The Acid-Sensing Ion Channel (ASIC)

[a]L. Schild, [a]Y. Gauchi, [b]S. Bertrand & D. Bertrand[b]

[a]Department of Pharmacology, Lausanne, Switzerland; [b]HiQScreen, 6 rte de Compois, 1222 Vésenaz, Geneva, Switzerland

Introduction

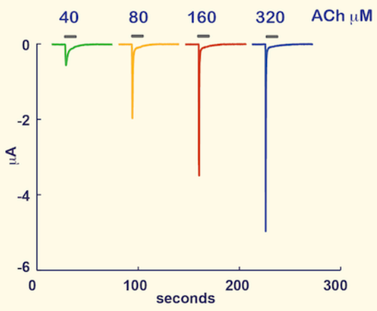
Acid-sensing (proton-gated) ion channels belong to the ENaC/degenerin superfamily of sodium (Na+) channels (Lv et al., 2011) that includes the epithelial Na+ channel (ENac). ASIC channels are multimeric channels made of homologous subunits; the subunits ASIC1-4 are encoded by four different genes ACCN1-4 genes. Evidence support the role of ASICs in pain sensation, expression of fear, neurodegenerarion after ischemia (see for review Wemmie 2013). Spontaneous mutations in ASIC1a have been associated to genetically transmissible diseases such as temporal lobe epilepsies. Activated by low pH, ASIC's display a fast response onset and rapid desensitization. Sharing similarities to other ligand gated such as the neuronal nicotinic acetylcholine receptors, ASIC1 are inactivated (desensitized) by sustained exposure to a condition that otherwise produces activation. Namely, desensitization occurs at pH that are higher (more alkaline) than those causing its activation, which results in activation and desensitization profiles that do not necessarily overlap or yield the so call window currents. Probing ASIC channels function was conducted using heterologous expression in Xenopus oocytes as well as in patch clamp in transfected cells (Vallet et al., 1998; Waldmann et al., 1997; Li et al., 2012). Development of HiClamp, an automated electrophysiological system using expression in Xenopus oocytes offers fast drug application and minimal compound volume allowing multiple measurements in 230 µL of solution and was shown to be optimum for functional investigation of ligand gated channels. In this work we present the latest developments of automated recordings for ASIC1a channels expressed in Xenopus oocytes and revisit physiological and pharmacological properties of this important class of ion channels. Offering new possibilities for functional screening of these rapidly desensitizing channels this work illustrates how to probe effects of libraries of compound in an unattended manner.

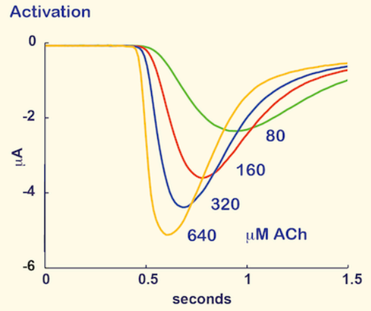
The HiClamp Principle

A Fully Automated System

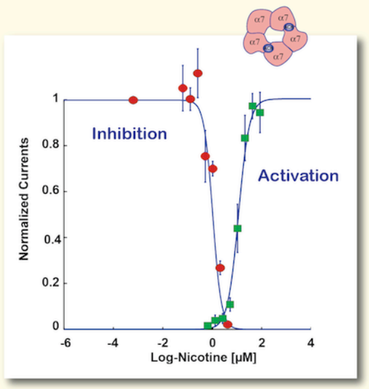
Measurements were carried out using the HiClamp

Functional properties of human nAChRs α7





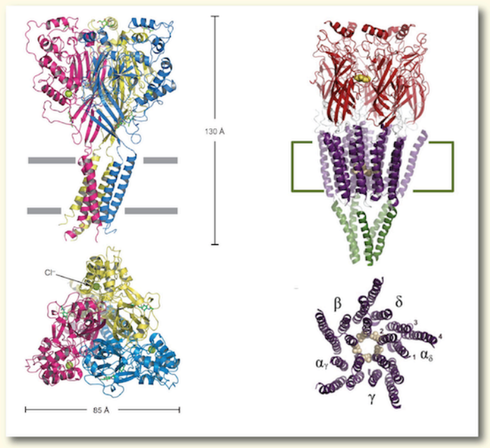
Activation of the α7 nAChR by brief ACh test pulses (5 s) evokes inward currents displaying a rapid onset and fast desensitization. Plot of the amplitude of the peak current as a function of the ligand concentration yields a typical dose-response profile. Sustained exposure to the agonist desensitizes the receptors at concentrations lower to those causing its activation.



Conclusions

Data presented herein illustrate the feasibility of recording of ASIC channels using the fully automated recording system HiQClamp. Advantages of this system which can be used unattended and for screening of hundreds of compounds also include the non-destructive use of small volume and fast drug exposure. Recording of the activation and desensitization time course of ASIC1a and the α7 nAChR reveals the faster responses of the later. The similitude of results obtained with a conventional two-electrode voltage clamp and HiClamp further underline the capacity of this system.

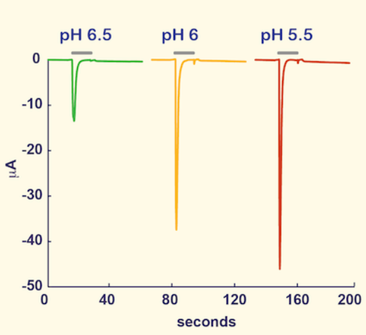
Structure comparison of the ASIC and nAChRs

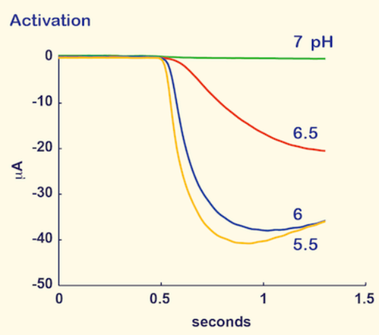


Jasti et al., Nature 2007

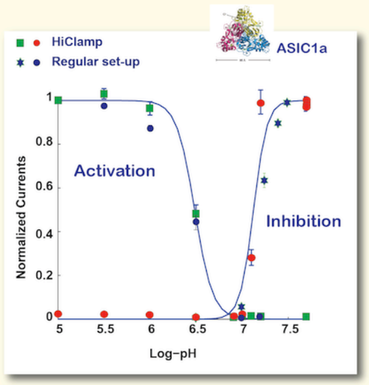
Baezinger et al., Neuropharmacol 2010

Functional properties of the human ASIC1a





Activation of the ASIC channels was obtained by brief steps at lower pH. Time course of the responses show the rapid onset of the current reflecting receptor opening. Plot of the peak inward current as a function of the pH yields the dose-response activation curve. Desensitization caused by sustained exposure to pH close to 7 reveals a high steepness. Blue dots were recorded with a conventional system.



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