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Nicotine inhibits hippocampal and striatal acetylcholinesterase activities, and demonstrates dual action on adult neuronal proliferation and maturation

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ABSTRACT

Aim: The present study investigated the effects of nicotine on acetylcholinesterase (AChE) activities in the hippocampus and striatum; and on immunoreactivity of certain neurogenic markers in the dentate gyrus (DG) of the hippocampus.
Methods: Male rats were given daily subcutaneous injections of nicotine at doses of 0.25, 2 or 4 mg/kg body weight for 28 days. Animals were euthanized by cervical dislocation at the end of administration. Brains were excised and processed for histochemical demonstration of AChE and immunohistochemical studies of Ki67, GFAP and NSE.
Results: There was significant decrease ($P < 0.001$) in AChE positive cells in the hippocampus and striatum following 2 and 4 mg/kg nicotine but not at 0.25 mg/kg. Nicotine treatment at 0.25 and 4 mg/kg significantly decrease ($P < 0.05$) immunoreactivity of Ki67 and NSE in DG. Contrastingly, 2 mg/kg nicotine did not alter Ki67 immunoreactivity but rather significantly increased ($P < 0.05$) NSE immunoreactivity in DG compared to control.
Conclusion: This study suggests that nicotine may inhibit AChE activities in the brain, thereby having a direct or indirect influence on prevention of central acetylcholine degradation, as well as either improve or retard maturation adult born neurons in DG, at different doses.

1. Introduction

Nicotine, the natural alkaloid found in *Nicotiana tabacum*, is the most studied constituent of tobacco smoke, and has been shown to demonstrate extensive pharmacological effects [1]. Nicotine interacts with a broad range of neuronal nicotinic acetylcholine receptors (nAChR), in the brain [2]. Nicotine have been considered to have many adverse effects, but in the last decade it has been the subject of potential therapeutic value for the management of neurologic and neurodegenerative diseases [3–7]. This has been linked to its activating action on nAChR. Data largely suggest enduring effects of nicotine and nicotinic agonists that indicate a neuroprotective ability of nAChR activation, involving mainly α7 and α4β2 nAChR subtypes, as demonstrated using selective nAChR agonists [8]. A variety of cellular mechanisms which range from the production of growth factors to inactivation of toxins and antioxidant properties of nicotine have been suggested to trigger the nAChR-facilitated neuroprotection in vivo and in vitro [9].

However, the effect of nicotine on the 'naturally occurring' process of neurogenesis in the adult brain has been deemed unfavourable [10]. Neurogenesis is a process of generation of new neurons in the brain from neural stem cells and neuronal differentiation of newly formed cells. Although most neurogenesis occurs during initial development (prenatally), certain regions of the brain maintain neurogenesis into adulthood and throughout life. These include the subgranular zone (SGZ) of the dentate gyrus (DG) of the hippocampus and the subventricular zone (SVZ) lining the lateral ventricles, whose neurons migrate to the olfactory bulb [11].

Cholinergic neurotransmitter activities in the hippocampus and striatum are implicated in a variety of brain functions [12–15]. Also cholinergic neurotransmission within these brain regions are

Abbreviations: nAChR, neuronal nicotinic acetylcholine receptors; SGZ, subgranular zone; DG, dentate gyrus; SVZ, subventricular zone; ACh, acetylcholine; AChE, acetylcholinesterase; Ki67, protein Ki,67 (Ki67); GFAP, glial fibrillary acidic protein; NSE, neuron specific enolase; CPu, caudate-putamen; CA, *Cornu Ammonis*.

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therapeutic targets of various neurodegenerative diseases [1,16]. Acetylcholine (ACh), the cholinergic neurotransmitter in the brain is removed from synaptic cleft in the nervous system by the action of acetylcholinesterase (AChE), which splits ACh into choline and acetate [17]. Chang et al., observed that nicotine administration may reduce AChE activities in the hypothalamus [18].

Therefore, this study has investigated the effects of nicotine on AChE activities in the hippocampus and striatum, with a view to demonstrate additional action of nicotine on the cholinergic systems apart from its known activiation of nAChR. Also, the study has also evaluated the effect of nicotine on activities of certain neurogenic markers; protein Ki-67 (Ki67), glial fibrillary acidic protein (GFAP) and neuron specific enolase (NSE), with a view to demonstrate action of nicotine on adult neuronal proliferation and maturation.

2. Materials and methods

2.1. Animal care and treatment

Adult male albino strain Wistar rats (150–200 g) were housed in clean plastic cages in a clean environment of natural day/light cycle, at room temperature. Animals were allowed free access to standard laboratory rat chow and water. All animals were handled in accordance with the guidelines for animal research as detailed in the NIH Guidelines for the care and use of laboratory animals [19]. Animals were randomly grouped into 4 groups of 6 animals each. Animals were given daily subcutaneous injections of nicotine dissolved in saline at graded doses of 0.25, 2 and 4 mg/kg body weight to 3 groups, with a control group receiving normal saline, for 28 days. Nicotine was obtained in free base form as (−)-Nicotine also called (−)-1-methyl-2-(3-pyridyl) pyrrolidine from Sigma Chemicals, USA. The selection of nicotine dose is based on previously published studies [1,20,21]. At the end of administration, rats were sacrificed by cervical dislocation, and their brains were rapidly excised and fixed in either cold 10% formol calcium (for AChE histochemistry) or 10% neutral buffered formalin (for Ki67, GFAP and NSE immunohistochemistry). All tissues were processed for rapid routine paraffin embedding, sectioned at 3 μm and stained via histochemical and immunohistochemical describe below.

2.2. AChE histochemistry

AChE activities were demonstrated by the use of acetylthiocholine iodide as substrate in a solution containing cuprous and ferric sulphate, as previously described (16), with slight modification. After incubation, sections were rinsed rapidly in 20% aqueous silver nitrate solution followed by rapid rinsing in 10% neutral buffered formalin to enhance staining and reduce background color.

2.3. Immunohistochemistry of neurogenic markers

Immunohistochemical studies for Ki67, GFAP and NSE were performed using the Novocastra™ NovoLink™ Polymer Detection System (Leica Biosystems, UK) and appropriate primary monoclonal antibodies. All antibodies were Novocastra™ Liquid Mouse Monoclonal Antibodies (Leica Biosystems, UK) for the appropriate antigen (Ki67, GFAP and NSE) that was demonstrated. A detail of protocol using this Detection system is found in manufacturer's instruction manual. In brief, following antigen retrieval, peroxidase blocking was performed using Novolink™ peroxidase block. Then sections were treated with Novolink™ protein block, and incubated in appropriate primary antibodies. This was followed by secondary incubation in Novolink™ post primary block, and a subsequent incubation in Novolink™ polymer. Sections were finally treated with Novolink™ DAB (3,3′-diaminobenzidine) working solution before counter staining in Haematoxylin. Sections were rinsed between major steps in either buffer solution (phosphate buffer) or distilled water as appropriate.

2.4. Image analysis and cell count

Stained sections were viewed under a Leica DM750 Digital Light microscope and digital photomicrographs were taken by an attached Leica ICC50 camera. The CA3 region and DG of the hippocampus and caudate-putamen (CPu) of the striatum were located using landmarks from the rat brain stereotaxic atlas [22]. At least four measurements were taken from non-overlapping areas of the striatum and hippocampus. Cell count was done at x400 magnification using the cell counter tool on Image Analysis and Processing for Java (ImageJ) program, a public domain software sponsored by the National Institute of Health (USA). ImageJ cell counter tool was used to identify and count per unit area, the number of AChE positive cells, and number of Ki67, GFAP and NSE immunoreactive positive cells.

2.5. Statistical analysis

Data obtained were analysed using One-way ANOVA, followed by Student Newman-Keuls (SNK) for post hoc. GraphPad Prism 5 (Version 5.03, GraphPad Software, USA.) was the statistical package used for data analysis. Student's *t*-test was employed for analysis of between group differences, when appropriate. Statistical significance was set at $P < 0.05$.

3. Results

3.1. AChE activity

Treatment with 2 and 4 mg/kg nicotine, significantly decreased ($P < 0.001$) the number of AChE positive (+ve) cells in CA3 region of hippocampus when compared to both control and 0.25 mg/kg nicotine treatment groups [Control = 41.72 ± 1.09; 0.25 mg/kg = 49.16 ± 4.66; 2 mg/kg = 18.59 ± 3.58; 4 mg/kg = 9.50 ± 2.71] (Fig. 1). Similarly, 2 and 4 mg/kg nicotine treatment also significantly decreased ($P < 0.001$) the number of AChE +ve cells in the CPu of striatum when compared to both control and 0.25 mg/kg nicotine treatment groups [Control = 99.56 ± 1.09; 0.25 mg/kg = 97.08 ± 4.37; 2 mg/kg = 46.27 ± 1.80; 4 mg/kg = 37.18 ± 7.54] (Fig. 2).

3.2. Neurogenic markers immunoreactivity in DG of hippocampus

Immunohistochemical studies showed significant decrease ($P < 0.05$) in Ki67 +ve immunoreactivity following 0.25 and 4 mg/kg of nicotine treatment compared to control, but no significant difference was observed in Ki67 immunoreactivity following 2 mg/kg of nicotine treatment compared to control. Rather, 2 mg/kg treatment significantly increased ($P < 0.01$) Ki67 positive immunoreactivity compared to both 0.25 and 4 mg/kg treatment [Control = 13.01 ± 2.84; 0.25 mg/kg = 2.27 ± 0.90; 2 mg/kg = 21.52 ± 4.44; 4 mg/kg = 2.48 ± 0.72] (Fig. 3). There was no significant difference in GFAP +ve immunoreactivity between control and all nicotine treated groups [Control = 9.21 ± 0.37; 0.25 mg/kg = 9.92 ± 2.15; 2 mg/kg = 11.67 ± 1.19; 4 mg/kg = 8.99 ± 1.71] (Fig. 4). There was significant decrease ($P < 0.05$) in NSE immunoreactivity following 0.25 and 4 mg/kg compared to control. Contrastingly, 2 mg/kg treatment significantly increased ($P < 0.05$) NSE positive immunoreactivity compared