GENERAL INTRODUCTION

In most animal tissues, glutamine and glutamic acid are predominant among the amino acids undergoing active metabolism. The acid and the amide constitute upto 60% of the free α-amino nitrogen in the mammalian brain. In brain the greater part of the deamidation is catalyzed by the enzyme glutaminase (1). Glutamic acid, produced as the result of deamidation, plays a great role in normal functioning of brain cells (2). Glutamic acid gives rise to the formation of γ-aminobutyric acid (GABA) which also plays a very significant role in normal functioning of complex neuronal network. Moreover, the brain, more than any other organ, is subjected to sudden spurts of activity resulting in a high rate of glycolysis and in localized acidosis. In these circumstances glutaminase might act as an accessory buffering mechanism although it might be recognized that the hydrolysis of glutamine produces an acid as well as a basic group. However, the newly formed acid may be more mobile or more rapidly metabolized or more suited to sequestration than the product of glycolysis. On the other hand, it might be the function of glutaminase to maintain the already high level of glutamic acid in the nerve cell. The function of the counterpart enzyme glutamine synthetase is understandable in the context of ammonia disposal and transport, but glutaminase reverses the synthesis and seems to undo a vital reaction of neuronal metabolism (8).

It was Krebs (3) who first distinguished two types of glutaminase viz. (a) brain type and (b) liver type. The brain type glutaminase was shown to be responsible for the hydrolytic phosphate activated deamidation and is associated with mitochondrial fraction. It was named subsequently as glutaminase I. The liver type glutaminase known as glutaminase II appears to be exclusively involved in liver function, found in hepatic tissues of all age. It is water soluble and active in presence of sodium pyruvate and not in presence of phosphate.

Recent research has indicated that the transport of ions across the mitochondrial membrane is a reaction of fundamental importance and is intimately dependent on the structural and metabolic integrity of the mitochondria (13,30).

Present investigation deals with the conditions influencing glutaminase activity in intact mitochondria. This may provide information on the physiological function in brain of this enzyme which is not usually functioning except under some special conditions produced in vivo (2). To create physiological condition enzyme activity is determined at the physiological pH viz. pH 7.4.

Some special features of brain mitochondria

Since the brain utilizes more than 25% of the total body oxygen and glucose, the concentration of mitochondria in brain tissue would be expected to be unusually great. About 15% of the total protein of whole rat brain is mitochondrial, although this figure may be as high as 25% in cerebral grey matter. Mitochondria are present throughout the perikaryon, cytoplasm, dendrites, axons and nerve endings. Such regions as the nerve endings, the axon hillock and nodes of Ranvier are particularly abundant. Mitochondria are especially concentrated in those regions of the neuron where excitatory events are most frequent, as at the synapses and the axon hillock (16).

Brain, as well as most other tissues, mitochondria are elongated cytoplasmic organelles, 0.3 - 1.0 μ in width and upto 5 μ in length, comprising of a surrounding and inner membranous structure.

Rat brain mitochondria contain about 35% lipid (dry weight), 57% protein and 5% ash (magnesium, calcium, sodium and potassium salts). Of the lipid, about 75% is phospholipid, 20% is cholesterol and remainder is diglycerides plus triglycerides (17, 18, 19). The major phospholipids are lecithin and serine phosphatides (phosphatidal and phosphatidyl); cardiolipin, is present in brain mitochondria in about the same concentration as in liver or heart

mitochondria (17). Analysis of brain mitochondria reveals 40% to be saturated and 60% unsaturated fatty acids. The fatty acid composition of brain and liver mitochondria is quite similar, the outstanding difference being the much higher concentration of linoleic in liver and C 22:6 in brain mitochondria. The fatty acid composition of the diet to a limited degree can influence the composition of brain mitochondria, particularly in the case of polyunsaturated acids (20). The high concentration of polyunsaturated fatty acids and low cholesterol help maintain mitochondrial membranes in the liquid-expanded state essential for rapid transport.

The turnover of mitochondrial phospholipid and protein increases sharply at the time of neuronal maturation and myelination in the rat brain (age 10-15 days) (19). Although the number of mitochondria also increases rapidly during this period, as evident by the increase in mitochondrial enzyme, there does not appear to be any marked change in the enzymic concentration of structural composition of mitochondria (21). As mitochondria first appear in the embryonic brain, they seem to have their full complement of enzymes, furthermore, their lipid composition does not vary significantly during the period of neuronal maturation (19). At this stage in neuronal maturation, where anaerobiosis is replaced by aerobiosis and oxidative enzymes appear, mitochondria become visible in the neuronal cytoplasm. Prior to this stage oxidative enzymes and mitochondria are sparse.

Although neuronal mitochondria are present in late embryonic development, their number greatly increases just after birth prior to the onset of myelination and neuronal maturation. Since lipid and protein metabolism greatly diminish in the brain after myelination and neuronal maturation, it is to be expected that the enzymic profile of brain mitochondria would reflect this change. The rate of amino acid incorporation by brain microsomal systems is greater in the presence of mitochondria from immature rat brain as compared to mitochondria of mature brain or even liver mitochondria (22). Furthermore, while thyroxine inhibits protein synthesis in the presence of mitochondria from mature brain, it actually stimulates with mitochondria from immature brain and liver (23). Evidently an unidentified mitochondrial factor present only in immature brain, interacts with thyroxine to stimulate the transfer of soluble RNA-amino acid into ribosomes. From turnover studies of mitochondrial lipids, the half life of brain mitochondria was estimated to be as long as 2 years (24).
