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Role of the ectodomain serine 275 in shaping the binding pocket of the ATP-gated P2X3 receptor

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Abstract

ATP-activated P2X3 receptors expressed in nociceptive sensory neurons play an important role in pain signaling. Basic properties of this receptor subtype, including very strong desensitization, depend on the rate of dissociation of the agonist from the binding site. Even though the rough structure of the ATP binding site has been proposed on the basis of the X-ray structure of the zebrafish P2X4 receptor and mutagenesis studies, the fine subunit-specific structural properties predisposing the receptor to tight capture of the agonist inside the binding pocket have not been elucidated. In this work, by exploring in silico the functional role for the left flipper located in the ectodomain region, we identified within this loop a candidate residue S275, which could contribute to the closure of the agonist-binding pocket. Testing of the S275 mutants using the patch-clamp technique revealed a crucial role for S275 in agonist binding and receptor desensitization. The S275A mutant showed a reduced rate of onset of desensitization and accelerated resensitization and was weakly inhibited by nanomolar agonist. Extracellular calcium application produced inhibition instead of facilitation of membrane currents. Moreover, some full agonists became only partial agonists when applied to the S275A receptor. These effects were stronger with the more hydrophobic mutants S275C and S275V. Taken together, our data suggest that S275 contributes to the closure of the agonist-binding pocket and that effective capture of the agonist provided by the left flipper in calcium-dependent manner determines the high rate of desensitization, slow recovery, and sensitivity to nanomolar agonist of the P2X3 receptor.

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