on the nicotinic control of glycine (GLY) release. The acute administration of nicotine in vivo was able to evoke endogenous GLY release in the rat hippocampus. The specific nicotinic agonists 5-IA85380 dihydrochloride (5IA85380), selective for the of α4β2 nAChR subtype, administered *in vivo* also elicited GLY release in a similar extent while the α7 agonist PHA-543613 hydrochloride (PHA543613) was less potent. Nicotine elicited GLY overflow also from hippocampal synaptosomes in vitro. This overflow was Ca^{2+} dependent and inhibited by methyllycaconitine (MLA) but not modified by dihydro-beta-erythroidine (DHBE, 1 μM). Choline(Ch)-evoked GLY overflow was Ca^{2+} dependent, unaltered in presence of DHβE and blocked by methyllycaconitine (MLA). Also 5IA85380 elicited a GLY overflow which was Ca^{2+} dependent, significantly inhibited by DHβE but unaffected by MLA. The GLY overflow produced by these nicotinic agonists resembles quantitatively that evoked by 9 mM KCl. The effects of a high concentration of 5IA85380 (1 mM) in presence of 2 μM DHβE on the release of GLY was also studied comparatively to that on glutamate and aspartate release. The nicotinic agonist 5IA85380 tested at high concentration (1 mM) was able to produce a stimulatory effect of endogenous release of the three aminoacids also in presence of 2 μM DHβE indicating the existence of a DHβE insensitive, α4β2 nAChR subtype with a functional role in the modulation of GLY, aspartate and glutamate release. Our results show that in the rat hippocampus the release of GLY is, at least in part, of neuronal origin and is modulated by the activation of both α7 and α4β2 (low and high affinity) nAChR subtypes.

doi:10.1016/j.bcp.2011.07.028

1.27

Ethanol interactions with nicotinic receptors in brainstem cholinergic centers

John McDaid[1,*], Shannon S. Wolfman[2], Keith Gallagher[1], Daniel S. McGehee[1]

[1] *Department of Anesthesia and Critical Care, University of Chicago, Chicago, IL, USA*
[2] *Committee on Neurobiology, University of Chicago, Chicago, IL, USA*

Nicotine and ethanol are two of the most widely co-abused drugs. Ethanol impairs motor activity at doses that also mediate rewarding effects and one theory is that the stimulant effects of nicotine may offset some of the ethanol induced motor impairment. Alternate hypotheses suggest that nicotine enhances the value of drug-associated cues, but the mechanisms underlying these interactions remain unclear. A major challenge in understanding the behavioral effects of ethanol is the identification of molecular targets that mediate those behaviors. Ethanol has been shown to modulate nicotinic acetylcholine receptors (nAChRs) in cell culture, but no such studies have been carried out in brain slices. To investigate this interaction, we tested the effect of bath applied ethanol on nAChR-mediated currents using whole cell patch clamp recording in tissue slices including a brainstem cholinergic center, the lateral dorsal tegmental nucleus (LDTg) from adult rats. The LDTg contributes to motor control and motor learning, as well as reward related circuitry. The majority of nAChR responses in LDTg neurons were completely blocked by the selective α7* antagonist MLA (10 nM). Bath application of ethanol at low, physiologically relevant levels (1–10 mM) caused a profound reduction in the magnitude of the α7* nAChR responses. Interestingly, the inhibitory effect of ethanol on α7* nAChRs was blocked by either the PKA inhibitor H89 or the adenylate cyclase inhibitor SQ22536 (introduced via the recording electrode solution). Bath application of PKA activators potentiated LDTg α7* nAChR currents, while inhibitors suppressed these currents. Bath application of the α7* nAChR positive allosteric modulator PNU120596, which interferes with α7* nAChR desensitization, eliminated the modulatory effects of ethanol on α7* nAChRs. Thus, ethanol may inhibit α7* nAChRs by enhancing desensitization through inhibition of the PKA pathway. Nicotine increased the frequency of miniature EPSCs in the mediodorsal thalamus, a brain region involved in motor control that receives extensive cholinergic input from the LDTg. This presynaptic effect of nicotine was significantly reduced either by MLA or 10 mM ethanol. Finally, using an accelerating rotarod to assess motor performance, we found that intra-cerebroventricular injection of PNU120596 reduced the motor impairment with systemic ethanol administration. These findings suggest that the motor impairment by ethanol is mediated, at least partially by a reduction in α7* nAChR-mediated excitation.

doi:10.1016/j.bcp.2011.07.029

1.28

Nicotinic cholinergic receptors in dorsal root ganglion neurons include the α6β4* subtype

Arik J. Hone[1,3,*], Erin L. Meyer[2,3], Melissa McIntyre[2], J. Michael McIntosh[1,2,3]

[1] *Interdepartmental Program in Neuroscience[1], University of Utah, Salt Lake City, UT, USA*
[2] *Department of Biology, University of Utah, Salt Lake City, UT, USA*
[3] *Department of Psychiatry, University of Utah, Salt Lake City, UT, USA*

Dorsal root ganglia (DRG) neurons express a variety of receptors and ion channels including nicotinic acetylcholine receptors (nAChRs). Reverse-transcription polymerase chain reaction (PCR) analysis indicates that DRG neurons may express nAChRs that contain α2–α6, α7, α9, α10 and β2–β4 subunits and pharmacological analysis supports the expression of receptors with an α7-, α3β4*-, and α4β2*-like composition [1–4] (* denotes the possible presence of additional subunits). However, given the variety of subunits present, we hypothesized that DRG neurons may express additional nAChR subtypes not previously reported. α-Conotoxins (α-Ctx) are small peptides isolated from the venom of carnivorous marine snails. Many of these peptides show a remarkable selectivity for individual nAChR subtypes. Using whole-cell voltage-clamp electrophysiology of isolated rat DRG neurons we exploited the selectivity of these conopeptides to characterize the nAChR subtypes expressed by these neurons.

Cultured lumbar DRG neurons from male Sprague–Dawley rats (25–45 days old) were studied using whole-cell voltage-clamp electrophysiology. The neurons were stimulated by brief applications of acetylcholine (ACh), exposed to various toxin antagonists, and the responses to ACh reassessed. Two broad types of responses were observed. The first type was rapidly desensitizing and blocked ($94.5 \pm 1.5\%$, $n = 9$; ±, SEM) by α-Ctx ArIB[V11L; V16D] (Fig. 1A), a highly selective antagonist of the α7 nAChR subtype. This result is consistent with previous reports demonstrating that a subpopulation of DRG neurons primarily expresses the α7 nAChR subtype [4]. The second type was characterized by responses that contained a substantial, more slowly desensitizing component. In these neurons, α-Ctx ArIB[V11L; V16D] blocked only $11.0 \pm 2.8\%$ ($n = 18$; Fig. 1B) of the response. In the presence of α-Ctx ArIB[V11L;V16D], α-Ctx PnIA, an antagonist of α3β2* and α6β2* nAChRs, inhibited $22.3 \pm 3.4\%$ ($n = 8$) of the remaining response (Fig. 1B). A combination of α-Ctx ArIB[V11L;V16D], α-Ctx PnIA, and dihydo-β-erythroidine was used to isolate responses mediated by α3β4* and α6β4* nAChRs. Exposure to this cocktail of antagonists inhibited $20.5 \pm 4.2\%$ ($n = 16$; Fig. 1C) of the response. The subsequent

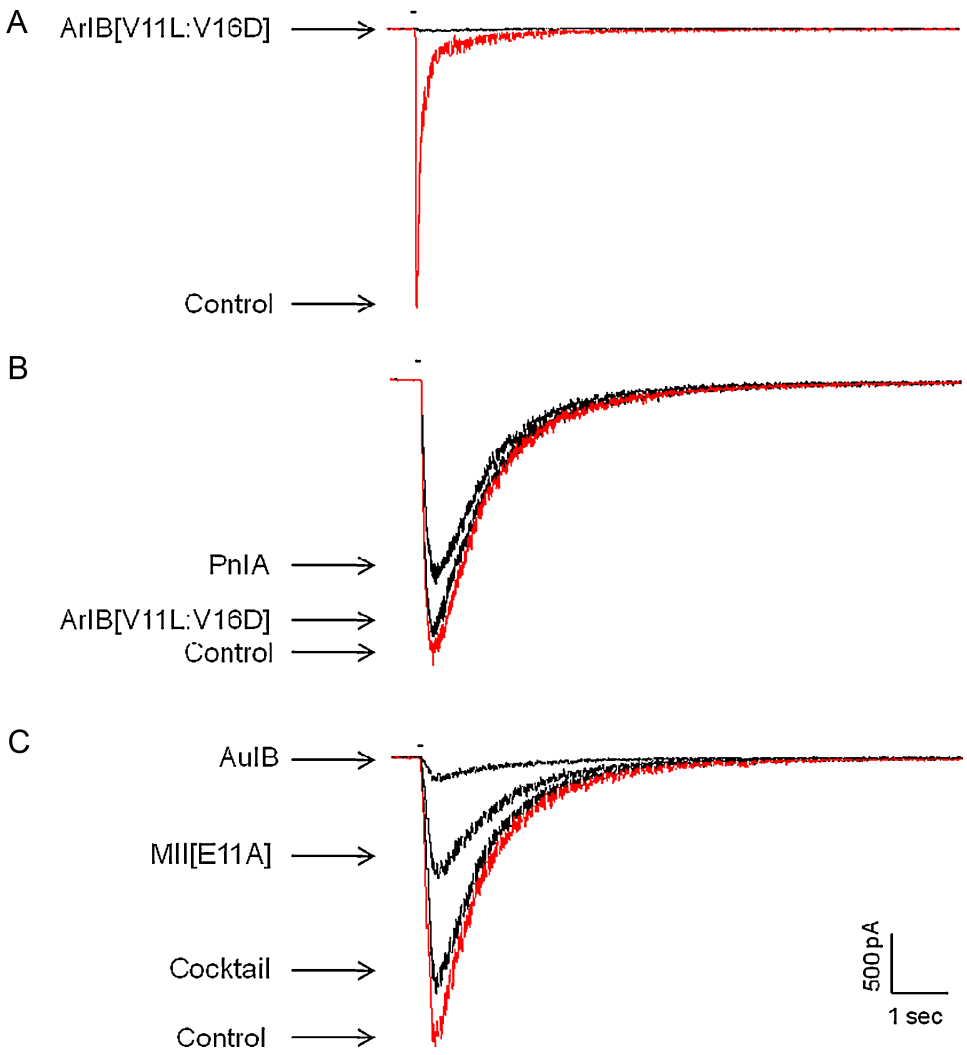

Fig. 1. Dorsal root ganglion neurons were voltage-clamped at a holding potential of −80 mV using a Multiclamp 700B amplifier and the signals digitized uing a Digidata 1440A (Axon Instruments, Sunnyvale, CA). The signals were sampled at 5 kHz and filtered at 1 kHz and the data analyzed offline using Clampfit 10 analysis software. To evoke responses, the neurons were stimulated by 100 ms applications of 1 mM acetylcholine every 2 min using a Picospritzer at 6 p.s.i. (General Valve Corporation, Pine Brook, NJ). After a steady response baseline was achieved, the neurons were exposed to various toxin antagonists. (A) Representative current traces before and after exposure to 200 nM α-Ctx ArIB[V11L; V16D]; (B) control response before exposure to antagonists and after application of α-Ctx ArIB[V11L; V16D] followed by 500 nM α-Ctx PnIA; C, control response followed by the application of a cocktail of antagonists (200 nM α-Ctx ArIB[V11L; V16D], 500 nM α-Ctx PnIA, and 1 μM dihydo-β-erythroidine), to isolate responses mediated by α3β4* and α6β4* nAChRs, followed by 200 nM α-Ctx MII[E11A] then 10 μM α-Ctx AuIB. Toxins were applied sequentially and additively. Scale bars apply to A, B, and C.

addition of α-Ctx MII[E11A], which under these conditions selectively blocks α6β4* vs. α3β4* nAChRs, further inhibited 36.1 ± 5.6% (n = 8) of the remaining response providing evidence for expression of α6β4* receptors (Fig. 1C). Finally, the remaining response was inhibited by 76.9 ± 5.2% (n = 5) upon addition of α-Ctx AuIB indicating the expression of α3β4* receptors (Fig. 1C). These results taken together with PCR data support the conclusions of other investigators that DRG neurons express multiple nAChR subtypes and to our knowledge, this is the first report of α6β4* nAChRs in DRG.

Acknowledgements: This work was supported by NIH grants MH53631 and GM48677 to J.M.M.

Reference

[1] Genzen, et al. J Neurophysiol 2001;86:1773.
[2] Haberberger, et al. Auton Neurosci: Basic Clin 2004;113:32.
[3] Lips, et al. Neuroscience 2002;115:1.
[4] Rau, et al. J Neurophysiol 2005;93:1358.

doi:10.1016/j.bcp.2011.07.030

1.29

Investigations into nicotinic Stat3 signaling using a luciferase reporter plasmid

Ralph H. Loring*, Abishek Chandrashekar, Tom Koperniak, Sharath Madasu

Department of Pharmaceutical Science, Northeastern University, Boston, MA, USA

Although better known as ion channels, nicotinic α7 receptors in cells involved in inflammation or the immune system also signal through the Jak/Stat pathway when activated by nicotine or other agonists [1]. We recently showed that α4β2 receptors also signal in part through Jak/Stat [2], and this signaling pathway may be involved in nicotine's neuro-protective and anti-inflammatory actions. In order to investigate nicotine-driven Jak/Stat signaling, we generated a custom reporter plasmid consisting of a Stat3 promoter driving a secreted luciferase reporter gene. Nicotine has no effect on luciferase expression in SH-EP1 and GH4C1