Post-mortem neurochemistry of schizophrenia

M C ROYSTON AND M D C SIMPSON

Introduction

The notion that schizophrenia has an organic basis has been considered likely for as long as the syndrome has been accepted. In a paper written in 1915 Bleuler\(^1\), responsible for much of the form of our current usage of the term ‘schizophrenia’ prophetically captured the ideas underpinning contemporary hypotheses of the organic aetiology of schizophrenia:

One must acknowledge that at least the great majority of clinical pictures which are now collected under the name *Dementia praecox* rest on some toxic action or anatomical process which arises independently of psychic influences.

In the ensuing years there have been many studies searching for the abnormal anatomy and toxic substances. These two lines of study examine complementary issues of brain abnormalities apparent at the levels of structural and neurochemical anatomy. In the study of the post-mortem schizophrenic brain there are many important questions which are being addressed by modern neuropathology and neurochemistry. Until recently, there had been little advance in either field but in the last 20 years technical advances and the refinements of research methodologies have produced a new body of evidence which has begun to fit together the pieces which form the jigsaw of schizophrenia.

In neurochemical studies, the initial approach did not focus on the post-mortem brain, but rather on the search for a naturally occurring ‘psychomimetic’ chemical in the various body fluids of schizophrenic patients. This produced many candidate chemicals and putative markers of aberrant biochemistry all of which have subsequently been dismissed following rigorous scientific enquiry. The endogenous psychotogenic ‘pink spot’ is a good example of such an endeavour\(^2\).

Post-mortem neurochemistry, in which the substrate of investigation is the...
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brain itself, has proved more illuminating. The investigation of the mode of action of those classes of drugs having a beneficial effect in the treatment of schizophrenia has led to the major neurochemical theory of schizophrenia, namely the dopamine theory, which will be considered in detail in this chapter. More broadly than an individual theory, the reversal of at least some of the symptoms of schizophrenia by a drug carries the implication that there is a modifiable chemical abnormality in the brain. This has been a major impetus to the research and development of "neurochemical" theories of schizophrenia. This approach integrates basic information concerning the chemical and electrical activities, as evidenced by neurotransmitter function, with the higher psychological functioning of the brain, providing a link with the disturbances of thought and perception which characterize the clinical picture of schizophrenia. Over the last 30 years there have been enormous technical advances in the field of analytical biochemistry allowing the chemical identification and quantification of an increasing number of transmitter substances in the central nervous system, many of which have been subsequently implicated in schizophrenia.

The development of complementary techniques for the analysis of neurotransmitter receptors has also been important in stimulating new post-mortem neurochemical studies. These methodologies are dependent on the pharmacological principles of ligand binding to specific receptor sites in the tissue studied. By using a ligand labelled with a radioisotope, generally tritium, the sensitive detection technique of radio-assay may be used. Studies may be performed on dissected portions of tissue from post-mortem or more rarely biopsy samples and the pharmacological characteristics of a receptor defined. An image of the distribution of receptors may be made using thin sections of tissue, incubated with a radioligand, and exposed to photographic film. The radiation from the receptor-bound ligand will then produce an image on the film, the intensity of which is in proportion to the tissue concentration of the receptor.

There has also been considerable progress in both neuroimaging and neuropathological studies (reviewed in a later chapter) which provide compelling evidence of a specific structural abnormality of the brain in schizophrenia. Studies of neurochemical pathology can provide complementary "functional" data to be interpreted in terms of structural pathological findings. Taken together, these lines of evidence support the now prevailing view that schizophrenia is "no longer a functional psychosis".

Neurotransmitter studies
Three major neurotransmitter systems will be discussed in detail in this review; the dopaminergic system, the neuropeptides and the amino acid neurotransmitters (glutamate and gamma amino butyric acid, GABA). The value of the results from neurochemical studies is critically dependent on the
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steps taken to eliminate a number of confounding variables which are inherent in post-mortem studies. These include the effects of agonal state, the deterioration of tissue from the time of death to freezing, the length of storage time, age of subjects, psychiatric diagnosis and selection of control subjects and the drug status of subjects before death with regard to psychoactive medications. Each of these factors may affect adversely the results of the study. For instance, a reduction of GABA content in the amygdala of schizophrenic subjects was reported by Perry et al[1] and by Spokes et al[2] but not by Cross et al[3]. Brain GABA levels were found to rise rapidly after death, thus measurement of the amount of transmitter is an unreliable marker for GABAergic functioning due to dependence on the post-mortem delay. Thus, there have been difficulties in validating findings and the interpretation placed on those findings. More recent studies, paying particular regard to these factors, have produced a degree of convergence in some important areas.

Dopamine

Since the early 1970s, hypotheses implicating abnormalities of central neurotransmission in the aetiology of schizophrenia have focused in turn on each of the major forebrain monoamine systems. However, no substantial evidence has been produced relating to dysfunction of either the noradrenergic or serotonergic pathways. In contrast, there has been support for overactivity in central dopamine-operated neurones[4]. The ‘dopamine hypothesis’ has emerged as the primary neurochemical concept, and has been a major influence on biological studies of schizophrenia.

Whilst persuasive, much of the evidence relating to dopamine overactivity in schizophrenia is indirect. This is true of the clinical observations which underpin the hypothesis, the most important of which is the finding that the antipsychotic potency of the neuroleptic drugs is directly related to their affinity as antagonists at the dopamine D2 receptor[5], and the converse observation that drugs which increase central dopamine function such as amphetamine and L-dopa exacerbate the positive symptoms of schizophrenia[6,7]. The inference which has been drawn is that the symptoms of schizophrenia are the consequence of an overactivity of certain dopaminergic pathways[8,9] and that this overactivity is a primary pathophysiological feature of the disease.

Post-mortem examination of the dopamine system has failed to produce unequivocal support for the idea of dopamine dysfunction in schizophrenia. However, for technical reasons the majority of studies have focused on the major target areas of the subcortical dopamine tracts within the basal ganglia, which contain high levels of dopamine, its metabolites and receptors. Whilst a case has been made for an involvement of the basal ganglia in schizophrenia[10], much evidence points to the pathophysiological involvement of the limbic forebrain and cerebral cortex. However, limbic and cortical areas
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receive a more diffuse dopamine innervation and are less amenable to study. Further technical advances, enabling examination of the mesolimbic and mesocortical dopamine systems at a detailed anatomical level may reveal highly localized and subtle, but critically important, abnormalities.

Dopamine receptor antagonism itself induces adaptive changes at several sites associated with the dopamine neurone. This has been a major difficulty in post-mortem studies of the dopamine system in schizophrenia, since the schizophrenic subjects have almost invariably received neuroleptic medication. The problem then arises of dissociating those effects which are primary to the pathology from those which arise as a result of drug therapy.

Whilst neurotransmitter turnover has been inferred post-mortem from tissue concentrations of transmitters and metabolites, the profound effects of dopamine receptor blockade on dopamine metabolism are a major confounding factor in studies of the brain concentrations of dopamine, its metabolites and associated enzymes. Several studies of this type have been reported, with occasional reports of significant abnormalities within the basal ganglia, but no general consensus. Early reports of increased dopamine concentrations in the caudate nucleus and nucleus accumbens were not confirmed in other studies, and particularly not in those patients who were drug-free at the time of death. Similarly, there are no marked or consistent changes in the tissue concentration of the major dopamine metabolite homovanillic acid (HVA) in the basal ganglia.

Dopamine metabolism in areas external to the basal ganglia has been less widely examined. However, the report of a unilateral increase in the dopamine concentration of the left amygdala has aroused much interest. Whilst Svendsen et al failed to replicate this finding, a later study by Reynolds confirmed the asymmetric increase in dopamine, with a smaller increase in HVA concentrations. A functional abnormality in amygdalar dopamine would have clear implications, since the amygdala is a key limbic structure, and is a major termination field for the mesolimbic dopamine pathway which has previously been implicated in the production of psychosis and antipsychotic drug action. However, the possibility that this may be a drug-induced effect can not be excluded; Bacoupolos et al described an increase in HVA concentrations in the frontal cortex which was related to previous neuroleptic exposure, and which persisted after long-term exposure to neuroleptic drugs.

Dopamine receptor supersensitivity has repeatedly been proposed to be the substrate for enhanced brain dopamine function. The search for an increased dopamine D2 receptor density in the striatum and nucleus accumbens of schizophrenic subjects has been widely confirmed, and was thought to be a primary pathological mechanism, indicating a hypersensitivity of dopaminergic synapses. However, neuroleptic administration to rodents was
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itself shown to induce an increase in dopamine D2 receptor density. The question of whether drug-naive or drug-withdrawn subjects exhibit dopamine D2 receptor hypersensitivity has been the subject of much research effort. Early studies included reports of either increased or normal striatal dopamine D2 receptor density in unmedicated schizophrenic subjects. More recently, Kornhuber et al in a group of 27 patients demonstrated an increase in D2 density only in those who had received neuroleptics during the 3 months prior to death. However, Seeman et al demonstrated a bimodal distribution of striatal D2 receptor density in 92 schizophrenic subjects, all of whom had received neuroleptic treatment prior to death, concluding that the higher receptor density mode could not be accounted for as an effect of neuroleptic administration alone. The issue remains incompletely resolved, despite the advent of in vivo receptor imaging methods in drug-naive subjects.

Dopamine D1 receptor density has been less widely examined, mainly because of the lack of suitable selective radioligands. Whereas an enhanced activity of the striatal adenylate cyclase associated with the D1 receptor has been reported, Carenzi et al found no such abnormality. Several radioligand binding studies of the striatal D1 receptor have failed to demonstrate any significant abnormality, although Hess et al reported a 50% reduction in striatal D1 sites labelled by [3H]-SCH23390. One further possibility which has been considered is that the functional interaction between D1 and D2 receptor activation may be abnormal. Recently, Seeman et al described a direct interaction between the binding of dopamine D1 and D2 selective ligands which was absent in over 50% of the schizophrenic striata examined.

Neuropeptides

As the number of peptide substances identified as candidate neurotransmitters in the central nervous system has increased so the list of potential aetiological factors in schizophrenia has grown. Approximately 25 separate neuropeptides have now been shown to be present within central neurones. Many of these have well-defined and highly specific patterns of distribution within the brain, and clearly serve important roles as central neuroregulators. Fewer than 10 of these substances have been subject to detailed investigation in post-mortem studies of schizophrenic brain.

An interest in the role of particular neuropeptides in schizophrenia has arisen from suggestions that some of these substances may be co-localized in the same neurones as other transmitter substances, and may modulate the effect of the primary transmitters. In particular, there is evidence that CCK is co-localized with tyrosine hydroxylase and may exhibit a functional relationship with dopamine. Neurotensin may be another dopamine co-transmitter. Both of these peptides have been shown to inhibit dopamine agonist-driven
behaviours by an action which does not involve direct antagonism of dopamine receptors. Abnormalities of dopamine–neuropeptide interactions are therefore potential pathogenetic mechanisms in schizophrenia. Moreover, distribution studies of certain neuropeptides within the brain have associated them particularly with the limbic cortex, being localized in well-defined neuronal networks. In view of the evidence which implicates limbic system dysfunction in schizophrenia, studies of neuropeptide markers are of great interest, and may provide clues to putative abnormalities of the dopamine system in limbic and cortical areas.

The few studies of peptide transmitters in schizophrenic post-mortem brain which have provided evidence of specific deficits suggest a focus of abnormalities within the temporal lobe. Whilst Kleinman et al. and Perry et al. reported no abnormalities in CCK concentrations in the entorhinal region, an association between reductions in CCK concentrations in the hippocampus and amygdala and the deficit state or type II symptoms of schizophrenia has been reported. These findings may provide a correlate to neuropathological findings in the temporal lobe, since the deficit state may be particularly associated with ventricular enlargement. Furthermore, CCK receptors have also been shown to be reduced in the hippocampus. However, there is little support for an abnormality of neuropeptide–dopamine interactions in the dopamine termination fields of the basal ganglia. Neurotensin concentrations have been found to be normal, and, whilst neurotensin receptors were found to be increased in the substantia nigra, this appears to be an effect of neuroleptic medication.

Amino acids

Glutamate is the putative excitatory neurotransmitter of the cortical pyramidal cells, which constitute the cortico-fugal projection to the basal ganglia and limbic system, defined pathways within the hippocampus, together with the intra-cortical and callosal pathways which serve to integrate and interconnect the two cerebral hemispheres. GABA is the major inhibitory transmitter of the cerebral cortex, being associated primarily with short axon interneurons.

The early interest in glutamate in schizophrenia followed the report of Kim et al. of low cerebrospinal fluid glutamate levels in chronic schizophrenic subjects. Although this finding was not replicated by other workers, Kim et al. put forward a hypothesis in which dysfunction of glutamatergic neurones was held to be of primary importance in schizophrenia. One important tenet of this hypothesis is the functional interrelation of glutamate and dopamine. In an early post-mortem brain study by Perry, the glutamate content was assayed in 6 brain regions and no differences between control and schizophrenic subjects could be found. In a larger study of 33 schizophrenic and 25 control subjects, Korpi et al. examined a wide range of brain areas...
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again finding no significant differences. However, the absolute level of glutamate reflects both the metabolic pool of glutamate and the neurotransmitter pool which constitutes the smaller of the two. An alternative strategy has been to use the radioligand $[^3\text{H}]$-D-aspartate which has a high affinity for the glutamate uptake site and can thus be used as a marker for glutamatergic neurones58. In a study of 14 schizophrenic and matched control subjects a variety of limbic structures together with temporal and frontal cortical areas were studied using this radioligand60. There was a regionally specific, highly significant bilateral increase in $[^3\text{H}]$-D-aspartate binding in the orbito-frontal cortex, the authors suggest this abnormally dense glutamatergic innervation may result from a failure in the normal developmenta l ‘pruning’ process which re-models the immature callosal/temporal projections to the frontal cortex. The results for the polar temporal cortex showed interesting lateralized effects; although in the overall statistical analysis performed the predicted reduction in $[^3\text{H}]$-D-aspartate binding fell short of statistical significance, when the data from each side of the brain were compared for individuals within this area it was found that in 8 of the 14 schizophrenic subjects but only 3 of the 14 control subjects had lower levels of binding on the left.

The post-synaptic glutamatergic receptor system has been well characterized. Three receptor sub-types have been identified according to their affinity for exogenous ligands59; N-methyl-D-aspartate (NMDA receptor), kainic acid (kainate receptor) and the alpha-amino-3-hydroxy-5-methylisoxazol-4-propionic acid (AMPA / quisqualate receptor). There is a growing data base of information concerning these receptors in post-mortem investigations of schizophrenia. Studies have focused on temporal structures, including the limbic components and the frontal cortex.

The results from these studies form a complex picture. In attempting a synthesis it is prudent to note that all the studies were performed on relatively small groups, that by Deakin et al60 had the largest number of subjects at $n = 14$. However, an overall picture emerges in which it would seem that there is evidence that in medial temporal structures there is a reduction in both pre- and post-synaptic markers for the glutamatergic system whereas there is an increase in pre- and post-synaptic markers in medial pre-frontal cortical areas. The significant findings in these studies are summarized in Table 1.

The positive findings in the temporal lobe show an interesting lateralization, with greater reductions in the left hippocampus60 and in BA3860, whereas in the frontal regions, when both hemispheres are considered separately, the increases are bilateral. It is of note that these 2 regions have a significant functional relationship; glutamatergic association fibres from the polar temporal cortex project from the contralateral hemisphere67. Investigation of the NMDA receptor complex in schizophrenia is of particular interest following the reports that a single dose of phencyclidine (PCP)
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Table 1. Summary of the significant findings in investigations of the pre- and post-synaptic markers of glutamate neurons

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<td>Post-synaptic glutamate receptors</td>
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can precipitate a schizophrenia-like psychosis in normal subjects. Moreover, it can result in a prolonged exacerbation of illness in previously stabilized patients. Unlike the amphetamine-induced psychosis, that produced by PCP demonstrates both positive and negative / deficit symptoms of schizophrenia. Several lines of evidence now indicate that the highly specific receptor site for the PCP drugs is a site within the ion channel gated by the NMDA-receptor. Preliminary studies attempting the pharmacological manipulation of this system have, so far, not produced evidence of clinical efficacy. However, the NMDA receptor forms part of an intricate complex and a clear understanding of the inter-relationship of the individual components will be necessary before the system can be successfully manipulated.

Summary of post-mortem neurochemistry in schizophrenia
It is clear from the preceding review that the neurochemistry of schizophrenia is complex and that a simple ‘transmitter deficit’ model analogous to the striatal dopamine loss in Parkinson’s disease is not likely. Detailed functional
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Inter-relationships exist between major transmitter systems, e.g. dopamine/glutamate and CCK/dopamine. Any abnormalities must therefore be considered in terms of their effect in a dynamic system. Thus, the findings which underpin the ‘dopamine’ theory of schizophrenia are being reviewed and integrated with findings from studies of peptide and amino acid neurotransmitters.

Contemporary with the development of neurochemical theories of schizophrenia in the last 20 years has been the renewed interest in the neuropathology of schizophrenia. This topic is the subject of review in Chapter 2. Subtle structural abnormalities, focused in temporal limbic components, have been demonstrated and have resulted in 2 important issues. First, do the structural abnormalities result from a degenerative or dysplastic/developmental process. Secondly, the question of abnormal cerebral lateralization of structure in schizophrenia. In considering the significance of these findings and their relationship to disturbed brain function in schizophrenia, it is pertinent to integrate neurochemical information.

A developmental disturbance in the normal process of neuronal migration and elimination to produce the final adult cortical arrangement will be reflected in abnormal patterns of receptor distribution. Techniques such as autoradiography which permit the acquisition of neurochemical data at an anatomical level will be able to study this directly. Moreover, studies of the complex sequential pattern of transmitter/receptor expression during development and their relationship to the developing structure of the brain, when compared to the pattern of structural/neurochemical abnormalities seen in schizophrenia, may provide clues to the timing and nature of the causal insult.

The development of cerebral asymmetries, both structural and neurochemical, are a late evolutionary development. Recent neuropathological studies, neurochemical and imaging studies in schizophrenia have suggested that there is an abnormal pattern of cerebral asymmetry in schizophrenia. To address this question, careful methodological design of studies is necessary. Ideally, paired data from the left and right hemisphere of each individual should be considered.

Conclusion

In the last 15 years, studies of post-mortem neurochemistry in schizophrenia have not produced a simple ‘neurochemical theory’ for schizophrenia. The complex interplay between the structure and function of the brain in the production of the clinical phenomena of schizophrenia is gradually emerging. Future studies which adopt an integrated approach to the study of the neurochemical anatomy of schizophrenia may reveal the ‘toxic action or anatomical process’ which Bleuler predicted.
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