EPIGENETIC ANALYSIS AND FUNCTIONALITY OF DOPAMINE D2, ADENOSINE A1 AND A2A RECEPTORS IN SCHIZOPHRENIA: MOLECULAR MECHANISMS INVOLVED

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1. Abstract

Schizophrenia is a psychiatric disorder whose etiology and categorization are not well defined yet. The heterogeneity of results from different research projects and from clinical practice, which tends to define phenotypic subgroups of patients, points to schizophrenia as a syndrome, a cluster of symptoms which are produced by different causes. The most accepted theory in explaining the etiology of schizophrenia is the hyperdopaminergic hypothesis. It has been described in postmortem tissue that dopamine receptors are more active in schizophrenia. A dysfunction of the glutamatergic system has also been proposed. Finally, there is a hypoadenosinergic hypothesis that has the potential to integrate the preceding two: interaction between the adenosine A1 (A1R) and A2A (A2AR) receptors in the presynaptic neuron which controls the glutamate and dopamine release, and the interaction between adenosine and dopamine receptors which regulates dopaminergic signal sensitivity in striatal neurons. The hypoadenosinergia hypothesis could be due to reduced levels of adenosine in the brains of schizophrenia patients, which could imbalance A1R-A2AR mediated control of glutamate release and lead in turn to a decreased glutamate level. A decreased dopamine release would also be expected; however, a lower A2A activation could be responsible for a hypersensitivity of the D2 dopamine receptor. Likewise, the hypoadenosinergia could be related to a variation in the levels or activity of adenosine receptors. Therefore, a main goal of the project was the study of adenosine and dopamine receptors in postmortem brain samples from patients with schizophrenia. Thus we analyzed the expression levels of the A1, A2A and D2 receptors and their intracellular signaling pathways, which are differentially expressed depending on the brain area analyzed.

Schizophrenia has been previously associated with epigenetic alterations, especially considering the existence of monozygotic twins discordant for the disease. Some genes whose expression is altered in schizophrenia have been associated with hypermethylation. In fact, DNA methyltransferases are overexpressed in various brain regions of schizophrenia and could be responsible for gene repression in this disease. At the end of this project,
we have shown that DNA methylation is involved in the differential expression of A$_{2a}$R and D$_2$R.

2. Results

2.1. Adenosine A$_1$ receptor (A$_1$R) analysis.
We observed that this receptor is differentially affected in patients with schizophrenia according to the brain area. In this sense, it was detected that A$_1$ receptor levels are decreased in the temporal and parietal cortex of schizophrenic patients, whereas their expression levels are not altered in the putamen. However, the low levels of the receptor did not correlate with changes in its mRNA levels, suggesting the involvement of post-transcriptional mechanisms in the pathological expression observed in the cerebral cortex.

2.2. Adenosine A$_{2a}$ receptor (A$_{2a}$R) analysis
It was shown that approximately 50% of patients suffering schizophrenia showed a significant reduction in A$_{2a}$R levels in the putamen. The effect of this reduction could be similar to a decrease in adenosine levels, leading to overactivation of D$_2$R. Furthermore, a PANSS scale analysis showed motor disturbances in this subgroup of patients regardless of drug treatment received. Moreover, the reduced A$_{2a}$R levels in this subset of patients occur both at the protein and mRNA level, and are accompanied by an increase in the methylation profile of ADORA2A gene promoter, encoding the A$_{2a}$R. In agreement with the PANSS ratings, hypermethylation observed in the ADORA2A promoter showed no correlation with the type of treatment received.

Unlike the results obtained in putamen, we observed that expression level of this receptor seems to be preserved in temporal and parietal cortex of patients with schizophrenia. No significant changes in ADORA2A expression were detected in these brain areas. Therefore, the study of this receptor resulted in differential expression depending on the brain area tested.
2.3. **Dopamine D₂ receptor (D₂R) analysis**
Previous studies in lymphocytes, based on analysis of a CpG island located in the promoter region of the encoding gene, showed that DNA methylation is involved in the expression of D₂ receptor. At the end of this project we observed that the expression levels of this receptor are increased in the parietal cortex of patients with schizophrenia. However, this increase was not correlated with a loss in the percentage of methylation in the promoter region because we even detected an increase in the degree of methylation of DNA. Therefore, it is possible that hypermethylation could be due to an increase in hydroxymethylcytosine levels (activating mark) instead of methyl cytosine levels (repressing mark).

2.4. **Metabotropic glutamate 5 receptor (mGlu₅R) analysis**
Because of a possible colocalization and interaction of the adenosine and dopamine receptors with mGlu₅R, in the framework of this project we studied mGlu₅R levels in samples from parietal and temporal cortex from patients with schizophrenia. We observed a decrease in parietal cortex while temporal cortex was not affected, suggesting again a different alteration depending on the brain area analyzed.

2.5. **Ectonucleotidase activity analysis**
We have shown that ATPase and ADPase activities are decreased in the putamen of patients with schizophrenia independently of A₂AR levels. Both activities are responsible for the generation of AMP, which acts as substrate of CD73 (ecto-5'-nucleotidase). These results show that these enzymatic activities, responsible for the generation of extracellular adenosine, are decreased in patients with schizophrenia, supporting the hypoadenosinergic hypothesis as one of the mechanisms involved in the etiopathogenesis of the disease.

2.6. **Functionality of pathways mediated by A₁R, A₂AR, D₂R and mGlu₅R**
It was observed that the inhibitory adenylate cyclase pathway mediated by A₁R, is desensitized in parietal cortex while it is preserved in temporal cortex from schizophrenia patients. However, the stimulatory pathway,
mediated by A2AR, and the inhibitory pathway mediated by D2R are preserved in both brain areas. In addition, phospholipase C pathway mediated by mGlu5R is also preserved in both areas. Proximity ligation assays showed the heterodimer formation in schizophrenia samples.

3. Relevance and possible implications

The characterization of a subset of patients with decreased A2AR levels in the striatum (putamen) and their correlation with hyperkinetic motor disturbances support a potential clinical trial to establish a possible personalized treatment. Both first and second generation antipsychotics are ineffective for cognitive symptoms and produce unwanted side effects. Therefore, new alternative pharmacological treatments need to be investigated. In this way, A2AR can be detected by radio-imaging techniques and patients could be selected taking into account motor disabilities. A personalized therapy could be designed focusing on the adenosinergic system. If reduced levels of A2AR play a role in the etiology of the disease, the treatment with A2AR agonists plus antipsychotic drugs could be a potential therapeutic approach by potentiating the inhibitory effect of A2A over D2 signaling. Besides, the correlation found between increased methylation of ADORA2A and the reduced receptor levels is noteworthy. Accordingly, some treatments based on epigenetic manipulation are now in clinical trials, such as valproic acid, an inhibitor of histone acetyl dehydrogenases, which promotes DNA demethylation and gene expression. This proposed combination of therapies could represent a new pharmacological strategy to reduce the dose of antipsychotics in certain types of patients. Therefore, results in this project have generated the need to transfer this information to a clinical study.

Also noteworthy is the experimentally demonstrated reduction of ectonucleotidase activity in postmortem putamen from patients with schizophrenia. These results again support the hypoadenosinergic hypothesis and provide new evidence on the mechanisms involved in the etiopathogenesis of this neurological disease.
Finally, the differential alteration of dopamine, adenosine and metabotropic glutamate receptors together with heterodimerization processes of these receptors could help in developing new pharmacological strategies based on bifunctional drugs that simultaneously modulate the activity of these receptors in schizophrenia.

4. Articles published


