM-dependent amine methylases, and it was proposed that increased brain levels of CH_3FH_4 may be responsible for the abnormal methylation processes thought to occur in schizophrenia [54].

A number of discrepancies among reported results have led to a re-investigation of the specificity of this novel CH_3FH_4-dependent methylation reaction [55–57]. Lin and Narasimhachari [57] first noted that the amine reaction products differed from those produced by SAM-dependent methylation processes and they suggested that the products formed were not due to direct methylation of the acceptor amines. The specificity of identification provided by gas chromatography combined with mass spectrometry showed that the major product of the incubation of CH_3FH_4 and dopamine with enzyme extract was not *N*-methyl dopamine but 6,7-dihydroxytetrahydroisoquinoline [58]. Correspondingly, tetrahydro-β-carbolines were formed from CH_3FH_4 and tryptamine derivatives [59–62]. Such compounds are known to be formed by the non-enzymic condensation of formaldehyde with the parent amines, and the enzyme originally identified as a methyl transferase was subsequently shown to catalyse the release of formaldehyde from CH_3FH_4 [63].

Current evidence suggests that this "formaldehyde-forming activity" is identical with the generally distributed enzyme of one-carbon metabolism, methylene-FH_4 reductase [55, 56], although homogeneous preparations of the enzyme will be required to confirm this suggestion. The major physiological function of methylene-FH_4 reductase is presumed to be the synthesis of CH_3FH_4 (see Pearson and Turner [55] for discussion), and the central role of this enzyme in providing methyl groups for subsequent synthesis of SAM is indicated by the regulation of its activity by SAM levels [55]. However, in the presence of a suitable oxidant *in vitro* a reversal of the methylene-FH_4 reductase reaction can occur which results in the conversion of CH_3FH_4 to FH_4 and formaldehyde. The formaldehyde produced can then condense non-enzymically with a suitable acceptor such as a catecholamine or indoleamine. Since the amine does not enter directly into the enzymic reaction, previously quoted kinetic constants for amine substrates [51, 52] are of little significance. The analogous formation of tetrahydroisoquinoline alkaloids by the non-enzymic condensation of catecholamines with acetaldehyde or biogenic aldehydes has been reported to occur *in vivo* in mammalian tissues [64]. Such alkaloids have been implicated in the process of alcohol addiction and in some of the side-effects of DOPA therapy in Parkinson's disease (for discussion see ref. 64). It is therefore feasible that formaldehyde-derived tetrahydroisoquinoline and tetrahydro-β-carboline alkaloids may form *in vivo* in sufficient concentrations to modify the uptake or metabolism of neurotransmitters. These alkaloids could arise by the formation of "active formaldehyde" from CH_3FH_4 through the action of methylene-FH_4 reductase or from other formaldehyde-generating systems such as sarcosine dehydrogenase [1]. Recent studies by Ordonez and Villaroel [65] have indicated that the reversal of the methylene-FH_4 reductase reaction may occur under certain conditions and these studies suggest that alkaloid formation in brain merits further investigation.

The incorrect identification of a number of products of one-carbon transfer reactions [50–53] has indicated that any discussion of a role for methylated amines in schizophrenia based solely on thin layer chromatographic studies for identifying these compounds should be treated with caution. Future work should rely on more specific methods of product characterization, for example mass fragmentography. Such an approach has recently been used to show the presence of amine-*N*-methylase activity in lung using either SAM or CH_3FH_4 as methyl donor [66]. Therefore, although the majority of the activity originally identified as a folate-dependent amine *N*-methyl transferase appears to be due to the action of methylene FH_4 reductase, the presence of small amounts of a genuine folate-dependent methylase cannot be precluded.

Folates and glycine metabolism. Although a large body of evidence supports a role for glycine as an inhibitory neurotransmitter, particularly in the spinal cord [67], our present knowledge of the metabolism of glycine in the nervous system is limited. However, both the synthesis and degradation of glycine are known to involve folate coenzymes [67] and they represent further points of interaction between folates and neurotransmitter metabolism.

Neuronal glycine is probably provided from serine through the action of serine hydroxymethyltransferase rather than by transport from the blood [68]. Indeed,

the regional distribution of glycine within the rat central nervous system correlates well with the distribution of serine hydroxymethyltransferase [14]. The reversible reaction catalysed by this enzyme involves the transfer of the 3-carbon atom of serine to FH_4:

$$\text{Serine} + FH_4 \rightleftharpoons \text{glycine} + \text{methylene-}FH_4.$$

An alternative pathway to glycine exists from glyoxylate although the contribution of this route has been estimated to be less significant than that from serine [69]. Serine itself can be derived from glucose via 3-phosphoglycerate or glycerate with the former route predominating in brain *in vivo* [70].

Glycine catabolism may follow several different pathways [69]. Since the serine hydroxymethyltransferase reaction is readily reversible, glycine may be converted to serine in the presence of excess methylene-FH_4 although the presence of amines or other compounds that can sequester the active formaldehyde moiety may regulate this reaction. An alternative route for the removal of glycine is through its oxidation to glyoxylate by L-amino acid oxidase, an enzyme that is known to occur in brain [71]. However, the major mechanism for glycine catabolism, at least in peripheral tissues, is by the glycine cleavage system [69] which catalyses the folate-dependent decarboxylation of glycine according to the following equation:

$$\text{Glycine} + FH_4 + NAD^+ \rightleftharpoons CO_2 + NH_4^+ + \text{Methylene-}FH_4 + NADH.$$

The reversible glycine cleavage system in rat liver is a multi-enzyme complex involving four protein components [72], which may be closely linked to serine hydroxymethyltransferase. Little is known of the properties of the glycine cleavage system in brain tissue except that it is located intra-mitochondrially.

Both serine hydroxymethyltransferase and the glycine cleavage system provide a means for the intramitochondrial generation of methylene FH_4. Conversion of this folate derivative to CH_3FH_4 is catalysed by a cytosolic enzyme and requires the transport of methylene FH_4 across the inner mitochondrial membrane, which may involve a specific shuttle system for folates as mentioned above [17]. There is at present little available evidence concerning the compartmentation of folate-dependent enzymes either in distinct cell types or specific regions of brain which may relate to the role of glycine as an inhibitory neurotransmitter.

Implications for studies on mental illness. The recent interest in the metabolism of brain folates is a consequence of the increasing clinical use of blood folate measurements, which has revealed that a number of neurological syndromes may be a result of folate deficiency (for discussion, see ref. 3). For example, several of the inborn errors of folate metabolism that have been described show aspects of neurological abnormality [2]. Of particular interest with regard to the transmethylation hypothesis of schizophrenia is the description of a patient with methylene FH_4 reductase deficiency who exhibited schizophrenic symptoms that were relieved on treatment with folic acid [73]. The psychotic symptoms returned when the folate therapy was discontinued. However, the significance of these observations in terms of a biochemical description of schizophrenia is not yet clear.

Neurological abnormality and mental retardation have recently been described in a phenylketonuric patient despite the close, dietary control of phenylalanine intake [74]. In this patient, the defect was found not to be in phenylalanine hydroxylase but in pteridine reductase activity, which resulted in a significant reduction in the levels of the amine neurotransmitters. Neurological deterioration has also been described in several cases of hyperglycinaemia due to a deficiency in the glycine decarboxylase component of the glycine cleavage system [75]. The neurological effects of this syndrome are presumably related to increased brain levels of the inhibitory neurotransmitter glycine. Although methionine adenosyltransferase deficiency has been implicated in schizophrenia [76], the only known case of such a defect was reported to show normal mental development [77] although the possibility of future deterioration cannot be excluded. Neurological involvement in various other cases of inborn errors of folate metabolism are known and have recently been reviewed in detail [2].

A number of workers [78, 79] have described a relationship between the levels of anti-convulsants and folate derivatives during the treatment of epileptic patients. Anti-convulsant drugs, and in particular diphenylhydantoin, lower serum folate in a significant number of epileptics and it has been suggested that the anti-folate effect of these drugs may be an important factor in control of the epilepsy [80]. However, although folic acid and its derivatives act as convulsants when injected into the cerebral cortex of rats or cats, millimolar concentrations of these compounds are required to elicit such effects [81]. The mechanism by which anti-convulsants affect folate levels is unclear, as is the mechanism of action of the anti-convulsants themselves. Nevertheless, involvement of folates in epilepsy merits further consideration and stresses the need for serum folate measurements of patients on anti-convulsant therapy as well as in undiagnosed cases of neurological abnormalities.

Conclusions. This review has attempted to highlight the interactions of folate and pteridine derivatives with the metabolism of certain amino acid and amine neurotransmitters. There is little evidence to support an involvement of folates in the metabolism of other known transmitters. For example, although choline synthesis involves a SAM-dependent methylation reaction, it is probable that the majority of choline is synthesized peripherally and is transported into brain in a lipid-bound form for conversion to acetylcholine [82]. Folic acid has, however, been reported to inhibit the high affinity uptake of glutamate and GABA into rat brain slice preparations, possibly through an effect on membrane permeability [83]. Our knowledge of the enzymology of folate metabolism in brain and its regulation is limited, and no folate-interconverting enzyme has been isolated in a homogeneous form from brain and characterized extensively. The role of folates in neural function and their possible deficiencies in mental illness indicates the potential of such studies. The development of specific inhibitors of folate-metabolizing enzymes would provide useful tools for studying interactions between

folates, pteridines and neurotransmitter metabolism. A number of such applications have been reported in the literature but these could usefully be extended. For example, a specific inhibitor of pteridine reductase has been used to investigate the significance of this enzyme to catecholamine biosynthesis [38]. The anti-Parkinsonian drug DOPA can be used to modify brain methionine levels and has been applied to demonstrate that the *de novo* synthesis of methyl groups occurs to a significant extent in brain rather than by transport of pre-formed methionine from blood [84]. Anti-folate drugs such as methotrexate inhibit a number of folate-dependent enzymes although with a lower affinity than that for dihydrofolate reductase. Drugs of this general type might, however, prove useful in studies of brain folate metabolism since dihydrofolate reductase is absent from this tissue.

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