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