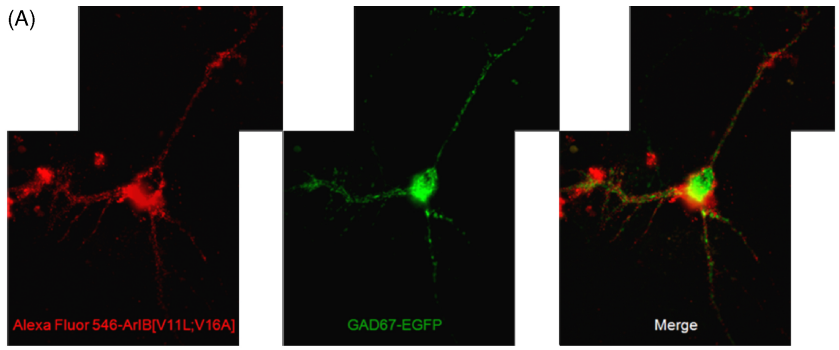

A, Cultured hippocampal neuron from the transgenic mouse strain FVB-Tg(GadGFP)45704Swn/J labeled with Alexa Fluor 546-ArIB[V11L;V16A] (red). The neuron was imaged live at 40x magnification using a cooled CCD camera. B, Cultured hippocampal neuron from Sprague Dawley rat showing neuronal processes labeled with Cy3-ArIB[V11L;V16A] (red) and immuno-labeled for synaptic vesicle protein-2 (green). Image was taken at 63x magnification using a cooled CCD camera.

Fig. 1.

1.10

Labeling α7 nAChRs on hippocampal neurons using fluorescent analogs of α-conotoxin ArIB[V11L;V16A]

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Hippocampal neurons are known to express several subtypes of nicotinic acetylcholine receptors (nAChRs). Among these, α4β2 and α7 are the most predominantly expressed subtypes. α7 nAChRs are expressed on several populations of neurons in the hippocampus but particularly on GABAergic interneurons where activation of α7 receptors induces the release of GABA. In the dentate gyrus of the hippocampus, presynaptic α7 receptors function at the mossy fiber-granule cell synapse to modulate glutamate release and thereby regulate granule cell activity. We evaluated the efficacy of two fluorescent derivatives of α-conotoxin ArIB[V11L;V16A] for detecting α7 nAChRs on cultured hippocampal neurons from mice and rats. ArIB[V11L;V16A] is a synthetic analog of a peptide isolated from the venom of the marine cone snail *Conus arenatus*. We conjugated ArIB[V11L;V16A] with two fluorescent dyes to produce Cy3-ArIB[V11L;V16A] and Alexa Fluor 546-ArIB[V11L;V16A]. Both fluorescent conjugates are ~1,500-fold more selective for α7 than for other nAChR subtypes as determined by functional studies of nAChRs heterologously expressed in *Xenopus laevis* oocytes. In addition, kinetic studies indicate that the binding of both conjugates is only slowly reversible. We used a combination of live-cell imaging and immunohistochemistry to evaluate the suitability of Cy3-ArIB[V11L;V16A] and Alexa Fluor 546-ArIB[V11L;V16A] for labeling α7 nAChRs. Hippocampal neurons from the transgenic mouse strain FVB-Tg(GadGFP)45704Swn/J that express EGFP as a reporter for glutamic acid decarboxylase-67 (GABAergic interneurons) were labeled with Alexa Fluor 546-ArIB[V11L;V16A] and imaged live. Neurons from Sprague Dawley rats were fixed, labeled with Cy3-ArIB[V11L;V16A], and stained with markers for either synaptic vesicle protein-2 (SV2) or postsynaptic density protein (PSD95). Labeling of neurons was observed using both fluorescent α-conotoxins and labeling was prevented by pre-incubation with α-bungarotoxin. The results demonstrate that Cy3-ArIB[V11L;V16A] and Alexa Fluor 546-ArIB[V11L;V16A] can be used to identify α7 nAChRs in cultured hippocampal neurons (Fig. 1).

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1.11

Radioligand binding characterization of [^{3}H]-A-998679: A novel positive allosteric modulator of α4β2 nAChRs

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Positive allosteric modulators (PAMs) have recently been identified that enhance responses of the α7 nAChR and other downstream events. Similarly, selective PAMs have also been identified for α4β2 nAChRs that do not possess intrinsic activity at the receptor on their own but potentiate the effects of agonists such as acetylcholine or nicotine. A-998679 is a close analog of NS-9283 (A-966933) which potentiates agonist responses at α4β2, but not at other heteromeric receptors. To further elucidate the interaction of this PAM with α4β2 nAChRs, A-998679 was radiolabeled. The present study characterized the ability of [^{3}H]-A-998679 to bind to native and recombinant nAChR α4β2 receptors. In membrane preparations