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MODULATION OF GLUTAMATE AND GABA CONTENTS BY QUETIAPINE IN NUCLEUS ACCUMBENS OF CHRONIC MILD STRESSED ALBINO RATS

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Abstract- *Aim:* Quetiapine is novel antipsychotic drug. However, there is limited clinical evidence regarding prescribing patterns for quetiapine when used as maintenance treatment for bipolar disorder. The present study investigated the changes in forced swimming test and the alteration of glutamate and GABA contents by this antidepressant drug in nucleus accumbens, as a part of the limbic system, from albino rats exposed to chronic mild stress(CMS)-induced anhedonia.

Methods: Albino rats were divided into 3 groups; Group-1 received vehicle without exposure to CMS, Group-2 received vehicle with exposure to CMS, Group-3 received quetiapine 10 mg/kg/day ip dissolved in DMSO for 3 weeks during exposure to CMS.

Results: Reversal of CMS-induced anhedonia after 3 weeks i.p. administration of 10 mg/kg/day quetiapine. It reduces the duration of immobility recorded by the forced swimming test (FST) as well as the modifies the contents of glutamate and GABA neurotransmitters in their isolated nucleus accumbens.

Conclusion: The present study proposes the presence of a possible GABAergic role of quetiapine in nucleus accumbens in the treatment of chronic mild stress-exposed albino rats.

Keywords- Chronic mild stress (CMS), nucleus accumbens, quetiapine, forced swimming test, albino rats

Running Title: Quetiapine and Selected Brain Amino Acids

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Introduction

Quetiapine (QUE) is a novel atypical antipsychotic drug that is widely used to treat schizophrenia and other psychotic disorders e.g. post-traumatic stress disorder [1,2]. It possesses neuroprotective effects, it seems to have a protecting action against cognitive impairments [3].

It was found that atypical antipsychotics are capapble to increase the action of the extracellular signal-regulated protein kinase (ERK), a member of the mitogen-activated protein kinase family (MAPK) [5]. The inhibition of this kinase enzyme, in prefrontal cortex and hippocampus, increases the liability to stressful stimuli and leads to cognitive and depressive disorders [6]. Thus, ERK may be involved in the pathogenesis of stress and anxiety as well as the therapeutic effects of these atypical antipsychotic drugs e.g. quetiapine [7].

Other neurotransmitter systems may be also involved in the pathogenesis of depression. The present findings suggest that, in addition to other neurotransmitter systems and biological aberrations, GABAergic influence may be implicated in the pathogenetic mechanisms of mood disorders [5].

Glutamate is the precursor of gamma amino-butyric acid (GABA). It is synthesized in a single step by glutamic acid decarboxylase enzyme. Turnover of GABA ocurrs through successive transamination and oxidation that lead to production of succinic semialdehyde and succinic acid, respectively. GABA plays an important role in relieving depressed mood. As it is a major inhibitory neurotransmitter in the brain, it has an important role in diminishing the activity of its target neurons e.g. neurons of limbic system that is involved in affective disorders. It interacts withthe activity of several neurotransmitters including dopamine, serotonin, and norepinephrine with a regulating effect on mood status [6].

The hypothesis of GABA mood regulating effect can be explained by its modulating effect of both norepinephrine (NE) and dopamine (DA) systems to the degree that control mood disorders [7]. This hypothesis is strongly supported b the finding of a reduction of plasma and cerebrospinal cord (CSF) GABA levels in depressed patients. It was reported that GABA induces a facilitatory effect of NE activity that helps in the elevation of depressive disorders [8].

The strongly expected relation of GABA and depression is thought to be supported by low plasma levels of GABA in different forms of mood disorders such as depression or bipolar disease [9]. Further

studies are needed to provide a strong evidence for this hypothesis using various antidepressant and antipsychotic drugs that might have a strong anti-anhedonic effect via this hypothesis of GABA-antidepressant effect.

Research and development of new antidepressant and antipsychotic drugs is not only dependent on the monoamine hypothesis of depression but also on hypothesis of neuroplasticity that focuses on neurons that carry the functions of injured or damaged neurons in affective disorders and in psychosis. Actually, there is a problem in explanation of the pathogenesis and hence in strategic treatment plan of a large numbers of patients with affective disorders who fail to reach complete cure of depressed mood or any other affective disorders. So, there is a developing beed to ameliorate plans of drug therapy of cases suffering from treatment-resistant depression by more development of new drugs with novel mechanisms of action such as antidepressant drugs acting on GABA transmission and its related pathways, depending on powerful understanding of pathogenesis of different forms of affective disorders [10].

This study is addressed to investigate whether quetiapine modulates glutamate and GABA concentrations in nucleus accumbens (N.Ac. as area of pleasure) in a rat model of chronic mild stress (CMS)- induced anhedonia simulating human depression with a high degree of face and predictive validity.

Material and Methods

Animals

Thirty-six albino rats weighing 180-200 g divided into 3 groups (number of rats in each group = 12 rats).

Materials

Quetiapine (AstraZeneca Pharmaceuticals, Macclesfield, UK) was dissolved in drinking water. The selected dose of quetiapine (10 mg/kg/day ip) [11].

Chemicals of HPLC

Gamma aminobutyric acid (GABA), L-glutamate and norvaline standards (Sigma chemicals Co), ethanol, (HPLC grade, MERCK), triethylamine ((TEA), MERCK), phenylisothiocyanate (PITC, Sigma chemicals Co.), hydrochloric acid (32%, MERCK), acetonitrile (MERCK), glacial acetic acid (Sigma chemicals Co), sodium acetate anhydrous (MERCK).

Ethics

All procedures were in accordance with the National Institute of Health's Guide for the Care and Use of Laboratory Animals, as well as the guidelines of the Animal Welfare Act.

Methods

Chronic Mild stress (CMS) Induced Anhedonia: Three-weeks Application of Stressors Procedure

It was adopted from Sanacora, et al [12]. The protocol consisted of the following stressors applied for 3 weeks without treatment to induce anhedonia simulating human depression in rats:

- 16 hrs. water deprivation (water bottles were removed from the cages during this time).
- 5 min. tail suspension (animals were held upside down by their tail with metal tongs).
- 1 to 2 hrs. restraint (animals were placed in a 50 ml conical tube with breathing holes).
- 30-45 min. paired housing (the mouse was placed in the cage of another mouse of the stress group, each week the home cage mouse was alternated).
- Soiled cage: 100 ml (16-18°C) water was poured into the cage.
- 5-min forced swim in cold water (16-18°C).

Each week, the stressors were presented in a different order and given at different times of the day.

The development of anhedonia in rats was tested by sucrose consumption test. The stressed animals consumed less sucrose when they become anhedonic comparing to the control group. Preliminary data have shown that control rats prefer a 2% sucrose solution over regular un-sweetened water (pilot study). Once each week, animals were given a bottle of 2% sucrose for a 1-h period, this occurs 6 hours after lights out (because the pilot study revealed that rats consumed more water during their active period), thereby, enhancing the chance of seeing a difference in sucrose consumption. After 1-hour, this bottle was removed and total sucrose consumption was calculated.

After exposure for 3 weeks stressors, rats were divided into 2 groups (each group=12 rats) with daily administration of saline or drugs for another 3 weeks, in addition to a control non-stressed non -treated group (n=12) as follows:

- *Group* 1: Control: neither exposed to CMS model nor to treatment, only ip injection of drinking water during the therapeutic period of treated groups.
- *Group* 2: exposed to CMS non-treated + ip injection of an equivelant volume of drinking water, as a solvent to quetiapine, during the therapeutic period of treated groups.
- *Group* 3: CMS-treated group with quetiapine 10 mg/kg/day ip [13].

Measurement of Immobility in Rats by the Forced Swimming Test (FST)

At the end of the study, the FST used here was essentially the same as described in detail elsewhere [13]. Swimming sessions were conducted by placing rats into individual glass cylinders (46 cm height, 20 cm diameter) containing 23-25°C water 30 cm deep, so that rats could not support themselves by touching the bottom with their paws.

Two training swimming sessions were conducted: an initial 15-min pretest followed 24 h later by a 5-min test. Following each swimming session, the rats were removed from the cylinders, dried with paper towels and returned to their home cages. A single observer, who was blind to the treatment conditions, did all the behavioral scoring.

The immobility is defined as floating in water without struggling, and doing only those necessary movements to keep the head above water; For each rat,the immobility time is calculated in sec. over a period of 5 minutes.

Determination of glutamate and GABA concentrations in nucleus accumbens homogenates of tested albino rats:

The glutamate and GABA levels in tissue homogenates of the nucleus accumbens were determined according to the method of Willner, et al [14].

High performance liquid chromatography (HPLC) with pre-column phenyl-iso-thio-cyanate (PITC) derivatization was used for determination of glutamate and GABA levels in homogenates of the nucle-

us accumbens of the brains of rats from different groups. Data are presented as nmol/mg of tissue protein.

The nucleus accumbens from each rat was homogenized and samples were centrifuged in a cooling (4°C) centrifuge at 15,000 rpm for 10 minutes. The supernatant was aspirated and transferred to an Eppendorf tube. The pellet was kept at -70°C until assayed for total protein content [15].

Each sample was derivatized by drying 100 µl of the aspirated supernatant in a centrivap under vacuum. The residue was dissolved in 20 µl of ethanol-water-triethylamine (2:2:1) and evaporated to dryness under vacuum. Thirty microliters of ethanol-water-triethylamine-phenylisothiocyanate (PITC) (7:1:1:1) was added to the residue and allowed to react for 20 min. at room temperature to form the PITC derivatives of the amino acids. Excess reagent was then evaporated under vacuum. The mobile phase of HPLC consisted of solvents A & B (solvent A: 0.1 M sodium acetate buffer (pH= 5.8); solvent B: acetonitrile:water (60:40, v:v)). A mixture of 80% solvent A and 20% solvent B was adjusted for the "isocratic" HPLC separations. Flow rate was set at 0.6 ml/min. The injected sample was 20 µl. The peaks were detected at a 254 nm wavelength. Standard curves for glutamate or GABA and norvaline were plotted using norvaline 2 nmol/20 µl as an internal standard. The ratio of the peak area of each concentration of each standard to the peak area of the internal standard was determined and entered against the concentration of the standard in a simple regression procedure.

Quantification of Total Tissue Protein

Total protein was measured according to the method of Detke, et al [15]. The aim of this procedure was to correlate glutamate and GABA concentrations to the total tissue protein amount.

Analysis of the Data

The data obtained are presented as mean ±SD (Standard deviation) and evaluated using one-way ANOVA, followed by Tukey's *post hoc* determination, using GraphPad Prism version 3.00 for Windows 97 (Graph Pad Software, San Diego, CA, USA).

Results

Effect of Quetiapine on Sucrose Consumption Test in CMS-Induced Anhedonia in Albino Rats

[Fig-1] demonstrates the reversal of anhedonia after 3 weeks i.p. administration of 10 mg/kg/day quetiapine to male albino rats continuously exposed to CMS protocol. Compared to the control-saline injected Group-1, the CMS Group-2 was associated with a (-86.59%) decrease in sucrose consumption (0.48±0.17 vs. 3.58±0.32 mL). This decrease was reversed with the administration of quetiapine (Group-3) to +33.58% of the control Group-1 level (2.68 ±0.42 mL versus the control value of 3.58±0.32 mL; mean±SD). The effect of quetiapine was statistically significant (p<0.05).

Significant Reduction in the Immobility Time (sec.) in Quetiapine Treated Group as Elicited by the Forced Swimming Test (FST)

A decrease in immobility time (in the FST) was recorded after treatment of albino rats exposed to CMS with quetiapine (Group-3) compared to CMS-exposed albino rats without treatment (Group-2). [Table-1].

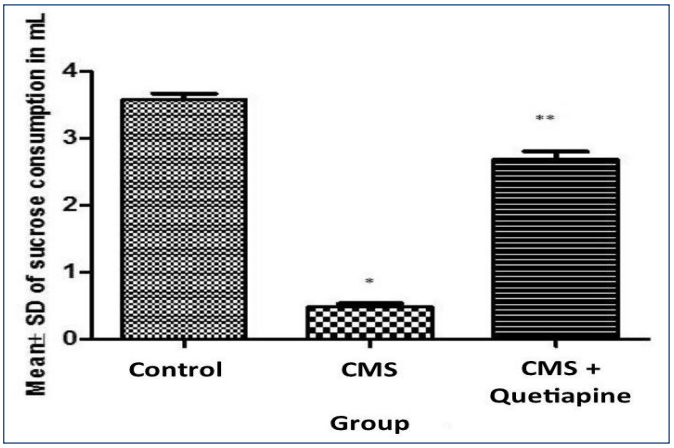

Fig. 1- Influence of exposure to chronic mild stress (CMS) on sucrose consumption in male albino rats of the different groups; control, chronic stress -with and without treatment. Data are means± SD from 12 animals per group.

**p<0.05= significant decrease Vs. control Group-1.*

*** p<0.05 = significant increase Vs. control-CMS Group-2.*

Table 1- changes in immobility time after 3 weeks of single daily ip administration of quetiapine starting from the end of the 3rd week up to the end of the 6th week of exposure to CMS protocol to albino rats.

Parameter	Control non-stressed (Group-1)	Control stressed non-treated (Group-2)	CMS+quetiapine (Group-3)
Duration of immobility (sec.)	65.15±1.25	175.5±2.5*	50.35±2.0**
% change from control		+ 169.38%	-22.72%
% change from CMS			-71.31%

** p<0.05= significant increase in immobility time (sec.) in Group-2 compared to the control non-stressed albino rats Group-1.*

*** p<0.05 = significant reduction in immobility time (sec.) in quetiapine-treated Group-3 compared to the CMS-non treated albino rats Group-2.*

Effect of 3-weeks Administration of Quetiapine on the Glutamate Level in the Homogenates of Nucleus Accumbens (N.Ac.) of Isolated Brains of Chronic Mild Stress Exposed Albino Rats

[Fig-2] represents the changes in glutamate concentration in the homogenates of the nucleus accumbens (N.Ac.) of the control, CMS, CMS+quetiapine administered to albino rats.

CMS significantly (p<0.05) increased the glutamate concentrations in the tested area of brains. Glutamate concentrations of CMS-treated rats was significantly (p<0.05) decreased by quetiapine treatment.

Effect of 3-weeks Administration of Quetiapine upon the GABA Concentrations in the Homogenates of Nucleus Accumbens (N.Ac.) of Chronic Mild Stress (CMS) Exposed Albino Rats

[Fig-3] represents the changes in GABA concentrations in the homogenates of nucleus accumbens (N.Ac.) of the control, CMS, CMS+ quetiapine- treated albino rats.

CMS significantly (p<0.05) decreased the GABA concentrations in the homogenates. GABA concentrations of CMS-exposed albino rats were significantly (p<0.05) increased by administration of quetiapine.

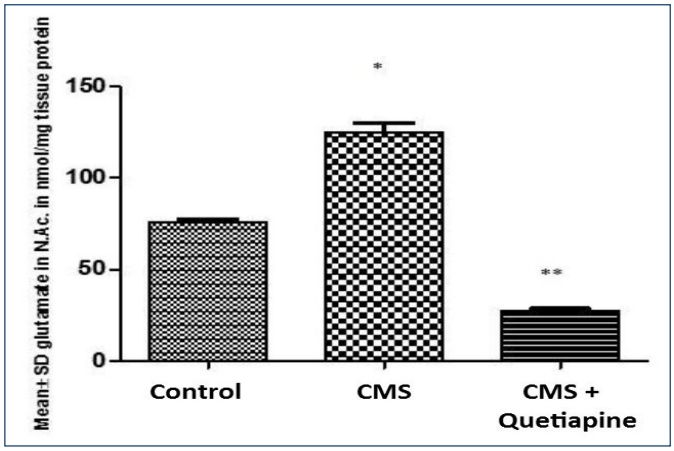

Fig. 2- Influence of CMS with and without administration of quetiapine on glutamate in homogenates of N.Ac. of albino rats of the different groups; control stressed and non-stressed as well as CMS-treated albino rats. Data are expressed as the mean ± SD from 12 animals per group.

**p<0.05 significant increase Vs. control-non-stressed Group-1.*

***p<0.05 significant reduction Vs. control stressed non-treated* group.

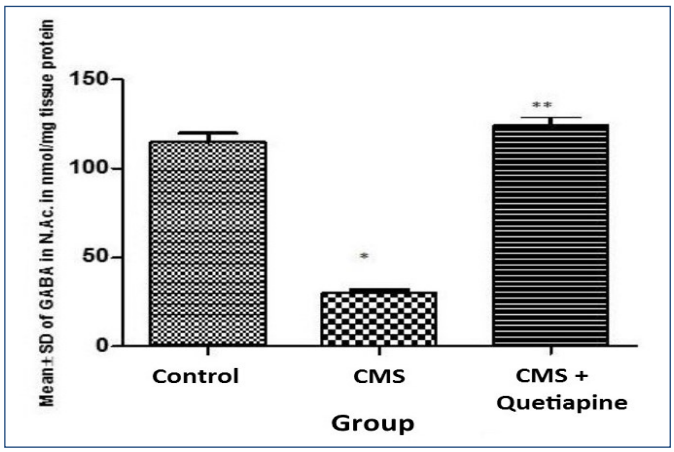

Fig. 3- Influence of CMS with and without administration of quetiapine on GABA concentrations in homogenates of N.Ac. of albino rats of the different groups; control stressed and non-stressed as well as CMS-treated albino rats. Data are expressed as the mean ± SD from 12 animals per group.

**p<0.05 significant reduction Vs. control-non-stressed Group-1.*

***p<0.05 significant increase Vs. control stressed non-treated Group-2.*

Discussion

In the present study, 3-weeks single daily dose of quetiapine induced a statistically significant increase in sucrose consumption by albino rats exposed to 6-weeks of CMS-induced anhedonia that simulates human depression. This means the reversal of anhedonia -simulating human depression by this drug's therapy for 3 weeks. It also increased the anti-immobility and struggling time in the FST as a behavioral despair screening test of drugs with ability to improve the depressed mood. Additionally, It augmented GABA and decreased glutamate concentrations of the homogenates of nucleus accumbens (N.Ac.) of the stressed albino rats. This is completely the reverse of what occurs in stressed non-treated Group-2 as a model of CMS-induced anhedonia.

Quetiapine is one of the new generations of antipsychotic and the aim of this study was to further investigate its possible action (s) on glutamate and GABA amino acid contents in N.Ac. of brains of rats, being a part of brain related to control of affective disorders that could accompanied any psychotic disease. Thus, Their concentrations were measured after 3 weeks of quetiapine treatment in a model of anhedonia simulating human depression with strong face and predictive validity.

The antidepressant action of quetiapine has been demonstrated in clinical and preclinical studies. Nevertheless, little information is known about its effectiveness in the treatment of stress-related disorder.

It was found that quetiapine augments the action of selective serotonin reuptake inhibitors (SSRIs). This is proven to be beneficial in neuropsychiatric disorders, especially in cases of psychosis associated with depressed mood. An experimental study was conducted on chronically stressed rats to determine the impact of combined administration of of quetiapine and escitalopram on their daily activities and their performance. These stressed rats were suffered from impairement of extra-dimensional (ED) cognitive acquisition. However *po* administration of quetiapine at a dose of 2.5 mg/kg was given to these rats prior to the restraint sessions. The repetition of administration of quetiapine completely prevented this stress-induced reduction in rats' cognitive performance and ameliorated their physical activities.

Additionally, single small oral dose administration of quetiapine either 0.63 or 1.25 or 2.5 mg/kg, before exposure to any stressor, reversed any inactivity or reduction in cognitive functions occuring in stressed rats. As well as, co-administration of small doses of quetiapine (0.63 and 0.3 mg/kg in control and stressed rats, respectively) and escitalopram, as an SSRI, (0.3 mg/kg, ip) facilitated all activities and daily performance in either control or stressed rats.

The above findings revealed that quetiapine administration either prevented or reversed stress-induced impairement in physical and cognitive activities in stressed rats. In addition to that, a beneficial interaction between quetiapine and escitalopram represents by a progressive improvement in cognitive acquisition in stressed rats.

These findings may have therapeutic implications for the treatment of stress-related psychiatric disorders in addition to the cognitive enhancement of stressed rats either by quetiapine administration alone or in addition to an SSRI e.g. escitalopram [18].

Enhancement of GABAergic activity in mood disorders, in parts of limbic system of the brain of stressed albino rats, in addition to, monoaminergic and serotonergic theories, point to the importance of the balance between multiple neurotransmitter systems in strategic plan of mood disorders either alone or in cases of psychosis accompanied b depressed mood. This is evidenced by reduction of GABA levels in plasma and cerebrospinal fluid (CSF) in patients with major depression below normal values [11]. Proton ((1)H) magnetic resonance spectroscopy (MRS) study measured low GABA concentrations in the occipital cortex of depressed patients but when they were treated with selective serotonin re-uptake inhibitor (SSRI), GABA concentration were comparable to that measured in normal subjects [11].

This is based on the fact that GABA release is dependent on this sIPSC in the prefrontal pyramidal neurons of the rat brain [19]. Many studies suggest the role of GABAergic system in therapy of depressed mood. A clinical study is conducted on cocaine-

dependent patients using venlafaxine treatment (SNRI). Its results showed an increase in GABA level in their prefrontal cortex. GABA concentration was low in these patients before venlafaxine treatment. This result was associated with improvement of cocaine abuse in such patients with a reduction in cocaine self-administration [20].

In conclusion, from the changes of GABAergic and glutamate concetrations in relation to anhedonic non-treated rats in addition to the shorter immobility period in the FST- induced by the tested drug, the present study reported a mood-regulating role of quetiapine via changes in concentrations of both glutamate and GABA neurotransmitters in the homogenates of nucleus accumbens of CMS-exposed albino rats that is a model of human depression with a high degree of validity.

Conflict of Interest : The author reports no conflicts of interest in this work.

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