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

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 to 10-100 and 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 effects 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, -aminobutyric acid A) receptors and (2) ASIC acid sensing ion channel types 1a and 3.

MATERIALS AND METHODS

Cells. Cultured HEK293 cells stably expressing GABA_A, ASIC 1a and ASIC 3 were used.

Patch-clamp system. The QPatch 16 performs patch-clamp experiments on up to 16 parallel patch-clamp positions. Solutions and compounds are applied by 8-pipette generating robot. Culture plates are basenivected in culture medium in an on-bottom-sealed chamber up to 4 hours. Prior to testing, the cells are automatically transferred to an on-bottom-sealed configuration. Subsequently, the culture plate is mounted in the QPatch 16 and the pipette is positioned for simultaneous recording. Subsequently increased suction leads to the whole-cell configuration. Subsequent increased suction also leads to the whole-cell configuration. Subsequent increased suction and the intracellular Ringer's solution consisted of (in mM): 120 K+, 1.8 Mg2+, 123.6 Cl-, 10 EGTA, 10 HEPES (pH 7.2). For ASIC experiments, the extracellular Ringer's solution consisted of (in mM): 110 Na+, 1.8 K+, 4 Ca2+, 10 Cl-, 10 HEPES (pH 7.2), and the intracellular Ringer's solution consisted of (in mM): 100 Cs+, 10 Na+, 10 Cl-, 10 HEPES (pH 7.2) in a chamber. Liquid flows between well-matched time constants in the range 50-100 ms. After application, all fluids are collected in a built-in waste reservoir (150 μl). Whole-cell currents were measured at holding potentials of -60 mV.

Data analysis. Recorded channel whole-cell currents were stored in an integrated database (Origin). Drug effects were analyzed as function of concentration (dose-response relationships) or in control experiments (control-vehicle relationship). Data analysis was accomplished with the QPatch assay software.

ASIC1a

Figure 1. Compound application protocols

Increasing current amplitudes indicate increasing compound concentrations. Red color indicates agonist, blue color indicates antagonist, and green color indicates activator.

Figure 2. Effect of GABA_A Receptor (α1β2γ)

Original recordings of GABA_A receptor currents in response to increasing concentrations of 1 μM BIG (left). Based on the recorded peak currents the concentration-response relationship was constructed (right). IC_{50} = 6.3 μM (literature values 5.8-6.5).

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 (right). Based on the recorded peak currents the concentration-response relationship was constructed (right). IC_{50} = 1 μM (literature values 5-10 μM).

Figure 4. Effect of phorbol 12-myristate 13-acetate (PMA)

Original recordings of GABA_A receptor currents in response to increasing concentrations of PMA from 1 μM to 10 μM (left). Based on the recorded peak currents the concentration-response relationship was constructed (right). IC_{50} = 1.8 μM (literature values 1.5-2 μM).

Figure 5. Effect of pH

Original recordings of ASIC1a currents elicited by reduction of extracellular pH from 7.3 to 4.3 in the presence of increasing concentrations of the antagonist amiloride (left). Based on the recorded peak currents the concentration-response relationship was constructed (right). IC_{50} = 4.5 μM (literature values 4-5 μM).

Figure 6. Effect of extracellular Ca2+ removal

Original recordings of ASIC1a currents elicited by reduction of extracellular Ca2+ from 2 mM to 0 mM (left). Based on the recorded peak currents the concentration-response relationship was constructed (right). IC_{50} = 1.6 μM (literature values 2.5-3 mM).

Figure 7. Effect of gadolinium

Original recordings of ASIC1a currents elicited by reduction of extracellular Ca2+ from 2 mM to 0 mM (left). Based on the recorded peak currents the concentration-response relationship was constructed (right). IC_{50} = 1.6 μM (literature values 2.5-3 mM).

Figure 8. Effect of pH

Original recordings of ASIC3 currents in response to increasing concentrations of pH from 7.3 to 4.3 (left). Based on the recorded peak currents the concentration-response relationship was constructed (right). IC_{50} = 4.5 μM (literature values 4-5 μM).

Figure 9. Effect of amiloride

Original recordings of ASIC3 currents elicited by reduction of extracellular pH from 7.3 to 4.3 in the presence of increasing concentrations of the antagonist amiloride (left). Based on the recorded peak currents the concentration-response relationship was constructed (right). IC_{50} = 4.5 μM (literature values 4-5 μM).

Figure 10. Effect of amiloride

Original recordings of ASIC3 currents elicited by reduction of extracellular pH from 7.3 to 4.3 in the presence of increasing concentrations of the antagonist amiloride (left). Based on the recorded peak currents the concentration-response relationship was constructed (right). IC_{50} = 4.5 μM (literature values 4-5 μM).

Figure 11. Effect of bicuculline

Original recordings of ASIC3 currents elicited by reduction of extracellular pH from 7.3 to 4.3 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} = 4.5 μM (literature values 5-10 μM).

SUMMARY

Three types of ligand-gated ion channels were profiled pharmacologically using the automated QPatch 16 patch-clamp system. The effects of agonists, antagonists and activators were tested and the IC_{50} or IC_{90} values determined. The values obtained were generally similar to published values obtained with conventional patch-clamp.