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NICOTINE-INDUCED PLASTICITY IN THE RETINOCOLLICULAR PATHWAY: EVIDENCE FOR INVOLVEMENT OF AMYLOID PRECURSOR PROTEIN

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Abstract—During early postnatal development retinocollicular projections undergo activity-dependent synaptic refinement that results in the formation of precise topographical maps in the visual layers of the superior colliculus (SC). Amyloid Precursor Protein (APP) is a widely expressed transmembrane glycoprotein involved in the regulation of several aspects of neural development, such as neurite outgrowth, synapse formation and plasticity. Stimulation of cholinergic system has been found to alter the expression and processing of APP in different cell lines. Herein, we investigated the effect of nicotine on the development of retinocollicular pathway and on APP metabolism in the SC of pigmented rats. Animals were submitted to intracranial Elvax implants loaded with nicotine or phosphate-buffered saline (vehicle) at postnatal day (PND) 7. The ipsilateral retinocollicular pathway of control and experimental groups was anterogradely labeled either 1 or 3 weeks after surgery (PND 14 or PND 28). Local nicotine exposure produces a transitory sprouting of uncrossed retinal axons outside their main terminal zones. Nicotine also increases APP content and its soluble neurotrophic fragment sAPPα. Furthermore, nicotine treatment upregulates nicotinic acetylcholine receptor α7 and β2 subunits. Taken together, these data indicate that nicotine disrupts the ordering and topographic mapping of axons in the retinocollicular pathway and facilitates APP processing through the nonamyloidogenic pathway, suggesting that sAPPα may act as a trophic agent that mediates nicotine-induced morphological plasticity.

Key words: nicotine, APP, superior colliculus, plasticity, development.

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Abbreviations: Aβ, amyloid-β; AD, Alzheimer's disease; APP, Amyloid Precursor Protein; CNS, central nervous system; EGTA, ethylene glycol tetraacetic acid; HRP, horseradish peroxidase; nAChRs, nicotinic acetylcholine receptors; OT, optic tectum; PBS, phosphate-buffered saline; PND, postnatal day; SC, superior colliculus; SGS/SO, stratum griseum superficiale/stratum opticum; TBS, Tris-buffered saline; TBS-T, TBS + 0.1% Tween 20; TMB, tetramethylbenzidine.

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INTRODUCTION

Nicotine, a natural alkaloid, is considered to be the primary psychoactive chemical substance in tobacco and exerts its pharmacological effects by binding to neuronal nicotinic acetylcholine receptors (nAChRs). These receptors are ligand-gated cation channels consisting of different α and β subunits which associate into functional homopentamers (α7, α8 or α9) or heteropentamers, comprised of two α and three β subunits. nAChRs are widely distributed throughout the central nervous system (CNS) and have been implicated in several events that shape the brain connectivity during development (Paterson and Nordberg, 2000; Albuquerque et al., 2009). Multiple subtypes of nAChRs are present throughout the visual system, playing important roles not only in the retina but also in retinal target tissues, such as the superior colliculus (SC) (Feller, 2002).

In the visual system, the uncrossed retinocollicular projection has been extensively used to study the specification of sensory neuronal circuits during early postnatal development (Campello-Costa et al., 2006; Tavares Gomes et al., 2009; Espírito-Santo et al., 2012). In adults, axon terminals are found restricted to well-defined terminal zones at the stratum griseum superficiale/stratum opticum (SGS/SO) border of the SC. This pattern gradually emerges from a diffuse innervation observed in newborns (Serfaty and Linden, 1994). This developmental shift in synaptic convergence ultimately represents the strengthening and growth of appropriately targeted connections and the weakening of inappropriate synapses with the elimination of the processes which bear them (Lo and Mize, 2002). The activation of nAChRs has been implicated in activity-driven sharpening of retinocollicular projections. Indeed, disturbances of axonal remodeling have been found in a null mutation of a specific nicotinic receptor subunit (Rossi et al., 2001) or exposure of the tectum to nicotinic receptor antagonists (Yu et al., 2003).

Functional assays have revealed that Amyloid Precursor Protein (APP) plays critical roles in synaptic plasticity, neurite outgrowth and synaptogenesis (for review, Müller and Zheng, 2012). Interestingly, APP and its homologs, the APP-like proteins APLP1 and APLP2, are developmentally regulated and comprise most of the protein mass that is rapidly transported through the optic nerve during the peak period of axon extension and synapse formation (Moya et al., 1994; Lyckman et al., 1998). APP is a type I transmembrane glycoprotein that

undergoes proteolytic processing by enzymes termed secretases in two opposing cleavage pathways. In the amyloidogenic pathway, sequential cleavages of APP by β- and γ-secretases result in the release of soluble amino-terminal fragment sAPPβ and amyloid-β (Aβ) peptide, the main component of senile plaques found in Alzheimer's disease (AD). Alternatively, in the nonamyloidogenic pathway, α-secretase cleaves APP within Aβ sequence, which not only precludes Aβ formation but also yields the soluble amino-terminal fragment sAPPα. While Aβ is highly aggregation prone and forms neurotoxic species such as oligomers and protofibrils, sAPPα is known to have a wide range of neurotrophic and neuroprotective functions (for a review, Gralle and Ferreira, 2007; Chasseigneaux and Allinquant, 2012).

Epidemiological studies about smoking and AD are somewhat contradictory. Recently data point that smoking is an important modifiable risk factor of dementia (Zhong et al., 2015; Cho et al., 2015). However, other groups have shown an inverse association between cigarette smoking and the onset of AD (Lee, 1994; Fratiglioni and Wang, 2000). Indeed, reduced levels of Aβ and lower senile plaque density have been found in the brain of smoking AD patients (Hellström-Lindahl et al., 2004; Ulrich et al., 1997). Accordingly, considerable data have emerged showing a link between nAChR activity and the metabolism of APP (Mousavi and Hellstrom-Lindahl, 2009; Nie et al., 2010).

Altered APP expression and processing have been linked to retinal abnormalities associated with AD (Koronyo-Hamaoui et al., 2011) and optic neuropathies that lead to visual impairment (Löffler et al., 1995). Visuoperceptual deficits have also been related to cigarette smoking (Hepsen and Evereklioglu, 2001). However, whether nicotine influences the structural organization of visual connections during early postnatal development and the metabolism of APP in the visual layers of the SC have not been described yet.

Herein we investigated the effect of local exposure to nicotine on the development of the retinocollicular pathway, on the content of both α7- and β2-containing nAChRs and in the APP metabolism. We found that nicotine induced a transitory disruption of the retinocollicular topography and an upregulation of both nAChRs subunit proteins and sAPPα levels. Taken together, these data suggest a role for APP in the nicotine-induced retinocollicular morphological plasticity.

EXPERIMENTAL PROCEDURES

Animals

Lister Hooded rats at different ages, from postnatal day (PND) 7 to PND 28, were obtained from the breeding colony at the Fluminense Federal University, Niteroi, BR and used for *in vivo*, biochemical and neuroanatomical experiments. Dams and their litters were housed in individual cages under humidity and temperature-controlled conditions, with a 12-h light–dark cycle and with *ad libitum* access to water and food. All animal research was in agreement with The National Institutes of Health Guide for the Care and Use of Laboratory Animals and was approved by the local Animal Care Committee (Protocol No. 00205-CEPA-UFF). The minimum number of animals sufficient to provide statistically significant results and to answer the scientific questions and goals was used for each neuroanatomical and biochemical study. The exact number of animals per group in each experiment is presented inside graphic bars in each figure.

Preparation of Elvax

The local delivery of nicotine was performed through intracranial implants of drug-impregnated ethylene vinyl acetate polymer (Elvax, Dupont). Nicotine (Nicotine hydrogen tartrate salt-Sigma) was embedded into Elvax in a concentration of 100 mM in phosphate-buffered saline (PBS). Elvax containing only vehicle (PBS) was also prepared. Elvax polymers were processed according to methods previously described (Smith et al., 1995). Briefly, Elvax beads were washed in 95% alcohol for a week and then dissolved in dichloromethane (100 mg Elvax/ml). Afterward, nicotine or PBS was then added to the Elvax mixture at the required concentration. Fast green, 0.01% was also added to aid the visualization of Elvax slices and implant procedures. The solution was vortexed, frozen in a dry ice/acetone bath, stored for at least 7 days at −20 °C and placed under a mild vacuum for 1 day to remove the solvent. 120-μm-thick slices were obtained using a cryostat. Release profile of nicotine from Elvax has been well described (Yan et al., 2006).

Surgical implantation

Rats were submitted to an intracranial implant of either a nicotine or vehicle-loaded Elvax at PND 7. Under isoflurane anesthesia (3% induction and 1.5% maintenance) an incision was made along the midline and the skin retracted to expose the skull. After a limited craniotomy and removal of the dura mater, the surface of the midbrain was exposed and an Elvax slice was carefully placed exactly over the SC, which ensures a slow, local release of nicotine with a low systemic level (Yan et al., 2006). The skull was then closed and cyanoacrylate gel was applied to the skin cut. Animals were monitored continuously until fully recovered from anesthesia and returned to their home cage. Correct Elvax placement was confirmed at dissection.

Horseradish peroxidase (HRP) histochemistry

Under inhaled isoflurane anesthesia, animals at either PND 13 or PND 27 received a single unilateral intraocular injection of 5 μl of a solution of 30% (HRP, Sigma, type VI) in 2% dimethylsulfoxide (DMSO) in NaCl 0.9%, in the right eye, with a Hamilton microsyringe, to label the uncrossed retinocollicular pathway. Approximately 24 h later, animals were deeply anesthetized with an overdose of isoflurane and transcardially perfused with saline (0.9% NaCl) followed by a mixture of 1% paraformaldehyde and 2% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2. Brains were removed, placed in a solution of 20%