Brain function in the elderly: role of vitamin B_{12} and folate

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Vitamin B_{12} (cobalamin) deficiency associated neuropathy, originally called sub-acute combined degeneration, is particularly common in the elderly. The potential danger today is that with supplementation with folic acid of dietary staples such as flour, that the incidence of this disease could rise as folic acid, as opposed to natural folate ($N_5CH_3HFGlu_1$), enters the cell and the metabolic cycle by a cobalamin independent pathway. This chapter briefly describes the clinical presentation of the disease, which unless treated will induce permanent CNS damage. The biochemical basis of the interrelationship between folate and cobalamin is the maintenance of two functions, nucleic acid synthesis and the methylation reactions. The latter is particularly important in the brain and relies especially on maintaining the concentration of S-adenosylmethionine (SAM) which, in turn, maintains the methylation reactions whose inhibition is considered to cause cobalamin deficiency associated neuropathy. SAM mediated methylation reactions are inhibited by its product S-adenosylhomocysteine (SAH). This occurs when cobalamin is deficient and, as a result, methionine synthase is inhibited causing a rise of both homocysteine and SAH. Other potential pathogenic processes related to the toxic effects of homocysteine are direct damage to the vascular endothelium and inhibition of N-methyl-D-aspartate receptors.

Vitamin B_{12} (cobalamin) deficiency leads to a neuropathy, which was previously called sub-acute combined degeneration of the cord. Since it affects the brain and peripheral nervous system, as well as the spinal cord, it seems more appropriate to call this cobalamin deficiency associated neuropathy. Recent events have suggested that an understanding of the pathogenic mechanisms involved may also lead to improved management of other brain diseases, especially vascular and Alzheimer's dementia. Furthermore, recent and pending decisions on folic acid fortification of food staples such as flour make a reappraisal of the topic opportune.

The current US Food and Drug Administration (FDA) has decided to fortify grain products with folic acid (1.4 mg/kg flour) in an effort to prevent the occurrence of neural tube defects and reduce the incidence

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of cardiovascular disease in the community[1]. It is estimated that this will deliver an additional 100 μg folic acid/day to the average US diet, although consumers of high amounts of grain products are likely to be exposed to higher levels[2]. This begs the question as to how much folic acid (FA) can be added to food, which will be completely converted to 5-methyltetrahydrofolate ($N^5CH_3THFGlu_1$), the normal form of folate circulating in the plasma following its absorption from the intestinal lumen. The point is that folic acid (FA), unlike $N^5CH_3THFGlu_1$, can be absorbed by cobalamin-deficient cells and can when given in large doses on a continuous basis maintain intracellular DNA synthesis, even when cobalamin is deficient (*vide infra*; Fig. 1). Cobalamin deficiency, which occurs most commonly in the elderly, causes both megaloblastic anaemia and the neuropathy. Thus, the megaloblastic anaemia which results from impaired DNA synthesis is corrected by folic acid therapy; however, the neuropathy which is caused by an impaired methylation process is not[3] (Fig. 1). This allows the progressive development of the neuropathy, which might otherwise have been diagnosed and treated appropriately with cobalamin[3]. The levels of folate fortification of flour, as recommended by the FDA, and at present in place in America since 1998 seem unlikely to pose a problem[4], since at this level all of the folic acid will be converted to $N^5CH_3THFGlu_1$. However, the levels recommended by the Folic Acid Working Party Group at the Centers for Disease Control and Prevention in America[5] could produce a potential danger, since, at the doses recommended, free FA would be present in the blood stream after

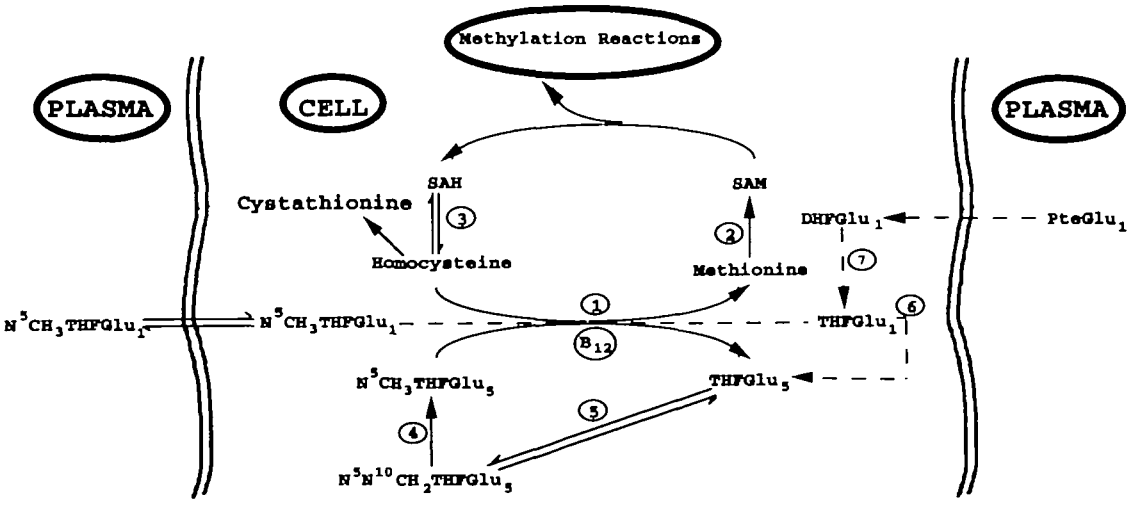

Fig. 1 Control of entry of folate into cells and the interrelation of intracellular folate and cobalamin. Reactions: 1, methionine synthase; 2, S-adenosylmethionine synthetase; 3, S-adenosylhomocysteine hydrolase, 4, methylene tetrahydrofolate reductase; 5, serine hydroxy methyl transferase; 6, tetrahydrofolate glutamate ligase; 7, dihydrofolate reductase.

a meal[4]. As this is a non-physiological compound it could have both short- and long-term deleterious effects. This chapter will review the basis of folate and cobalamin interrelationships, and their effects on brain function with particular reference to the elderly, since they seem to be the most at risk in society today[6].

Clinical diseases caused by vitamin B_{12} (cobalamin) and folate deficiency

Folate and cobalamin are both members of the so-called B-complex of vitamins. These B vitamins act as co-factors for specific enzymes and, thereby, enable them to carry out their metabolic functions. Cobalamin deficiency may result in two major clinical conditions, namely megaloblastic anaemia and cobalamin deficiency associated neuropathy (cobalamin neuropathy). Cobalamin neuropathy occurs on its own in roughly a third of patients, in association with megaloblastic anaemia in a further third and as megaloblastic anaemia on its own in the remaining third. The megaloblastic anaemia is reversible on treatment, whereas the neuropathy, once established, leaves permanent damage. It usually affects people over the age of 70 years, but up to 20% may develop these diseases in earlier years[6]. It was thought to be commoner among Caucasians than Africans, but this seems unlikely when modern diagnostic techniques are employed[7].

Folate deficiency results in a megaloblastic anaemia, which is morphologically identical to that of cobalamin deficiency and occurs in all subjects with severe deficiency[8]. Whether folate deficiency causes neurological changes remains debatable. It does seem occasionally to occur in patients with chronic severe deficiency[9] and in others may be a factor in the production of depression[10].

Cobalamin neuropathy affects the cerebral cortex, spinal cord and peripheral nerves. Not surprisingly, therefore, it produces a wide variety of clinical signs and symptoms in different patients, which have been described in detail elsewhere[6]. The classical presentation is one of symmetrical paraesthesia in the feet and hands, leading on to gait ataxia. The dominant site is usually the feet, but occasionally the hands or even the pelvic area may be affected initially. Muscle weakness and paralysis are late signs and are usually irreversible. Other rarer features may be poor vision, impotence and urinary and faecal incontinence.

The onset of signs tends to occur after the symptoms and includes loss of cutaneous sensation in a stocking and glove distribution. Vibration and proprioceptive sensation impairment is common, especially in the legs. This leads on eventually to impairment of corticospinal tract

function with spastic paralysis. Usually the reflexes are absent due to the dominant peripheral neuropathy and this is one of the differential causes of absent reflexes and the presence of the Babinski sign (others include tabo-paresis, motor-neurone disease and Friederick's ataxia).

Cerebral syndromes are difficult to assess. They include global dementia, depression, hypomania, diminished intelligence and memory loss going on occasions to frank psychosis and personality changes. Mental impairment of some form is universal in elderly patients with cobalamin deficiency[6].

Failing to establish the diagnosis of cobalamin deficiency means that the symptoms and signs tend to progress over a period of months. The rate of progression varies from patient to patient. The commonest effect is for distal symptoms to extend proximally, but new symptoms may also appear. Most patients remain functionally independent, but some can become bed-ridden from cobalamin deficiency alone. The prevention of permanent neurological sequelae from cobalamin deficiency is dependent on early diagnosis and prompt treatment. The degree of residual disability following full treatment with parenteral cobalamin correlates with the duration and severity of the signs and symptoms prior to diagnosis.

Recent evidence has shown that neuropsychiatric disorders, which are reversible on cobalamin therapy, may occur in elderly subjects without evidence of anaemia or macrocytosis[11]. Such deficiencies are especially common amongst elderly institutionalised subjects[11].

Pathology

The dominant lesions affect the cervical and upper thoracic spinal cord and the cerebrum, which is mildly atrophic. The microscopic picture shows spongiform changes mainly in the white matter due to loss of myelin and axons, which in turn produces vacuoles. These are surrounded by myelin-laden macrophages. This appearance was termed a 'lachen felden' (field of holes) by the German physicians who described the condition in the mid-nineteenth century[3]. The changes predominantly affect the dorsal columns, the lateral and anterior corticospinal and spino-cerebellar tracts. The peripheral nerves show marked changes, especially in the distal sensory nerves, which include demyelination and loss of axons[12].

Causes of cobalamin and folate deficiency

The commonest causes of cobalamin or folate deficiency are either nutritional deficiency or malabsorption.

Nutritional deficiency

The normal dietary intake of cobalamin is about 5 μg/day. Dietary deficiency of both cobalamin and folate is especially common amongst alcoholics and the elderly. Since cobalamin is synthesised by bacteria, it only occurs in animal matter, thus meat and fish are good sources. On the other hand, it is not present in fruit or vegetables and, accordingly, deficiency may also occur in strict vegetarians (or vegans)[13]. Folate, on the other hand, is mainly present in green vegetables[14].

Malabsorption

The best known cause of cobalamin malabsorption is pernicious anaemia, a disease which is caused by an immunologically-mediated atrophy of the gastric mucosal lining, leading to loss of parietal and peptic mucosal cells[13]. These are responsible for the production of acid, pepsin and intrinsic factor. Intrinsic factor (IF) is required for the normal absorption of cobalamin. Dietary cobalamin is first bound by a non-specific R binder found mainly in saliva. In the alkaline medium of the duodenum IF displaces this R binder from cobalamin and the resultant IF-B_{12} complex is then taken up by a specific receptor on the luminal surface of ileal mucosal cells. Type I gastric atrophy which induces achlorhydria and loss of IF is caused by a genetically mediated immune mechanism. This must be dissociated from type 2 gastric atrophy, which occurs as a result of chronic infestation of the gastric mucosa by *Helicobacter pylori*.

Recently, it has been suggested that cobalamin deficiency in the elderly may also occur due to a combination of dietary deficiency and failure to mobilise organically bound cobalamin from food. This is thought to be due to hypochlorhydria occurring as a consequence of partial gastric mucosal atrophy[15].

Folate deficiency is a common presenting feature of coeliac disease or gluten-sensitive enteropathy, which causes folate malabsorption and does occasionally present in the elderly[16].

Metabolic roles of cobalamin and folate

Three hours after absorption, cobalamin is transported in the blood from the ileum to the brain bound to transcobalamin 2 (TC2)[13]. The cobalamin–TC2 complex crosses the blood brain barrier and enters the brain cells by a process of adsorptive endocytosis using a specific high affinity cell surface receptor. TC2 is degraded by lysosomal proteases

and the released cobalamins are converted to their methyl and adenosyl forms. Methyl-cobalamin is bound to methionine synthase in the cytosol, while adenosyl cobalamin is bound to methyl malonyl-CoA mutase in the mitochondria. The synthase and mutase enzymes are the only mammalian enzymes, which are known to be cobalamin dependent[13].

Folate is carried in the blood as $N^5CH_3THFGlu_1$ under physiological circumstances. It crosses the blood brain barrier by an active transport process since it enters against a blood/CSF concentration gradient. It is taken up by a specific cell wall receptor and enters the cell cytoplasm where it must either be demethylated using homocysteine and cobalamin-dependent methionine synthase to produce $THFGlu_1$ which is the reduced metabolically active form of folate, or it will exit the cell back into the CSF/bloodstream. For $N^5CH_3THFGlu_1$ to remain in the cell, it must be polyglutamated; however, $N^5CH_3THFGlu_1$ unlike $THFGlu_1$ is a poor substrate for the ligase enzyme, which is responsible for the addition of glutamates (Fig. 1). FA, on the other hand, on entering the cell is reduced *via* $DHFGlu_1$ to $THFGlu_1$ which is then polyglutamated in the usual way (*i.e.* it does not have to pass through a cobalamin-dependent pathway[14]; Fig. 1).

The function of folate in the cell is to act as a conveyor of carbon one moieties which, in turn, maintain two metabolic processes: the synthesis of nucleic acids for cell division and the methylation reactions for internal metabolism (Fig. 2)[14]. Carbon moieties are extracted from serine and formate by tetrahydrofolate pentaglutamate ($THFGlu_5$) to produce 5,10-methylene tetrahydrofolate pentaglutamate ($N^5N^{10}CH_2$-$THFGlu_5$) and 10-formyltetrahydrofolate penta glutamate ($N^{10}CHOTNFGlu_5$) respectively, which are responsible for the synthesis of pyrimidines and purines (Fig. 2). In particular, $N^5N^{10}CH_2$-$THFGlu_5$ is in a pivotal position because it can either supply its carbon moiety to deoxyuridine monophosphate to synthesise thymidylate *via* thymidylate synthetase for pyrimidine biosynthesis, or is reduced by methylene tetrahydrofolate reductase (MTHFR) to 5-methyl tetrahydrofolate pentaglutamate ($N^5CH_3THFGlu_5$) (Fig. 2). The latter is used to remethylate homocysteine to produce methionine and reform $THFGlu_5$. The latter is then re-available to receive another carbon moiety for distribution. Methionine maintains the methylation cycle, which returns to homocysteine (Fig. 2). Thus, $N^5N^{10}CH_2$-$THFGlu_5$ can either be used to maintain DNA synthesis in rapidly dividing cells like the bone marrow and intestinal mucosa or the methylation reactions in cells involved in internal metabolism[3] such as the liver and brain.

Since cell replication is of a low order in the adult brain[17], purine and pyrimidine requirements are minimal. The main function, therefore, of folate and cobalamin in the brain is the maintenance of the methylation reactions by synthesising S-adenosyl methionine *via* $N^5CH_3THFGlu_5$ and methionine (Fig. 2).

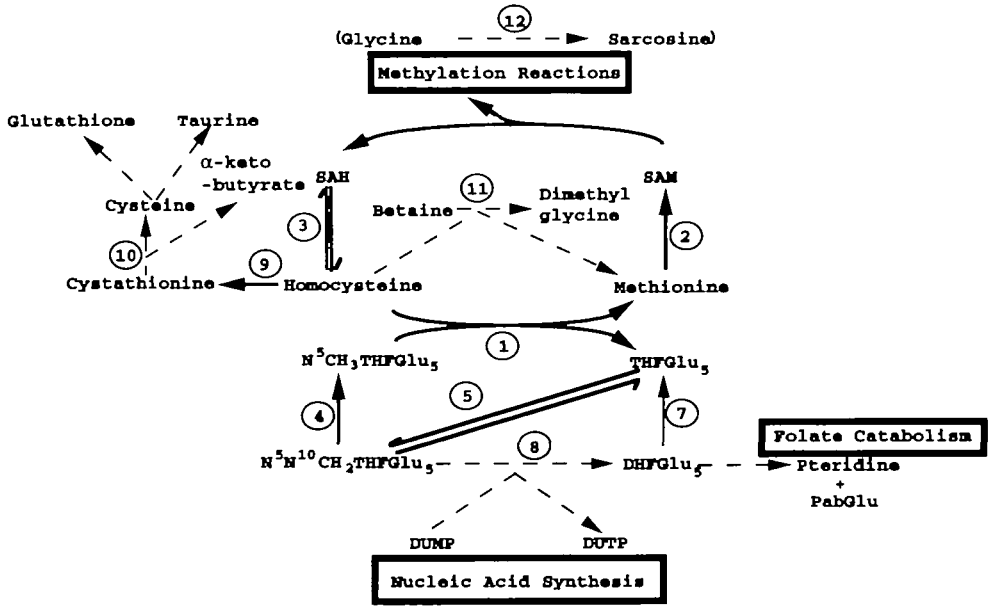

Fig. 2 Reactions involving folate and cobalamin interrelationships with particular reference to the brain. Reactions present and active in the brain have solid lines, those not present or inactive in the brain are shown as dotted lines. Reactions 1–7 are as in the caption to Figure 1. Other reactions: 8, thymidylate synthetase; 9, cystathionine synthetase; 10, cystathionine lyase; 11, betaine methyl transferase; and 12, glycine methyl transferase.

Since $N^5CH_3THFGlu_5$ has a much higher affinity for methionine synthase than $N^5CH_3THFGlu_1$, the latter on entering the cell from the plasma will only be demethylated and polyglutamated if there is a deficiency of $N^5CH_3THFGlu_5$. This will only occur under conditions of either cell division or of MTHFR deficiency (Fig. 1)[3].

The methylation reactions

The principal mechanism whereby cobalamin and folate metabolism influences brain function is through the methylation cycle. These comprise the use of a large number of methyltransferases, which are important regulators of internal metabolism and whose functions vary widely[18]. They include small molecules like DOPA and large compounds such as lipids and proteins. The compound responsible for maintaining these reactions is S-adenosylmethionine (SAM), also termed the universal methylator, which passes a methyl group onto the relevant methyltransferase enzyme. The main inhibitor of this process is the product of the reaction, S-adenosylhomocysteine (SAH). Accordingly, in

order to maintain the methylation reactions, the cellular cytoplasmic concentration of SAM has to be kept at a level that is several times higher than that of SAH[3]. This is processed by the action of two enzymes:

1 SAM synthetase, an ATP-dependent enzyme, which synthesises SAM from methionine and adenosine. There are in fact three such enzymes, one which is present throughout the body and has a low K_m, one which is present only in the liver and has a high K_m, and a third that appears to be of importance only in fetal life (Fig. 2)

2 SAH hydrolase which converts SAH to adenosine and homocysteine. Since the direction of this enzyme favours the back reaction to SAH[19], it is imperative that both homocysteine and adenosine are rapidly metabolised. Both the hydrolase and synthetase enzymes must always be more potent than the methylation requirement in order to ensure that the methylation ratio remains significantly above unity[3].

Adenosine is removed by its rapid conversion to inosine, while homocysteine, a highly reactive substance, can be removed in four ways. The two important modes of control are: (i) the re-methylation of homocysteine using $N^5CH_3THFGlu$ *via* cobalamin-dependent methionine synthase to produce methionine (Fig. 2); and (ii) the transsulphuration reaction where homocysteine is catabolised by cystathionine synthase and cystathionine lyase to produce cystathionine and cysteine respectively, both of which are pyridoxine dependent (Fig. 2). Cysteine then produces taurine, sulphate and pyruvate that can be used to provide energy. Homocysteine is thus at a pivotal position; at times of excess supply of methionine the catabolic reaction occurs, while at times of relative deficiency homocysteine is re-methylated. Under normal circumstances, the re-methylation reaction is used more frequently than the catabolic transsulphuration pathway, usually at a ratio of 2–3 to 1, since methionine synthase has a lower K_m than cystathionine synthase. Thus, the transsulphuration reaction only comes into operation under conditions of a high methionine load, as may occur following a meal[3].

The other two methods of eliminating homocysteine are to use the betaine methyltransferase pathway to re-methylate homocysteine to methionine, this however is not present in the brain (*vide infra*) or for homocysteine to leak out of the cell into the blood stream.

Control mechanisms for the methylation reactions

The cell has a variety of methods to ensure that the SAM to SAH ratio is maintained at the most effective level. When SAM levels are low, the endogenous synthesis of methionine and SAM are maintained by the production of $N^5CH_3THFGlu_5$. This is accomplished by the reduction of

$N^5N^{10}CH_2$-$THFGlu_5$ *via* MTHFR. The sole function of $N^5CH_3THFGlu_5$ is to re-methylate homocysteine since, under physiological conditions, folate reduction *via* MTHFR is not reversible. When SAM levels are high, SAM inhibits MTHFR allosterically, thus reducing the endogenous synthesis of $N^5CH_3THFGlu_5$, methionine and SAM. At the same time, the elevated levels of both SAM and SAH enhance the activity of cystathionine synthase, thus increasing the rate of transsulphuration and the catabolism of SAM and its sulphur skeleton[20]. The excess methyl groups of the SAM molecule about to be catabolised are removed *via* glycine methyl transferase, which converts glycine to sarcosine, an inert end product of internal metabolism. Glycine methyl transferase is minimally affected by SAH inhibition, but is inhibited by high levels of $N^5CH_3THFGlu_5$. Build up of the latter occurs in situations associated with methionine synthase dysfunction, as may occur with cobalamin deficiency or following nitrous oxide (N_2O) anaesthesia[21].

Relationship to the central nervous system

While these control systems are present in many organs, especially the liver, their effect on the methylation cycle in the central nervous system (CNS) is limited. There is little evidence that cystathionine lyase is present in the CNS. Thus, under circumstances of SAM excess, homocysteine cannot be catabolised to cysteine and accordingly accumulates as cystathionine in the brain[22].

Equally, there is no alternative method whereby homocysteine can be re-methylated to methionine, since betaine methyl transferase is not present in the brain[23]. Thus, the brain is totally reliant on methionine synthase to metabolise homocysteine and SAH (Fig. 2).

Cobalamin deficiency and methionine synthase inhibitors

When either cobalamin is deficient or defective, or methionine synthase, the enzyme for which it is a cofactor, is inhibited by the anaesthetic gas N_2O, SAM levels will tend to fall. Under these circumstances, SAM levels can only be maintained by increased uptake of methionine from the blood, which, in turn, will be derived either from dietary methionine or by endogenous synthesis in those organs which, unlike the brain, do possess betaine methyltransferase[23]. At the same time, homocysteine levels will rise, since in the brain there is no alternative means of re-methylating homocysteine and, since the transsulphuration pathway is incomplete, homocysteine cannot be degraded beyond cystathionine[22]. Under these circumstances, homocysteine in the presence of adenosine

will be reformed to SAH, as the hydrolase enzyme favours the back reaction[19]. As a result, SAH levels in the brain rise dramatically and SAM levels will tend to fall[24], the so-called methylation ratio of SAM/SAH falls and the SAM-mediated methylation reactions are inhibited[25]. In fact, there is a close correlation between the level of the methylation ratio and the activity of methionine synthase in the brain[26], which demonstrates that the level of the ratio is markedly dependent on normal function of methionine synthase.

In contrast, in rapidly dividing cells such as the bone marrow, when methionine synthase is inhibited, the re-synthesis of $THFGlu_5$ from $N^5CH_3THFGlu_5$ is blocked and this impairs DNA synthesis. This is the basis of the 'methyl folate trap hypothesis' of cobalamin induced megaloblastic anaemia. However, as explained above, cell division is minimal in the brain, which accordingly has a minimal demand for DNA synthesis.

Thus although there are intricate mechanisms in place to ensure that SAM levels are maintained in situations of potential folate deficiency, the cell has no fall-back position for cobalamin deficiency which it does not expect to occur.

Characteristics of SAH induced inhibition of SAM methylation reactions

It is important to realise that SAH induced methylation inhibition is not a uniform phenomenon. The following characteristics have been demonstrated:

1 The degree of SAH induced inhibition for any given level of SAH varies for each methyltransferase enzyme.

2 The pattern of inhibition induced by rising values of SAH varies for any given enzyme.

3 The degree of inhibition for given SAH values of any particular enzyme may vary from organ to organ.

4 For any enzyme, in any particular body organ, and for any given level of SAH there is a marked species variation. For instance, the degree of inhibition of protein 'O' or 'N' methylation in the brain is lower in rats than in pigs, which in turn is lower than in humans.

These conclusions derive from experiments performed on pigs using N_2O anaesthesia to inhibit methionine synthase[27]. Nevertheless, when a series of transmethylation enzymes which are present in the brain were assessed in the presence of 50 μM SAH, a level of SAH which has been shown to be present in the brain of pigs following N_2O anaesthesia and

which compares with the normal 5 μM in air[28], there was at least 50% inhibition of the function of all the enzymes. This was especially so for protein 'O' and 'N' methylase enzymes and, accordingly, these enzymes are particularly appropriate for investigating the significance of SAH induced inhibition under different experimental conditions, both in the pig[28] and human brain[29].

Potential toxic effects of SAH inhibition of methylation reactions

The effect of SAH inhibition of methylation reactions potentially gives a wide variety of pathogenic effects. The classical explanation for cobalamin neuropathy is that there is a failure of myelin synthesis, through hypomethylation of post-transcriptional myelin basic protein, at the 107 arginine site. This it was considered would destabilise the protein and cause neuropathy[30]. However, it is perfectly possible that hypomethylation might affect other tertiary protein structures. Recently, it has been suggested that tumour necrosis factor (TNF) induced cell death may be mediated by a non-classical pathway (*i.e*, not *via* free oxygen radical induction) which is controlled by a transmethylation enzyme, carboxy-O-methyltransferase. This in turn controls the methylation of RAS, which when methylated switches off RAS induced cell death *via* specific ICE proteases. Carboxy-O-methyltransferase is in turn inhibited by SAH[31]. Thus, in the presence of high SAH levels, carboxy-O-methyltransferase will be switched off and the alternative TNF induced cell death pathway is activated.

Potential toxic effects of hyperhomocysteinaemia

It is also possible that the pathogenic agent in brain diseases associated with hyperhomocysteinaemia may in fact be homocysteine itself. Elevated levels of homocysteine are associated with and induced by low levels of cobalamin, folate and pyridoxine, which are cofactors for the three enzymes responsible for homocysteine metabolism, namely methionine synthase, MTHFR and cystathionine synthase, respectively. Genetic defects in MTHFR and cystathionine synthase and inherited abnormalities of cobalamin also cause hyperhomocysteinaemia[32]. Deficiency of these vitamins are common in demented patients, whether due to vascular dementia or to Alzheimer's disease (AD)[33,34]. Vascular disease due to atherosclerosis is well known to be associated with hyperhomocysteinaemia[35], it may also be associated with AD[34]. The cause of the pathogenic

process, which relates hyperhomocysteinaemia to dementia whether due to diffuse cerebro-vascular disease in vascular disease or affecting specific areas of the brain such as the hippocampus in AD[34], is still debated. It may be related to microvascular disease[36, 37] or to a neurotoxic effect causing activation of N-methyl-D-aspartate receptors which leads to cell death[38]. Homocysteine may also be converted to homocysteic acid which has an excitotoxic effect on neurons[39] which in turn is neurotoxic. Whatever the mechanism, hyperhomocysteinaemia, in association with low levels of cobalamin pyridoxine and folate, is known to induce impairment of cognitive function[40].

Animal models of cobalamin/folate induced brain damage

Animal models of cobalamin related neuropathy have been of three varieties: (i) dietary cobalamin deficiency[41]; (ii) N_2O anaesthesia[22]; and (iii) total gastrectomy[42,43]. Dietary deficiency is difficult to maintain, takes a long time to induce the disease and tends to give variable results. Nitrous oxide has produced lesions in monkeys[22], fruit bats[44] and pigs[24]. However, in a large majority of the animals tested, including rodents, it has failed to induce the lesion[26]. Recently, it has been demonstrated in rats that total gastrectomy will produce cobalamin deficiency associated neuropathy in rats within weeks[42,43]. This has the potential to offer a commercially viable model of the brain disease, which could further define the biochemical basis of the disease.

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