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Nicotinic receptor involvement in regulation of functions of mouse neutrophils from inflammatory site

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ABSTRACT

Participation of nicotinic acetylcholine receptors (nAChRs) in functioning of polymorphonuclear neutrophils (PMNs) isolated from inflammatory site of mice and expression of different nAChR subunits were studied. Nicotine and acetylcholine (ACh) modified respiratory burst induced by a chemotactic peptide N-formyl-MLF in neutrophils of male (but not female) mice. Antagonists of nAChRs α-cobratoxin (αCTX), α-conotoxins MII and [A10L]PnIA at concentrations of 0.01–5 μM, 0.2 μM and 1 μM, respectively, eliminated nAChR agonist effects. ACh also affected adhesion of PMNs, this effect was also prevented by αCTX (100 nM) and MII (1 nM). Neutrophils of female mice after chronic nicotine consumption acquired sensitivity to nAChR agonists. Changes of free intracellular Ca^{2+} concentration in neutrophils under the action of nAChR ligands were analyzed. In cells with no Ca^{2+} oscillations and relatively low resting level of intracellular Ca^{2+}, nicotine triggered Ca^{2+}-spikes, the lag of the response shortened with increasing nicotine concentration. A nicotinic antagonist caramiphen strongly decreased the effect of nicotine. RT-PCR analysis revealed mRNAs of α2, α3, α4, α5, α6, α7, α9, β2, β3, and β4 nAChR subunits. Specific binding of [^{125}I]-α-bungarotoxin was demonstrated. Thus in view of the effects and binding characteristics the results obtained suggest a regulatory role of α7, α3β2 or α6* nAChR types in specific functions of PMNs.

1. Introduction

PMNs participate in innate and adaptive immunity (Ishikawa and Miyazaki, 2005; for review see Jaillon et al., 2013; Scapini and Cassatella, 2014; Takashima and Yao, 2015). They are potent in microbicidal action, production of cytokines and pattern recognition molecules. PMNs, developed in bone marrow and released into circulation, express numerous proteolytic enzymes and cytotoxic proteins, as well as a set of membrane and cytoplasmic subunits of NADPH oxidase to produce superoxide, a predecessor of other reactive oxygen species (ROS). PMNs are the quickest and the most powerful effectors of the acute stage of inflammation (Jaillon et al., 2013; Rigby and DeLeo, 2012; Segal, 2005). Inadequate activity of PMNs may lead to uncontrolled inflammatory reaction. A proper intensity of neutrophil immune reactions is regulated by different membrane receptors: G-protein-coupled, Fc-, adhesion, cytokine and innate immune receptors (for review, see Futosi et al., 2013). nAChRs have been also revealed in PMNs. High affinity binding of their specific agonists [^{3}H]-nicotine and [^{3}H]-epibatidine was observed in human neutrophils (Benhammou et al., 2000; Lebargy et al., 1996), the latter was shown for smokers (Cormier et al., 2004). α-Bungarotoxin (α-Bgt) binding sites were found in PMNs (Cormier et al., 2004) that indicated expression of α7 or α1 nAChR subunits. mRNAs coding α4, α3, β2 and β4 subunits and corresponding proteins were disclosed in human PMNs suggesting the existence of α4β2 and α3β4 nAChR types (Benhammou et al., 2000). α7 nAChRs

Abbreviations: ACh, acetylcholine; BSA, bovine serum albumin; αBgt, α-bungarotoxin; $[Ca^{2+}]_i$, intracellular free Ca^{2+} concentration; αCTX, α-cobratoxin; fMLF, *N*-formyl-L-methionyl-L-leucyl-L-phenylalanine; HBSS, Hanks' balanced salt solution; IL-8, interleukin-8; LPS, lipopolysaccharide; MIP-2, macrophage inhibiting protein-2; MMP-9, matrix metallopeptidase-9; nAChR, nicotinic acetylcholine receptor; NF-kB, nuclear factor-kB; PKC, protein kinase C; PMSF, phenylmethylsulfonyl fluoride; PMN, polymorphonuclear neutrophilic granulocyte; ROS, reactive oxygen species; RT-PCR, Real-Time Reverse-Transcription Polymerase Chain Reaction; TNF-α, tumor-necrosis factor-α.

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