from HEK-293 cells expressing ferret α4β2 nAChRs, [^{3}H]-A-998679 bound to a high affinity site with a K_d of 2.8 nM and a B_{max} of 6405 fmol/mg. Unlabeled A-998679 (and related analogs) displaced binding with a K_i value of 7 nM. Association and dissociation curves were monophasic, with extremely fast on-rate and relatively slow off-rate. We also evaluated binding interactions using membranes from native tissues. In membranes prepared from human frontal cortex, [^{3}H]-A-998679 showed saturable binding with a K_d of 60 nM and a B_{max} of 2900 fmol/mg protein. However, specific binding was relatively poor in rat membranes, which bound [^{3}H]-cytisine with high affinity—the basis of this difference remains to be elucidated. In summary, our studies demonstrate, for the first time, that [^{3}H]-A-998679 is a relatively high affinity binding tool that may be useful in further examining interactions of PAM with α4β2 nAChRs.

doi:10.1016/j.bcp.2009.06.040

1.12

α3* and α7* nAChR mediated Ca^{2+} transient generation in neuroblastoma IMR-32 cells

H. Ween[2], K. Thorin-Hagene[2,*], E. Andersen[2], J.H. Gronlien[2], L. Lee[1], M. Gopalakrishnan[1], J. Malysz[1]

[1] *Neuroscience Research, Global Pharmaceutical Research and Development, Abbott, Abbott Park, IL, United States*
[2] *Neuroscience Research, Global Pharmaceutical Research and Development, Oslo, Norway*

α3* and α7* nAChRs are members of cys-loop ligand gated ion channel family implicated in the control of intracellular Ca^{2+} signaling regulation. Both subunits are also expressed in human neuroblastoma IMR-32 cells. In this study, we investigated and compared the intracellular global Ca^{2+} transient generation evoked by selective activation of α3* and α7* nAChR pathways in IMR-32 cells using Ca^{2+} imaging (FLIPR), and examining the effects of various inhibitors (all tested at 10 μM except as noted) of ER Ca^{2+} ATPase pump (CPA and 1 μM thapsigargin), Ca^{2+} induced Ca^{2+} release (ryanodine and dantrolene), Ca^{2+} channels (nitrendipine, diltiazem, and 100 μM Cd^{2+}), nAChRs (100 nM MLA and mecamylamine), and removal of extracellular Ca^{2+}. The activation of α3* pathway was obtained by agonists with the following rank order of potencies (pEC_{50}): epibatidine (7.6) > varenicline (5.9) > nicotine (5.0) > cytisine (4.7) in a concentration-dependent manner. As reported previously [1], the addition of selective α7 agonists alone had no effect on basal Ca^{2+}. In the presence of an α7 PAM (A-867744 or PNU-120596), α7 agonists concentration dependently evoked Ca^{2+} transients with the following rank order (pEC_{50}): A-795723 (8.7) > NS6784 [2] (7.3) ≈ PNU282987 (7.2). The effects of various inhibitors on the α3* and α7* mediated Ca^{2+} transient generation were examined on the responses evoked by varenicline (10 μM) and NS6784 (1 μM + α7 PAM), respectively. Removal of extracellular Ca^{2+} and pre-addition of MLA, but not CPA, thapsigargin, ryanodine, dantrolene, nitrendipine, diltiazem, Cd^{2+} or mecamylamine, attenuated or diminished the α7* agonist evoked Ca^{2+} transients. In contrast, removal of extracellular Ca^{2+}, diltiazem, nitrendipine, and mecamylamine inhibited the α3* mediated Ca^{2+} transients. Other compounds tested: Cd^{2+}, CPA, thapsigargin, ryanodine, dantrolene, and MLA had no effect. The effects of the Ca^{2+} channel blockers were also examined in HEK-293 cells, lacking endogenous Ca^{2+} channels, expressing human α3β4 nAChRs by Ca^{2+} imaging and in IMR-32 cells by patch clamp. Nitrendipine and diltiazem, but not Cd^{2+}, directly inhibited α3* agonist evoked responses. In summary, this study shows that α3* and α7* nAChR agonist evoked global Ca^{2+} transient generation in IMR-32 cells does not involve Ca^{2+} channels, intracellular Ca^{2+} stores, or Ca^{2+} induced Ca^{2+} release. However, these mechanisms may still be involved in other forms of nAChR mediated Ca^{2+} signaling.

References

[1] Gopalakrishnan S, et al.Society of biomolecular sciences meeting. 2008 [Poster #P15006].
[2] Briggs CA, et al.Nicotinic acetylcholine receptors as therapeutic targets symposium. Biochemical Pharmacol 2007;74:SMA-25–6.

doi:10.1016/j.bcp.2009.06.041

1.13

Positive allosteric modulation of α7 neuronal nicotinic acetylcholine receptors: Lack of mechanism-based evidence for cytotoxicity in PC12 cells and rat primary cortical neurons

Min Hu*, Murali Gopalakrishnan, Jinhe Li

Neuroscience Research, Global Pharmaceutical Research & Development, Abbott Laboratories 100, Abbott Park Road, Abbott Park, IL 60064-6125, United States

α7 nicotinic acetylcholine receptors (α7 nAChRs) play an important role in cognitive function. Positive allosteric modulators (PAM) amplify effects of α7 nAChR agonists and demonstrate potential as an approach for treatment of cognitive deficits in neuropsychiatric diseases. PAMs can either predominately affect the apparent peak current response (type I) or increase both the apparent peak current response and duration of channel opening due to prolonged desensitization (type II). The delay of receptor desensitization by type II PAMs raises the concern about the possibility of Ca^{2+}-induced toxicity through prolonged activation of α7 nAChRs. The present study addresses whether type I PAM [N-(4-chlorophenyl)]-alpha-[(4-chloro-phenyl)-amino methylene]-3-methyl-5-isoxazoleacet-amide (CCMI) and type II PAM 1-[5-Chloro-2,4-dimethoxy-phenyl]-3-[5-methyl-isoxazol-3-yl]-urea (PNU-120596), or 4-[5-(4-Chloro-phenyl)-2-methyl-3-propionyl-pyrrol-1-yl]-benzenesulfonamide (A-867744) could reveal differential cytotoxicity profiles. Studies were conducted using in vitro cell culture models—PC12 and rat cortical neuronal cells expressing endogenous α7 nAChR. Our results showed that neither type I nor type II PAMs had any detrimental effect on cell viability or cytotoxicity. In particular, type II PAMs did not affect neuron number and neurite outgrowth under conditions when nAChR activity was measured by α7 ligand sensitive Ca^{2+} influx and ERK1/2 phosphorylation. This study demonstrated that both type I and type II α7 nAChR selective PAMs, although exhibiting differential electrophysiological profiles, do not exert cytotoxic effects in cells endogenously expressing α7 nAChRs.

doi:10.1016/j.bcp.2009.06.042

1.14

A novel nicotinic antagonist protects the function of hippocampal slices against neurotoxic organophosphates

P.A. Ferchmin*, Dinely Perez, A. Henrique Martins, Brenda L. Cuadrado, Marimee Carrasco, Vesna A. Eterovic

Department of Biochemistry, University Central Del Caribe, School of Med, Bayamon, Puerto Rico

Our group described that cembranoids, cyclic diterpenoids, of marine or terrestrial origin are noncompetitive nicotinic antagonists. The tobacco cembranoid (1S,2E,4R,6R,7E,11E)-cembra-2,7,11-

triene-4,6-diol (4R) protects acute hippocampal slices against excitotoxicity via a nicotinic mechanism. The data shows that 4R protects against the neurotoxic organophosphates paraoxon (POX) and diisopropylfluorophosphate (DFP) suggesting that cembranoids could be novel antidotes against these neurotoxins. Exposure to organophosphate (OP) insecticides or sublethal doses of OP war nerve toxins cause health impairment. The best-documented detrimental effects involve deficits in behavioral performance and abnormalities in nerve function. Many of the chronic symptoms associated with OP insecticide exposure are indistinguishable from those reported by Gulf War veterans allegedly exposed to OP nerve toxins. Current postexposure medical countermeasures against nerve agents (atropine, oximes, reversible AChE inhibitors and benzodiazepines) are useful in preventing mortality but are not sufficiently effective as far as protecting the CNS against apoptotic neuronal death. We used acute hippocampal slices to study the toxicity of POX and DFP and the protection by 4R. Acute hippocampal slices are a choice preparation to quantitatively measure early neurotoxic and neuroprotective events. This model has been successfully used for more than two decades by others and by us to study the effect of anoxia, oxygen and glucose deprivation, and excitotoxic amino acids. The main parameter measured is the loss of synaptically evoked population spikes (PS), which reflects the sum of axon potentials from a population of neurons and is an early predictor of neuronal apoptosis. Routinely, POX and DFP were superfused for 10 min and washed off for 30 min. Afterwards antidotes were applied for 60 min, and the PS were recorded. Our results show that 50–100 μM POX decreased the PS area by 60-80%; a higher concentration, up to 200 μM POX, did not increase the damage. The effect of POX developed with a half-life of 2 min; the maximum effect was reached by 10 min and remained unchanged for up to 1 hour. Ten μM POX completely inhibited AChE activity in the slice. The classical antidote, 200 μM pralidoxime, applied 30 min after POX provided an almost total remission of the damage caused by POX. One μM atropine, the main antidote against OPs presently used, was not significantly neuroprotective against POX when used alone. As POX, DFP inhibited the activity of AChE; but contrary to POX, DFP caused a concentration dependent loss of PS. 4R, at 2 to 10 μM applied together with 1 μM atropine 30 min after exposure to POX or DFP, protected nearly 100% and after 1 hour 70% of PS area.

Acknowledgements: Grant support: 1UO1NS0635551; NCRR RCMI G-12RR03035.

doi:10.1016/j.bcp.2009.06.043

1.15

From acetyl bispidine to an extended bispidine amide framework: Synthesis and structure–affinity relationships for nicotinic acetylcholine receptors (nAChRs)

D. Gündisch*, C. Eibl

Department Pharm. Sci., College of Pharmacy, University of Hawaii at Hilo, HI, United States

Cytisine discovered in the 19th century is an invaluable template in the development of bioactive compounds. Especially its bispidine framework which is fused to a 2-pyridone moiety has been used as a core structure for the synthesis of ligands for numerous biological targets including nAChRs. It is accessible by double Mannich reaction from N-*t*Boc-4-piperidone, formaldehyde and benzylamine and subsequent reduction of the carbonyl group yielding N-benzyl-N′-*t*Boc-bispidine. The N-protected bispidine, especially N-*t*Boc-bispidine after cleavage of the N-benzyl protecting group, served as starting material for the synthesis of diverse bispidine analogs. N-*t*Boc-bispidine itself interacts with nAChRs (e.g. Ki: 45 nM for α4/β2*). The obtained bispidine amides were tested for their affinities for different nAChR subtypes by competition assays with [^{3}H]epibatidine α4/β2*, α3/β4*, muscle type) and [^{3}H]MLA (α7*), respectively, using membrane fractions of native tissues (rat brain, calf/pig adrenals and Torpedo californica electroplax). The simplest analog, acetyl bispidine, displayed high affinity for α4/β2 (Ki: 5.6 nM). Compounds showed a broad affinity spectrum (e.g. Ki values from 1.2 nM to >10,000 nM for α4/β2*), which provided important insight into structure–affinity relationship.

doi:10.1016/j.bcp.2009.06.044

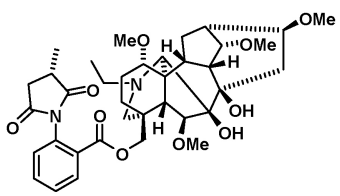
1.16

Probing the non-competitive binding site within the n-terminal region of α4β2 nicotinic receptors

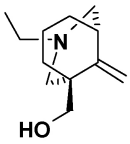
Gracia X.J. Quek[1,*], Jill I. Halliday[2], Malcolm D. McLeod[2], Mary Chebib[1]

[1] *Faculty of Pharmacy, The University of Sydney, Australia*
[2] *Research School of Chemistry, The Australian National University, Australia*

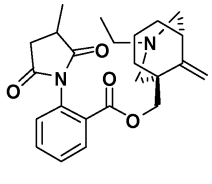
Novel nicotinic acetylcholine receptor (nAChR) antagonists have been derived from methyllycaconitine (MLA). AE Alcohol analogue 1 [(1*S**, 5*S**)-(3-ethyl-9-methylidene-3-azabicyclo[3.3.1]non-1-yl)methanol] is a truncated version and displays non-competitive binding on α4β2, α3β4 and α7 nAChRs. AE Succinimide analogue 2 [(3-ethyl-9-methylene-3-aza-bicyclo[3.3.1]nonan-1-yl)methyl 2-(3-methyl-2,5-dioxopyrrolidin-1-yl)benzoate] contains an anthranilate ester side-chain displaying mixed competitive and non-competitive binding at these receptors.



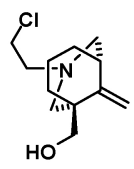
Methyllycaconitine (MLA)



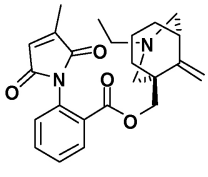
Analogue 1



Analogue 2



Analogue 1 Probe



Analogue 2 Probe

Mutation of the acetylcholine binding protein (AChBP) subunits to mimic the binding site of mammalian nAChRs combined with radioligand binding studies and X-ray crystallography has provided