The present study aimed at exploring the pharmacological profile of a different compound, NS206, discovered to act as an α4β2 nAChR PAM. Using Xenopus oocyte electrophysiology, NS206 was shown to act as a potent and efficacious α4β2 nAChR PAM not only at the LS, but also at the HS-isoform. Moreover, the pattern of modulation observed at HS-α4β2 was distinct in the sense that NS206 acted by augmenting maximal ACh efficacy (peak current amplitude) without affecting ACh potency (EC_{50}). By comparison, modulation of LS-α4β2 was mediated through an increase in functional ACh potency (i.e. decreased EC_{50}), while maximal ACh efficacy was unchanged, similar to the mode-of-action observed with the LS-α4β2-selective PAM NS9283. The PAM-selectivity profile at a number of other nAChR subtypes was also characterized and will be presented. Using point mutated nAChR subunits, engineered to abolish the binding site for LS-α4β2 PAMs, loss of PAM activity was confirmed for NS9283. Interestingly, however, NS206 retained full PAM activity in the mutated receptor constructs. Collectively, these findings suggest NS206 to present a novel class of α4β2 nAChR PAM which acts through a receptor binding site separate from that of LS-α4β2 PAMs (e.g. NS9283) and to possess a distinct pattern of PAM activity, involving modulation of both LS- and HS-α4β2 nAChRs. The discovery of NS206 (and related molecules) provides an important pharmacological tool that may enable a deeper understanding of α4β2 nAChR PAM in vivo pharmacology and at broader level, insight into the physiological significance of the HS- and LS-isoforms of the α4β2 nAChR.

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1.24

Ascorbic acid is a positive modulator of α9α10 nicotinic cholinergic receptors

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Inhibitory activity of efferent cholinergic fibers projecting from the brainstem and contacting cochlear hair cells can ameliorate acoustic trauma. This inhibitory synapse is mediated by α9α10 nicotinic receptors and the subsequent activation of an SK2 type K^+ current that hyperpolarizes the hair cell. Hence, increasing α9α10-mediated responses pharmacologically could have a potential therapeutic use in noise-induced hearing loss. In this work we endeavored to identify new positive modulators for this receptor. Using the two-electrode voltage clamp technique we studied the effect of ascorbic acid (ASC) on acetylcholine (ACh) evoked responses in *Xenopus* oocytes expressing the rat α9α10 receptor. Responses to 10 μM ACh were potentiated by ASC in a concentration-dependent manner: at 3 mM ASC, an 81 ± 6% ($n = 7$) potentiation was observed. Potentiation was more pronounced at lower (305 ± 40%, 3 μM ACh, $n = 8$) than at higher (138 ± 35%, 1 mM ACh, $n = 8$) ACh concentrations. Neither 3 mM dihidroascorbate nor 3 mM D-iso-ascorbate had an effect on 10 μM ACh-evoked responses. These results suggest that the reduced L form of ASC is the active compound. The extracellular cysteines 192 and 193 (*Torpedo* α numbering) are not involved in the effect of ASC since mutating them to serine did not abolish the potentiating effect. ASC did not modify responses to ACh of rat α7 and α4β2 receptors expressed in oocytes. Altogether, our results show that ASC potentiates α9α10-mediated responses and thus has a potential therapeutic use in noise-induced hearing loss.

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1.25

A structure–activity study of 4R-cembranoid reversal of diisopropylfluorophosphate-inflicted functional impairment in hippocampal slices

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Diisopropylfluorophosphate (DFP) is an organophosphate insecticide used in many studies as a surrogate for more toxic chemical warfare nerve agents. DFP produces neurodegeneration in vivo and irreversibly decreases the area of population spikes (PS) recorded from the CA1 region of the acute hippocampal slice preparation. Tobacco-derived (1*S*,2*E*,4*R*,6*R*,7*E*,11*E*)-2,7,11-cembratriene-4,6-diol (4R) is a neuroprotective natural product that reverses DFP-induced damage both in vivo and in the hippocampal slice. The objective of this study was to define the molecular features of the cembranoid molecule that lead to high potency against DFP, concomitantly with no intrinsic toxicity, using the hippocampal slice assay. Thirteen 4R analogues were obtained by semisynthetic or bacterial biocatalytic transformations of the natural product scaffold. Acute hippocampal slices were divided into three groups: (*a*) the DFP control (slices exposed to 100 μM DFP for 10 min), (*b*) neuroprotection by the cembranoid (slices exposed to 100 μM DFP for 10 min, washed for 30 min and then exposed to 10 μM of each tested cembranoid for 1 h), and (*c*) toxicity control (slices exposed to 10 μM cembranoid for 1 h). Population spikes (PS) were measured before and after the treatment. The results are expressed as %Protection (=100 × (%Recovery in *b* − %Recovery in *a*)/(100 − %Recovery in *a*). Two analogues displayed marginal toxicity when applied in the absence of DFP; these were excluded from the subsequent analysis. Exposure to 100 μM DFP for 10 min reduced the PS to approximately 30% of the original value. Superfusion with 10 μM of the parent 4R 30 min after DFP reversed the effect of DFP by 80%. Similar protective activity was observed with the 6-keto, 9β-OH, 10α-OH and 10β-OH analogues. On the other hand, the 4*S*-epimer of 4R and 4R-*O*-methyl analogues were totally devoid of protective activity but the activity was restored in the 4R-*O*-methyl-6-keto analogue. These results suggest that the oxygens in positions 4 and 6 are crucial for the 4R binding to its target, which triggers the protection against the organophosphate toxicity in hippocampus slices.

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1.26

Different presynaptic nicotinic receptor subtypes modulate *in vivo* and *in vitro* the release of glycine in the rat hippocampus

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In the present study, using an *in vivo* approach (microdialysis technique associated to HPLC with fluorimetric detection) and in vitro purified hippocampal synaptosomes in superfusion, we investigated on the glycinergic transmission in hippocampus, focusing

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.

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1.27

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 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.

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1.28

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