Nicotine produces structural plasticity in dopaminergic neurons: evidence from mouse neuronal cultures and human IPSC-derived neurons

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Converging findings, mostly obtained in animal studies, indicate that activation of nicotinic acetylcholine receptors (nAChR) critically affect the functional output of mesencephalic dopaminergic neurons. While electrophysiological and neurochemical changes have been extensively studied, less information is available on structural and molecular mechanisms of plasticity. In addition, the translational relevance of these findings in animal models has not been fully addressed, a critical point in quest for novel pharmacological treatments. Using an *in vitro* model of primary cultures of mouse mesencephalic dopaminergic neurons, we recently characterized cellular and molecular mechanisms involved in these phenomena. Exposure to low-dose nicotine increases dendritic arborization and soma size via activation of alpha4-beta2-containing nAChR; this effect depends upon the availability of functional dopamine D3 receptor (D3R) and activation of the ERK and Akt-mTOR intracellular pathways known to be involved in cellular growth (Collo et al., 2013). In the present study, using the same primary culture model, we studied the role of the alpha6-containing nAChR in mediating structural plasticity produced by nicotine. Pharmacological blockade with alpha-conotoxin PIA and preparations from alpha6 null mutant mice were associated with the lack of nicotine-induced structural plasticity. These results are in keeping with the results obtained with alpha4 null mutant mice and pharmacological treatments with beta-erythroidine, suggesting a key role for the alpha4-alpha6-beta2-containing nAChR. The in vivo relevance of this observation was obtained using a prenatal nicotine exposure model in wildtype and nAChR subunit null mutant mice. In newborns the size of dopaminergic neurons was morphometrically assessed between P1 and P15. Nicotine significantly increased the soma size of DA neurons in wildtype but not in nAChR subunit null mutant mice when compared to vehicle-exposed mice. To address the issue of translational relevance of these findings to humans we have extended our study to dopaminergic neurons differentiated from human inducible pluripotent stem cells (hiPSC). hiPSC from healthy donors (Devine et al. 2010) were differentiated into dopaminergic neurons following the protocol of Kriks et al. 2011 with modifications. Terminal differentiation was achieved at about 80 days in culture, as assessed by the expression of dopaminergic neuron-specific molecular markers and dopamine release and uptake. Exposure to nicotine (1-10 uM) produced structural plasticity, increasing dendritic arborization and soma size, effects blocked by nAChR antagonists. These findings indicate that nicotine can affect cellular structure and plasticity in dopaminergic neurons at doses comparable to those of tobacco smokers. In particular, data from hiPSC-derived neurons suggest that nicotine-induced structural plasticity in dopaminergic neurons could occur also in humans. These observations underlie the translational relevance of hiPSCs for pharmacological studies, opening the way to the development of novel therapeutics with a reduced inter-species translational risk.

Collo G. et al. (2013) Mol. Pharmacol. 83:1176-1189
Devine M.J. et al. (2010) Nat. Commun. 2:440
Kriks S. et al. (2011) Nature 480:547-51