Problems and prospects in neurochemistry

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The importance of neurochemistry in understanding the functional basis of the nervous system was emphasized. Attention was drawn to the role of lipids, particularly the sphingolipids, whose metabolic abnormalities lead to 'sphingolipidosis' in the brain and to gangliosides, which show growth-promoting and neuritogenic properties. Several questions that remain to be answered in this area were enumerated. It was pointed out that neurons make a large number of proteins, an order of magnitude higher than other cells, and several of these are yet to be characterized and their functional significance established. Myelination and synaptogenesis are two fundamental processes in brain development. Although much is known about myelin lipids and proteins, it is not known what signals the glial cell receives to initiate myelin synthesis around the axon. In fact, the process of myelination provides an excellent system for studying membrane biogenesis and cell–cell interaction. Great strides were made in the understanding of neurotransmitter receptors and their function in synaptic transmission, but how neurons make synapses with other specific neurons in a preprogrammed manner is not known and requires immediate study. In this context, it was stressed that developmental neurobiology of the human brain could be most profitably done in India. The importance and complexity of signal transduction mechanisms in the brain was explained and many fundamental questions that remain to be answered were discussed. In conclusion, several other areas of contemporary research interest in the nervous system were mentioned and it was suggested that a 'National Committee for Brain Research' be constituted to identify and intensify research programmes in this vital field.

ABOUT four years ago, I was asked to review the past and present of neurochemical research activities in India. There I wrote: 'All phenomena which characterize life processes should ultimately be amenable to explanation in chemical and physical terms. The functioning of the nervous system, the most challenging one among the biological phenomena, is no exception. Even higher functions of the brain such as thought and memory will have to be explained eventually in terms of the chemistry of the constituent molecules and their physical properties. Therefore, neurochemistry – the chemical approach to the study of the nervous system – would be vital to our understanding of the nervous system[1]. Even 'consciousness' is likely to have a molecular basis.

The chemical exploration of the brain began at the turn of the century. The basic biochemical components of the nervous system are now identified and we can account for almost 100% of the mass of the neuron. It is unlikely that any new class of organic molecules peculiar to the nervous system will be discovered in future. This means that the special functions of the nervous system will have to be explained in terms of the molecules that are already known. Much has been discovered regarding the biochemistry of the brain. Its continuous dependence on the blood supply of glucose is now well known and the metabolic pathways of carbohydrates are also well established. Brain contains a very large amount of lipid and a bewildering variety of lipid components exist in it[2]. While no particular lipid component is characteristic of the nervous tissue, some lipids occur in large amounts in the brain. Their functional significance is unknown at present. The sphingolipids occur in high concentrations in the brain and their abnormal metabolism, usually due to the lack of a particular catabolic enzyme, leads to their accumulation specifically in the nervous tissue, causing a number of diseases, commonly known as 'sphingolipidosis', which result in severe mental retardation. Why should the accumulation of a single sphingolipid component lead to mental retardation? What role does it play in neural communication and eventually in thought and higher functions of the brain?

Ethanolamine and choline plasmalogens are major phospholipid components in the nervous tissue and in muscle. Do they have any role in excitability of the membranes? Similar questions can be asked of many other lipids. Interestingly, gangliosides – another major group of lipids in the brain, have been implicated in cellular recognition as adjuncts at receptor sites and show growth-promoting and neuritogenic properties. Much remains to be discovered about the function of these complex lipids in the brain. By far, proteins are the most important molecules with an array of biological functions. It appears that gene expression is maximal in neurons among the various cells, i.e. species of mRNA that are found in neurons are about an order of magnitude higher than in other cells. This means that a very large number of proteins are made and most of these are yet to be identified. While several of them are

for house-keeping functions, undoubtedly there are many proteins specific to the nervous tissue. Their isolation, characterization and determination of functional significance remains a challenging task for the neurochemist.

Myelination and synaptogenesis are two fundamental neurobiological processes on which the ultimate functional ability of the brain depends. In the last two decades, significant progress has been made in the molecular biology of myelin. We have established the biosynthesis of ethanolamine plasmalogens, the main phospholipid components of the myelin membrane, and in this context demonstrated for the first time the ability of the developing brain to synthesize long-chain alcohols which are precursors of these ether lipids. The enzymes for the biosynthesis of ether lipids as well as that of other myelin lipids such as sulfatides and cerebrosides increase enormously during the active period of myelination. In many of the myelin-deficient mice such as the quaking, Jimpy Shriverer mutants, myelin lipids are not synthesized at adequate rates. Excellent procedures for myelin isolation are now available which have helped in the isolation of myelin proteins and study of their synthesis. A great deal of information is now available on the structure of myelin proteins, their genes and the various gene products obtained by alternate splicing[3]. However, it is not known what signals the glial cell receives to initiate myelin synthesis around the axon. Our recent experiments have indicated that the Wolfgram protein may have a role in the initiation of myelin synthesis, but many important questions remain to be answered on myelination and on the causes of demyelination. In addition, we now know that several myelin proteins are post-translationally modified. They undergo phosphorylation, acetylation and acylation. What is the importance of these alterations in myelin function? For a long time myelin was believed to be an inert membrane merely acting as an insulating material around the axon, facilitating saltatory conduction. This view is no more true. A large number of enzymes which include some of the signal transduction pathways and some receptors have recently been found in myelin, necessitating a radically new look at myelin function. In fact, the process of myelination provides an excellent opportunity to understand membrane biogenesis, cell–cell interaction and many other fundamental phenomena.

Crucial to the function of neural network is synaptogenesis and the events that occur at the synapse. In fact, this is one of the most active and promising areas of current research. Several neurotransmitters are now known, and their metabolism has been worked out. The mechanistic details of their release, re-uptake or degradation are also known. It is the variety of receptors with which neurotransmitters bind to elicit their action that is now attracting the attention of the neurochemist. For each neurotransmitter a set of distinct receptors have now been recognized and their molecular biology is beginning to be understood. Great strides have been made in the recent past in delineating the structure–activity relationship and in localizing them within the synaptic membrane. Simultaneously, similar information is also becoming available on ion channels such as the sodium channel of the neural membrane. The application of molecular biology techniques to this area is yielding valuable information. However, how neurons make synapses with other specific neurons in a seemingly preprogrammed manner is still unclear and this is an area that requires immediate attention. In this context, we observed a few years ago that much of the muscarinic cholinergic receptor ontogeny in the human brain occurs in the third trimester of foetal life. During this period the high affinity dopaminergic receptors also arise in the human brain. Such studies on human foetal brains are extremely important. In fact, developmental neurobiology of the human brain could be most profitably done in India as facilities such as foetal brain repositories have already been initiated.

For some time it has been known that there are two main pathways for signal transduction, viz. the cyclic AMP pathway and the phosphatidylinositol pathway. The latter is now recognized as more versatile and the recent advances made in this area are very fascinating and extremely complex. In this pathway, in addition to the receptor proteins, GTP-binding proteins (G proteins), phospholipase C, diglyceride kinase, protein kinase C and an intracellular receptor for inositol-triphosphate (IP_3) participate. In the neural tissues, it has now been shown that in addition to IP_3, a number of inositolpolyphosphates are generated depending upon the brain area and the neurotransmitter. However, so far, a receptor for only IP_3 has been identified. What then is the role of all the other inositolpolyphosphates generated within the cell? How do they regulate the intracellular calcium level? There appear to be several G proteins – some of them are characteristic of specific areas of the brain. Similarly, several isoenzymes of phospholipase C, diglyceride kinase and protein kinase C have now been identified. They have a specific distribution within the nervous system. Recent studies on the structure of the IP_3 receptor within the brain have shown that several isomeric proteins arise due to alternate splicing of a single gene and these show distinct differences in their binding properties with IP_3 and show characteristic distribution in various areas of the brain[4]. Therefore, an enormous number of permutations and combinations of the various elements of the phosphatidylinositol signal pathway is possible and the expression of a particular set in a given neuron would make it unique. This area continues to be an active area for further research.

In conclusion, many other areas of contemporary research interest may be mentioned. Some of these are:

1. Molecular biology of early response genes and transcriptional regulation of CNS development and function.
2. Molecular determinants of neural regeneration.
3. Cytokines: their relation to neurotrophic and neurite growth.
4. Molecular signalling for cell growth.
5. Role of protein tyrosine phosphorylation in neural tissues.
6. Calcium dynamics and neural function.
7. Molecular biology of myelin: the glial–neural relationship.
8. Excitability – its regulation by eicosanoids and other lipid mediators.
9. Monitoring cellular biochemistry in intact neuronal systems.
10. Ageing of the brain.

Unfortunately, the research effort in any of these areas in India is subcritical. There is no need to emphasize the importance of brain research. I wish to suggest that a 'National Committee for Brain Research' be constituted to identify and encourage research programmes in this vital field.

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X-Ray studies on the bilayer structure of trypsin-treated rat brain myelin

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Trypsin-treated rat brain myelin was subjected to biochemical and X-ray studies. Untreated myelin gave rise to a pattern of three rings with a fundamental repeat period of 155 Å consisting of two bilayers per repeat period, whereas myelin treated with trypsin showed a fundamental repeat period of 75 Å with one bilayer per repeat period. The integrated raw intensity of the $h = 4$ reflection with respect to the $h = 2$ reflection is 0.38 for untreated myelin. The corresponding value reduced to 0.23, 0.18, 0.17 for myelin treated with 5, 10, 40 units of trypsin per mg of myelin, respectively, for 30 min at 30°C. The decrease in relative raw intensity of the higher-order reflection relative to the lower-order reflection is suggestive of a disordering of the phosphate groups upon trypsin treatment or an increased mosaicity of the membrane or a combination of both these effects. However, trypsin treatment does not lead to a complete breakdown of the membrane. The integrated intensity of the $h = 1$ reflection, though weak, is above the measurable threshold for untreated myelin, whereas the corresponding intensity is below the measurable threshold for trypsin-treated myelin, indicating a possible asymmetric to symmetric transition of the myelin bilayer structure about its centre after trypsin treatment.

SEVERAL studies have been made on the susceptibility of the proteins in the myelin membrane to various proteases since proteases are thought to be involved in the etiology of demyelinating diseases such as multiple sclerosis and experimental allergic encephalomyelitis causing disruption of the myelin structure. Supporting this contention, Cammer *et al.*[1,2] have demonstrated that neutral proteases secreted by macrophages degrade the basic proteins in the myelin membrane. Myelin basic proteins were also shown to be markedly hydrolysed by endogeneous neutral proteases of the brain[3], myelin[4,5], serum[6] and leukocytes[7]. Therefore, it would be of interest to study the effect of proteases on the bilayer structure of myelin.

X-ray diffraction studies on a variety of myelinated nerves from both the peripheral nervous system (PNS) and the central nervous system (CNS) have been reported[8]. Similar studies on PNS myelin at 10 Å

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