SOPHION BIOSCIENCE

Application Report

RD(TE671)nAchR α1 on QPatch

Endogenous nicotinic channels

Summary

The human cell line RD(TE671) endogenously expresses the nicotinic acetylcholine alpha 1 receptor (nAChRα1). The QPatch can reliably measure ligand-gated RD(TE671) currents and was used to confirm the specific activation of nAChRα1 due to acetylcholine addition on the QPatch.

- Stable seals rates above 80% were repeatedly obtained
- QPatch performed reliable dose-response experiments on the fast ligand-gated channel nAChRα1
- It was confirmed that the observed currents indeed are due to the activation of acetylcholine receptors

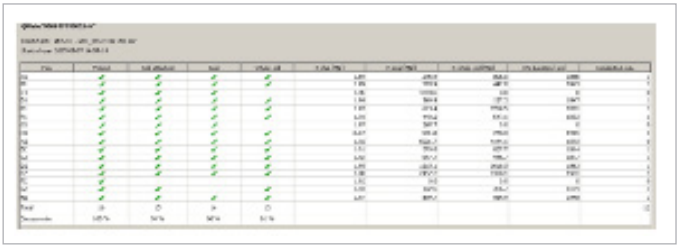

Fig. 1: Typical overview of success rates with the RD(TE671) cell line.

Results and discussion

The RD(TE671) cells performed well on QPatch with a seal rate constantly above 80% (Figure 1). Cells were homogenous in size around 15 pF and all exhibited significant nAChRα1 current upon stimulation with acetylcholine (ACh).

Treated with different concentrations of acetylcholine, the cells reliably produced currents in the nanoampere range (Fig. 2).

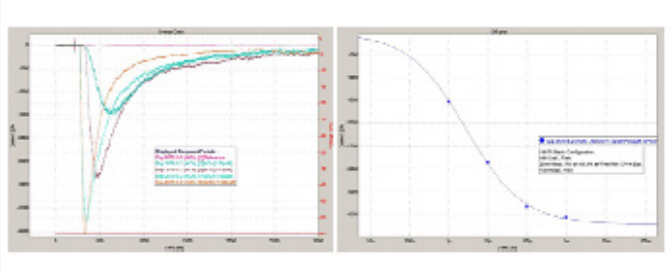

Fig. 2: Left: raw traces with increasing ACh concentration. Right: Agonist (ACh) Hill fit.

Exposure to an increased concentration of ACh showed dose dependency with an EC_{50} of 2.67 ± 1.1 µM (n=11) (7.8 µM, 2) (Fig. 2).

Using cells directly from the freezer gives a lot of flexibility and less day to day variation. In this set of experiments, the frozen cells were used in an antagonist concentration-response experiment using gallamine, a blocker of nAChRα1 (3), to investigate whether the obtained current was directly mediated via the acetylcholine receptor α1.

Gallamine blocked the current in a concentration-dependent manner and had an IC_{50} value of 5.32 ± 0.25 µM (n=5) (Fig. 3).

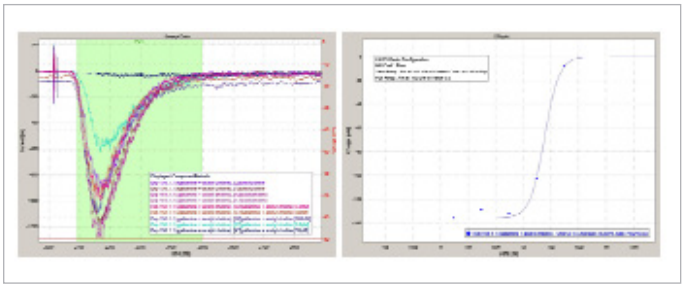

Fig. 3: Left: Raw current traces with increasing concentrations of gallamine with constant ACh concentration. Right: Hill fit on gallamine data.

Methods

The cells are grown according to the Sophion standard operating procedure (SOP) for RD(TE671) cells. Physiological Ringer's solution was used on QPatch. When washing, extracellular Ringer's solution with 2 unit/ml acetylcholineesterase was used for washing.

Assay

A whole cell protocol modified from a standard CHO protocol was used to obtain the whole cell configuration. During the addition of acetylcholine, the cells were kept at two different potentials, –60 and -90 mV and the channels were opened with a 200-sec interval.

Conclusion

These experiments demonstrate that the QPatch is an ideal platform for reliable dose-response experiments with a fast ligand-gated channel. Using a specific agonist and antagonist, it was confirmed that the observed currents indeed are due to the activation of acetylcholine receptors.

The workload involved in creating a cell line expressing a specific gene of interest can sometimes set the limit for initiating a set of pilot experiments. To be able to work with receptors being endogenously expressed in well-known cell lines can be not only cost-reducing but also instrumental in running initial studies of certain receptor targets. We have in this study examined the RD(TE671) cell line for its endogenous expression of nAChRα1 and validated its capability to be used on the QPatch platform. We conclude that the overall success rates in obtaining stable seals and completed experiments are at a level that clearly identifies the RD(TE671) assay as feasible and meaningful on the QPatch.

References:

1. Morris A et al. 1991. Two isoforms of the muscle acetylcholine receptor alpha-subunit are translated in the human cell line TE671. FEBS Lett 295:116-8
2. Shao Z et al. 1998. Potentiation and inhibition of nicotinic acetylcholine receptors by spermine in the TE671 human muscle cell line. J Pharmacol Exp Ther 286:1269-76
3. Benson JA. 1992. Electrophysiological Pharmacology of the Nicotinic and Muscarinic Cholinergic Responses of Isolated Neuronal Somata from Locust Thoracic Ganglia. J Exp Biol 170: 203-233

Author:

Rasmus B Jacobsen, Application scientist

Sophion Bioscience A/S, Baltorpvej 154, 2750 Ballerup, Denmark
Phone: +45 4460 8800, E-mail: info@sophion.com

sophion.com