

P011 Role of the Conserved Glutamine 291 in the rat γ -Aminobutyric Acid Transporter rGAT-1

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The role of the conserved glutamine 291 in the function of the rat γ -aminobutyric acid transporter GAT-1 has been investigated. Q291 mutants are unable to transport GABA and to give rise to transient, leak and transport-coupled currents although targeted to the plasma membrane. Coexpression experiments of WT and Q291 mutants suggest that GAT-1 is a functional monomer although it requires the oligomeric assembly for the surface expression. We have determined the accessibility of Q291 by the impact of impermeant sulfhydryl reagents on cysteine residues engineered in close proximity to Q291. The effect of these reagents indicates that Q291 faces the external aqueous milieu. The introduction of a steric hindrance close to Q291 by MTSET modification of C74A/T290C, severely altered the mutant affinity for cations. All together, these results suggest that this irreplaceable residue is involved in the interaction with sodium or in maintaining the cation accessibility to the transporter.