Qube - 384-channel patch clamp: Characterization of the liquid flow channel

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Introduction

The Qube is the new gigaseal-based 384-channel planar patch clamp system developed by Sophion Bioscience A/S. The system is equipped with 384 individual amplifiers providing continuous recordings at a sampling rate up to 50 kHz from all wells simultaneously. The consumable for the Qube is built on Sophion's tried-and-tested silicon technology for optimal giga-seal formation and has been designed to have an efficient liquid flow system which is necessary for testing fast ligand-gated ion channels (LGIC) such as the nicotine acetylcholine receptors (nAChR).

In this work we present recordings obtained using the Qube system from two LGICs: ASIC1a and nAChRα1.

The acid-sensitive channel (ASIC) belongs to the ENaC/DEG family. ASIC1a is expressed in the brain and in the peripheral nervous system where it is involved in modulating response to pain (1). The nicotinic acetylcholine receptor alpha 1 (nAChRα1) belongs to the Cys-loop family and is expressed both centrally and in the peripheral nervous system. In the periphery nAChRα1 modulate e.g. the synaptic transmission at the neuromuscular junction (2).
Here we show activation of these LGICs by their respective ligand (H^+ and acethylcholine (ACh), respectively) and pharmacological block by known reference compounds (amiloride and tetracaine, respectively). These data demonstrate: 1) the design of the Qube consumable allows fast liquid exchange for recordings of fast LGICs and 2) the design of the Qube consumable allows multiple liquid additions to the same recording unit.

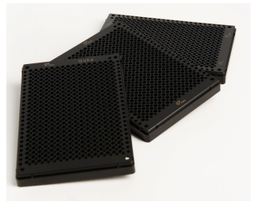
Methods

Cells
ASIC1a currents were measured in a HEK-293 cell line stably overexpressing the ion channel (kindly provided by Neurosearch A/S), nAChRα1-currents were measured in TE-671 cells (ATCC cat# CRL-8805) and $K_ir2.1$ currents were measured in RBL-2H3 cells (ATCC cat# CRL-2256).

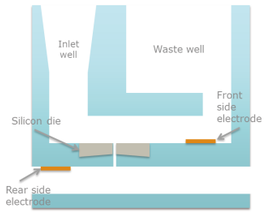
Solutions
The composition of the intracellular solution was (mM): CsF 140, EGTA 1, HEPES 10, NaCl 10 (pH 7.2 with CsOH) and the extracellular (mM): $CaCl_2$ 2, $MgCl_2$ 1, HEPES 10, KCl 4, NaCl 145, glucose 10 (pH 7.4 with NaOH). Low pH solution for ASIC was buffered with MES. In the liquid exchange experiments the intracellular solution was (mM): KCl 120, $CaCl_2$ 5.37, $MgCl_2$ 1.75, EGTA 10, HEPES 10, Na_2ATP 4 (pH 7.2 with KOH, $[CaCl_2]_{free}$ 115 nM).

All experiments were carried out at ambient temperature using Qube consumables with a single patch-hole per recording unit. Analysis was performed using the Sophion Assay Software and Origin 7.5 (OriginLab, MA).

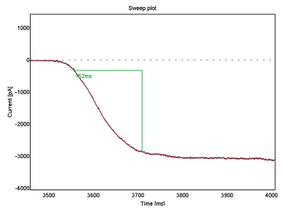
Figure 1 The Qube consumable



A) Photograph of the 384-well consumable for the Qube.

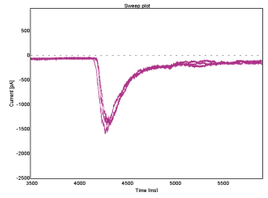


B) Schematic drawing of an individual recording unit. As can be seen the electrodes are integrated in the design which minimizes variability between runs and eliminates the need for maintenance chlorination. The waste can be efficiently emptied enabling cumulative dose-response studies. The Qube consumable will be offered as single-hole and multi-hole units.

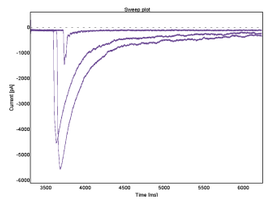


C) The rise time for the liquid exchange was measured using the Kir2.1 expressing cell line RBL and alternating solutions containing high and low concentration of K^+. The average 10-90% rise time across the plate was 170 ± 9 ms.

Figur 3 Acetylcholine (ACh)-induced membrane currents in TE-671 rhabdomyosarcoma cells natively expressing nAChRα1

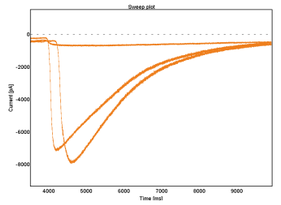


A) Current traces from three consecutive additions of ACh (300 µM) with wash-out of the agonist between recordings. The time between recordings was 200 sec.

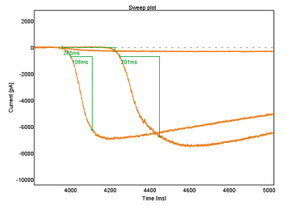


B) Pharmacological block of the ACh-induced current (300 µM) by tetracaine (10 µM). The cell was stimulated with ACh, then incubated with tetracaine and stimulated with ACh and finally tetracaine was washed out and the cell was stimulated again with ACh. The average block by 10 µM tetracaine was 83 ± 2 %. This value, if extrapolated, corresponds to an IC_{50} of approximately 2 µM (4).

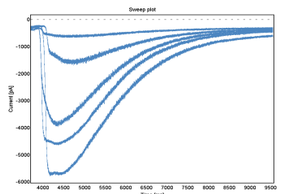
Figure 2 Stimulation of ASIC 1a with low pH



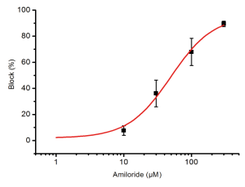
A) Single cell perfused three times at low pH (pH 5.3) and rinsed with standard saline (pH 7.4) between stimulations. During the second stimulation amiloride (300 µM) was added to the perfused solution to block the current.



B) Current rise times determinations of ASIC1a currents in the absence and presence of amiloride ($t_{10-90\%}$ 108, 265 and 201 ms respectively) in the same experiment. The average rise time of the pH 5.3-induced current across the plate was 117 ± 13 ms.



C) Antagonist study. Current traces for control (lowest sweep) and four concentrations of amiloride (10, 30, 100 and 300 µM) recorded on the same recording unit.



D) The resulting dose response relationship for amiloride. The IC_{50} for amiloride was 50 µM (3).

Conclusion

In this work we have examined the liquid exchange properties of the consumable developed for the new Qube instrument – the very first giga-seal-based 384-channel planar patch clamp system.

We have convincingly shown that the consumable for the Qube can support multiple additions with high reproducibility. The liquid exchange properties of the recording unit allow for reliable and fast delivery of activating and blocking compounds that enable quality measurements of currents from ligand-gated ion channels such as ASIC1a and nAChRα1. We have shown that pharmacological block of ASIC1a currents by amiloride yields an IC_{50} value comparable to values in the literature. The effect of amiloride was reversable upon wash.

The Qube system is aimed at the high through-put segment of ion channel screening where data quality still matters - with the data presented here – we are on the right track.

References
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4. Gentry and Lukas. J Pharm Exp Ther. 1038-48, 2001