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Nicotine restores monoamine neurotransmitter changes in the cortex and hippocampus of reserpinized rats as a model of depression

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Abstract. – *Background and Objectives:* **A number of studies have shown that nicotine has an antidepressant-like effect. The prevalence of smoking is much higher in people suffering from depression. In addition, the administration of nicotine from transdermal nicotine patch can exert antidepressant activity in nonsmokers and the continuous infusion of nicotine to rats attenuates learned helplessness, a putative behavioral model of depression. The aim of the present study is to elucidate the neurochemical effect of nicotine on monoamine levels in the cerebral cortex and hippocampus of reserpinized rats as a model of depression.**

Materials and Methods: **In the present study, rats were divided into control animals treated with saline and reserpinized group which received a daily i.p injection of reserpine for 15 days to establish the animal model of depression. Starting from the 16th day, the reserpinized rats were divided into reserpinized rats, and reserpinized rats treated daily with nicotine (0.4 mg/kg) for 15 and 30 days. After decapitation, the cerebral cortex and hippocampus of each rat were dissected out. The levels of monoamine neurotransmitters (serotonin, norepinephrine and dopamine) were measured in each area using a spectrofluorimeter.**

Results: **The daily i.p injection of reserpine induced a significant decrease in monoamine levels in the cortex and hippocampus. Nicotine administration restored the changes in monoamine neurotransmitters induced by reserpine in both areas after 30 days.**

Discussion: **The data of the present study suggest that the antidepressant-like effect of nicotine could be mediated by the effect of nicotine on monoamine neurotransmitters in the cortex and hippocampus of rat brain.**

Key Words:

Nicotine, Reserpine, Monoamines, Hippocampus, Cortex, Depression.

Introduction

A clinical association between smoking and depression has been reported in a number of studies[1,2]. The prevalence of smoking among the depressed patients was viewed as a self-medication to alleviate some symptoms of depression[3]. Moreover, nicotine patch can improve mood in nonsmoking depressed patients[4,5] while smoking cessation frequently precipitates depressive symptoms that are reversed upon relapse to smoking[6].

It is known that nicotinic acetylcholine receptors are involved in major depression and effects of antidepressant drugs[3]. In addition, the antidepressant-like effect of acute and chronic nicotine is completely blocked by mecamylamine, a nicotine antagonist, suggesting an involvement of nicotinic receptor systems[2,7]. It has been demonstrated that acute and chronic intermittent administration of nicotine in Flinders sensitive Line rats, an animal model of depression[8], alleviated the depressive symptoms as determined by reduction of their immobility in the forced swimming test[9]. Indeed, an antidepressant-like effect of nicotine has been demonstrated in a variety of animal[7,9] and some human studies[4].

The majority of hypotheses suggest that depression arises from the disregulation of one or more neurotransmitters or neuromodulators in areas of the brain involved in the mood regulation e.g. cerebral cortex and limbic system[10,11], where the hippocampus is an area of the limbic system of the brain involved in emotion and memory[12,13].

Several reports have demonstrated that the volume of the hippocampus and prefrontal cortices are decreased and the structure imaging studies have shown reduced grey matter volumes in depressed patients[14,15].

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It has been postulated that the debilitating and often chronic symptoms of depression result from a perturbation in serotonin (5HT), norepinephrine and/or dopamine transmission. This hypothesis spawns from work done in the late 1950s showing that monoamine oxidase inhibitors and tricyclic antidepressants which elevate the levels of monoamines by preventing their metabolism and blocking their reuptake respectively were effective antidepressants[16]. Further support for the chemical hypothesis of depression is based on clinical data where the side effects of reserpine as an antihypertensive agent in 1960s suggested that depleting brain monoamines had detrimental effects on mood[17].

Reserpine is known to induce hypothermia, hypomotility, ptosis and catalepsy and to slow the frequency and increase the amplitude of electroencephalogram (EEG) waves by deleting intracranial monoamines such as norepinephrine, serotonin and dopamine[18,19]. Because these actions of reserpine are antagonized by tricyclic antidepressive agents, the reserpine-induced changes are considered as a model of depression and are used frequently for the evaluation of the antidepressive agents[20]. The use of reserpine-reversal as a screening test for antidepressants is well documented[21,22]. Jancsàr and Leonard[22] concluded that the animal model of depression which results from chronic administration of a low dose of reserpine was more valuable than the acutely reserpinized animal for the detection of the antidepressant activity. In addition, Iritani et al[23] observed that reserpine treated rats showed reduced locomotion, increased anhedonia, and have low feeding day by day during 14-day i.p. treatment. These behavioral changes indicate the symptoms of depression, suggesting the usefulness of these rats for a model of mood disorder.

The present study aims to investigate the antidepressant-like effect of nicotine on the reserpinized rat model of depression through the impact of chronic nicotine treatment on the monoamine levels in the cortex and hippocampus of reserpinized rats.

Materials and Methods

The animals used in the present study were adult male albino rats (*Rattus norvegicus*) weighing 160-180 g. The animals were obtained from the animal house of the National Research Center, Egypt. They were maintained on stock diet and kept under fixed appropriate conditions of housing and handling. All experiments were carried out in accordance with the research protocols established by the Animal Care Committee of the National Research Center – Egypt.

Chemicals

Reserpine (Mallinckrodlt. Inc, Martin Luther King Jr Blvd, Paris-Kantucky) was dissolved in glacial acetic acid (1 µg/µl) and then completed to 25 ml with distilled water. Nicotine hydrogen tartrate (Sigma, Hamburg – Germany) was dissolved in physiological saline solution and neutralized to pH 7 by using drops of sodium hydroxide.

Norepinephrine bitartrate salt (Sigma, Taufkirchen – Germany), dopamine hydrochloride and serotonin (Fluka – Sigma – Aldrich, Taufkirchen – Germany) were used for standard solution.

n-heptane (Labscan Ltd., Dublin, Ireland), 1-butanol (POCH SA, Gliwice, Poland), acetic acid, hydrochloric acid, ethyl alcohol (EDWIC, El-Nasr Pharmaceutical Chemicals Co., Egypt), Sodium acetate (Fluka, Buchs, Switzerland), Iodine (Panreac, Barcelona Spain), ethylenediamine tetraacetic acid (EDTA) (S.D. fine – Chem Ltd. Mumbai, India), sodium sulfite and O-phthalaldehyde (OPT) (Merck, Schuchardt, Germany) were used for the quantitative determination of monoamines in the selected brain areas.

Design of the Experiment

At the beginning of the experiment, the rats were divided randomly into control and the rest was injected intraperitoneally (i.p.) with reserpine (0.1 mg/kg/day) for 15 days to establish the animal model of depression according to Janscar and Leonard[22].

At the 15th day a group of the reserpinized rats was sacrificed to examine the depleting effects of reserpine on the monoamine levels. Starting from the 16th day, the reserpinized rats were divided into two groups. The first group received a daily i.p. injection of reserpine followed by a subcutaneous (s.c.) injection of nicotine (0.4 mg/kg, free base) with one hour interval between the two drugs for 15 and 30 days. The second group received a daily i.p injection of reserpine followed by a s.c. injection of saline with one hour interval between them for 15 and 30 days.

After decapitation, the cortex and hippocampus of each rat was dissected out, weighed and kept frozen until analysis.

The quantitative determination of monoamines levels (norepinephrine, dopamine and serotonin) was carried out using a spectrofluorometer (Jasco FP-777, with a source of xenon arc lamp 150 watt, Jasco Ltd., Tokyo, Japan) according to the method described by Ciarlone[24].

Statistical Analysis

Differences between the control group and reserpinized rats were analyzed by the independent t-test. The comparisons between the control, reserpinized rats and reserpinized rats treated with nicotine were carried out using one way analysis of variance (ANOVA), followed by post hoc test using Scheffe test. Differences were considered significant when p values were less than 0.05. Statistical analyses were performed using Statistical Package for Social Sciences (SPSS, Chicago, IL, USA).

Results

As shown in Table I, the daily i.p. injection of reserpine for 15 days induced a significant decrease in the levels of serotonin, norepinephrine and dopamine in the cortex and hippocampus of rats.

Table II shows the effect of the daily s.c. injection of nicotine on the monoamine levels in the cortex of reserpinized rats (depressed animals). Statistical analysis (ANOVA) revealed significant changes in the cortical levels of serotonin, norepinephrine and dopamine between the control, reserpinized rats and reserpinized rats treated with nicotine for 15 and 30 days. In the cortex of reserpinized rats, a significant decrease was recorded in the levels of the three monoamines, below the control values, after 15 and 30 days of daily reserpine administration. When the reserpinized rats were treated daily with nicotine for 15 days and 30 days, the monoamines levels showed non-significant changes from the control values at the two time intervals.

In the hippocampus, significant changes in the levels of monoamines were recorded among the controls, reserpinized rats and reserpinized rats treated daily with nicotine for 15 days and 30 days except for the level of norepinephrine after 30 days that showed non-significant changes among the three tested groups, (Table III). Both serotonin and dopamine levels showed a significant decrease in the hippocampus of reserpinized rats injected daily with reserpine for 15 and 30 days. This decrease continued in the reserpinized rats treated daily with nicotine for 15 days in case of serotonin while, dopamine showed non-significant decrease as compared to control rats. However, the daily treatment of the reserpinized animals with nicotine for 30 days resulted in non-significant changes in the levels of serotonin and dopamine as compared to the control values. Norepinephrine showed a significant decrease below the control value after 15 days in the hippocampus of reserpinized rats and reserpinized rats treated daily with nicotine. However, after 30 days non-significant changes were recorded in norepinephrine level among the three tested groups.

Discussion

It has been observed that chronic administration of nicotine or selective nicotine agonist in the learned helplessness paradigm in rats may al-

Table I. Effect of daily reserpine administration (o.1 mg/kg) for 15 days on the concentrations of serotonin, norepinephrine and dopamine (µmol/g fresh tissue) in the cortex and hippocampus of rat brain.

Brain area	Monoamines	Controls	Rats treated with reserpine	%d
Cortex	Serotonin	0.944 ± 0.056 (6)	0.712 ± 0.074 (6)	-24.576*
	Norepinephrine	2.419 ± .060 (6)	1.593 ± .105 (6)	-37.081*
	Dopamine	2.523 ± 0.106 (6)	1.819 ± 0.077 (6)	-27.903*
Hippocampus	Serotonin	2.779 ± 0.331 (6)	1.257 ± 0.240 (6)	-54.767*
	Norepinephrine	5.873 ± 0.457 (5)	4.498 ± 0.292 (6)	-23.412*
	Dopamine	6.092 ± 0.448 (6)	4.783 ± 0.375 (6)	-21.487*

Mean ± SEM is presented with number of animals between brackets. n.s. = nonsignificant; * = significant at $p< 0.05$; %d = Percentage difference from the controls.

Table II. Effect of daily s.c. nicotine injection (0.4 mg/kg) for 15 and 30 days on the concentrations of serotonin, norepinephrine and dopamine (µmol/g fresh tissue) in the cortex of rat brain.

	Injection period	Controls	Rats treated with reserpine#	%d	Rats treated with reserpine and nicotine#	%d	F ratio	Sig
Serotonin	15 days	0.944 ± 0.056 (6)	0.630 ± 0.025 (6)	-33.262*	0.857 ± 0.075 (6)	-9.216 ns	8.546	0.003
	30 days	0.944 ± 0.056 (6)	0.766 ± 0.024 (6)	-18.855*	0.888 ± 0.035 (6)	-5.932 ns	4.870	0.023
Norepinephrine	15 days	2.419 ± 0.060 (6)	1.769 ± 0.115 (6)	-26.870*	2.182 ± 0.109 (6)	-9.797 ns	11.196	0.001
	30 days	2.419 ± 0.060 (6)	2.080 ± 0.054 (6)	-14.014*	2.545 ± 0.093 (6)	5.208 ns	11.319	0.001
Dopamine	15 days	2.523 ± 0.106 (6)	2.052 ± 0.073 (6)	-18.668*	2.678 ± 0.166 (6)	6.143 ns	7.158	0.007
	30 days	2.523 ± 0.106 (6)	2.169 ± 0.043 (6)	-14.031*	2.516 ± 0.083 (6)	-0.277 ns	6.041	0.012

Mean ± SEM is presented with number of animals between brackets. n.s. = nonsignificant; * = significant at $p < 0.05$; %d = Percentage difference from the controls. # = Reserpine treatment started 15 days before the listed time intervals to establish the animal model of depression.

Table III. Effect of daily s.c. nicotine injection (0.4 mg/kg) for 15 and 30 days on the concentrations of serotonin, norepinephrine and dopamine (µmol/g fresh tissue) in the hippocampus of rat brain.

	Injection period	Controls	Rats treated with reserpine#	%d	Rats treated with reserpine and nicotine#	%d	F ratio	Sig
Serotonin	15 days	2.779 ± 0.331 (6)	1.818 ± 0.067 (6)	-34.581*	1.881 ± 0.173 (6)	-32.313*	6.047	0.012
	30 days	2.779 ± 0.331 (6)	1.952 ± 0.062 (6)	-29.759*	2.267 ± 0.112 (6)	-18.424 ns	4.150	0.037
Norepinephrine	15 days	5.873 ± 0.457 (6)	4.280 ± 0.382 (6)	-27.124*	4.196 ± 0.126 (6)	-28.554*	7.211	0.006
	30 days	5.873 ± 0.457 (6)	5.128 ± 0.230 (6)	-12.685 ns	5.412 ± 0.305 (6)	-7.849 ns	1.191	0.331
Dopamine	15 days	6.092 ± 0.448 (6)	4.764 ± 0.290 (6)	-21.799*	4.948 ± 0.227 (6)	-18.779 ns	4.601	0.028
	30 days	6.092 ± 0.448 (6)	4.818 ± 0.069 (6)	-20.913*	5.965 ± 0.240 (6)	-2.085 ns	5.599	0.015

Mean ± SEM is presented with number of animals between brackets. n.s. = nonsignificant; * = significant at $p < 0.05$; %d = Percentage difference from the controls. # = Reserpine treatment started 15 days before the listed time intervals to establish the animal model of depression.

leviate their depressive symptoms[25]. Moreover, it has been suggested that nicotine receptors could be suitable targets for the development of novel pharmacotherapy for the treatment of depression[26]. The aim of the present study was to investigate the ability of nicotine to restore the reserpine induced depletion in monoamine levels in the cortex and hippocampus of rat brain.

The monoamine hypothesis of depression postulates that the etiology and pathogenesis of depression arises from the central deficiencies in serotonin, norepinphrine and/or dopamine[27]. As reserpine is highly lipid soluble, it rapidly penetrates the cell membranes of amine containing synaptic vesicles in the nerve terminals and prevents the uptake of amines into the intracellular storage vesicles, so that depletion occurs and ultimately there is a failure of monoamine neurotransmission[28,29]. This may explain the decrease in monoamine levels in the cortex and hippocampus of reserpinized rats recorded throughout the present experiment. Furthermore, the decrease in monoamine levels induced by reserpine permits the use of this model of depression as a screening test for the antidepressant effect of nicotine.

Our findings showed that in the cortex the daily nicotine injection for 15 and 30 days restored the significant decrease in monoamines levels induced by reserpine to non-significant changes as compared to control levels.

The neurons in the dorsal raphe can be suggested as the primary source of serotonin in the brain[30]. From cell bodies concentrated in dorsal and caudal raphe nuclei, widespread serotonergic projections extend to a considerable variety of brain areas believed to be associated with the symptoms of depression including hypothalamus, amygdala, cortex, hippocampus, basal ganglia and brain stem[31]. In addition, the most important noradrenergic projections with regard to psychological functions arise from the locus ceruleus and ascend from the brain stem to innervate the thalamus, dorsal hypothalamus, hippocampus and cortex[32].

In the dorsal raphe nuclei, a brain structure known to be the major of the forebrain serotonergic innervation, the presence of nicotinic acetylcholine receptors have been demonstrated in mice[33], rats[34-36] and humans[37]. Both $\alpha 4\beta$ and $\alpha 7$nicotinic acetylcholine receptors have been identified on the serotonergic neurons in the dorsal raphe nuclei of rats[38]. *In vitro* nicotine stimulates the discharge of serotonergic neurons in the dorsal raphe nuclei[39,40]. Similarly *in vivo* electrophysiological recordings have shown the stimulatory effect of systemic nicotine on serotonergic neurons in the dorsal raphe nuclei[41].

The hippocampus receives its primary serotonergic innervation from the median raphe nucleus, that provides the sole serotonergic innervation to the dorsal hippocampus[42].

Noradrenergic neurons in the locus coerulous also contain $\alpha 4\beta 2$[43,44] and $\alpha 7$ nicotinic acetylcholine receptors[45] and these neurons are activated by nicotine *in vitro*[46] as well as *in vivo*[47,48].

Presynaptic nicotinic acetylcholine receptors have a well documented role in facilitating neurotransmitter release[49,50]. Specially nicotine stimulates the release of serotonin[51-53] and norepinephrine in various forebrain regions[51,54]. Accordingly, the recovery in the levels of monoamine neurotransmitters in the cortex of reserpinized rats treated with nicotine could be attributed to the stimulatory effect of systemic nicotine on the nicotinic acetylcholine receptors located in the raphe and locus ceruleus nuclei. This may also explain the late recovery in the monoamine levels observed after 30 days in the hippocampus of reserpinized rats treated daily with nicotine.

Supporting this explanation, it has been reported that the antidepressant like effect of acute and chronic nicotine is completely blocked by mecamylamine, a nicotinic antagonist, suggesting the involvement of nicotinic receptor systems[7]. It has been suggested that the down regulation of β-adrenoreceptor in the cortex may reflect an adaptation to nicotine-induced increase in norepinephrine availability in the synaptic cleft[55].

Nicotine is known to promote dopamine synthesis and release[56,57]. Nicotine also promotes catecholamine biosynthesis by activating tyrosine hydroxylase, the first major rate-limiting enzyme[58,59]. In addition, nicotine not only increases gene expression of tyrosine hydroxylase but also other subsequent catecholamine biosynthetic enzymes (dopamine-β-hydroxylase and phenyl ethanolamine N-methyltransferase) in the periphery and many catecholaminergic regions of the central nervous system[59,60].

The hippocampus receives dopaminergic innervation from raphe nuclei and/or ventral tegmental area[61]. Nicotine stimulates the dopamine secreting cells that project from the ventral tgmental area to the limbic structures[62,63].

In the light of the previously mentioned studies the restoration of dopamine levels in the cortex and hippocampus of reserpinized rats treated

with nicotine to the control values may arise from the increase in the synthesis of this catecholamine by nicotine administration and the stimulatory effect of nicotine on the dopamine secreting cells in the raphe and ventral tegmental area.

In conclusion the data of the present study showed that nicotine has the efficacy to restore the depletion in the levels of the three monoamine neurotransmitters underlying the etiology of depression illness. Therefore, the antidepressant like effect of nicotine on reserpine induced depression may be mediated by the effect of nicotine on monoamine neurotransmitters.

As almost all of the available antidepressant drugs act on these neurotransmitters, the importance of the nicotinic receptors as a target in the therapy of depression must be pointed out.

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