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Effect of Nicotine on Brain Gaba levels in Depressed rats.

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ABSTRACT

Effect of nicotine on brain GABA levels in depressed rats. The present study was planned: to study effect of nicotine on brain GABA levels in depressed rats. to compare the effect of nicotine and imipramine on brain GABA levels. Isolation induced hyperactivity model was used to induce depression in rats. Five groups of 10 rats each were taken. Vehicle (D/W) treated rats before and after isolation were considered as baseline reading. Compared results of depression induced animal with results of animal without depression. Following drug treatments were administered: Rats from natural habitat was considered as before isolation. This group was used for normal GABA levels in rat brain. Vehicle (D/W) (1ml/kg) and imipramine (10mg/kg) were administered intraperitoneally. Nicotine was administered in a dose of 0.4mg/kg and 0.2mg kg by subcutaneous or inhalational route respectively. Brain GABA levels were estimated by fluorimetric method. In this model of depression, vehicle treated rats after isolation significantly reduced brain GABA levels as compared to vehicle before isolation. Results of imipramine treated rats after isolation showing significantly increased in brain GABA levels as compared to vehicle treated rats after isolation. Nicotine administered by inhalational route showed increase in brain GABA levels as compared to vehicle treated rats after isolation. Nicotine administered subcutaneously increased brain GABA levels as compared to vehicle treated rats after isolation. Imipramine and nicotine (inhalation) showed comparable results with normal GABA level i.e. before isolation rats. GABA level reduced in depressed rats. Imipramine, nicotine(inhalation) and nicotine(sc) increased brain GABA level in depressed rats.

Keywords : Depression, Isolation induced hyperactivity, Brain GABA level, Nicotine

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INTRODUCTION

The potential role of GABAergic dysfunction in mood disorders was first proposed by Emrich et al based on the efficacy of valproate in the treatment of bipolar patients[1]. GABA is an inhibitory neurotransmitter present almost exclusively in the central nervous system (CNS), distributed across almost all brain regions, and expressed in interneurons modulating local circuits. GABA transmission is present in interneurons modulating local neuronal circuitry, including noradrenergic, dopaminergic, and serotonergic neurons[2]. After Emrich's hypothesis, several animal and human studies have evaluated the potential role of GABAergic abnormalities in the pathophysiology of mood disorders. Preclinical studies have suggested that GABA levels may be decreased in animal models of depression, and clinical studies reported low plasma and CSF GABA levels in depressed patients [3].

GABAergic modulation of neuronal activity

GABA may activate the dopaminergic system, depending upon the brain region and the duration of GABA stimulation. It has been reported that muscimol, which is a GABA agonist, may reduce the immobility time in the behavioral despair model for depression by activating the rat dopaminergic system [4,5].

Animal studies have reported a complex interaction between GABAergic and noradrenergic transmissions. It has been reported that GABA, progabide, and fengabine induce norepinephrine neuronal activity in rat brains[6-9]. $GABA_A$ and $GABA_B$ receptor activation may increase and decrease norepinephrine release in rat cortex and hippocampus respectively[10-12].

GABA-serotonin relationship may be more complex. It has been reported that serotonin release is increased by stimulation of GABA receptors in rat suprachiasmatic areas[13].

MATERIAL AND METHODS

Experimental protocol was approved by Institutional Animal Ethical Committee (IAEC).Wistar rats weighing 200-250gm housed in polypropylene cages (single rat/cage) were used. They were fed pellet diet and water *ad-libitum*. The rats were maintained under standard conditions of temperature ($25^0C \pm 5^0C$) and relative humidity (55±10%). Rats of either sex were used. Rats were divided into 10 groups.10 rats in each group.

Evaluation of antidepressant activity in rats[14]

Antidepressant action of nicotine was studied in 'isolation induced hyperactivity model' in rats.

Drugs

Imipramine HCl (Sun Pharmaceutical Industries Ltd, Mumbai), Nicotine Hydrogen Tartrate

(Sigma-Aldrich, Poland) were dissolved in distilled water (vehicle).The study treatment were administered as follows: vehicle 1ml/kg (intraperitoneal) imipramine10mg/kg (intraperitoneal), nicotine 0.4mg/kg (subcutaneous),nicotine 0.2mg/kg (inhalational).As shown in **Table -1.**

Table -1 Study treatment design group

Groups	Treatment
Group 1	Vehicle control (before isolation)
Group 2	Vehicle control (after isolation)
Group 3	Imipramine (10mg/kg i.p.) for 7 consecutive days (after isolation)
Group 4	Nicotine (0.4mg/kg s.c.) single dose (after isolation)
Group 5	Nicotine (0.2mg/kg inhalation) single dose (after isolation)

Design of experiments

In this model of depression, adult Wistar rats were socially deprived for a period of 15 days. Rats were housed singly in cages (38cm × 26cm × 20cm) without any visual or auditory contact with their normally housed counter parts for 15 days. After isolation, rats became hyperactive. This increase in locomotor activity was measured by using digital photoactometer[14].

Biochemical evaluation by estimation of brain GABA

Depressed rats were sacrificed before and after completion of drug treatment period. Brain was isolated immediately and transferred to homogenization tube containing 5 ml of 0.01 M hydrochloric acid and homogenized. Brain homogenate was transferred to bottle containing 8 ml of ice cold absolute alcohol and kept for 1 h at 0 ^{o}C . The content was centrifuged for 10 min at 16 000 rpm, supernatant was collected in petridish. Precipitate was washed with 5 ml of 75% alcohol for three times and washes were combined with supernatant. Contents in petridish were evaporated to dryness at 70 ^{o}C on water bath under stream of air. To the dry mass 1 ml water and 2 ml chloroform were added and centrifuged at 2000 rpm. Upper phase containing GABA (2.0 ml) was separated and 10 µl of it was applied as spot on Whatman paper (No.41).

The mobile phase consisted of n-butanol (50 ml) acetic acid (12 ml) and water (60 ml). The chamber was saturated for half an hour with mobile phase. The paper chromatogram was developed with ascending technique. The paper was dried in hot air and then spread with 0.5% ninhydrin solution in 95% ethanol. The paper was dried for 1 h at 90 ^{o}C. Blue color spot developed on paper was cut and heated with 2 ml ninhydrin solution on water bath for 5 min. Water (5.0 ml) was added to solution and kept for 1h. Supernatant (2.0 ml) was decanted and absorbance was measured at 570 nm on fluorimetric detector[15].

Statistical Analysis

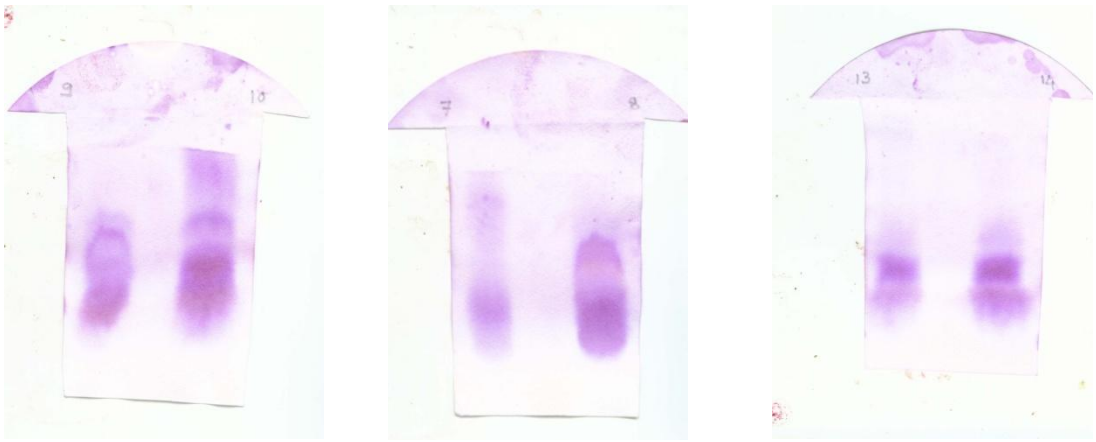
In estimation of brain GABA level, data was analyzed by one- way analysis of variance

(ANOVA) followed by Tukey test, using Primer of Biostatistics. P <0.05 was considered as significant[16].

RESULTS AND DISCUSSION

Brain GABA level in rat before isolation was 53.28ng/g of brain tissue. Level of GABA was reduced to 21.47ng/g of brain tissue after isolation. Level of GABA in brain after isolation was significantly reduced as compared to before isolation(p <. 0.001) shown in **Table -2* Figure 2**.

GABA levels in the vehicle treated group after isolation was compared with the three study treatment group i.e. imipramine, nicotine(sc) and nicotine(inhalation) after isolation. GABA levels were significantly increased in imipramine treated groups to 48.18ng/g of brain tissue. This increase was highly significant as compared to the GABA levels in vehicle treated groups after isolation. Difference between results of vehicle and imipramine treated rats were highly significant(p <. 0.001).This is depicted in Figure 1 and Figure 2.



(A) (B) (C)

Figure 1: – Comparison of effect

A. Vehicle control Vs Imipramine on GABA level in depressed rats.
B. Vehicle control Vs Nicotine (inhalation) on GABA level in depressed rats.•
C. Vehicle control Vs Nicotine (sc) on GABA level in depressed rats.

Nicotine administered by subcutaneous and inhalational route increased in GABA level to 29ng/g and 42.92ng/g of brain tissue respectively. Nicotine administered by subcutaneous and inhalational route showed significantly increased brain GABA level as compared to vehicle treated rats after isolation i.e. in depressed rats. This is shown in **Figure 1(B /C)** and **Table 2**.

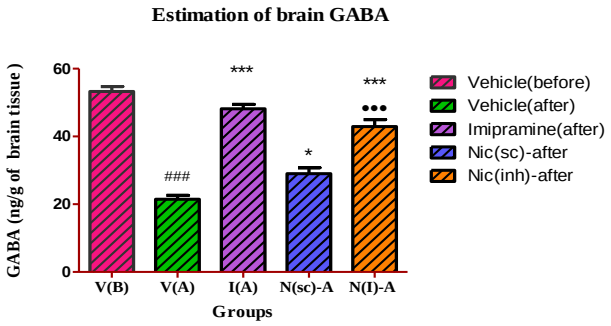


Figure 2: Brain GABA level in depressed rats after study treatment

= Vehicle control (before isolation) Vs Vehicle control (after isolation) (p<0.001)

***= Vehicle control (after isolation) Vs imipramine (after isolation) (p<0.001)

*** = Vehicle control (after isolation) Vs Nicotine(inhalation) after isolation (p<0.001)

••• = Nicotine(sc) after isolation Vs Nicotine(inhalation) after isolation (p<0.001)

* = Vehicle control (after isolation) Vs Nicotine(sc) after isolation (p<0.05)

Table 2: Results of Brain GABA level after study treatment in depressed rats

Groups	Treatment	Brain GABA level in depressed rats
Group 1	Vehicle control (before isolation)	53.28±3.57
Group 2	Vehicle control (after isolation)	21.47±2.9###
Group 3	Imipramine (10mg/kg i.p.) for 7 consecutive days (after isolation)	48.18±3.17***
Group 4	Nicotine (0.4mg/kg s.c.) single dose (after isolation)	29±4.29*
Group 5	Nicotine (0.2mg/kg inhalation) single dose (after isolation)	42.92±5.04*** •••

= Vehicle control (before isolation) Vs Vehicle control (after isolation) (p<0.001)

***= Vehicle control (after isolation) Vs imipramine (after isolation) (p<0.001)

*** = Vehicle control (after isolation) Vs Nicotine(inhalation) after isolation (p<0.001)

••• = Nicotine(sc) after isolation Vs Nicotine(inhalation) after isolation (p<0.001)

* = Vehicle control (after isolation) Vs Nicotine(sc) after isolation (p<0.05)

Imipramine and nicotine administered by inhalational route in depressed rats showed comparable results with GABA level in rats before isolation.

GABA level of nicotine treated by subcutaneous route was significantly less as compared to GABA level of imipramine treated rats as well as nicotine administered by inhalational route(p <. 0.001).

The role of GABAergic dysfunction in mood disorders was first proposed 20 years ago. Preclinical studies have suggested that GABA levels may be decreased in animal models of depression, and clinical studies reported low plasma and CSF GABA levels in mood disorder patients[3]. Also, antidepressants, mood stabilizers, electroconvulsive therapy, and GABA agonists

have been shown to reverse the depression-like behavior in animal models and to be effective in unipolar and bipolar depression patients[2]. The hypothesis of reduced GABAergic activity in mood disorders may complement the monoaminergic and serotonergic theories, proposing that the balance between multiple neurotransmitter systems may be altered in these disorders[3].

The previous study by the authors revealed the antidepressant effect of nicotine on isolation induced model of depression in rats. Rats with 15 days of isolation period demonstrated characteristic symptoms like increase in locomotor activity. The increase in locomotor activity was measured by using digital photoactometer in the previous study. Locomotor activity was significantly increased in control i.e. vehicle treated group after isolation. Locomotor activity was reduced with imipramine and nicotine treated rats in depression as compared to vehicle treated rats after isolation[17].

Reduced GABA levels in rat nucleus accumbens, brain stem, and cortex have been reported after a session of forced swimming test. In learned helplessness model after suffering from an inescapable foot shock, animals are not able to perform simple escape tasks in a shuttle box, resembling the psychomotor impairment present in human depression. Sherman and Petty demonstrated that GABA injection into frontal neocortex and hippocampus reversed the learned helplessness reaction[2].

In present study, brain GABA level of vehicle treated groups after isolation was reduced as compared to before isolation. Seven days treatment of imipramine and single dose of both nicotine administered by subcutaneous as well as inhalational route increased the brain GABA level in depressed rats.

Effect on brain level of GABA in imipramine and nicotine administered by inhalational route was more pronounced as compared to nicotine administered by subcutaneous route. Hence, it can be concluded that nicotine elucidates its antidepressant effect in isolation-induced hyperactivity model of depression through modulation of brain GABA level in rats. This result is consistent with findings observed in depressed rats and the present results suggest that deficits in the brain GABA level are related to depression.

CONCLUSION

In conclusion present study suggest that the increased brain GABA level after nicotine in depressed rats may be useful to some extent at least. In addition, study on site-specific action of nicotine on brain GABA level needed.

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