5.4

Nicotine suppresses hyperexcitability of inflamed colonic sensory neurons

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Controlled clinical trials suggest an involvement of neuronal nicotinic acetylcholine receptors (nAChRs) in ulcerative colitis (UC). UC has been found to occur largely in nonsmokers and a remission in the disease can be induced by administration of nicotine patch. Notable improvements occur in both global clinical grade as well as in abdominal pain. Previously, we reported that UC is accompanied by alterations in nAChRs expressed in colonic sensory neurons with an increased and predominant activity of α7 nAChR subtype. To better understand a role of nAChRs in the sensory regulation of colonic inflammation, we examined the effect of nicotine on action potential firing in colonic neurons in a mouse model of experimental colitis. Based on disease activity index dose-response, adult C57Bl/J6 and α7 knock-out male mice were treated for 5–7 days with 5% and 2.5% dextran sulphate sodium (DSS), respectively, in drinking water provided *ad libitum*. After 5 days of treatment the mice showed a significant loss of weight, developed signs of diarrhea and rectal bleeding. Dorsal root ganglia of colonic origin were isolated from DSS treated mice that exhibited the above described signs of colonic inflammation. Prelabeled colonic neurons were tested using whole-cell current-clamp recording. Colonic neurons from DSS treated α7 knock-out mice showed a lower threshold for action potential firing than those from C57Bl/J6 mice. Bath application of 1 μM nicotine suppressed action potential firing in inflamed colonic neurons isolated from the DSS treated C57Bl/J6 mice but not from the DSS treated α7 knock-out mice. These data suggest that nicotine interaction with α7 nAChRs mediates a suppression of the hyperexcitability of sensitized colonic DRG neurons.

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5.5

Anti-inflammatory effects of α4β2 nicotinic receptor activation revealed through microarray analysis of nicotine-induced gene changes

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Epidemiological data suggest that smoking, although dangerous overall, confers some protection against neurodegenerative diseases. Cholinergic activation of α7 nicotinic receptors gives protection against inflammation in the peripheral nervous system, but few anti-inflammatory actions are associated with α4β2 receptors, the major high-affinity nicotinic receptor subtype in brain. Surprisingly, 10 μM nicotine treatment of SH-EP1 cells stably expressing human α4β2 receptors altered gene expression for 18 genes associated with inflammation or immune responses (detected using Affymetrix arrays), but had no effect on these genes in wild-type cells. Quantitative RT-PCR corroborated eight gene expression changes including cytokines IL-1β, IL-6 and IL-11, the chemokine CXCL2, and the redox-protective enzyme SOD2. We concentrated further evaluation on the pro-inflammatory cytokines (PICs) IL-1β and IL-6. The nicotinic antagonists dihydro-β-erythroidine and mecamylamine blocked α4β2-mediated suppression of PICs, indicating that receptor activation is required. Nicotine exposure prevented NFkB translocation (part of the pro-inflammatory induction pathway), but not in wild-type cells, and these changes correlate to decreases in PIC production measured by ELISAs. Further, nicotine blocked cytokine production and NFkB translocation caused by stimulation of α4β2 SH-EP1 cells with the endotoxin lipopolysaccharide (see figure). Preliminary data suggest that PIC mRNAs are significantly increased in three of four brain regions tested in α4 knockout mice compared to wild-type and heterozygote mice. This unexpected finding that nicotine suppresses PICs by α4β2 receptor-mediated NFκB suppression suggests that nicotinic activation of α4β2 receptors promotes previously unknown anti-inflammatory effects that may explain epidemiological evidence for neuroprotective effects of smoking against Parkinson's and Alzheimer's disease (Fig. 1).

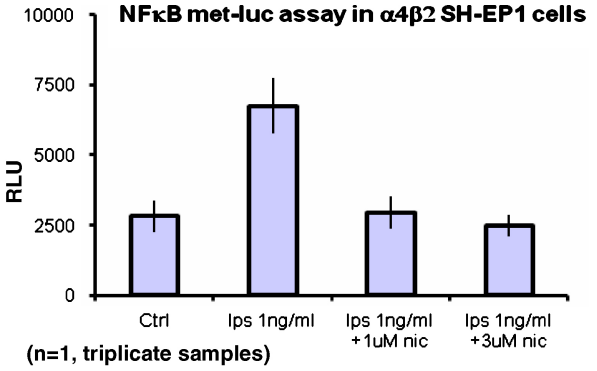

Fig. 1. Nicotine blocks NFκB translocation in lipopolysaccharide-treated α4β2 SH-EP1 cells.

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5.6

Role of α7 nicotinic acetylcholine receptors in regulating tumor necrosis factor-α (TNF-α) as Revealed by subtype selective agonists

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Immunological responses to protect against excessive inflammation can be regulated by central nervous system (CNS) through the cholinergic anti-inflammatory pathway wherein acetylcholine (ACh) released upon stimulation from vagus or splenic nerves in innervated tissues can inhibit inflammatory cytokines. Although a role for α7 nicotinic acetylcholine receptor (α7 nAChR) in mediating the cholinergic anti-inflammatory pathway has been suggested, pharmacological modulation of this pathway by selective agonists remains to be further elucidated. In this study, the role of α7 nAChRs in TNF-α release was investigated using high affinity and selective α7 nAChR agonists. In mouse peritoneal macrophages,

lipopolysacharide (LPS)-induced TNF-α release *in vitro* was inhibited in a concentration-dependent manner by the α7 selective agonist A-833834, which was attenuated by methyllycaconitine. The inhibitory effect of A-833834 on LPS-induced TNF-α was also observed in human whole blood *ex vivo*. *In vivo*, i.v. LPS-stimulated TNF-α release was attenuated following acute administration of A-833834 and A-585539, another α7 nAChR agonist with limited brain penetration, the latter suggesting that these effects are not centrally mediated. The plasma levels for achieving *in vivo* efficacy in this model were found to be closer to the functional EC_{50} values for α7 nAChR activation than that to the binding affinity of these ligands. A-833834 was also efficacious in suppressing TNF-α release in a dose-dependent manner following oral administration in another *in vivo* model of zymosan-induced peritonitis. These studies collectively demonstrate that selectively targeting peripheral α7 nAChRs could offer a novel therapeutic modality to treat acute and chronic inflammatory disease states.

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