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A PROPOSED PATHOLOGICAL MODEL IN THE HIPPOCAMPUS OF SUBJECTS WITH SCHIZOPHRENIA

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SUMMARY

1. The hippocampal formation plays an important role in the normal functioning of the brain, being implicated in cognition and sensory gating, both of which are affected in schizophrenia. The hippocampal formation receives information from the association cortices, which is processed by glutamatergic transmission within the hippocampus. Dopamine, noradrenaline, 5-hydroxytryptamine (5-HT), acetylcholine and GABA, all of which have been proposed to play a role in the neurobiology of schizophrenia, can affect this transmission.

2. The advent of the 'atypical' antipsychotics, with their broad pharmacological spectra and improved therapeutic outcome, has revitalized research into neurotransmitter dysfunction other than that of dopamine. In particular, there has been interest in the serotonergic and cholinergic systems within the hippocampal formation because these are two of the transmitter systems targeted by clozapine and olanzapine.

3. From the study of these systems, using tissue obtained post-mortem from subjects with schizophrenia, we propose that there is a hyperserotonergic state in the hippocampal formation of some subjects with schizophrenia caused by a conformational change in the 5-HT transporter. The model we propose allows us to construct further studies that will test the consequences of such a hyperserotonergic state in the hippocampal formation. This model has the potential to open new avenues in schizophrenia research.

Key words: acetylcholine, hippocampus, 5-hydroxytryptamine, schizophrenia, serotonin.

INTRODUCTION

It is hypothesized that some of the key symptoms of schizophrenia, such as delusions and hallucinations, could be due to altered hippocampal control of cognitive function and sensory gating.[1] The presence of functional and structural abnormalities in the hippocampal formation is supported by data from neuroimaging studies[2] and studies using tissue obtained post-mortem.[3,4] However, the associations of such changes in the hippocampal formation with the pathology of schizophrenia have yet to be fully clarified.

Historically, the proposal that 5-hydroxytryptamine (5-HT) has a role in the pathology of schizophrenia arose from the fact that lysergic acid diethylamide, which is an agonist at serotonergic receptors, causes psychoses that are similar to some of the positive symptoms of schizophrenia.[5] However, there has been renewed interest in the involvement of 5-HT in the pathophysiology of schizophrenia because the newer antipsychotic drugs, such as clozapine, olanzapine and risperidone, are all antagonists at serotonergic receptors.[6] Furthermore, 5-HT has increasingly been implicated in some of the functions that appear to be disturbed in schizophrenia, such as perception, cognition and mood regulation.[7]

HIPPOCAMPUS

The hippocampus classically consists of the cornu Ammonis (CA1–3),[8] although most studies include parts of the medial temporal lobe, such as the subiculum, dentate gyrus, entorhinal cortex and the parahippocampal gyrus, which is referred to as the hippocampal formation. The trisynaptic pathway, which is the circuit through which information enters, passes through and leaves the hippocampal formation and the majority of the sensory efferents from the entorhinal cortex to the hippocampal formation are glutamatergic in nature, using both ionotropic and metabotropic receptors.[8] The remaining neurons in the hippocampal formation are interneurons, capable of affecting neurotransmission around the trisynaptic pathway. These interneurons are GABAergic and use the fast-acting ionotropic $GABA_A$ and slower-acting metabotropic $GABA_B$ receptors.[8] It has been reported that up to 97% of these interneurons express muscarinic receptors.[9] This suggests that acetylcholine (ACh) has the ability to influence the activity of these interneurons, thereby also controlling the flow of information around the trisynaptic circuit. In addition to the cholinergic projections from the septal nuclei and the diagonal band of Broca,[10] the hippocampal formation receives projections from a number of other distally located brain nuclei. Serotonergic projections from both the medial and dorsal raphe nuclei are known to innervate the horn of Ammon, the subiculum and the dentate

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gyrus in a diffuse manner.[11] The presence of a number of serotonergic receptors in the hippocampal formation suggests that these projections also have the capacity to influence the processing of information within the hippocampal formation. In turn, it seems likely that the atypical antipsychotic drugs could influence hippocampal function by acting on both the cholinergic- and serotonergic-innervating systems. It could also be predicted that changes in the innervating serotonergic and cholinergic systems could be involved in the pathology of schizophrenia because both play a role in cognitive function,[7,12] which has been shown to be altered in the illness.

INDICATOR OF CHANGED HIPPOCAMPAL SEROTONERGIC FUNCTION IN SCHIZOPHRENIA

To begin to investigate markers of serotonergic function, we measured both affinity and density of [^{3}H]-paroxetine binding to the serotonin transporter (SERT) in brain tissue from schizophrenic and non-schizophrenic subjects. We found that there was an approximate 50% decrease in the affinity of [^{3}H]-paroxetine binding to SERT in the hippocampal formation of subjects with schizophrenia.[13,14] There were no changes in the affinity of [^{3}H]-paroxetine binding in either the frontal cortex or caudate–putamen in these subjects, nor were there any changes in the density of [^{3}H]-paroxetine binding in the three regions studied. The SERT, located on presynaptic neurons, is responsible for regulating extraneuronal levels of 5-HT.[15] In turn, extraneuronal levels of 5-HT activate serotonergic receptors. Therefore, for this finding to be functionally significant, it is necessary to show that a change in the affinity of [^{3}H]-paroxetine binding to SERT would be associated with a change in the synaptic levels of 5-HT and, therefore, a change in serotonergic receptors.

There are several lines of evidence that suggest a decrease in the affinity of [^{3}H]-paroxetine binding to SERT would result in a change in the synaptic levels of 5-HT. First, it has been reported previously that a relationship exists between the affinities of paroxetine and other 5-HT uptake inhibitors and their ability to reduce 5-HT uptake.[15] Second, we have shown that there is a direct relationship between the affinity of [^{3}H]-paroxetine binding and [^{3}H]-5-HT uptake in human platelets.[16] Finally, we have shown that treating neonatal rats with 5,7-dihydroxytryptamine results in a decreased affinity of [^{3}H]-paroxetine binding to hippocampal SERT of a magnitude similar to that seen in the hippocampus from schizophrenic subjects, but no change in the density of [^{3}H]-paroxetine binding to SERT in the hippocampal formation of the adult rat. Most importantly, we have also shown a marked reduction (approximately 65%) in [^{3}H]-5-HT uptake by synaptosomal preparations from the same rats that had been treated with 5,7-dihydroxytryptamine.[17] This demonstrates that a reduction in the affinity of [^{3}H]-paroxetine binding to SERT, of a similar magnitude to that seen in the hippocampal formation of some subjects with schizophrenia, would be associated with a decrease in 5-HT uptake. Combining these data, it would seem that a decrease in affinity for [^{3}H]-paroxetine binding of the size observed in the hippocampal formation from schizophrenic subjects would result in an approximate 65% reduction in 5-HT uptake. A reduction in 5-HT uptake of this order of magnitude would be likely to cause an increase in extraneuronal levels of 5-HT.

MARKERS FOR A HYPERSEROTONERGIC STATE

Due to rapid post-mortem degradation of neurotransmitters, direct measurement of 5-HT in tissue collected post-mortem is not viable. However, given that levels of neurotransmitters have the capacity to regulate receptor expression, changes in the levels of 5-HT receptors could be used as a marker for changes in the levels of 5-HT at the synapse. If a decrease in serotonergic uptake does exist in the hippocampal formation of schizophrenic subjects, it is reasonable to assume that the resulting increase in synaptic 5-HT levels would cause downregulation of the post-synaptic 5-HT receptors. However, the existence of at least 11 human serotonergic receptors and the variation in their pre- and/or post-synaptic localization between brain regions means that it is not possible to easily prioritize which receptors would be affected by changes in SERT.

It is well documented that the 5-HT and ACh systems in the hippocampal formation are highly interactive.[18] Furthermore, animal studies have shown that direct application or administration of 5-HT causes the release of ACh.[19–21] Therefore, an increase in synaptic concentrations of 5-HT would be associated with increased levels of extraneuronal ACh. In turn, increased levels of ACh would cause a reduction in the density of post-synaptic $M_{1/4}$ receptors; a phenomenon that can be investigated in tissue obtained post-mortem. Furthermore, because both 5-HT and ACh have the capacity to control glutamatergic transmission throughout the hippocampal formation, potential interactions between 5-HT and ACh in the hippocampal formation could be important in the pathological processes associated with schizophrenia.

Significantly, we have shown that there is a decrease in hippocampal $M_{1/4}$ receptors in most areas of the hippocampal formation from schizophrenic subjects.[22] Furthermore, there is an inverse relationship between affinity of [^{3}H]-paroxetine binding and the levels of $M_{1/4}$ receptors as measured using [^{3}H]-pirenzepine (Fig. 1). These data suggest that the increased levels of 5-HT, as a result of changes in SERT, have resulted in an increase in ACh release and, thus, a downregulation of the $M_{1/4}$ receptors. The 5-HT receptors that are known to play a role in modulating the release of ACh include the 5-HT_{1A}, 5-HT_{2A} and 5-HT_4 receptors.[19–21] Therefore, these receptors are now prime candidates for investigating the effects of a

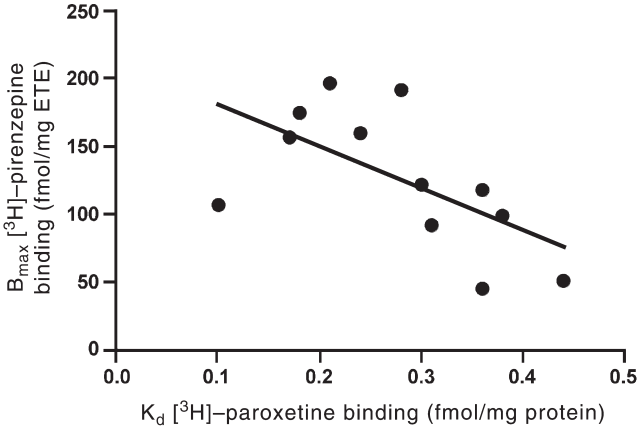

Fig. 1 The relationship between the affinity of [^{3}H]-paroxetine binding to the serotonin transporter (SERT) and the density of [^{3}H]-pirenzepine binding in the hippocampus ($r = 0.61$; $P < 0.05$). ETE, estimated tissue equivalents.

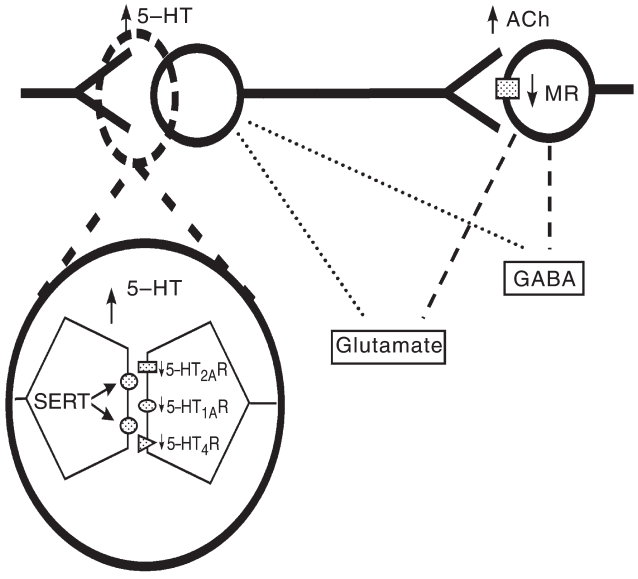

Fig. 2 A schematic representation of the model we propose to be critical in the pathology of schizophrenia in the hippocampus. For the sake of simplicity, we have shown the 5-hydroxytryptamine (5-HT) receptors to be localized on a single neuron rather than multiple neurons. R, receptor; SERT, serotonin transporter; ACh, acetylcholine; MR, muscarinic receptor.

hyperserotonergic state at the synapse in the hippocampus from subjects with schizophrenia.

THE MODEL

From existing hippocampal data we have developed a model to define some of the pathological processes involving the hippocampal formation in schizophrenia (Fig. 2). This model predicts that changes in SERT cause an increase in extraneuronal 5-HT. As a result of the increase in 5-HT levels at the synapse, there has been a resulting increase in ACh release, which, in turn, has caused a decrease in $M_{1/4}$ receptors. The 5-HT receptors that most potently modulate ACh release are the 5-HT_{1A}, 5-HT_{2A} and 5-HT_4 receptors. Therefore, our model predicts that these receptors will be downregulated in the hippocampus from subjects with schizophrenia. Furthermore, the restricted diffusion of neurotransmitters from their point of release means that the changes in the affinity of binding to SERT and changes in 5-HT receptor densities would be spatially colocalized within the hippocampal formation.

We have now shown that [^{3}H]-citalopram binding in the hippocampus is most dense in the stratum lacunosum-moleculare of CA1 and CA2 (Fig. 3). In the subiculum, CA1, CA2 and CA3, the binding is of a medium density and diffuse across all layers. There is relatively low-density binding in the dentate gyrus. From these data, we would expect to find a decrease in the density of 5-HT_{1A}, 5-HT_{2A}

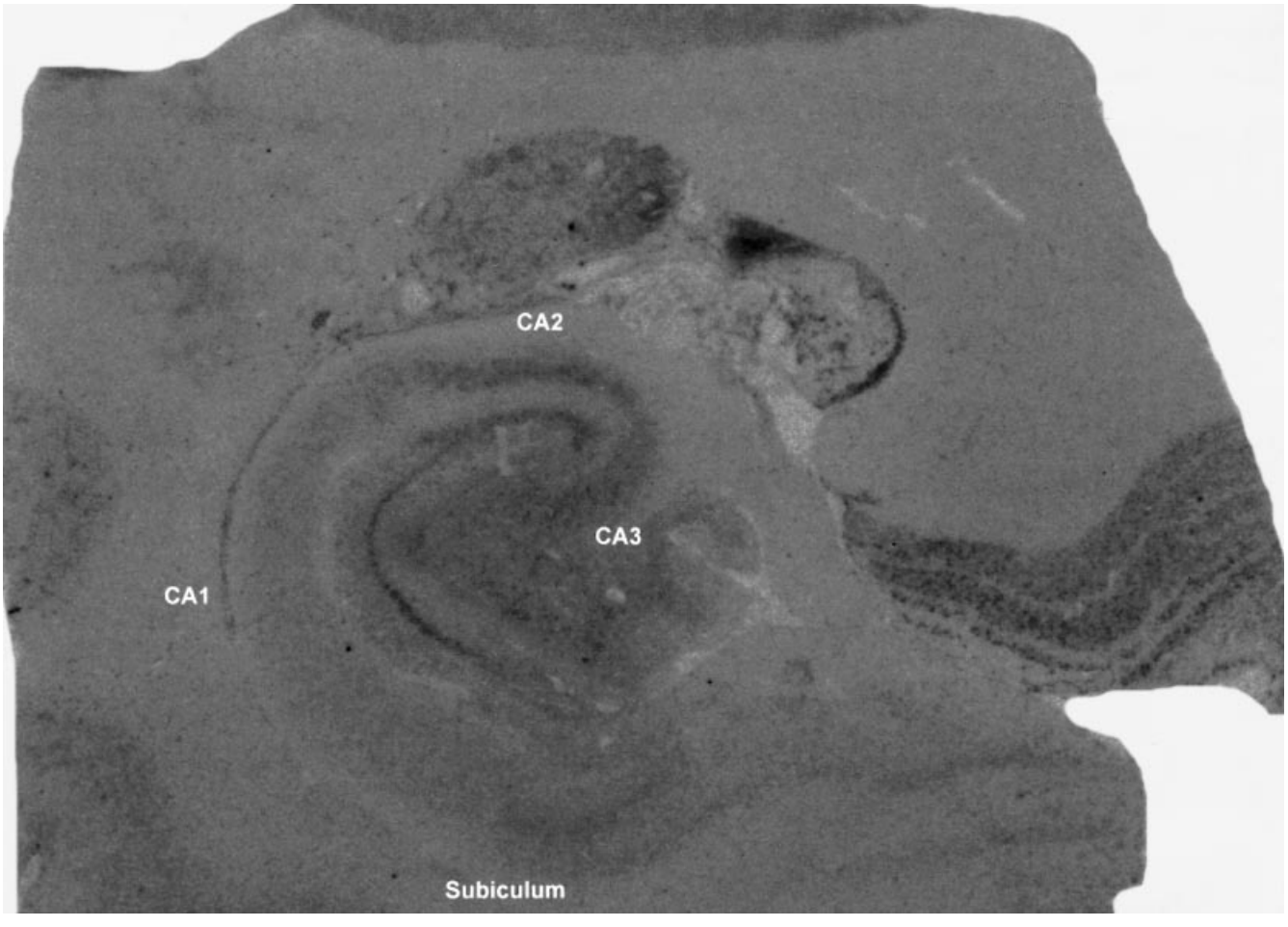

Fig. 3 An autoradiograph showing the distribution of [^{3}H]-citalopram binding in human hippocampus.

or 5-HT_4 receptors in the regions where SERT is at the highest concentrations. There are a number of other factors related to our hypothesis that require further investigation. These include measurement of the components of the brain that control the activity of SERT, because changes in such factors may be involved in a hyperserotonergic state and, thus, associated with the pathology of schizophrenia. Because the hippocampal formation and frontal cortex are innervated by collateral projections from the medial raphe nucleus, we would predict that it is most likely that the changes seen in hippocampal SERT are specific to the neurons innervating the hippocampal formation and are due to local influences rather than changes in the expression of SERT in the raphe. Initially, to obtain some idea of the nature of the change in the control of SERT, we need to assess whether the conformational change we have proposed is due to changes in the glycosylation or phosphorylation states of the transporter. These post-translational modifications could be important because glycosylation has been reported to be essential for the assembly and expression of SERT at the cell surface[23] and phosphorylation has been shown to be important in modulating SERT activity.[24] The proposed model also raises the potential for antipsychotic drugs with combined serotonergic and cholinergic antagonism to have an additive effect in the hippocampal formation. This could partially account for the increased clinical effectiveness of the atypical antipsychotic drugs, such as clozapine and olanzapine. However, it is possible that designing drugs that better target these systems in the hippocampal formation may lead to the development of more effective antipsychotic agents with fewer side effects.

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