α7 Nicotinic Receptor Mediation of CNS Inflammatory Response Examined by Magnetic Resonance Imaging and Bioluminescence Imaging

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Introduction

Nicotinic acetylcholine receptors (nAChRs) are members of a diverse family of ligand-gated ion channels that serve as targets for acetylcholine and nicotine [1]. They play critical roles throughout the brain and body by mediating cholinergic excitatory neurotransmission, modulating the release of neurotransmitters, and having longer-term effects on gene expression and cellular interactions [1]. In addition to their function in neuromuscular junctions and in neurons, studies have shown that many immune cell types express nAChR subunits and that binding of nicotine or acetylcholine to α7-nAChR leads to a suppression of inflammation [2-5]. Nicotine administration has been shown to attenuate inflammation in an experimental autoimmune encephalomyelitis (EAE) mouse model of multiple sclerosis [6]. Although the involvement of α7-nAChR in CNS autoimmune disease has been suggested, the extent to which α7-nAChRs mediate the effect of nicotine on clinical and pathological hallmarks of EAE has not been explored. This study used a combination of in vivo MRI and bioluminescence imaging to examine the effect of nicotine on EAE in α7-nAChR knockout mice.

Methods

C57BL/B6 wild-type ($\alpha7^{+/+}$) and nAChR α7 knockout mice ($\alpha7^{-/-}$) were injected with 200 μg of MOG_{35-55} peptide containing 500 μg of non-viable, desiccated Mycobacterium tuberculosis. On the day of and 2 days after immunization, the mice were inoculated with 200 ng of pertussis toxin intraperitoneally. Mice received 100 mg/ml nicotine (Nic) or PBS (n=6-8/group) for 28 days upon EAE induction through continuous infusion from osmotic minipumps (Durect Corp, Cupoertino, CA). The resulting nicotine plasma levels (~40 ng/ml) were consistent with those in human smokers. Mice were monitored daily for symptoms and scored on the following scale with 0.5 increments: 0, no symptoms; 1, flaccid tail; 2, hindlimb weakness; 3, complete hindlimb paralysis; 4, complete hindlimb paralysis with forelimb weakness; 5, moribund or deceased.

In vivo MRI was performed on a 7 Tesla small-animal scanner (Bruker BioSpin, Billerica, MA). Coronal fat-suppressed T2-weighted images were acquired over the entire brain of each animal (RARE; TE1=14.5 ms, TE2=65.5 ms, TR=4500 ms, 0.5 mm slice thickness, Matrix 256x256, FOV=2.8 cm, eight averages, 40 coronal slices, scan time 28 minutes). For imaging of ROS generation in brain, bioluminescence images in live mice were captured with a 1 min acquisition time using a cooled IVIS imaging system (Xenogen IVIS-200, Alameda, CA) after injection of 27 mg/kg DHE (Molecular Probes, Eugene, OR).

Results

Differences in clinical scores are significant ($p<0.05$) at day 10 post-immunization between the $\alpha7^{+/+}$ Nic group and the other groups, but not across both PBS groups and the $\alpha7^{-/-}$ Nic group (Figure 1, A). Visualization and quantification of brain inflammation by in vivo bioluminescence imaging at day 14 post-immunization and PBS/nicotine treatment reveals significant ROS differences between $\alpha7^{+/+}$ Nic and both PBS groups. There is only a moderate, non-significant, difference between the $\alpha7^{-/-}$ Nic and PBS groups (Figure 1, B). T2 weighted periventricular images, obtained 14 days after immunization plus Nic/PBS treatment, are shown in Figure 1C. Arrows indicate focal lesions located around the lateral ventricles and increased signal intensity. Nicotine exposure diminished EAE-induced brain lesion volume in $\alpha7^{+/+}$ mice ($p<0.01$) but had only a partial effect in $\alpha7^{-/-}$ ($p=0.051$).

Conclusion

The principal findings in this study are that although α7-nAChR deficiency prevents nicotine from protecting against clinical manifestations in EAE mice, many parameters relating to inflammatory and autoimmune response affected by nicotine exposure are only partially attenuated in $\alpha7^{-/-}$ mice. These results indicate that cholinergic modulation of inflammation involves not only α7-nAChR alone but also likely involves several nAChR subtypes.

References

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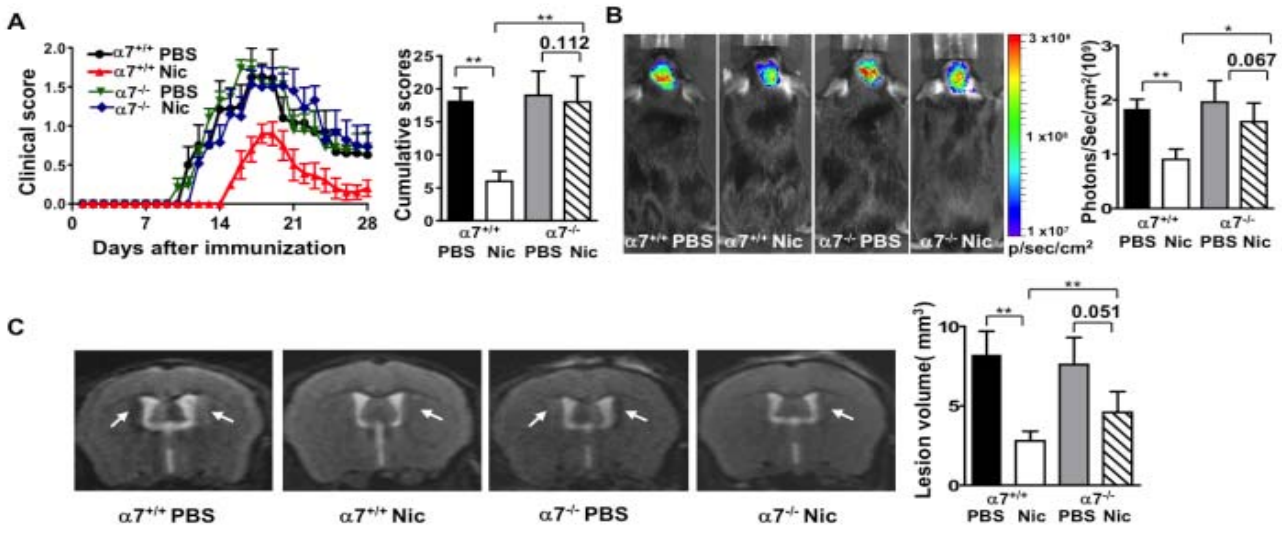

Figure 1. A) Clinical scores reveal protective effect of Nic in $\alpha7^{+/+}$ mice but not in $\alpha7^{-/-}$. B) No significant difference in ROS signal was found in $\alpha7^{-/-}$ mice given Nic and PBS. C) MRI reveals that nicotine diminished lesions in $\alpha7^{+/+}$ mice but had only a partial effect in $\alpha7^{-/-}$.