effect of these substances. The therapeutic consequences in cases of poisoning by organophosphorus compounds has yet to be tested.

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Temperature-Dependence of Allosteric Modulators of Nicotinic Ach-Receptors

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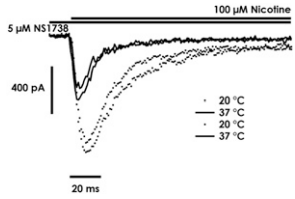
Nicotinic acetylcholine receptors (nAChRs) belong to the superfamily of ligand-gated ion channels. The most abundant homomeric nAChR in the mammalian brain are the pentameric α7 nAChRs, which consist of five α7 subunits. Positive allosteric modulators (PAMs) of nAChRs have been implicated to be putative therapeutics in the pharmacological treatment of Alzheimer's disease, schizophrenia and Parkinson's disease.

Recently, Sitzia et al. (2011) showed a striking temperature dependence for positive allosteric modulation of PNU-120596 on nAChRs. The aim of our study was to investigate the temperature dependence of other PAMs on nACHRs with automated patch clamp techniques. For this study, we have used GH4C1cells expressing human nAChR α7 subunits. Electrophysiological recordings were performed on the Patchliner, a robotic patch clamp device allowing temperature controlled recordings from up to 8 cells simultaneously.



First experiments on GABA A receptors allude to a similar but weaker effect on the temperature dependence of PAMs.

These data suggest that experiments on the efficacy of PAMs conducted at room temperature have to be interpreted with caution.

Sitzia et al (2011) FrontPharmacol;2:81.

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VIP Enhances Acetylcholine-Activated Potassium Current via a cAMP/PKA Transduction Pathway

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Background: Recent studies have indicated that various components of cardiac nerve network may play important role in atrial fibrillation (AF). Vagal stimulation shortens atrial effective refractory period, predominantly via the activation of the acetylcholine-activated potassium channel (IKACh) that shortens action potential duration and increases the vulnerability to AF. Vasoactive intestine polypeptide (VIP), a neural polypeptide co-released with acetylcholine (ACh) largely from intrinsic cardiac neurons during vagal stimulation, was shown to contribute to the vulnerability to AF. However, the underlying mechanism remains unclear. The aim of this study is to investigate the effect of VIP on IKACh in the presence of ACh in rat atrial myocytes.

Methods: The effects of VIP on IKACh in isolated adult rat atrial myocytes were investigated with voltage patch-clamp recording techniques. IKACh were recorded as an inwardly rectifying current activated by muscarinic agonist carbachol (CCH, 1µM). Furthermore, the post-synaptic signal pathway was investigated with Rp-cAMP (10 µM), a competitive antagonist of cyclic AMP (cAMP), H-89 (0.1µM), PKA inhibitor, and forskolin (0.1µM), an activator of adenylyl cyclase.

Results: VIP (1µM) potentiates IKACh at a holding potential of −115 mV (VIP: −19.34 ± 5.22 pA/pF, n=12, vs. baseline: −15.85 ± 2.66 pA/pF, n=14, $p<0.05$). The enhancement of IKACh by VIP could be mitigated by Rp-cAMP (VIP plus Rp-cAMP: −8.24 ± 1.44 pA/pF n= 6; $p<0.05$ vs. baseline), and could be inhibited by H-89 (VIP plus H-89: −13.41 ± 2.95 pA/pF n=6; p=0.59 vs. baseline). Furthermore, these enhancements could be mimicked by an activator of adenylyl cyclase, forskolin (−27.35 ± 4.82pA/pF n= 5; $p<0.01$ vs. baseline).

Conclusion: VIP enhanced IKACh in atrial myocytes which might contribute to vagal-induced APD shortening and is likely mediated by a cAMP-PKA dependent pathway.

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Study of the Interaction between General Anesthetics and a Bacterial Homologue to the Human Nicotinic Receptor

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Pentameric Ligand Gated Ion Channels (pLGICs) are membrane receptors widely spread in the animal kingdom that play a key role in the nervous signal transduction. The discovery and the crystallization of bacterial homologues, such as GLIC from Gloeobacter violaceus in 2008 (Bocquet et al., Nature, 2008, 457:111; Hilf & Dutzler, Nature, 2008, 457:115) and of a novel locally-closed form (Nury et al. Nature, 2011, 469:428) provided new insights in the understanding of the operating mechanism of these channels.

General anesthetics (GAs) such as propofol and desflurane could be co-crystallized with GLIC (see for example Nury et al., Nature, 2011, 469:428). The potential existence of several binding sites for GAs, alcohols and ions that modulate the activity of the channel is currently debated.

Here, we intend to study the dynamic properties of the interactions between GLIC-desflurane and GLIC-propofol by means of molecular dynamics simulations, on the basis of existing crystal structures of open and locally-closed forms of the channel. We computed more than one hundred all-atom simulations of the ligand-bound GLIC system inserted in a membrane to obtain significant statistics on the exploration of the cavity by desflurane and propofol. We complete this study by free energy calculations to estimate the affinity of propofol and desflurane to both the inter- and intra-subunit cavities. This approach may allow us to explore the ligand-filled cavity rather exhaustively and provide a sound background for deriving hypotheses on GAs.

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Ligand Gated Ion Channels on the Qube

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Drug discovery on ion channel targets has been an under-explored territory because suitable screening tools for patch clamp were missing. This changed during the 2000's with the introduction of automated patch clamp (APC) devices. Still, the throughput and running cost of APC devices did not allow for the employment of the patch clamp assays in primary screening. Because of this bottleneck, APC has remained a secondary screening tool. We are now presenting data from the first true gigaseal-based 384-channel planar patch clamp system, the Qube, capable of providing the throughput needed for primary screening. The Qube is a collection of years of experience with planar patch clamp devices: High quality, reliability and easy to use concepts are the core in both the tried-and-tested silicon technology of the consumable, and in the technologies used in the instrument. The Qube offers GΩ seals and efficient integrated liquid flow in 384-well format.

We show here that the Qube provides exceptional data quality for electrophysiological assays with fast desensitizing ligand-gated ion channels. The fast liquid exchange permits accurate XC_{50} estimations. We have tested the acid sensing ion channel (ASIC1) and the nicotinic acetylcholine receptor (nAchRα1) on the Qube in both agonist and antagonist configurations, showcasing the instrument's broad flexibility in terms of assay design.

The results prove that: 1) Repetitive agonist applications result in reproducible current responses. 2) The agonist can be applied and washed off an unlimited number of times as long as the cell is stable. 3) XC_{50} values correspond to expected values (QPatch and literature).

Collectively, our results show that the design of the flow channels on the Qube consumable enables recordings on ligand-gated ion channels with the highest throughput capacity seen so far, without compromising data quality, reproducibility or reliability.

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Assessing Time-Dependent Changes in Relative Ca^{2+} Permeability using Patch Clamp Photometry

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Time-dependent changes in monovalent cation permeability have been reported for P2XR, TRPV1 and TRPA1. An important physiological question is whether relative Ca^{2+} permeability (P_{Ca}/P_{Na}) also changes during prolonged agonist exposure. Previous evidence obtained using current reversal potential (E_{Rev}) measurements suggests this to be the case for TRPV1, but it is not known for P2XRs. In our hands, both TRPV1- and P2X-transfected HEK293 cells exhibited time-dependent changes in N-methyl-D-glucamine permeability during prolonged exposure to capsaicin (10 microM) or ATP (30 microM) respectively, which occurred immediately and was complete within 10-15 s. Next we used patchclamp photometry to study time-dependent changes in fractional Ca^{2+} current (Pf%). These experiments were problematic due to Ca^{2+} entry often saturating the pipette Ca^{2+} chelator, fura-2, even under conditions of low external Ca^{2+}. Nevertheless, in experiments where fura-2 saturation did not occur within the first 5-10 seconds of stimulus, the relationship between total charge influx and change in fura-2 fluorescence was linear for both channels,