

Nicotinic $\alpha 3\beta 4$ receptors recorded on Nanion's Patchliner

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Summary

The nicotinic acetylcholine receptor (nAChR) is a member of the ligand-gated ion channel superfamily which includes GABA_A, 5HT₃, NMDA and glycine receptors. It is a cation-permeable ion channel activated by the neurotransmitter acetylcholine and the natural alkaloid, nicotine. Neuronal nAChR are pentameric and functional channels are formed from a repertoire of nine α ($\alpha 2$ to $\alpha 10$) and three β subunits ($\beta 2$ to $\beta 4$). Most nAChR exist as heteromers with the stoichiometry 2 α to 3 β , however some α subunits function as homomers, these being $\alpha 7$ or $\alpha 9$ (for reviews see Refs. 1 & 2). nAChR have been proposed to play a role in many neurological disorders such as Alzheimer's Disease, Parkinson's, Tourette's Syndrome and depression. mRNA for the $\alpha 3$ and $\beta 4$ subunits is found in the mammalian CNS and PNS, in particular autonomic ganglion cells and chromaffin cells. nAChR containing the $\alpha 3$ subunit are essential for mediating fast synaptic transmission in the autonomic nervous system and is essential for survival³. Block of nicotinic $\alpha 3\beta 4$ receptors by methadone has also been suggested to play a potential role in analgesia⁴.

Here we present data collected on a 4- or 8-channel Patchliner showing the potential use of the Patchliner to record nAChR $\alpha 3\beta 4$ currents activated by nicotine. Nicotine activated $\alpha 3\beta 4$ receptors in a concentration-dependent manner with an EC₅₀ similar to those reported in the literature^{5,6}. Nicotinic $\alpha 3\beta 4$ receptors could be repetitively activated by nicotine and blocked by mecamylamine, a ganglionic blocker with clinically relevant hypotensive actions, with an IC₅₀ in good agreement with the literature^{4,5,7}.

Results

Current responses of an individual cell expressing $\alpha 3\beta 4$ receptors to increasing concentrations of nicotine are shown in Figure 1. A concentration response curve revealed an EC₅₀ for nicotine activation of $31 \pm 2 \mu\text{M}$ ($n = 11$), in excellent agreement with the literature^{5,6}.

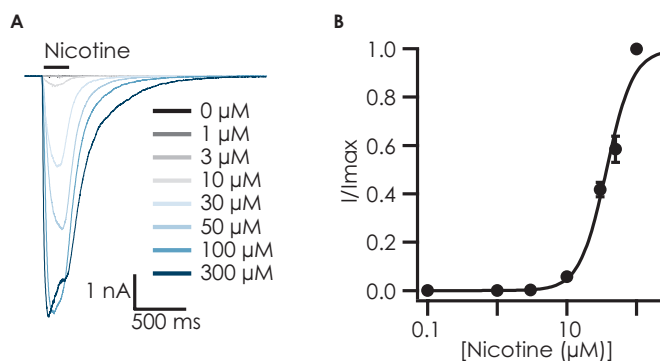


Figure 1:

A Activation of nicotinic $\alpha 3\beta 4$ receptors by increasing concentrations of nicotine. **B** Concentration response curve for nicotine activation, EC₅₀ = $31 \pm 2 \mu\text{M}$ ($n = 11$).

Application Note

Figure 2 shows the repetitive activation of nicotinic $\alpha 3\beta 4$ receptors. Currents were activated with a similar peak amplitude when challenged 10 times with 50 μM nicotine.

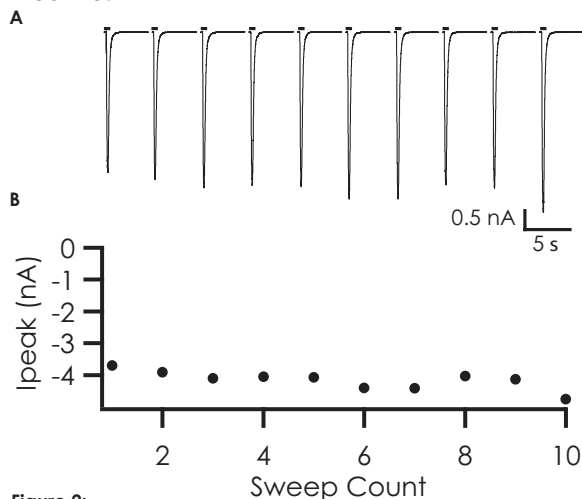


Figure 2: **A** Nicotinic $\alpha 3\beta 4$ receptors could be repetitively activated by 50 μM nicotine. **B** Timecourse of the experiment. Nicotinic $\alpha 3\beta 4$ currents of approx. 4 nA were activated 10 times reproducibly in the same cell.

A full concentration response curve to mecamylamine was performed (Fig. 3). Mecamylamine blocked the nicotine activated current with an IC_{50} of $2.1 \pm 0.7 \mu\text{M}$ ($n = 4$), in good agreement with the literature^{4,5,7}.

References

1. Colquhoun *et al.*, in Burger's Medicinal Chemistry, Vol II Drug Discovery and Drug Development, Chapter 11, pp 357-405. Ed. Abraham, D., J. Wiley, New York., S
2. Albuquerque *et al.*, 2009. *Physiol Rev.* 89: 73–120
3. Xu *et al.*, 1999. *PNAS.* 96: 5746-5751
4. Xiao *et al.*, 2001. *JPET.* 299: 366-371
5. Xiao *et al.*, 1998. *Mol. Pharmacol.* 54: 322-333
6. Colquhoun & Patrick., 1997. *J. Neurochem.* 69: 2355-2362
7. Papke *et al.*, 2001. *JPET.* 297: 646-656

Methods

Cells

HEK293 cells stably expressing nicotinic $\alpha 3\beta 4$ were used.

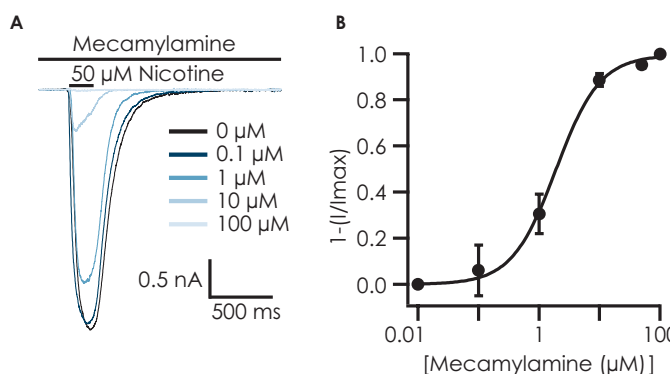


Figure 3: Block of nicotinic $\alpha 3\beta 4$ receptors by mecamylamine. Mecamylamine at increasing concentrations (0.1 μM - 100 μM) was pre-incubated and then co-applied with 50 μM nicotine. Some recovery from block was achieved upon washout (approx. 20%; data not shown). **B** Concentration response curve for mecamylamine block, $\text{IC}_{50} = 2.1 \pm 0.7 \mu\text{M}$ ($n = 4$), in good agreement with the literature^{4,5,7}.

In summary, nicotinic $\alpha 3\beta 4$ receptors stably expressed in HEK293 cells can be reliably activated by nicotine and blocked by the non-specific nAChR blocker, mecamylamine. The data shown here agrees well with published literature for nicotinic $\alpha 3\beta 4$ receptors¹⁻⁷. Therefore, the Patchliner provides a viable, higher throughput alternative to conventional patch clamp for the discovery of agonists and antagonists of nicotinic $\alpha 3\beta 4$ receptors as potential therapeutics for analgesia and Tourette's Syndrome, amongst others.

Cell culture

Cells were cultured and harvested according to Nanion's standard cell culture protocol.

Electrophysiology

Whole cell patch clamp recordings were conducted according to Nanion's standard procedure for the Patchliner. Cells were held at a holding potential of -70 mV. To achieve short exposure times, solutions were stacked in the robotic pipettor. First, wash solution (185 μl) was aspirated followed by aspiration of the agonist-containing solution (40 μl) and then rapidly applied to the cell at a speed of 171 $\mu\text{l/s}$. Wash solutions contained the antagonist in the mecamylamine experiments. The cells were pre-incubated in mecamylamine before co-application with 50 μM nicotine.