Application Note

Channel: nAChR a3β4
Cells: HEK293
Tools: Patchliner®

Nicotinic a3β4 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 $GABA_A$, $5HT_3$, 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 (α2 to α10) and three β subunits (β2 to β4). Most nAChR exist as heteromers with the stoichiometry 2α to 3β, however some α subunits function as homomers, these being α7 or α9 (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 α3 and β4 subunits is found in the mammalian CNS and PNS, in particular automomic ganglion cells and chromaffin cells. nAChR containing the α3 subunit are essential for mediating fast synaptic transmission in the autonomic nervous system and is essential for survival[3]. Block of nicotinic α3β4 receptors by methadone has also been suggested to play a potential role in analgesia[4].

Here we present data collected on a 4- or 8-channel Patchliner® showing the potential use of the Patchliner® to record nAChR α3β4 currents activated by nicotine. Nicotine activated α3β4 receptors in a concentration-dependent manner with an EC_{50} similar to those reported in the literature[5,6]. Nicotinic α3β4 receptors could be repetitively activated by nicotine and blocked by mecamylamine, a ganglionic blocker with clinically relevant hypotensive actions, with an IC_{50} in good agreement with the literature[4,5,7].

Results

Current responses of an individual cell expressing α3β4 receptors to increasing concentrations of nicotine are shown in Figure 1. A concentration response curve revealed an EC_{50} for nicotine activation of 31 ± 2 µM (n = 11), in excellent agreement with the literature[5,6].

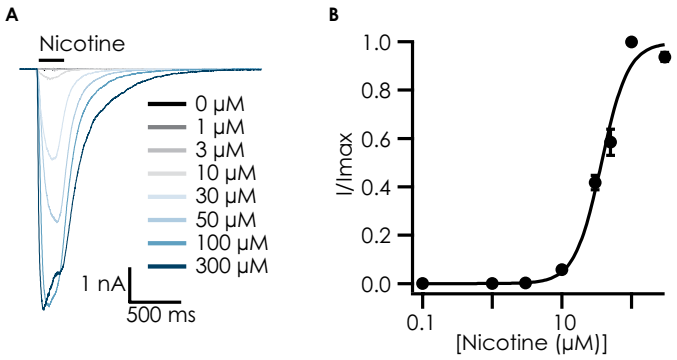

Figure 1:
A Activation of nicotinic α3β4 receptors by increasing concentrations of nicotine. **B** Concentration response curve for nicotine activation, EC_{50} = 31 ± 2 µM (n = 11).

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Figure 2 shows the repetitive activation of nicotinic a3β4 receptors. Currents were activated with a similar peak amplitude when challenged 10 times with 50 µM nicotine.

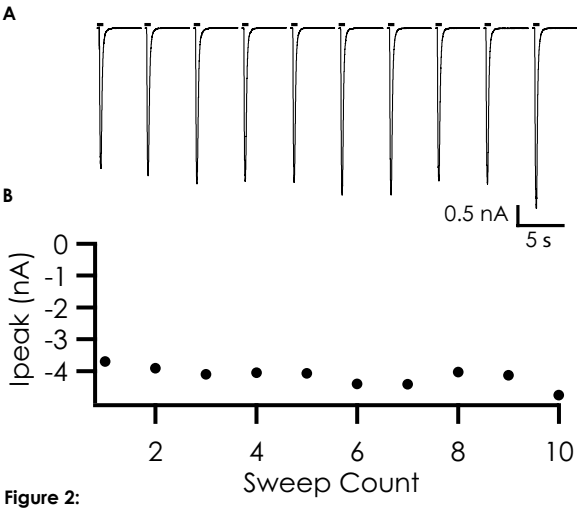

Figure 2:
A Nicotinic a3β4 receptors could be repetitively activated by 50 µM nicotine. **B** Timecourse of the experiment. Nicotinic a3β4 currents of approx. 4 nA were activated 10 times reproducibly in the same cell.

A full concentration response curve to mecamylamine was performed (Fig. 3). Mecamylamine blocked the nicotine activated current with an IC_{50} of 2.1 ± 0.7 µM (n = 4), in good agreement with the literature[4,5,7].

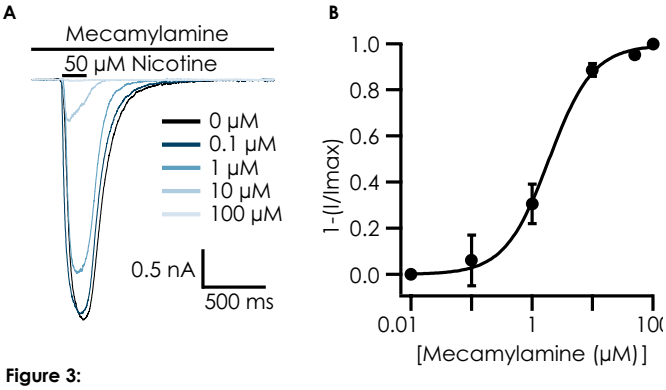

Figure 3:
Block of nicotinic a3β4 receptors by mecamylamine. Mecamylamine at increasing concentrations (0.1 µM - 100 µM) was pre-incubated and then co-applied with 50 µM nicotine. Some recovery from block was achieved upon washout (approx. 20%; data not shown). **B** Concentration response curve for mecamylamine block, IC_{50} = 2.1 ± 0.7 µM (n = 4), in good agreement with the literature[4,5,7].

In summary, nicotinic a3β4 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 a3β4 receptors[1-7]. Therefore, the Patchliner® provides a viable, higher throughput alternative to conventional patch clamp for the discovery of agonists and antagonists of nicotinic a3β4 receptors as potential therapeutics for analgesia and Tourette's Syndrome, amongst others .

References

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Methods

Cells

HEK293 cells stably expressing nicotinic a3β4 were used.

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 µl/s. Wash solutions contained the antagonist in the mecamylamine experiments. The cells were pre-incubated in mecamylamine before co-application with 50 µM nicotine.

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