Dynamic behavior of surface CB1R in cortical neurons in vitro

ABSTRACT

Presynaptic cannabinoid type 1 receptors (CB1R) are major mediators of retrograde synaptic plasticity at both excitatory and inhibitory synapses and participate to a plethora of physiological functions. Whether presynaptic receptors, such as CB1R, display functionally relevant movements at the surface of neuronal membrane is not known. We analyzed the lateral mobility of native CB1R in cortical neurons, using single quantum dot imaging. We found that CB1R are highly mobile and rapidly diffuse in and out of presynapses. Agonist-induced desensitization reduced the number of surface CB1R and drastically decreased the membrane dynamic of the CB1R that remained at the presynaptic surface. Desensitization specifically excluded CB1R from synapses and increased the number of immobile receptors in the extrasynaptic compartment. The results suggest that decrease of mobility may be one of the core mechanisms underlying the desensitization of CB1R, the most abundant G-protein coupled receptor in the brain.

METHODOLOGY

Single particle tracking and surface diffusion calculation: To specifically track surface CB1Rs in real time, polyclonal antibodies directed against an extracellular N-terminus domain of CB1R were coupled to anti-rabbit F(ab′)2 conjugated with Quantum Dots 655 nm (Qdots). Synapses were visualized with a fluorescent marker of active mitochondria, MitoTrackerGreen, which colocalizes with presynaptic synaptotagmin clusters. A real-time video of CB1R–Qdots was recorded in 9- to 10-days-in-vitro cortical neurons (32°C). Instantaneous diffusion coefficient (*D*), percentage of mobile receptors, synaptic dwell time, percentage of synaptic receptors, and mean square displacement (MSD) were all calculated from reconstructed CB1R–Qdot trajectories.

CONCLUSIONS

Our data shed light on the dynamic behavior of native presynaptic CB1Rs and suggest that subtle regulations of CB1R movements participate in the complex molecular cascade underlying agonist-induced desensitization. The presently revealed mechanism has significant implications for the study of mechanisms underlying tolerance of other presynaptic GPCRs.

Keywords: receptor mobility, CB1R, G-protein coupled receptor