Qube: 384-channel patch clamp screening system
Ligand-gated ion channel applications

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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 50 kHz from all wells simultaneously. The consumable for the Qube, "QChip384" 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 have used the Qube system to record currents from two desensitizing LGICs: the acid-sensing channel 1a (ASIC1a) and the nAChRα1. ASIC belongs the ENaC/Deg family and ASIC1a is expressed in both the central nervous system (CNS) and in the peripheral (PNS). In the PNS it is among other things involved in modulating responses to pain (1). The nicotinic acetylcholine receptor alpha 1 (nAChRα1) belongs to the Cys-loop family and is also expressed in the CNS and as well as in the PNS. In the PNS nAChRα1 modulate e.g. the synaptic transmission at the neuromuscular junction (2).

Here we show that repetitive activation of these LGICs by their respective ligand (H^+ and ACh respectively) result in reproducible current responses and that the evoked currents can be blocked pharmacologically by known reference compounds (amiloride and Gd^{3+} for ASIC1a and tetracaine for nAChRα1). These data demonstrate: 1) the design of the QChip384 allows fast liquid exchange on the Qube for recordings of fast LGICs and 2) the design of QChip384 allows multiple liquid additions to the same recording unit.

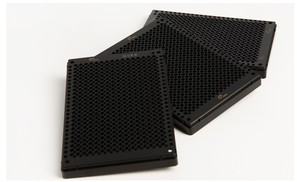
Methods

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_v2.1 currents were measured in RBL-2H3 cells (ATCC cat# CRL-2256).

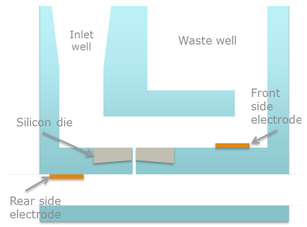
Solutions
The composition of the intracellular solution was (mM): CsF 140, EGTA 1, HEPES 10, NaCl 10 (pH 7.2 with CsOH) and the extracellular (mM): $CaCl_2$ 2, $MgCl_2$ 1, HEPES 10, KCl 4, NaCl 145, glucose 10 (pH 7.4 with NaOH). Low pH^+ solution for ASIC was buffered with MES. In the liquid exchange 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).

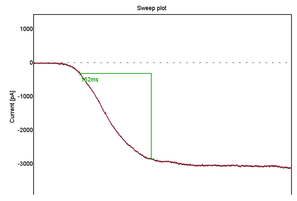
Figure 1 The Qube consumable



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

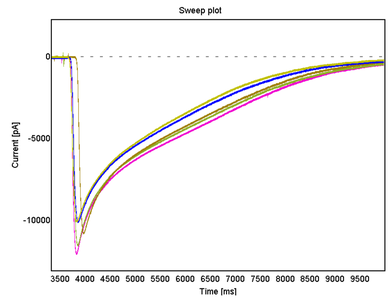


B) Schematic drawing of an individual recording unit. As can be seen the electrodes are integrated in the design which minimizes variability between runs and eliminates the need for maintenance chlorination. The waste can be efficiently emptied enabling cumulative dose-response studies. The Qube consumable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

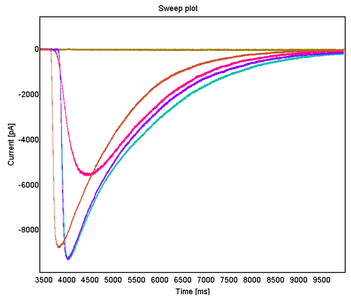


C) The rise time for the liquid exchange was measured using the Kir2.1 expressing cell line RBL and alternating solutions containing high and low concentration of K^+. The average 10-90% rise time across the plate was 170 ± 9 ms.

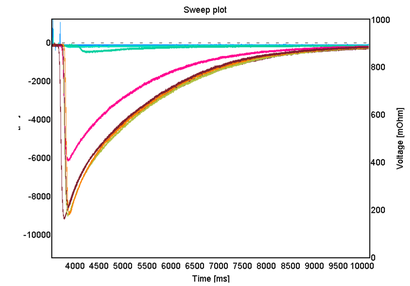
Figure 2 Activation and steady-state inactivation of ASIC1a



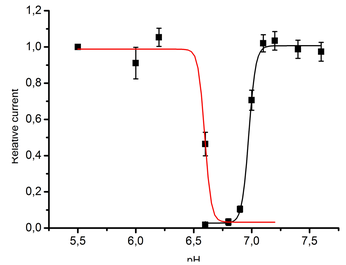
A) Current traces from a single cell stimulated with repetitive pulses of pH 6.2.



B) Current traces evoked by stimulation with increasing concentrations of extracellular protons (pH 6.8, 6.6, 6.0, and 5.5). The 10-90% rise times (t_{10-90}) for the currents were 0.35 ± 0.01 at pH 6.6, 0.15 ± 0.01 at pH 6.2, 0.14 ± 0.01 at pH 6.0 and 0.10 ± 0.01 at pH 5.5 seconds, respectively (no attempt to measure at pH 6.8 was made due to the small currents).

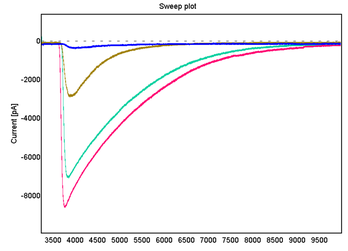


C) ASIC1a currents in response to pH 6.2 after pretreatment with different pH (7.6, 7.4, 7.2, 7.1, 7.0, 6.9, 6.8, and 6.5).

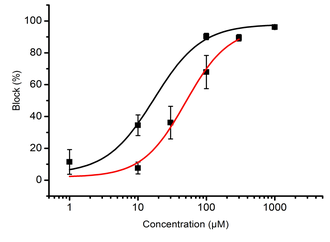


D) Activation (red line) and inactivation (black line) relationships for ASIC1a. EC_{50} for activation was pH 6.6 and for inactivation pH 6.9 (3).

Figure 3 Dose-response relationships for ASIC1a

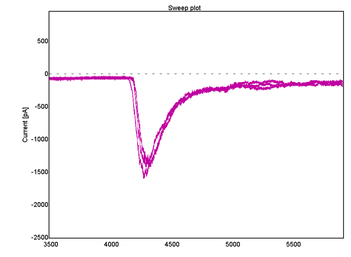


A) ASIC1a current traces in response to pH 6.2 in the presence of increasing concentrations of Gd^{3+} (0, 1, 10 and 100 µM).

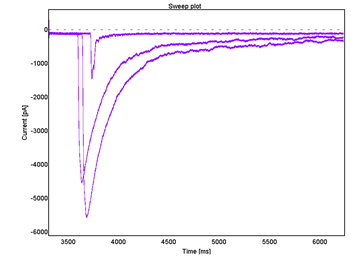


B) Dose-response relationships for Gd^{3+} (black line) and amiloride (red line). The IC_{50} for Gd^{3+} and amiloride were 20 and 50 µM, respectively. In the amiloride experiments the cells were stimulated using pH 5.5 in the presence of increasing concentrations of amiloride (10, 30, 100 and 300 µM). Currents were normalized to currents evoked by pH 6.2 and pH 5.5, respectively in the absence of inhibitor.

Figure 4 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 (6).

Conclusion

In this work we have examined the liquid exchange properties of the QChip384 consumable developed for the new Qube instrument – the very first giga-seal-based 384-channel planar patch clamp system.

We have shown that the QChip384 supports multiple liquid additions with high reproducibility. The liquid exchange properties of the recording unit allow reliable and fast delivery of activating and blocking compounds which enable quality measurements of currents from ligand-gated ion channels such as ASIC 1a and nAChRα1. Further, we have also shown pharmacological block of ASIC1a by amiloride and Gd^{3+} yield IC_{50} values in expected literature ranges (4, 5) and that an efficient wash-out of pharmacological compounds can be obtained.

The Qube system is the next generation patch clamp platform aimed at the high throughput segment of ion channel screening. With the Qube you get the highest data quality which accelerates your lead finding process. The Qube is real patch clamping, real fast.

References

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