

Neurochemical Effects of Nicotine on Albino Rat's Brain

Anshu Tewari^{1,*}, Mahdi Hasan¹, Rahul Misra², Anita Rani¹

¹Department of Anatomy, King George's Medical University, Lucknow, India

²Department of Anaesthesia, GSVM Medical College and Hospital, Kanpur, India

*Corresponding author: Anshu Tewari, Department of Anatomy, King George's Medical University, Lucknow, India. Tel.: +91-9605365043, Fax: +91-5222391657, E-mail: anshutewari21@gmail.com.

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Background: In the recent era, smoking has become fashionable. Seventy percent of the Indian population is continuously being exposed to smoking. Cigarette smoking produces various quantities of nicotine that is readily absorbed by the physiological system of smokers.

Objectives: Adolescence is the period in which smokers typically begin their nicotine addiction. To date, no attempt has been made to estimate changes in cholinergic and DA-D2 receptors (Dopaminergic D2 receptors) following nicotine administration, although literature review has revealed available publications regarding changes in dopamine concentration following nicotine. Hence the present experiment was conducted to study the alteration of cholinergic and DA-D2 receptors in hippocampus and corpus striatum respectively following nicotine exposure by using neurochemical receptor binding techniques.

Materials and Methods: In the present study an experiment was done on male albino rats. Nicotine was administered for eight weeks orally via a cannula, using dose rate (5 mg/d, 10 mg/d). The results were compared with control adult rats, given vehicle in an identical manner. After exposure, we assessed [³H] Quinuclidinyl Benzilate as a specific ligand for cholinergic receptors in the hippocampus and DA-D2 receptors binding in corpus striatum using [³H] Spiperone as a specific ligand for DA-D2 receptor.

Results: Chronic nicotine treatment induced the up-regulation of cholinergic receptors in the hippocampus and down-regulation of DA-D2 receptors in the striatum.

Conclusions: Such knowledge is important for understanding nicotine dependence and the consequences of nicotine administration for the treatment of neurological disorders. These effects may underlie long-term behavioral changes associated with adolescent nicotine exposure.

Keywords: Nicotine; Receptors, Cholinergic; Quinuclidinyl Benzilate; Hippocampus; Corpus Striatum; Receptors, Dopamine D2

1. Background

Much of the attention of nicotine research is centered on its addiction issues and less focus is placed on its neurotoxicity. Generally, smoking habit develops in adolescence (1, 2) and gradually consumption of cigarettes increases and probability of quitting it decreases with age (3, 4), this includes response to nicotine (5). But still the effects of nicotine, which is one of the most frequently abused drug, in susceptible adolescents is not clearly understood. However, norepinephrine and dopamine are controlled by cholinergic receptors (6) and therefore CNS effects of adolescent nicotine treatment are likely to be more widespread than simply those on cholinergic synapse.

2. Objectives

In the current study, we have used models of adolescent nicotine to examine binding of receptors of norepinephrine in the hippocampus; a finding that may shed new light in understanding the complex mechanism of

nicotine dependence. There has yet to be direct comparison between simultaneous changes in extracellular neurotransmitter levels and changes in radiotracer binding level in mammalian brain. These data would provide an important measure of sensitivity (that is the amount of neurotransmitter reflected in radiotracer binding changes), which would allow inference about the magnitude of its response in the clinical populations.

3. Materials and Methods

3.1. Sample Size

Male Drukey rats (150-200 g) were obtained from the Industrial Toxicology Research Centre and were offered pellet diets under standard animal house conditions. Nicotine was administered orally beginning on Postnatal day (PND) 40. Stock solution of nicotine was prepared with (-)-Nicotine hydrogen tartrate (Lancaster Hysel Pharmaceuticals) dissolved in normal saline. Total number of albino rats used for this study was 24, which were grouped

Implication for health policy/practice/research/medical education:

Harmful effects of nicotine addiction

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in control, experimental group I (E1), and experimental group II (E2). Each group had six animals. E1 were given 5 mg/kg and E2 were given 10 mg/kg of nicotine solution for two weeks. Control rats were given normal saline equivalent to the nicotine volume. This paradigm activates central nicotinic receptors and produces plasma nicotine levels similar to those in typical smokers, approximately 25 ng/mL (7). Nicotine was removed from the animals 24 hours prior to killing to allow its metabolism (8).

3.2. Estimation of DA-D2 receptor binding

Assay of DA-D2 receptor binding was carried out in the ipsilateral striatal region of control and experimental groups following the method (8). Crude synaptic membrane fraction was prepared by homogenizing the tissue in pre-chilled 0.32 M sucrose followed by centrifugation at 50000 rpm for 10 minute. The pellet was re-homogenized in 5 mM TrisHCl buffer at pH 7.4, in the same volume and centrifuged at a similar speed. The pellet was finally suspended in 40 mM TrisHCl buffer at pH 7.4 and stored at 20°C until used for the assay. The binding incubation was carried out in triplicates at 37 °C for 15 minutes using synaptic membrane fraction with 1 nM [³H] Spiperone as specific ligand for the DA-D2 receptor. Parallel triplicate assay using high concentration of unlabelled haloperidol (DA receptor antagonist) was used to determine non-specific binding. After 15 minutes of incubation at 37 °C, the reaction was determined by cooling in ice and filtered through a glass microfiber filter (GF/C) under vacuum using the Brandel cell harvester. The filter papers were washed twice with the same buffer, dried and radioactivity counted in LKB Rack β Liquid.

3.3. Scintillation Counter

Estimation of binding of cholinergic receptors was done by a standard method using [³H] Quinuclidinyl Benzilate as specific ligand (9). Radioactivity was assessed by beta scintillation counter (10). Result was expressed in picomole bound per gram (pmol bound/g) of protein.

3.4. Statistical Analysis

Mean significant difference in the treatment groups was determined using one way analysis of variance (ANOVA). The level of significance was analyzed by calculating the least significant difference. Values of $P < 0.05$ were considered to be statistically significant.

4. Results

It was observed that the consumption of food by the control group of animals was good while nicotine fed rats (experimental group) had diminished food intake. During the first few days, the movement and activity of the experimental group was less. Later they showed signs of excitation and hyperactivity marked by repeated

jumping. Subsequently after seven weeks, these animals became lethargic.

4.1. Morphological Observation

The corpus striatum was soft, friable, laminated and pale in color. There was no significant morphologic change in control and experimental groups.

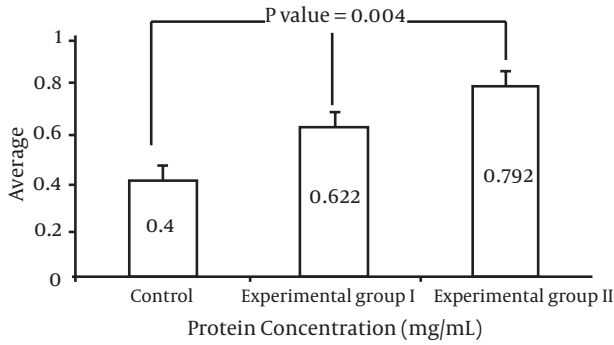
4.2. Neurochemical Observation

As shown in Figures 1, 2, 3, and 4, the hippocampal cholinergic muscarinic receptors binding showed statistically significant changes following prolonged nicotine exposure. The number of cholinergic muscarinic receptors was significantly increased in E1 (310.33 pmol bound/g protein) and E2 (331.57 pmol bound/g protein) as compared to control rats (249.78 pmol bound/g protein) and the effect was intensified with dose (Figure 1 and 2). The total membrane protein concentration was increased significantly in E1 (5 mg/kg Nicotine treated) and E2 (10 mg/kg Nicotine treated) groups as compared to the control group (Figure 3). In the present study, we found [³H] Spiperone (specific ligand for DA-D2 receptors) binding in the corpus striatum of treated animals was significantly ($P < 0.05$) reduced. This decrement was by 45.11% in E1 (5 mg/kg Nicotine treated) and 81.57% in E2 (10 mg/kg Nicotine treated) in comparison to the control animals (Figure 4).

5. Discussion

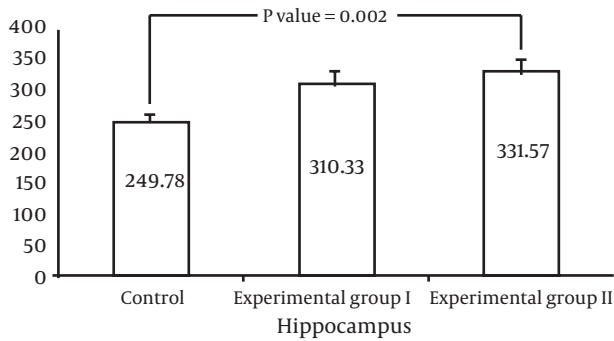
Adolescence is a period in which active cell modeling and replication occurs (10). Certain areas like cerebellum, hippocampus, and corpus striatum etc. show proliferation of axon terminals, dendritic projection and alteration in neurotransmitter receptors (11). Consistent with a previous report, the current study also showed that the adolescence period is highly vulnerable to nicotine effects, as maturation occurs in this phase only (12). Previous studies identified particular target regions for adverse effects of nicotine; for example delayed cell damage in hippocampus is characterized by increase in total membrane protein indicative of decrease in overall cell size (7, 12). It is therefore worthwhile to examine the effects of adolescence nicotine treatment on this region to see if there is any homology between the effects on the nicotinic receptors, catecholamine system and neurobehavioral changes. We found that DA-D2 receptors were reduced following chronic nicotine exposure in both experimental groups. This down-regulation of [³H] Spiperone binding receptors in the dopaminergic regions of rats treated chronically with nicotine suggests two possibilities; either a decrease in the number of DA-D2 receptors or their affinity for radio-ligand. We can correlate dopamine outflow with the number of DA-D2 receptors with the evidence from a previous report (13). He demonstrated that relatively small binding changes reflect large

Figure 1. Concentrations of Total Membrane Protein of Hippocampus



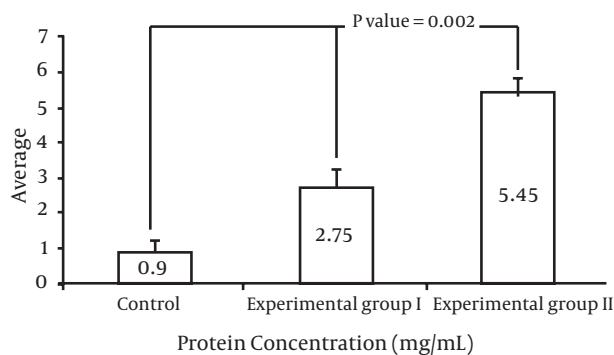
The concentrations of total membrane protein of hippocampus in control, experimental group I, and experimental group II are being shown. Each group had six animals.

Figure 2. Comparative Study of Change in Cholinergic Receptors in Hippocampus



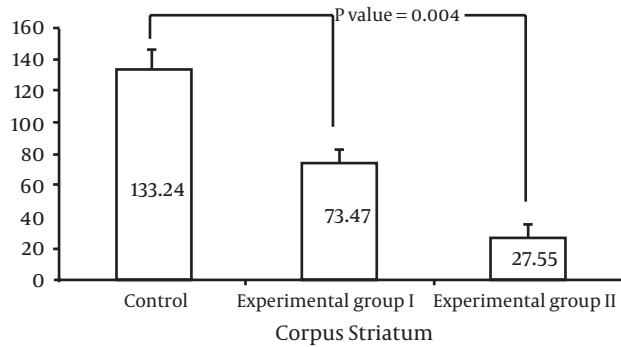
Receptors and pmol bound/g protein were measured using [3H] QNB. Values represent Means ± SEM of six animals per group. Significant difference from the control group is shown using the ANOVA post hoc Bonferoni test.

Figure 3. Estimation of Protein in Corpus Striatum



Estimation of protein (mg/mL) in corpus striatum in control, experimental group I (5 mg/kg nicotine treated), experimental group II (10 mg/kg nicotine treated) following nicotine exposure. Each group had six animals.

Figure 4. Comparative Study of Down-Regulation of DA-D2 Receptors in Corpus Striatum



Comparative study of down-regulation of DA-D2 receptors (pmol bound /g protein) using [3H] Piperone. Decrease was found in ipsilateral corpus striatum. Values represent the Means ± SEM of six animals per group. Significant difference from the control group is measured using ANOVA. Bonferoni post hoc test result was significant with $P < 0.05$.

changes in dopamine outflow using C-11 Raclopride. Thus the reduction in DA-D2 receptors directly clicks towards the increase in dopamine release. Our results point towards auto-desensitization and resultant tolerance of the receptors to nicotine exhibiting an overall decrease in DA-D2 binding. We can correlate neurotransmitter outflow with the number of receptors (14). They demonstrated that relatively small binding changes reflect large changes in neurotransmitter outflow. The phenomenon can be explained on the basis of auto-desensitization (10). Definite evidence was obtained for roles of both nicotinic and muscarinic acetylcholine system in the hippocampus in working and spatial memory (15). Hippocampus is the store-house of memory, emotion and any damage to the hippocampal circuitry can lead to behavioral changes (16).

Another question that arises is whether the presence of residual nicotine in the brain is directly responsible for changes in norepinephrine release from striatal synapse following chronic nicotine exposure. This appears unlikely for several reasons. Firstly, nicotine was stopped approximately 24 hours prior to death to allow sufficient time for its metabolism (17, 18). Secondly, several dilution and washes of synaptic membrane were performed during release experiments that would most likely have removed residual nicotine. The present study thus supports the concept that adolescence represents an entirely separate period in which neurotoxicants elicit the effects that are unique from those seen with exposure in earlier or later period. Our result thus supports an emerging pattern, where adolescence nicotine exposure elicits hippocampal cell damage leading to abnormality of synaptic receptors and correspondingly behavior abnormalities. A recent report indicated that activation of nicotine receptors by low dose nicotine resulted in apoptotic cell death

in primary hippocampal progenitor cells (10). Eventually there was down gradation of cholinergic receptors at the same region. At the same time one study explained cellular deterioration and neuronal loss on the basis of oxidative damage in lipid, protein and DNA of brain cells (19). Similarly, the clinical data in a recently published study reported that autosensitization of DA-D2 receptors inhibit dopamine release at low firing rates. The final issue that needs to be addressed is whether the changes in synaptic receptors seen here have corresponding effects on behavior. Smokers exhibit a higher incidence of depression, which undermines tobacco cessation efforts (20-22). In addition women and adolescents both have more prominent withdrawal and greater difficulty in quitting smoking (23). Future behavioral research related to hippocampal function in the area of neurotoxicity of nicotine will shed some light on this issue. Our study provides information regarding indirect effects on cell maturation, proliferation and synapse formation.

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Authors' Contribution

Corresponding author: Dr. Anshu Tewari; Supervisor: Dr. Mahdi Hasan; Laboratory work: Dr. Anita Rani; Assistance in preparing manuscript: Dr. Rahul Misra.

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