ISSN: 2278-5213

RESEARCH ARTICLE

Nicotine induced modulation of muscarinic cholinergic receptors in hippocampus in animal model

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Abstract

Many studies are done emphasizing adverse effects of nicotine on fetal or adult period but very little work is done in field of nicotine exposure in adolescent period. In this study, experiments were done on adult male albino rats where, nicotine was administered for 60 d orally via cannula (5 mg and 10 mg/d). After exposure, muscarinic cholinergic receptor binding was assessed in the hippocampus; using (3H) QNB as specific radio-ligand and it was found that there was upregulation of muscarinic cholinergic receptors. The results were evidenced by assessing the hippocampal cell damage characterized by increase in total membrane protein concentration indicative of a decrease in overall cell size. Nicotine exposure causes substantial impact in adolescent period which may further contribute to additive habits and deficits in behavioural performance.

Keywords: Nicotine exposure, adolescence, muscarinic cholinergic receptor, hippocampal cell damage.

Introduction

Much of the attention of nicotine research is centered on its addiction issue and less focus is placed on its neurotoxicity. Smoking typically begins in adolescence and recent estimates indicates that over one third of US high school students smoke (Pierce and Gilpin, 1996). There is a strong correlation between the age at which smoking is initiated and the subsequent addiction liability (Jeff and Edward, 1996). Ninety percent of adult smokers begin during adolescence and the earlier at which smoking begins, higher the daily consumption of cigarette and lower the probability of quitting (Jeff and Edward, 1996). It is increasingly evident that adolescence is a unique developmental period in which the synaptic function and the response to stimulant or depressant drugs differ from that of younger or adult (Spear and Brake, 1986; Markwiese *et al.,* 1998) which includes response to nicotine (Kelly *et al.,* 1991). But still the effects of nicotine, which is one of the most popularly used substance, in susceptible adolescence is not clearly understood. However, nicotinic cholinergic receptors also control the release of other neurotransmitters especially nor-epinephrin and dopamine (Klink *et al*., 2001) and therefore, CNS effects of adolescent nicotine treatment are likely to be more widespread than simply those on cholinergic synapse. In this study, we have used models of adolescent nicotine to examine binding of receptors of nor-epinephrin in hippocampus, a finding that may shed new light in understanding the complex mechanism of nicotine dependence. They were chosen to encompass areas involved in cognitive performances, learning, memory, addiction. 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.

Materials and methods

Experimental animals: Male Drukrey rats (150-200 g) from Industrial Toxicology Research Centre (ITRC), Lucknow, Animal breeding colony were used in the study. The animals were kept on ad libitum pellet diet with free access to water under standard animal house conditions. The animal study model was approved by Animal house ethical committee, Pharmacology department, King George's Medical University.

Nicotine administration: Nicotine was administered orally beginning on post natal day 40. Stock solutions of nicotine were prepared with (-) nicotine hydrogen tartrate (Lancaster Hysel Pharmaceuticals) dissolved in normal saline.

Experimental groups: Total number of albino rats used for study was 24. They were grouped in control, Experimental group I (E1), Experimental group II (E2) containing 6 animals in each group. E1 were given 5 mg/kg and E2 were given 10 mg/kg of nicotine for 2 weeks. Control rats were given normal saline equivalent to nicotine volume. Nicotine vehicle paradigm demonstrably activates central nicotinic receptors and produces plasma nicotine level similar to those in typical smokers, approximately (Trauth *et al.,* 1999). Nicotine was removed from the animals 24 h prior to sacrifice to allow its metabolism (Schoedel *et al.,* 2003).

Cholinergic receptor binding assay: The cholinergic (muscarinic) receptor binding was assayed in the hippocampal synaptic membrane preparations following the method of Agarwal *et al.* (1981), using 3H-quinuclidinyl benzilate (3H-QNB) as the specific radio-ligand. The membrane preparation containing 250-300 g protein was incubated in 40 mM Tris–HCl buffer, pH 7.4 at 37°C for 15 min with 1 nM of 3H-QNB in triplicate in the presence (non-specific binding) and absence (total binding) of 1 M atropine sulfate. The reaction was stopped by cooling the reaction mixture in ice, followed by rapid filtration through glass micro-fibre (Whatman GF/C) filters. The filter papers were washed with 5 mL Tris–HCl buffer twice, dried and transferred to vials containing scintillation fluid (Aquasol). Radioactivity was counted in a LKB-Rack beta scintillation counter having an efficiency of approximately 50% for tritium. Specific binding was calculated by subtracting non-specific binding from total binding and the result was expressed in terms of pmoles bound/g protein. Protein was estimated by the method of Lowry *et al.* (1951).

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.

Results

Total membrane protein concentration was increased significantly in E1 and E2 groups as compared to control group (Fig. 1). In this study, we found that the hippocampal cholinergic muscarinic receptors binding showed statistically significant changes following prolonged nicotine exposure (Fig. 2). The number of cholinergic muscarinic receptors were significantly increased in E1 (310.33 pmoles bound/g protein) and E2 (331.57 pmoles bound/g protein) as compared to control rats (249.78 pmoles bound/g protein) and the effect was intensified with dose (Fig. 2). During the infusion period, males showed enhanced response to nicotine challenge but changes in post-treatment period were inconsistent.

Discussion

The active cell replication and remodeling of synapses feature prominently in adolescence period (Trauth *et al*., 1999). Certain areas like cerebellum, hippocampus and corpus striatum show proliferation of axon terminals, dendritic projection and alteration in neurotransmitter receptor in adolescence (Santana *et al.,* 1992). Consistent to the previous report by Trauth *et al.* (2000) this study finding indicated that the vulnerability of developing brain to nicotine extends to adolescent period in which catecholamine system undergo their final maturation. Previous studies (Trauth *et al.,* 1993, 2000) identified particular target regions for adverse effects of nicotine.

Fig. 1. Total membrane protein levels of control and experimental groups.

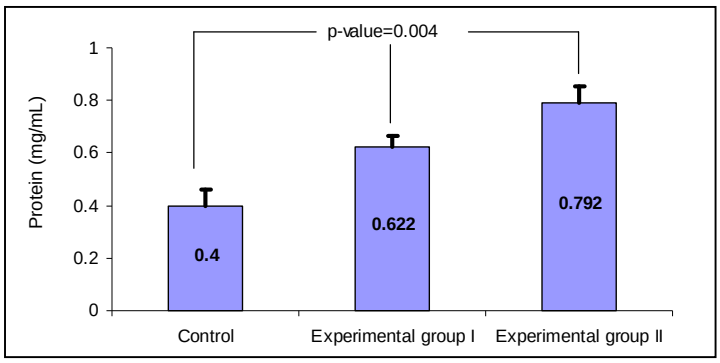
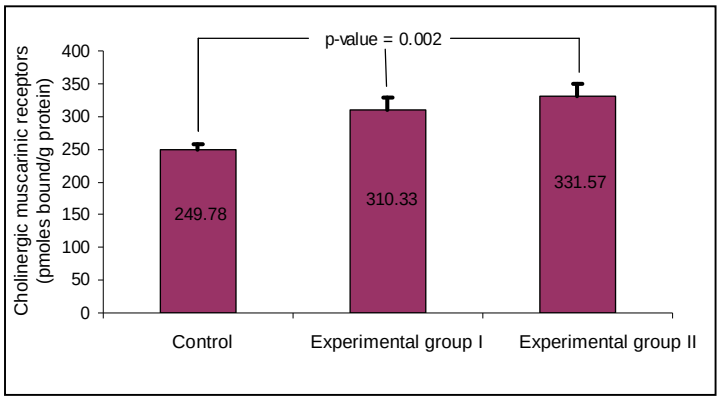


Fig. 2. Number of cholinergic muscarinic receptors of control and experimental groups.



Delayed cell damage in hippocampus is characterized by increase in total membrane protein indicative of a decrease in overall cell size. 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 neurobehavioural changes. We can correlate neurotransmitter outflow with number of receptors with the evidence (Breier *et al.,* 1997) which demonstrated relatively small binding changes reflect large changes in neurotransmitter outflow. The phenomenon can be explained on the basis of auto-desensitization. *In vitro* experiments indicated that even within a specified region, chronic nicotine treatment can enhance or repress dopamine turnover in different areas (Jennifer *et al.,* 2001). Desensitization and resultant tolerance to nicotine may be non-uniform with some neurons of hippocampus exhibiting overall decrease in their firing rates while others are activated. A third possibility for regional difference resides in different rates of adjustment of tyrosine hydroxylase activity, a rate limiting enzyme in catecholamine synthesis (Trauth *et al.,* 1999). In this regards also, there are differential rates for each process in different brain region. Chronic exposure of nicotine elicits an early peak of tyrosine carboxylase activity in the locus coeruleus, but later more sustained increases in the terminal fields within the hippocampus and thalamus effects which may persist for long after cessation of treatment.

Regardless of the cellular mechanisms underlying the effects of nicotine on catecholamine system, the effects observed in this study are likely to be specific to adolescence. One potential explanation of mechanism underlining the disparate pattern of effect of adolescence nicotine on different brain regions is heterogenous expression of nicotinic receptors subtypes and their differential propensity for desensitization. Definite evidence was obtained for roles of both nicotinic and muscarinic ach system in hippocampus in working and spatial memory (Jeff *et al*., 1986). The hippocampus possess information derived from associated brain regions involved in learning, memory, emotion and motivation and any damage to hippocampal cholinergic system may result in altered behavioural responses (Hurt *et al.,* 2000). The final issue that needs to be addressed is whether the changes in synaptic function seen here have corresponding effects on behaviour. For that purpose, we have to study the motor activity of animals. Another question that arises is whether the presence of residual nicotine in brain is directly responsible for changes in nor-epinephrin release from striatal synaptosome following chronic nicotine exposure. This appears unlikely for several reasons. For one, nicotine was stopped approximately 24 h prior to death to allow sufficient time for its metabolism (Schoedel *et al.,* 2003; Petersen *et al.,* 1984). Second, several dilution and washes of synaptic membrane were performed during release experiments that would most likely have removed residual nicotine. This study thus supports the concept that adolescence represents 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 adolescent nicotine exposure elicits hippocampal cell damage leading to abnormality of synaptic receptors and corresponding behaviour abnormalities. A report indicated that activation of nicotine receptors by low dose nicotine resulted in apoptotic cell death in primary hippocampal progenitor cells (Trauth *et al.,* 1999). Eventually there was down gradation of cholinergic receptors of same region. At the same time, Wu and Anthony (1999) explained cellular deterioration and neuronal loss on the basis of oxidative damage in lipid, protein and DNA in brain cell. Similarly, the clinical data in a 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 receptor seen here have corresponding effects on behaviour. Therefore, the gross measurements from MRI technique remains to be one of the most powerful and valuable methods to detect patho-physiology. Smokers exhibit a higher incidence of depression which undermines tobacco cessation efforts (Lowry *et al.,* 1952; Glassman *et al*., 1993; Leon, 1996).

In addition, women and adolescents both have more prominent withdrawal and greater difficulty in quitting smoking (Bjornson *et al*., 1995; Fant *et al*., 1996). Future behavioural research related to hippocampal function in the area of neurotoxicity of nicotine will shed some light on this issue. Our results may thus provide some of evidence for unique effects of adolescent nicotine exposure on synaptic function of catecholamine system that would contribute to these behaviour effects in smokers.

Conclusion

Our results provide evidence that the regulation of the density of the muscarinic receptors in cholinergic nerve processes which make appropriate synaptic contacts and respond to nicotine. Furthermore, upregulation of cholinergic receptors may cause addiction, drug abuse and behavioural deficits which needs to be reinforced by neurochemical, motor and histopathological studies.

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