trations, and compared their response to a fixed ACh test pulse. A significant reduction in the RG3487 (3 and10 nM) induced current potentiation was observed in cells expressing the α7β2 nAChRs compared to cells expressing the α7nAChR alone. These data illustrate that the presence of the β2 in the α7 receptor complex modifies the overall properties of the nAChRs and could result in a differential sensitivity to compounds as the α7 and β2 are co-expressed in some areas of the brain.

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1.11

A-582941, a pro-cognitive α7 nAChR agonist, differentially modulates mitochondrial membrane potential

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Alzheimer's disease involves multiple pathogenic processes such as abnormal amyloid deposition, tau phosphorylation, oxidative stress as well as mitochondria dysfunction leading to progressive impairment and loss of cognitive function. An assay was established in SK-N-SH cells using JC-1 dye to measure mitochondrial membrane potential (MMP), as an indicator of mitochondrial health, and the effects of compounds on MMP. A-582941, an α7 nAChR partial agonist, was able to enhance MMP with a potency of 11.4 μM and 45% efficacy after overnight serum starvation, which reflects the early stages of apoptosis based on cell viability. Dimebolin and donepezil, other pro-cognitive compounds, but not memantine, also preserved MMP with potency and efficacy values of EC_{50} = 4.6 μM (100%) and 2.2 μM (93%), respectively. Similar results were obtained using either kainic acid or ionomycin as insults. From previous studies, these four compounds exhibit either preclinical or clinical efficacy in models of memory consolidation and short-term recognition. In addition, these compounds are neuroprotective against Aβ insult or promoting neurite outgrowth in primary cortical cultures. Dimebolin and donepezil also increase the MMP over a relatively wide concentration range without compromising nuclear morphology or plasma membrane integrity, both of which are indications of irreversible cellular injury. This approach may allow for further differentiation of pro-cognitive compounds. Studies further demonstrated that concentrations of A-582941, which gave less than a 25% response in preserving MMP was significantly potentiated (to 75%) when cells were simultaneously treated with combinations of A-582941 and dimebolin. Studies are underway to compare the effects of other α7 nAChR agonists with different profiles. Preservation of MMP is an essential event in rescuing neurons from energy-depletion in neurodegenerative states and inhibiting release of pro-apoptotic components.

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1.12

Discovery of nicotinic acetylcholine receptor ligands in the chemical universe database GDB-13

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It is a dream for every medicinal chemist to examine how any possible molecule could interact with a given target. Using parallel processing, we recently reported the exhaustive computational enumeration of all possible organic molecules up to 13 non-hydrogen atoms (C, N, O, S, Cl) in form of the chemical universe database GDB-13 (www.gdb.unibe.ch) [1]. We also showed that a previous version of the database, GDB-11, could be used to design analogs of known nicotinic ligands for synthesis and testing [2]. Here we used the database GDB-13 to search for analogs of the natural product nicotine. A fast similarity classification [3] was used to select 5000 close analogs of nicotine in GDB-13. While several (ca. 150) of these analogs were known AChRs ligands, 50 compounds with no reported activity on AChRs were selected and purchased from commercial vendors. The compounds were probed at α7 neuronal nicotinic receptors expressed in *Xenopus* oocytes using the fully automated electrophysiology HiClamp (Multichannel System). Three of the most active molecules were characterized in detail by determination of the EC_{50}'s and/or IC_{50}'s. Moreover, the mode of action of inhibitors was analyzed in competition experiments. Such ligand-based similarity searching in GDB-13 should be generally useful to rapidly expand the pharmacology of acetylcholine receptors and should help to identify potent and subtype selective agonists and antagonists.

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1.13

Ligand-based QSAR modeling of neuronal nicotinic receptor data and its impact on drug design

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Neuronal nicotinic receptors (NNRs) belong to the Cys-loop family of ligand-gated ion channels and form from five subunits as homologous or heterologous, oligomeric receptors. NNRs are of interest as targets for the treatment of a variety of central and peripheral nervous system disorders, including Alzheimer's, Parkinson's, and schizophrenia, as well as for cessation of smoking and pain management. Consequently, designing subtype selective ligands, i.e., orthosteric agonists and antagonists, allosteric modulators, and channel modulators of NNRs, is an active area of pharmaceutical research. Work on membrane-bound NNR proteins has provided key information on both the structure and function of NNRs, but a lack of high resolution protein structures limits structural design efforts. However, much progress has been achieved

using ligand-based approaches. A large number of ligand-based studies on NNRs have been conducted to explore NNR quantitative structure-activity relationships, identify pharmacophoric elements, and design novel and subtype selective NNR agents. Our studies will highlight examples of ligand-based modeling strategies of NNR ligands using a variety of methodologies (random forest, k nearest neighbors, Bayesian, shape and similarity based pharmacophore, etc.) with data based on orthosteric agonists and antagonists at α4β2, α7, α3β4 and/or α6β2* NNR subtypes. We will also briefly discuss application to ligand development for therapy.

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1.14

The twin drug approach for novel nicotinic acetylcholine receptor (nAChR) ligands: Synthesis and structure–affinity relationships

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We have used known nicotinic acetylcholine receptor (nAChR) ligands, as well as important elements of their pharmacophores, to design and synthesize novel nAChR ligands using the twin drug approach. Either two identical or two non-identical pharmacological entities were combined in different ways (linker, no linker, overlap). For example, we generated heterodimer ligands with one part derived from antioxidants, NSAIDs, scaffolds for monoamine related targets, or for beta-amyloid interaction and the second part derived from a nicotinic ligand. In a first approach to evaluate the effect of these compounds on diverse nAChRs, the compounds synthesized were tested for their affinities for different nAChR subtypes using the radioligands [^{3}H]epibatidine (α4β2*, α3β4* and muscle type nAChRs) and [^{3}H]methyllycaconitine (α7* nAChRs). We also tested these compounds on membrane fractions from rat brain, pig adrenals, and Torpedo californica electroplax in competition assays. A broad spectrum of affinities (e.g. Ki values for α4β2*: <10 nM to >10,000 nM) provided important insights into structure–affinity relationships. These studies will result in novel compounds that could ultimately be useful for development of therapeutics to treat disorders involving nAChR dysfunction.

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1.15

Scanning mutagenesis of α-conotoxin AuIB reveals a critical residue for activity at the α3β4 nicotinic acetylcholine receptor

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α-Conotoxin AuIB, a disulfide-bonded peptide of 15 amino acids with a 4/6 intercysteine spacing, inhibits the α3β4 nicotinic acetylcholine receptor (nAChR) subtype, which is a predominant subtype in the peripheral nervous system [1,2]. The ribbon isomer of AuIB has been shown to be more potent than the native AuIB (globular isomer) and to discriminate between stoichiometries of α3β4 nAChRs expressed in *Xenopus* oocytes [3]. AuIB also inhibits high voltage-activated N-type calcium channels in rat DRG neurons via the activation of G protein-coupled $GABA_B$ receptors [4]. Interestingly, α-conotoxin AuIB possesses analgesic activity *in vivo* and, therefore, may be a potential drug lead for treating chronic and neuropathic pain. In order to develop improved drugs void of side effects, it is necessary to understand the molecular determinants of AuIB binding to its putative targets: $GABA_B$ receptor vs. α3β4 nAChR. The aim of the present study was to determine the critical amino acid residues of AuIB responsible for its interaction with α3β4 nAChRs. Alanine scanning mutagenesis of the native AuIB peptide was carried out to construct AuIB alanine-substituted analogues which were tested in *Xenopus* oocytes expressing rat α3 and β4 subunits. Two-electrode voltage clamp recording was used to assess the effect of AuIB and its analogues (3 μM) on the ACh-evoked current amplitude. Phenylalanine to alanine mutation at position 9 of AuIB abolished inhibition of α3β4 nAChRs, whereas substitution of glycine at position 1 with alanine significantly reduced inhibition ($18.0 \pm 10.5\%$, $n = 3$) compared to native AuIB ($48.5 \pm 6.9\%$, $n = 7$) ($p < 0.05$). Mutation of residues other than cysteine and proline, which are known to disrupt the tertiary structure of α-conotoxins, did not significantly reduce the inhibition of ACh-evoked currents compared to native AuIB. Subsequent homology modelling/docking simulation was performed using a homology model of the rat $(\alpha3)_2(\beta4)_3$ nAChR. The results suggest that interaction of AuIB Phe9 with Lys81 and Trp79 on the β4 nAChR subunit may be essential for AuIB binding/interaction on α3β4 nAChR. In conclusion, we have identified phenylalanine at position 9 as the critical residue for specific interaction of AuIB with the α3β4 nAChR. Future studies using site-directed mutagenesis of the β4 subunit are required to further dissect the mechanism of AuIB binding/interaction on α3β4 nAChR.

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1.16

Acetylcholine binding protein-nicotinic receptor chimeras for delineating structure and determinants of ligand selectivity

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Acetylcholine binding proteins have provided a wealth of information on structure of the extracellular domain of the Cys-loop ligand-gated ion channels since their initial report by Sixma and colleagues. The availability of high resolution X-ray crystal structures of these proteins in complex with various nicotinic ligands has provided an atomic resolution view of the determinants of ligand recognition. In turn, this has provided opportunities for