ENDOGENOUS ION CHANNELS OF MAMMALIAN CELL LINES CHARACTERIZED WITH THE QPATCH™

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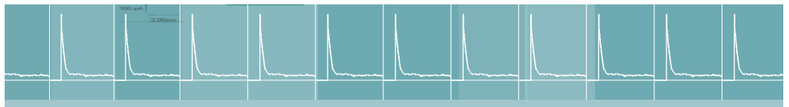
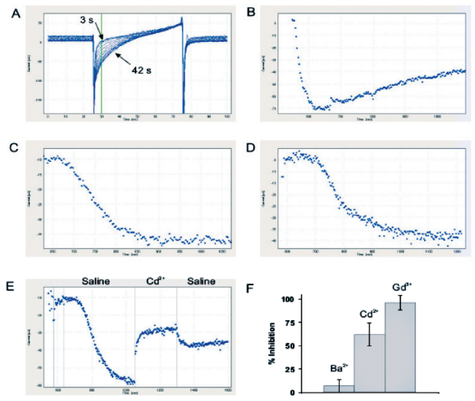


A large number of mammalian cell lines are commercially available to be used as expression systems for membrane or cytoplasmal proteins. A number of voltage and ligand gated ion channels of potential interest for the pharmaceutical industry are endogenously expressed in several CNS and non-CNS cell lines including TTX-sensitive Na^+ channels, Ca^{2+}-release activated Ca^{2+} (CRAC) channels, inward rectifier K^+ channels, acid-sensing ion channels (ASIC) and muscarinic alpha-adrenergic receptors mAChR). We have explored the applicability of five commonly employed cell lines from American Type Culture Collection (ATTC) for use with Sophions QPatch™ automated patch clamp systems (QPatch 16 and QPatch HT) and characterized the ion channel types that they endogenously express. Specificly we have explored:

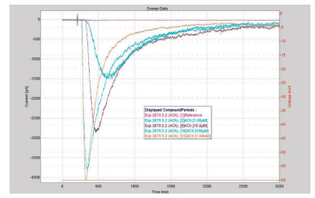
- Suitability for automated patch clamp studies ('patchability')
- Background ionic currents that may interfere with currents of experimentally expressed ion channels
- Possible use for characterizing ion channels of interest without the need to experimentally introduce their genes (expression)

The tests have led to the development of a number of simple standard operation procedures (SOPs) for employment of the cell lines in QPatch characterizations of ion channels.



CELL LINE: RBL-2H3

Figure 5.
Ca^{2+}-release activated Ca^{2+} (CRAC) channel currents (I_{CRAC}) in RBL-2H3 rat basophilic leukemia cells.
A: Family 14 of superimposed I_{CRAC} obtained after stimulation with 10 µM Ins(1,4,5)P_3 and 10 mM BAPTA. Time constant for current trace # 1 and 14 are indicated. **B:** Current amplitude at -80 mV for 224 current traces including those shown in panel A. **C:** I-t plot showing the activation of I_{CRAC} by 15 mM BAPTA. **D:** Activation of I_{CRAC} in the absence of external Na^+ (NMDG substitution). **E:** I-t plot showing inhibition of I_{CRAC} by $CdCl_2$. **F:** Efficacy of $BaCl_2$, $CdCl_2$ and $GdCl_3$ in blocking I_{CRAC} (Adapted from Schrøder et al., J Biomol Screen, 13:638-643, 2008).



CELL LINE: TE-671

Figure 6.
Raw current traces for the activation of mAChR currents of TE-671 human medullablastoma cells by 1-1000 µM acetylcholine (Ach).

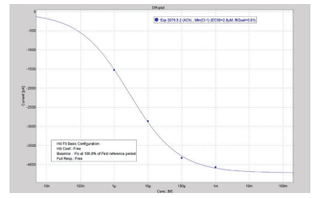


Figure 7.
Concentration-response plot for the activation of mAChR currents by 1-1000 µM Ach. EC_{50}=2.67±1.1 µM (7.8 µM, Shao et al., J Pharmacol Exp Theor, 286: 1269-1276, 1998).

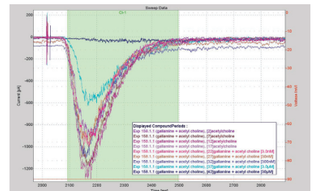


Figure 8.
Raw mAChR current traces showing successive inhibition with increasing concentrations of the antagonist gallamine (3 nM to 30 µM). Currents were elicited by exposure to 1 µM Ach.

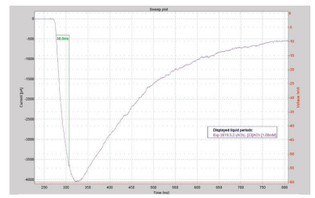


Figure 9.
Rise time of mAChR current with 1 mM of acethylcholine.

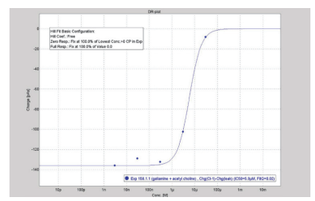


Figure 10.
Concentration-response plot for the inhibition of mAChR currents of TE-671 human medullablastoma cells by gallamine (3 nM to 30 µM). IC_{50}=5.9 µM.

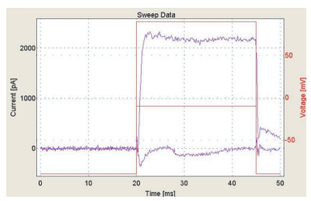
BASIC PROPERTIES OF FIVE CELL LINES: PATCHABILITY AND EXPRESSION OF ENDOGENOUS ION CHANNELS

Cell line	Origin	ATTC #	Basic patchability			Na^+ current		K^+ current		Ca^{2+} current		LGIC current	
			R_{seal} (GOhm)	$R_{wholecell}$ (GOhm)	Wholecells (%)	Expression (%)	I_{peak} (pA)	Expression (%)	I_{peak} (pA)	Expression (%)	I_{peak} (pA)	Expression (%)	I_{peak} (pA)
SH-SY5Y	Human neuroblastoma	CRL-2266™	3.42±1.01	2.08±1.01	38	28 (TTX insensitive)	-409±101	16 (K_v3.x)	1040±358	3	-180	-	-
PC-12	Rat adrenal gland	CRL-1721™	1.22±0.35	6.47±2.30	46	-	-	53	263±164	-	-	-	-
IMR-32	Human neuroblastoma	CCL-127™	6.36±2.26	4.31±1.75	33	74	-377±68	21	113±13	-	-	-	-
RBL-2H3	Rat basophilic leukemia	CRL-2256™	1.60±0.21	1.98±0.46	77	-	-	100 K_{ir}	-1087±79[1]	95 CRAC	-32±3	-	-
TE671	Human medullablastoma	CRL-8805™	0.87±0.16	0.87±0.13	85	100	1525±330	-	-	-	-	100 mAChR[2]	1190±149

Basic Properties
The table collects the following types of data for the five cell lines that were characterized:
1. patchability with QPatch™ based on gigaseal and whole-cell resistances and whole-cell success rate
2. expression level of the endogenous ion channel species as percentage of cell recordings that exhibited the specific ionic current
3. whole-cell current amplitudes of the identified ion channels. The nature of the ion channels were based on use of blockers, activators and in substitutions. The functional properties are presented in the figures below

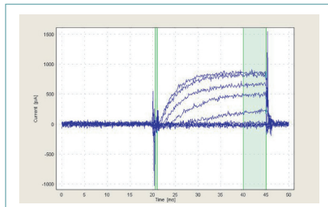
The patchability was tested with physiological Ringer's solutions and without specific optimization of test conditions. For all cell lines: n>40. Where the identity of ion channels is positively known the name is given without parenthesis. Ion channels listed in parenthesis indicate most likely identity.

1: In response to 140 mM K^+ at -40 mV
2: In response to 370 µM ACh



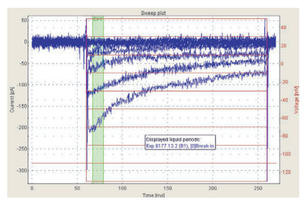
CELL LINE:SH-SY5Y

Figure 1.
Representative raw current traces from SH-SY5Y human neuroblastoma cells in response to two different pulse stimulations: 1. A large outward TEA sensitive K^+ current, and 2. a inward fast inactivating TTX blockable Na^+ channel current followed by a slowly inactivating Cd^{2+} sensitive Ca^{2+} channel current



CELL LINE: PC-12

Figure 2.
Raw traces of slowly activating K^+ currents in PC-12 rat adrenal gland cells in response to increasing depolarizing test pulses. The cell was held at -80 mV between the 50 ms test pulses that ranged from -80 to 80 mV.



CELL LINE: IMR-32

Figure 3.
Raw Ca^{2+} (or Na^+) current traces in response to increasingly depolarizing test pulses. Test pulses were 200 ms long stepping from a holding potential of -110 mV to -130 mV increasing to 50 mV. The Ca^{2+} currents were not seen in the initial experiments but were seen after the cells had been differentiated with neuronal growth factor.

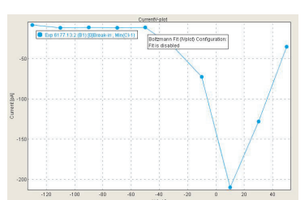


Figure 4.
I-V relationship of endogenous Ca^{2+} (or Na^+) channels in IMR-32 human neuroblastoma cells.

SUMMARY:

Five commercially available cell lines were tested for use with QPatch™ automated patch clamp systems and for their expression of endogenous ion channels. All cell lines proved to be well suited for exploration with the QPatch technology. It was found that each cell line exhibited a specific and characteristic expression of ion channels including sodium, potassium and calcium channels as well as ligand-gated cation channels. It is concluded that the selection of a proper cell line in several cases may eliminate the need for expression of specific ion channel genes in drug testing assays.
Standard SOPs for assays based on QPatch™ recordings of ion channel currents of the five tested, commercially available cell lines are available from Sophion Bioscience.