A2A-D2HETEROMERIZATION ON STRIATAL ASTROCYTES: EVIDENCE AND MODULATION OF GLUTAMATE RELEASE

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Introduction: The A2A and D2receptor-receptor interaction (RRI) at the striatal neuron has opened up new perspectives on the molecular mechanisms related to neuropsychiatric disorders (e.g. Parkinson's disease (PD), schizophrenia). In the last year several studies showed both adenosine A2A (A2A) and dopamine D2(D2) receptors expressed in adult striatal astrocytes. Since the importance of glial involvement in neuropsychiatric or neurodegenerative disease vulnerability is being increasingly recognized, we investigated the expression and the functional role of RRI on the astrocytes and on their processes (gliosomes) isolated from adult rat striatum. To this aim, we performed biochemical analysis and immunoassay to demonstrate the existence of the A2A-D2heteromers on striatal astrocytes and on gliosomes.

Materials and methods: Purified striatal astrocyte processes and slices were prepared from adult rat striatum. By immunofluorescence, we evaluated the expression of A2A-D2heteromers, astrocytic markers, and vesicular glutamate transporters. The release of glutamate from gliosomes was studied in superfusion apparatus, also in presence of the synthetic peptide VLRRRRKRVN (corresponding to the D2region involved in electrostatic interaction underlying A2A-D2heteromerization). Co-immunoprecipitation of the A2A and D2receptors in the astrocyte processes was carried out on plasma membranes and the proximity ligation assay (PLA) was performed on striatal slices.

Results: We observed the co-expression of the A2A and D2receptors on GFAP-, ezrin- and VGLUT1-positive astrocyte processes. Focusing on the glutamate release from gliosomes, we obtained evidence for A2A control of the D2-mediated modulation of the striatal gliotransmission; D2receptors inhibited the 4AP-evoked glutamate release, while activation of A2A receptors, per se ineffective, abolished the effect of D2receptor activation. The synthetic D2peptide VLRRRRKRVN abolished the ability of the A2A agonist to antagonize the D2-mediated effect. The co-immunoprecipitation of A2A and D2receptors in astrocyte processes demonstrated their co-expression on the plasma membrane and the PLA detected their strict co-localization on the astrocytes.

Discussion and conclusion: Our data indicate that both A2A and D2receptors were expressed in the same astrocyte processes. Evidence for the A2A-D2RRI was obtained by measuring the release of the gliotransmitter glutamate from the astrocyte processes and by biochemical and biophysical approaches: the A2A and D2receptors physically and functionally interact and they form heteromers. Therefore, these receptors could play a crucial integrative role at the striatal astrocyte processes: the astrocytic A2A receptors control the dopaminergic modulation of the striatal glutamatergic transmission through the A2A-D2heteromers. At the same time our findings, highlighting that a reduced D2-mediated control at striatal perisynaptic astrocyte processes might result in an increase in the glutamate level at the synapse, could help us to understand how astrocytes (and remodeling of astrocyte processes) contribute to the molecular mechanisms related to neuropsychiatric disorders.