

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 $\alpha 4\beta 2$ nAChR subtype, administered *in vivo* also elicited GLY release in a similar extent while the $\alpha 7$ 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- β -erythroidine (DHBE, 1 μM). Choline (Ch)-evoked GLY overflow was Ca^{2+} dependent, unaltered in presence of DHBE and blocked by methyllycaconitine (MLA). Also 5IA85380 elicited a GLY overflow which was Ca^{2+} dependent, significantly inhibited by DHBE 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 DHBE 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 DHBE indicating the existence of a DHBE insensitive, $\alpha 4\beta 2$ 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 $\alpha 7$ and $\alpha 4\beta 2$ (low and high affinity) nAChR subtypes.

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Ethanol interactions with nicotinic receptors in brainstem cholinergic centers

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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 $\alpha 7^*$ 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 $\alpha 7^*$ nAChR responses. Interestingly, the inhibitory effect of ethanol on $\alpha 7^*$ 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 $\alpha 7^*$ nAChR currents, while inhibitors

suppressed these currents. Bath application of the $\alpha 7^*$ nAChR positive allosteric modulator PNU120596, which interferes with $\alpha 7^*$ nAChR desensitization, eliminated the modulatory effects of ethanol on $\alpha 7^*$ nAChRs. Thus, ethanol may inhibit $\alpha 7^*$ 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 $\alpha 7^*$ nAChR-mediated excitation.

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Nicotinic cholinergic receptors in dorsal root ganglion neurons include the $\alpha 6\beta 4^*$ subtype

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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 $\alpha 2$ – $\alpha 6$, $\alpha 7$, $\alpha 9$, $\alpha 10$ and $\beta 2$ – $\beta 4$ subunits and pharmacological analysis supports the expression of receptors with an $\alpha 7$ -, $\alpha 3\beta 4^*$ -, and $\alpha 4\beta 2^*$ -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 ($94.5 \pm 1.5\%$, $n=9$; \pm , SEM) by α -Ctx Arib[V11L; V16D] (Fig. 1A), a highly selective antagonist of the $\alpha 7$ nAChR subtype. This result is consistent with previous reports demonstrating that a subpopulation of DRG neurons primarily expresses the $\alpha 7$ 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 $\alpha 3\beta 2^*$ and $\alpha 6\beta 2^*$ 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 dihydro- β -erythroidine was used to isolate responses mediated by $\alpha 3\beta 4^*$ and $\alpha 6\beta 4^*$ nAChRs. Exposure to this cocktail of antagonists inhibited $20.5 \pm 4.2\%$ ($n=16$; Fig. 1C) of the response. The subsequent