Biochemistry 2017 vol.56 N26, pages 3394-3402

Reconstructed Serine 288 in the Left Flipper Region of the Rat P2X7 Receptor Stabilizes Nonsensitized States

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Abstract

© 2017 American Chemical Society. Serine 275, a conserved residue of the left flipper region of ATP-gated P2X3 receptors, plays a key role in both agonist binding and receptor desensitization. It is conserved in most of the P2X receptors except P2X7 and P2X6. By combining experimental patch-clamp and modeling approaches, we explored the role of the corresponding residue in the rat P2X7 receptor (rP2X7) by replacing the phenylalanine at position 288 with serine and characterizing the membrane currents generated by either the wild-type (WT) or the mutated rP2X7 receptor. F288S, an rP2X7 mutation, slowed the deactivation subsequent to 2 and 20 s applications of 1 mM ATP. F288S also prevented sensitization (a progressive current growth) observed with the WT in response to a 20 s application of 1 mM ATP. Increasing the ATP concentration to 5 mM promoted sensitization also in the mutated rP2X7 receptor, accelerating the deactivation rate to typical WT values. YO-PRO1 uptake in cells expressing either the WT or the F288S P2X7 receptor was consistent with recorded membrane current data. Interestingly, in the human P2X7 (hP2X7) receptor, substitution Y288S did not change the deactivation rate, while the Y288F mutant generated a "rat-like" phenotype with a fast deactivation rate. Our combined experimental, kinetic, and molecular modeling data suggest that the rat F288S novel phenotype is due to a slower rate of ATP binding and/or unbinding and stabilization of nonsensitized receptor states.

http://dx.doi.org/10.1021/acs.biochem.7b00258

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