ASSESSING FUNCTIONAL PROPERTIES OF LIGAND-GATED ION CHANNELS WITH AUTOMATED WHOLE-CELL PATCH-CLAMP TECHNOLOGY

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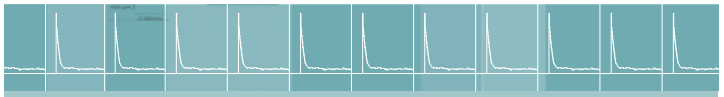

Most ligand-gated ion channels (LGICs) are characterized by fast transient currents in response to application of agonists. Typically the time constants for activation and subsequent desensitization amount to1-100 and 10-1000 ms, respectively. Consequently, recording of proper whole-cell LGIC currents with the patch-clamp technique requires a fast solution exchange system. Furthermore, characterization of LGIC blockers and modulators generally requires complex compound application protocols, because the effect of a test compound needs to be evaluated simultaneously with a transient application of the agonist. These requirements challenge a realistic electrophysiological characterization of LGICs. We employed the automated QPatch 16 patch-clamp system, to characterize the effects of agonists, antagonists and activators on two types of fast LGICs: (1) $GABA_A$ (γ-amino-butyric acid A) receptors and (2) ASIC (acid sensing ion channels) types 1a and 3.

MATERIALS AND METHODS

Cells. Cultured HEK293 cells stably expressing $GABA_A$, ASIC1a and ASIC3 were used.

Patch-clamp system. The QPatch 16 performs patch-clamp experiments on the disposable QPlate which contains 16 individual and parallel patch-clamp positions. Solutions and compounds are applied by a 4-pipette pipeting robot. Cells may be kept in culture medium in an on-board stirred reservoir for up to 4 hours. Prior to testing, the cells are automatically transferred to an on-board mini centrifuge, spun down and resuspended in Ringer's solution and washed before being applied to the pipetting wells in the QPlate. Gigaseals are formed upon execution of a combined suction/voltage protocol. Subsequent increased suction leads to the whole-cell configuration. Solutions and compounds are applied through integrated glass-coated microfluidic flow channels. Liquid flow is laminar with exchange time constants in the range 50-100 ms. After application, all fluids are collected in the built-in waste reservoir (100 µL). Whole-cell currents were measured at a holding potential of -90 mV.

Ringers. For $GABA_A$ experiments the extracellular Ringer's solution consisted of (in mM): 145 Na^+, 4 K^+, 2 Mg^{2+}, 155 Cl^-, 10 HEPES (pH 7.4), and the intracellular Ringer's solution consisted of (in mM): 120 K^+, 1.8 Mg^{2+}, 123.6 Cl^-, 10 EGTA, 10 HEPES (pH 7.2). For ASIC experiments the extracellular Ringer's solution consisted of (in mM): 140 Na^+, 3 K^+, 1 Ca^{2+}, 1 Mg^{2+}, 167.02 Cl^-, 5 MES, 5 HEPES (pH 7.3), and the intracellular Ringer's solution consisted of (in mM): 10 Na^+, 136 Cs^+, 10 Cl^-, 135 F , 1 EGTA, 10 HEPES (pH 7.3).

Protocols. Agonist protocol: Figure 1 (upper panel) shows the sequence of exposures to extracellular Ringer's solutions. The agonists were applied for 2 seconds. Increasing intensity of color (red) indicates increasing doses of agonist. Antagonist and modulator protocol: Figure 1 (middle and lower panel) shows the sequence of extracellular Ringer's solutions applied. Increasing color intensity indicates increasing concentrations of antagonist (blue) and activator (green). Agonist application is indicated in red.

Data analysis. Recorded ion channel whole-cell currents were stored in an integrated database (Oracle). Drug effects were analyzed as function of concentration (dose-response relationship). Data analysis was accomplished with the QPatch Assay Software.

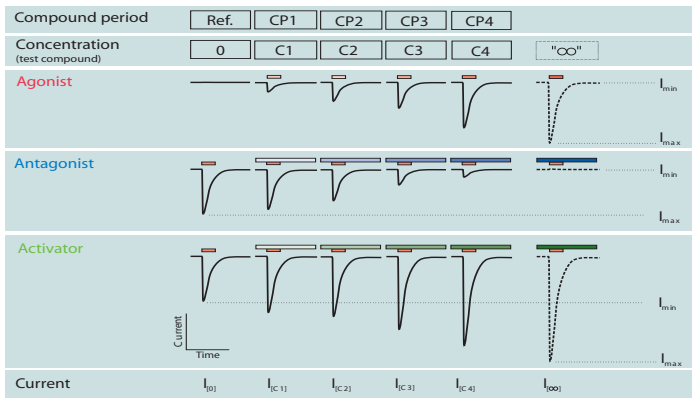

Figure 1. Compound application protocols
Increasing color intensities indicate increasing compound concentrations. Red color indicates agonist, blue color indicates antagonist, and green color indicates activator.

$GABA_A$ Receptor ($\alpha_1\beta_2\gamma_2$)

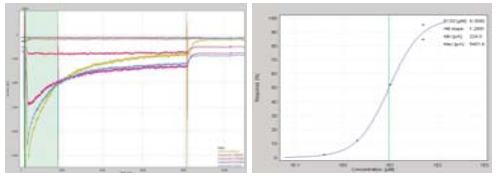

Figure 2. Effect of GABA
Original recordings of $GABA_A$ receptor currents in response to increasing concentrations of the agonist GABA (left). Based on the recorded peak currents the concentration-response relationship was constructed (right).
EC_{50} = 9.36 µM.
(Literature values: 9-18 µM).

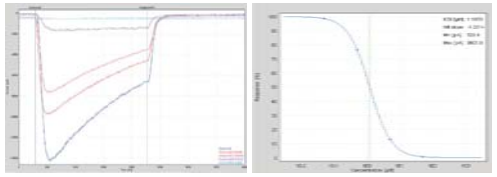

Figure 3. Effect of bicuculline
Original recordings of $GABA_A$ receptor currents in response to 10 µM GABA in the presence of increasing concentrations of the antagonist bicuculline (left). Based on the recorded peak currents the concentration-response relationship was constructed (right).
IC_{50} = 1.20 µM.
(Literature values: 1-3 µM).

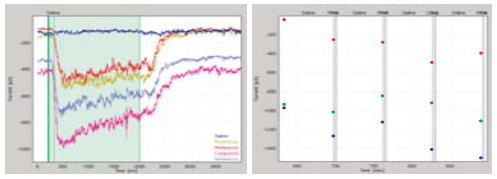

Figure 4. Effect of chlordizepoxide
$GABA_A$ receptor currents in response to 10 µM GABA in the absence or presence of 20 µM of the activator chlordizepoxide (left). I-t plot of the $GABA_A$ currents (right). Red dots represent leak-corrected currents. Blue and green dots represent uncorrected currents and leak currents, respectively. The effect of chlordizepoxide was at least partially reversible as seen by the return toward baseline values upon wash.

ASIC1a

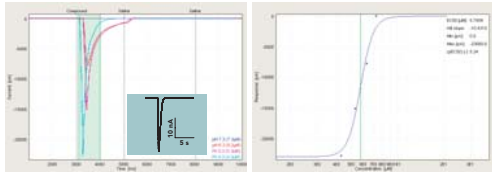

Figure 5. Effect of pH
Original recordings of ASIC1a currents in response to increasing degrees of acidification of the extracellular solution from pH 7.3 to 4.3 (left). Inset shows manual patch-clamp recording using the same protocol. Based on the recorded peak currents the pH-response relationship was constructed (right).
pH_{50} = 6.3±0.3 (n=4).
(Literature values: 5.8-6.5).

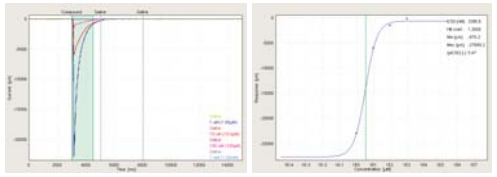

Figure 6. Effect of amiloride
Original recordings of ASIC1a currents elicited by reduction of extracellular pH from 7.3 to 4.3 in the presence of four increasing concentrations (1 µM to 1 mM) of the antagonist amiloride (left). Based on the recorded peak currents the concentration-response relationship was constructed (right).
IC_{50} = 13.3±4.0 µM (n=4).
(Literature values: 2.2-10 µM).

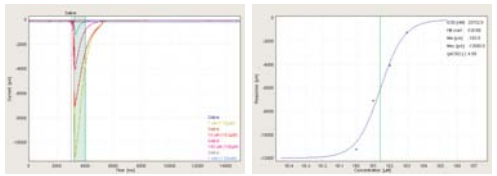

Figure 7. Effect of gadolinium
Original recordings of ASIC1a currents elicited by reduction of extracellular pH from 7.3 to 4.3 in the presence of four increasing concentrations (1 µM to 1 mM) of the antagonist gadolinium (left). Based on the recorded peak currents the concentration-response relationship was constructed (right).
IC_{50} = 29±14 µM (n=5).
(Literature values N/A).

ASIC3

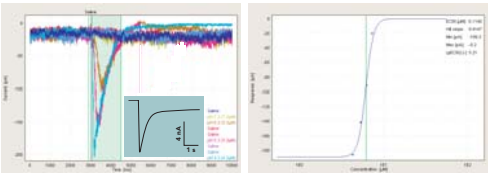

Figure 8. Effect of pH
Original recordings of ASIC3 currents in response to increasing degrees of acidification of the extracellular solution from pH 7.3 to 4.3 (left). Inset shows manual patch-clamp recording using the same protocol. Based on the recorded peak currents the pH-response relationship was constructed (right).
pH_{50} = 6.5±0.1 (n=9).
(Literature values: 4.2-6.7).

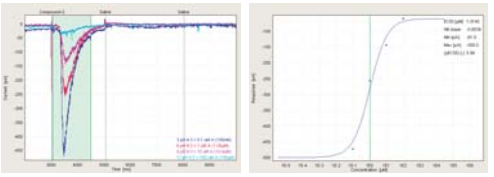

Figure 9. Effect of amiloride
Original recordings of ASIC1a currents elicited by reduction of extracellular pH from 7.3 to 4.3 in the presence of increasing concentrations (1 µM to 1 mM) of the antagonist amiloride (left). Based on the recorded peak currents the concentration-response relationship was constructed (right).
IC_{50} = 5.0±0.8 µM (n=8).
(Literature values: 16-63 µM).

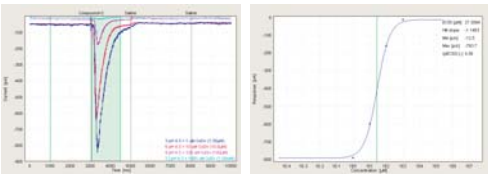

Figure 10. Effect of gadolinium
Original recordings of ASIC3 currents elicited by reduction of extracellular pH from 7.3 to 4.3 in the presence of increasing concentrations (1 µM to 1 mM) of the antagonist gadolinium (left). Based on the recorded peak currents the concentration-response relationship was constructed (right).
IC_{50} = 41±12 µM (n=3).
(Literature values N/A).

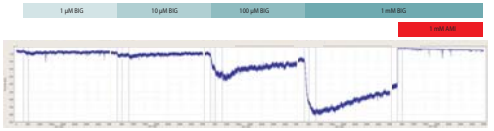

Figure 11. Effect of benzimidozolyl-guanidinium (BIG)
Effect of increasing concentrations (1-1000 µM) of BIG on unstimulated ASIC3 currents at pH 7.3. The channels are activated by BIG as seen by the sustained dose-dependent negative current. Amiloride (1 mM) blocked the current completely.

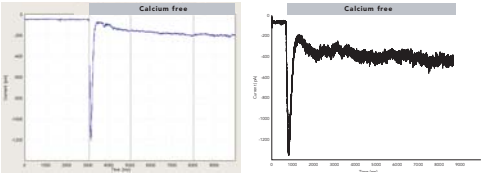

Figure 12. Effect of Ca^{2+} removal
Effect of extracellular Ca^{2+} removal on ASIC3 currents recorded at an extracellular pH of 7.3. After an initial current transient a sustained negative current develops. Left panel shows currents obtained with QPatch 16. Right panel shows currents obtained with conventional (manual) patch-clamp.

The sustained BIG activation of inward ASIC3 currents are similar to those elicited by removal of extracellular Ca^{2+} suggesting that BIG might act by displacing Ca^{2+}, which inhibits the channel, from an extracellular binding site. Interestingly, Ca^{2+} removal, but not BIG application, induces an inward current transient. No current stimulation in response to BIG and Ca^{2+} removal was seen for ASIC1a (data not shown).

SUMMARY

Three types of ligand-gated ion channels were profiled pharmacologically using the automated QPatch 16 patch-clamp system. The effects of agonists, an antagonists and activators were tested and the EC_{50} or IC_{50} values determined. The values obtained were generally similar to published values obtained with conventional patch-clamp.