Characterization of the rapidly-desensitizing α7 nicotinic acetylcholine receptor using the Qube

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Introduction

The ligand-gated α7 nicotinic acetylcholine receptor (α7 nAChR) plays an intricate role as part of the excitatory cholinergic response in the nervous system. Due to its major function in not only serving a primary immune response but also its direct link to multiple neurological disorders, α7-directed targeted therapies are critical in addressing an unmet need in health and human disease. However, the ability of the channel to rapidly desensitize after channel activation makes α7 one of the most difficult ion channels to study using both conventional as well as automated patch-clamp (APC) platforms. Recently, both Friis et Al. (2009) and Hao et Al. (2015) demonstrated that α7 can be studied on the QPatch. This study further demonstrates that the Qube, the 384-channel high-throughput APC platform, can also be used to record the α7 nAChR. We thus clearly show the Qube as an extremely versatile instrument both in drug discovery and studying various ion channel biophysics.

Materials and methods

The isosmotic extracellular solution (305 mOsm/kg) contained (in mmol/L): 2 CaCl2, 1 MgCl2, 10 HEPES, 4 KCl, 145 NaCl, 10 glucose, and sucrose was used to adjust bath solution osmolarity (pH 7.4; adjust with KOH). Standard intracellular solution contained: 120 KF, 20 KCl, 10 HEPES, 10 EGTA (pH 7.2; KOH). Stock solutions of acetylcholine (ACh; 100 mM, Sigma) and nicotine (Nicotine; 10 mM, Sigma) were prepared in extracellular solution or DMSO, respectively. CODA-4 was provided by Coda Biotherapeutics and prepared in extracellular solution, Coda Biotherapeutics also provided stable cell lines expressing homomeric α7 nAChR, Ric-3, and NACHO or different α7 nACHORD0g9/k8 chimeras (CODA-A or CODA-B) in recombinantly expressed HEK293 cells. The cells were cultured according to Sophion AG cell culture protocol in DMEM (D6434, Sigma) supplemented with 13% FBS, geneticin (1 mg/ml), and 0.6 µg/ml puromycin. Cells were detached using detachi (Gelantis) prior to the assay.

Conclusion

In conclusion, we present additional evidence that the Qube, the 384-channel high-throughput APC platform, can measure similar potencies of ACh on α7 (EC50 values ~71 µM), which previously was only obtained on the QPatch. We also present evidence that CODA-4 is a more potent agonist than acetylcholine. Moreover, we determined that CODA-A and CODA-B, two different α7/GlyR chimeras, show significantly less desensitization than the homomeric α7 when assayed on the Qube.

References


ACh induced traces and dose-response relationship obtained on QPath

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References