co-expressed with mouse α6 and β2 subunits. Nevertheless wild-type, human β3 subunits allowed for function when integrated into mouse α6β4*-nAChR. These findings indicate that gain of function mutants in human or mouse β3 subunits are only relevant in the context of α6β4*- but not α6β2*-nAChR. Moreover, the few differences in sequences between human and mouse β3 subunits are key to effects on function of mouse α6β4*-nAChR and are targets of further investigation. Overall, the ramifications of expression of dominantly negative, wild-type β3 subunits in α6*-nAChR remain to be determined. These studies are important because of the probable roles of α6*-nAChR in reward and nicotine dependence and toward discovery of therapeutic drugs selective for α6*-nAChR.

Acknowledgements: Supported by Philip Morris USA and Philip Morris International through the external research program.

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doi:10.1016/j.bcp.2009.06.036

1.8

Characterization of insect nicotinic receptors by heterologous expression: Insecticide selectivity and the influence of molecular chaperones

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Nicotinic acetylcholine receptors (nAChRs) are important excitatory neurotransmitter receptors in both vertebrate and invertebrate species. In insects, nAChRs are the target site for commercially important insecticides such as neonicotinoids and spinosad [1]. Ten nAChR subunits (Dα1-Dα7 and Dβ1-Dβ3) have been identified in the model insect species *Drosophila melanogaster* and a similar number have been identified in other species. Whereas early cloning and expression studies of insect nAChRs focused primarily on *Drosophila*, more recent studies have been extended to include economically important pest species such as the aphid *Myzus persicae*, the cat flea *Ctenocephalides felis* and the rice brown planthopper *Nilaparvata lugens*. Past studies have included the identification of nAChR point mutations associated with insecticide resistance insect populations, for example [2]. Unfortunately, however, considerable difficulties have been encountered in attempts to characterize insect nAChRs by heterologous expression studies. In almost all cases, successful heterologous expression of insect nAChRs has required approaches such as the expression of hybrid nAChRs (i.e. the co-expression of insect nAChR subunits with non-insect partner subunits) or the expression of artificial subunit chimeras. In addition to expression studies with several insect pest species, we have previously reported expression studies with 9 of the 10 identified *Drosophila* nAChR subunits. We have now isolated a full-length expressible clone of an additional *Drosophila* nAChR subunit (Dα5). As has been reported previously [3], Dα5 has an unusual structure, containing an N-terminal domain that is approximately 300 amino acids longer than that of other previously characterized nAChR subunits. Heterologous expression studies have revealed that, despite its atypical N-terminal domain, Dα5 can contribute to the formation of a high affinity nicotinic binding site. The pharmacological properties of insect nAChRs, including their sensitivity to insecticides will be discussed, as will be our attempts to achieve functional expression of insect nAChRs. A further strategy that we are examining is the role of nAChR-associated chaperone proteins such as RIC-3 [4]. We have reported the cloning of 11 alternatively spliced isoforms of the molecular chaperone RIC-3 from *Drosophila* [5]. The ability of these RIC-3 isoforms to influence of maturation of insect (and non-insect) recombinant nAChRs is being investigated.

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doi:10.1016/j.bcp.2009.06.037

1.9

Labeled peptide and protein neurotoxins for basic study on nicotinic acetylcholine receptors and for practical applications

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Different types of nicotinic acetylcholine receptors (nAChRs) were found in various organs and tissues of different systems (muscle, neuronal, immune and others). Malfunctioning of the definite nAChR subtypes is proved to be associated with muscle dystrophies, psychiatric diseases and neurodegeneration, therefore their detection and quantification at normal state and pathologies is an important task. The promising tools for solving this task would be peptide and polypeptide neurotoxins selectively targeting distinct nAChR subtypes. Among them are α-conotoxins (from *Conus* mollusks) that effectively discriminate between the different nAChR subtypes. We synthesized numerous analogs of various α-conotoxins (with single and multiple amino acid substitutions) specific either for muscle- or neuronal nAChRs. Some of them (SIA[D12K], PnIA[A10L,D14K]) were found to be more potent than the native peptides. The PnIA[A10L,D14K] analog (the first α-conotoxin crystallized with acetylcholine-binding protein, AChBP) was radioiodinated and retained its high potency (K_D 0.2-1.0 nM) in binding to AChBPs. Another radioiodinated derivative of the α-conotoxin ImII analog, namely [^{125}I]-ImII[W10Y] was bound (K_D 1.5-6.1 μM) to muscle-type nAChR from *Torpedo californica* but did not compete with α-cobratoxin (classical snake venom polypeptide antagonist of muscle-type and α7 nAChRs) suggesting interaction with an alternative binding site on this receptor. For detecting α7 nAChRs in tissues, we used Alexa Fluor488-labeled fluorescent derivative of α-bungarotoxin and showed the presence of α7 nAChR in the dorsal root ganglia and spinal cord of mice, additional evidence of specificity being the lack of staining in α7 knock-out mice.

doi:10.1016/j.bcp.2009.06.038