S-HT. The binding also did not depend on the anomeric conformation of the glycosidic linkage, as revealed by CMP-NeuAc (6) and several sialoglycoconjugates (6).

The analysis of the interactions has identified those features in both S-HT and sialic acids that are important for establishing the specificity of binding. These findings have implications for biological systems and, in addition, identify aspects of the specificity that affect the use of immobilized S-HT in the affinity chromatography of sialic acids.

Enzymes of carbohydrate and amino acid metabolism in the human brain

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The brain is potentially able to oxidize glutamine or glutamate by using the enzymes glutaminase, glutamate dehydrogenase and 2-oxoglutarate dehydrogenase (Yu et al., 1982; Tildon & Roeder, 1984). The products of glutamine metabolism, glutamate and 2-oxoglutarate, stimulate the phosphorylation of pyruvate dehydrogenase (Sieghart, 1981), and glutamine will compete with glucose for oxidation in dissociated brain cells (Roeder et al., 1984). These effects may be related to the reciprocal arrangement between the enzymes of glutamine metabolism and pyruvate dehydrogenase reported below.

Twenty-two neurologically normal putamen autopsy samples were supplied by the M.R.C. Brain Bank, Cambridge, and homogenized in 10 vol. of 0.32 M-mannitol (pH 7.4). Glutaminase (GLNASE) and glutamate dehydrogenase (GDH) were assayed in the homogenate. Glutamine synthetase (GS) was assayed in the supernatant, and pyruvate dehydrogenase (PDH) and 2-oxoglutarate dehydrogenase (2OGDH) in the resuspended pellet (10 vol. of 0.32 M-mannitol) of a 27 000 g centrifugation at 4°C. GLNASE and GS were assayed radiometrically, separating [14C]-glutamate and [14C]glutamine on Dowex 1×8 acetate columns (Pishak & Phillips, 1979). PDH and 2OGDH were assayed by trapping 14CO2 evolved from [14C]pyruvate or [14C]glutamate by the fluorimetric assay of Lowry & Passoneau (1972). Data for each sample were stored on disc together with data concerning the age of the patient, cause of death, time taken for the tissue to reach 4°C and 20°C, and the duration of storage at 70°C. Association between these factors could be visualized and analysed by using a linear regression program with a PDP12 computer. Age or post-mortem variables did not relate to the activity of any enzyme. GLNASE was adversely affected by pulmonary illness, which was the listed cause of death for three of 22 subjects (c.f. Butterworth et al., 1983). Removal of this group did not affect the nature of the relationships between the enzymes in the rest of the group and all data were included for analysis. It was not possible to categorize agonal status for other causes of death for the data available.

The activities of GS, GLNASE, GDH and 2OGDH were positively associated with each other (P < 0.05 for all permutations, linear regression), GS, GLNASE and 2OGDH activities were each inversely related with PDH activity (P < 0.05). The slope for PDH vs GDH was negative, but the association was not linear.

The results support a reciprocal arrangement between the enzymes of glutamine metabolism and pyruvate oxidation, and perhaps outline the enzymatic machinery through which oxidation of glutamine is able to inhibit the oxidation of glucose (Roeder et al., 1984). PDH activity is subject to feedback regulation by the products of tricarboxylic cycle metabolism (Jope & Blass, 1975) and the reciprocal relationship between the tricarboxylic cycle enzyme 2OGDH and PDH might be explained on this basis.

The synthesis (Norenberg & Martinez-Hernandez, 1979) and oxidation of glutamine are likely to be primarily an astrocytic phenomenon (Yu et al., 1979; Tildon & Roeder, 1984), but a proportion of the glutamate used by GS may be derived from glutamate which has been synthesized from glucose in neurons, which, after release, is taken up by astrocytes (Stewart & Rosenberg, 1979). Shank & Campbell (1984) have suggested that neuronal tricarboxylic acid cycle intermediates lost to glutamate synthesis may be replenished by malate or 2oxoglutarate synthesized in astrocytes and transported to neurons. Two tricarboxylic acid cycles in glia and neurons may thus be linked by glutamaterglutamate transfer of nialate/2-oxoglutarate, and neuronal to glial glutamic transfer, and by incorporation of malate into the tricarboxylic cycle, which might thus become an integral part of this expanded metabolic cycle which may explain their positive association with 2OGDH and inverse association with PDH. Oxidation of the carbon skeletons of the neurotransmitter amino acids may be necessary to counteract the problems associated with diversion of glucose metabolism into their synthesis. As the process is contained and cyclical, this does not detract from the concept that exogenous glucose is the major respiratory substrate of the brain, but illustrates the necessity of cerebral amino acid and carbohydrate metabolism. The situation has obvious analogies with the γ-aminobutyric acid shunt, where the carbon skeleton of γ-aminobutyric acid is recovered as succinate.

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Abbreviations used: GLNASE, glutaminase; GDH, glutamate dehydrogenase; GS, glutamine synthetase; PDH, pyruvate dehydrogenase; 2OGDH, 2-oxoglutarate dehydrogenase.

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Abnormal carbohydrate and amino acid metabolism in the Huntington disease brain

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By adopting facilities which allow the oxidation of the neurotransmitter amino acids, the brain is able to counteract the problems associated with diversion of glucose carbon into their synthesis. The brain is critically dependent upon a sustained supply of glucose, and insufficient use of this substrate might be expected to adversely affect neuronal function and viability (Siesjö, 1978). Huntington's disease is an adult-onset genetically determined degenerative disorder in which basal ganglia γ-aminobutyric acid neurons are particularly vulnerable (Bird et al., 1974). The reasons for this are not clearly understood, but attention has often been focused upon abnormal carbohydrate metabolism as an underlying factor. This is supported by reduced striatal glucose utilization in Huntington’s disease patients (Kuhl et al., 1982), and by reduced striatal activities of phosphofructokinase and pyruvate dehydrogenase at autopsy (Bird et al., 1977; Sorbi et al., 1983). Such observations, of course, do not discriminate between reduced oxidative capacity as a result or cause of cell death. However, the present study suggests that the relationships between amino acid and carbohydrate metabolism that normally ensure the efficient utilization of glucose are disrupted in Huntington’s disease. This may allow the hypothesis that cell death may be related to inefficient utilization of cerebral glucose, although the triggering factor remains unresolved.

Twenty-two Huntington’s disease (HD) putamen autopsy samples were age-matched with the control group (CON) previously described (Carter, 1985b) (mean age ± S.E.: 63.4 ± 2.2 years, HD 60.7 ± 1.8 years) and there was no difference between the autopsy intervals to 4°C (CON 3.0 ± 0.3 h, HD 3.0 ± 0.5 h) or −20°C (CON 52.8 ± 5.5 h, HD 44.7 ± 7 h). Glutamine synthetase (GS), glutaminase (GLNASE), glutamate dehydrogenase (GDH), 2-oxoglutarate dehydrogenase (20GDH) and pyruvate dehydrogenase (PDH) were assayed as in Carter (1985b) and in the same experiments as control tissue. The activities of each of these enzymes were significantly reduced in the Huntington’s disease samples (means ± S.E.; P < 0.05; Student’s t-test): PDH, CON 13.7 ± 1.3, HD 4.9 ± 0.6; 20GDH, CON 7.49 ± 0.6, HD 2.34 ± 0.42; GS, CON 487 ± 69, HD 142 ± 38; GLNASE, CON 6550 ± 1199, HD 1072 ± 440; GDH CON 818 ± 49, HD 558 ± 40 nmol/min/g of protein per h,1. The significance of reductions in GS (Carter, 1982), GLNASE (Butterworth et al., 1983), PDH (Sorbi et al., 1983) and 20GDH (Carter, 1985a) has previously been discussed, and the main emphasis of this study concerns the relationships between the activities of these enzymes.

As seen in normal tissue (Carter, 1985b), there was a linear positive association between 20GDH and GS, 20GDH and GLNASE, and GS and GLNASE (P < 0.05; Linear regression analysis). Other associations between 20GDH, GS, GLNASE and GDH, although positive, were not linear. In contrast with the inverse relationships between PDH and 20GDH, GDH, GLNASE and GS seen in normal tissue (Carter, 1985b), each of these associations was positive in the Huntington’s disease putamen, and linearly related (Carter, 1985b), to a positive association in Huntington’s disease tissue. Considerable care must be taken in the interpretation of these results. The listed cause of death for 12 of 22 of the Huntington’s disease samples was bronchopneumonia, whose chronic anoxic effects might reasonably be expected to influence cerebral energy metabolism. Because this was the listed cause of death in only three of 22 of the control group, valid analysis of the effects of this variable was not possible. In these three controls, there was no significant association between PDH and the enzymes of glutamine metabolism, although the slope for each association was positive as in Huntington’s disease tissue. Bronchopneumonia may therefore contribute to the reversal of relationships between these enzymes seen in Huntington’s disease.

In normal tissue, it was suggested that the reciprocal arrangement between the tricarboxylic acid cycle enzyme PDH and PDH could reflect the regulation of PDH by the functional status of other cycle enzymes (Carter, 1985b). The listed cause of death for 12 of 22 of the Huntington’s disease samples was bronchopneumonia, whose chronic anoxic effects might reasonably be expected to influence cerebral energy metabolism. Because this was the listed cause of death in only three of 22 of the control group, valid analysis of the effects of this variable was not possible. In these three controls, there was no significant association between PDH and the enzymes of glutamine metabolism, although the slope for each association was positive as in Huntington’s disease tissue. Bronchopneumonia may therefore contribute to the reversal of relationships between these enzymes seen in Huntington’s disease.

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