

Modulating Role of Mirtazapine on Concentrations of both Glutamate and GABA in Nucleus Accumbens of Chronic Mild Stressed Albino Rats

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Abstract

Aim: Mirtazapine is a noradrenergic and specific serotonergic antidepressant (NaSSA). It enhances both noradrenergic and 5HT₁ serotonergic transmission. The present study investigated the changes in forced swimming test and the alteration of 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 mirtazapine 16 mg/kg/day intraperitoneal (ip) dissolved in DMSO for 3 weeks during exposure to CMS.

Results: Reversal of CMS-induced anhedonia after 3 weeks ip administration of 16 mg/kg/day mirtazapine was observed. It modifies the behavioral changes recorded by the forced swimming test (FST) as well as the content of GABA neurotransmitter in their isolated nucleus accumbens.

Conclusion: The present study proposes the presence of a possible GABAergic role of mirtazapine in nucleus accumbens in the treatment of depressive disorders.

Keywords: Chronic Mild Stress (CMS); Nucleus accumbens; Mirtazapine; Forced swimming test; Albino rats

Introduction

Mirtazapine [1,2,3,4,10,14b-hexa-hydro-2-methylpyrazino [2,1-a] pyrido [2,3-c]benzazepin] is a tetracyclic compound with antidepressant activity in human [1]. Mirtazapine has a unique mechanism of action, different from that of the classical tricyclic antidepressants, the serotonin selective reuptake inhibitors and monoamine oxidase inhibitors, and could be described as a noradrenergic and specific serotonergic antidepressant, abbreviated as NaSSA [2]. The pharmacological profile of mirtazapine is characterized by potent presynaptic 2-adrenergic antagonistic activity, 5-HT₁ agonistic activity, and potent 5-HT₂ and 5-HT₃ antagonistic activities, as well as by a potent H₁ antagonistic activity. The blockade of presynaptic 2-adrenergic receptors is considered as a possible mechanism for antidepressant activity of mirtazapine [3].

New antidepressant drugs, with less adverse effects than imipramine derivatives, have been developed; they selectively block both the serotonin transporter and the noradrenaline (NA) transporter, (that is, mixed serotonin/nor adrenaline reuptake inhibitors, SNRIs, NaSSA e.g. venlafaxine, duloxetine, mirtazapine) [2].

The pharmacological profile of mirtazapine is characterized by potent presynaptic 2-adrenergic antagonistic activity, 5-HT₁ agonistic activity, and potent 5-HT₂ and 5-HT₃ antagonistic activities, as well as by a potent H₁ antagonistic activity [3]. The blockade of presynaptic 2-adrenergic receptors is considered as a possible mechanism for antidepressant activity of mirtazapine. The interactions of mirtazapine with 5-HT receptors were studied in vivo in experiments measuring selective 5-HT receptor subtype-mediated behaviors [4].

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].

Gamma amino-butyric acid (GABA) is the major inhibitory neurotransmitter in the brain and diminishes the activity of its target neurons. It is a major inhibitory neurotransmitter in the central nervous system and modulates the activity of several neurotransmitters including dopamine, serotonin, and norepinephrine. GABA is synthesized in a single step from its precursor glutamate by glutamic acid decarboxylase. GABA is metabolized by successive transamination and oxidation to yield succinic semialdehyde and succinic acid, respectively. As a part of the transamination reaction, a recycling system is formed in which α -ketoglutaric acid is converted to the GABA precursor glutamate by GABA-glutamic acid transaminase [6].

The cornerstone of the GABA hypothesis of bipolar disorder is that GABA provides inhibitory action to both norepinephrine and dopamine systems [7]. Although this widely expressed neurotransmitter has been thought to exert a tonic inhibitory effect on NE systems, GABA may facilitate NE activity and relatively reduced plasma GABA levels in depressed patients [8].

There is a well proven tendency for depressed and bipolar patients to have lower levels of GABA in their blood plasma. These low plasma levels are thought to reflect lower brain levels and a study suggested using GABA to treat depression in order to bring up its plasma level. Unfortunately this theory is too simplistic and needs more studies to emphasize it. The current theory of GABA and depression is that low

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plasma levels of GABA may identify an inheritable tendency for mood disorders such as depression or bipolar disease [9].

Additionally, there is a paradigm shift from a monoamine hypothesis of depression to a neuroplasticity hypothesis that focuses on glutamate and may represent a substantial advancement in the working hypothesis that directs research towards research and development of new antidepressant drugs. Although there are multiple classes of drugs with monoamine-based mechanisms of action, there remain a large percentage of patients who fail to reach a sustained remission of depressed mood. The ultimate need for improved drug therapy of cases suffering from treatment-resistant depression means there is a large space for the development of new compounds with novel mechanisms of action such as antidepressant drugs acting on glutamate transmission and its related pathways [10].

This study is addressed to investigate whether mirtazapine 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 as chronic stress acts as a predisposing and participating factor in the onset of depression in humans..

In order to reach the aim of the present study, it involves the use of stressed albino rats whose sucrose consumption is reduced by chronic exposure to stressors for 3 weeks with different order every week to avoid conditioning.

Intraperitoneal administration of the fresh drug solution every day for another 3 weeks of CMS to test its effect both weekly on sucrose consumption and at the end of the study on the duration of immobility in seconds as well as on GABA and glutamate contents in selected brain areas of tested albino rats.

Stressed albino rats =reduction in sucrose consumption, prolongation of immobility test and changes in selected brain amino acids.

Treated albino rats by mirtazapine=reversal of the above mentioned changes at least to levels comparable to control non-treated group.

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

Mirtazapine (ChemOrganic Limited, China) was dissolved in dimethyl sulfoxide (DMSO, 8 mg/mL). The selected dose of mirtazapine {16 mg/kg/day intraperitoneal (i.p.) for 3 weeks} [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

I. Chronic mild stress (CMS)-induced anhedonia: Three-weeks application of stressors procedure:

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

- a) 16-h water deprivation (water bottles were removed from the cages during this time)
- b) 5 min.-tail suspension (animals were held upside down by their tail with metal tongs)
- c) 1-to2-h restraint (animals were placed in a 50 ml conical tube with breathing holes)
- d) 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)
- e) Soiled cage: 100 ml (16-18°C) water was poured into the cage
- f) 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. Measurement of development of anhedonia as a result of exposure to CMS was done [12]. 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 unsweetened 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 saline during the therapeutic period of treated groups
- **Group 2:** exposed to CMS non-treated+i.p. injection of an equivalent volume of DMSO, as a solvent to mirtazapine, during the therapeutic period of treated groups
- **Group 3:** CMS-treated group with mirtazapine 16 mg/kg/day ip [11]

II. 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 [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 nucleus 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 and 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 [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, U.S.A.).

Results

I. Effect of mirtazapine on sucrose consumption test in CMS-induced anhedonia in albino rats

Figure 1 demonstrates the reversal of anhedonia after 3 weeks ip administration of 16 mg/kg/day mirtazapine to male albino rats continuously exposed to CMS protocol. Sucrose consumption in mL of the different groups (control, CMS and CMS+mirtazapine administration) was calculated. In comparison to the control-saline injected group 1, the CMS group 2 was associated with a (-85.34%) decrease in sucrose consumption (0.67 ± 0.16 vs. 4.57 ± 0.33 mL). This decrease was reversed with the administration of mirtazapine (group 3) to +15.75% of the control group (1) level (5.29 ± 0.67 mL versus the control value of 4.57 ± 0.33 mL; mean ± SD). The effect of mirtazapine was statistically significant (p<0.05).

II. Significant reduction in the immobility time (sec.) in mirtazapine-treated group as elicited by the forced swimming test (FST)

Reduction of immobility time (in the FST) was observed after treatment of albino rats exposed to CMS with mirtazapine (group 3) compared to CMS-exposed albino rats without treatment (group 2) (Table 1).

Table 1 changes in immobility time after 3 weeks of single daily

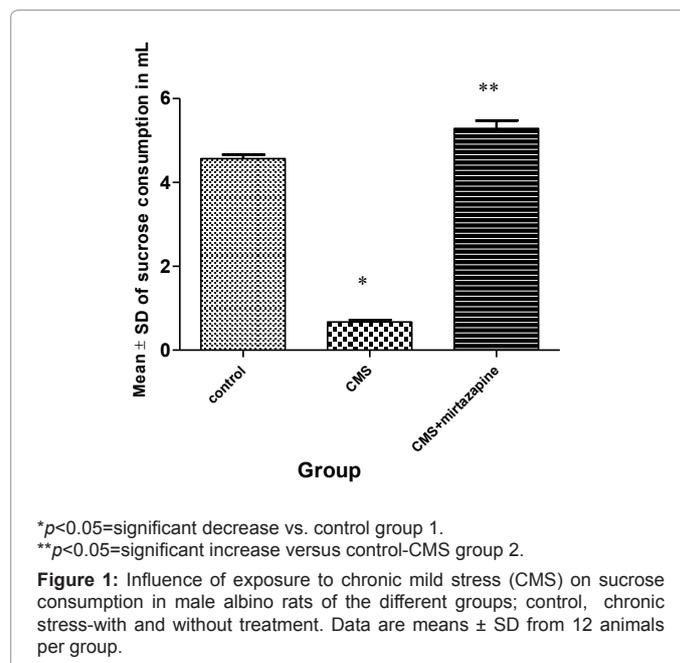


Figure 1: Influence of exposure to chronic mild stress (CMS) on sucrose consumption in male albino rats of the different groups; control, chronic stress-with and without treatment. Data are means ± SD from 12 animals per group.

Parameter	Control non-stressed Group (1)	Control stressed (Group 2)	CMS+mirtazapine (Group 3)
Duration of immobility (sec)	98.75 ± 1.41	169.8 ± 1.80*	84.75 ± 1.11**
% change from control		+71.95%	-14.18%
% change from CMS			-50.09%

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

* 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 mirtazapine-treated group (3) compared to the CMS–non treated albino rats group (2)

Table 1: Significant reduction in the immobility time measured in seconds by mirtazapine in CMS-exposed albino rats (group 3) compared to the control stressed group (2) as elicited by the forced swimming test (FST).

i.p. administration of mirtazapine starting from the end of the 3rd week up to the end of the 6th week of exposure to CMS protocol to albino rats.

- * $p < 0.05$ = 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 mirtazapine-treated group (3) compared to the CMS –non treated albino rats group (2)

III. Effect of 3-weeks administration of mirtazapine on the glutamate level in the homogenates of nucleus accumbens (N.Ac.) of isolated brains of chronic mild stress (CMS)-exposed albino rats

Figure 2 represents the changes in glutamate concentration in the homogenates of the nucleus accumbens (N.Ac.) of the control, CMS, CMS+mirtazapine 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 mirtazapine treatment.

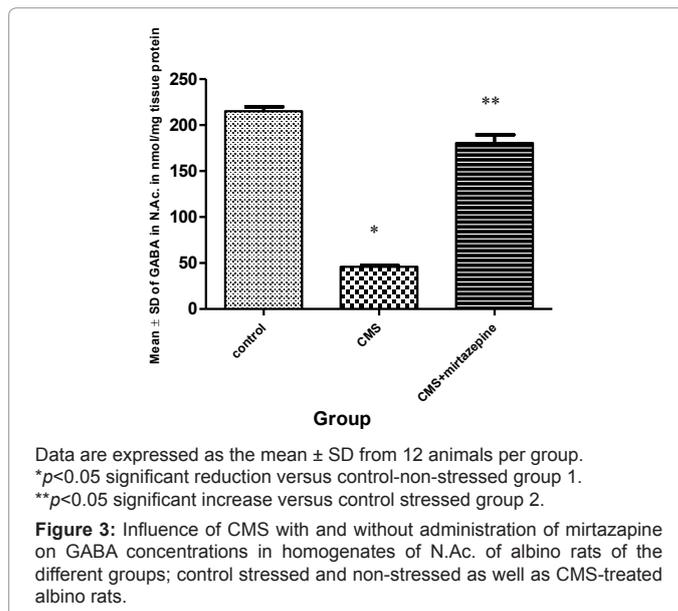
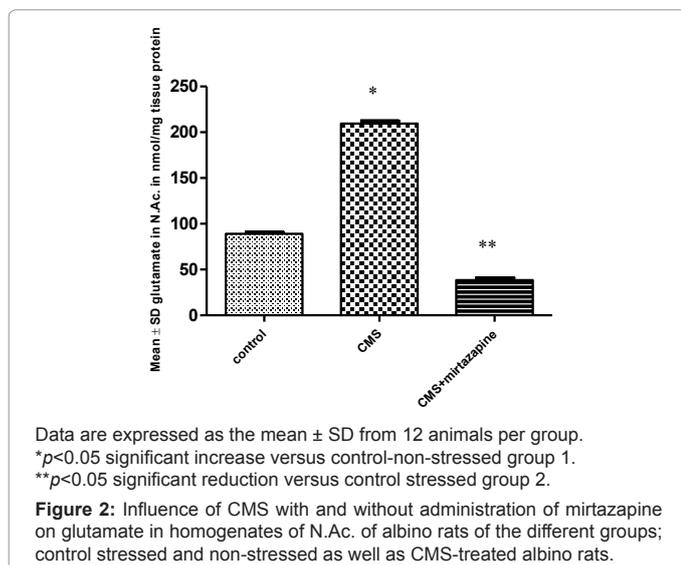
IV. Effect of 3-weeks administration of mirtazapine upon the GABA concentrations in the homogenates of nucleus accumbens (N.Ac.) of chronic mild stress (CMS)-exposed albino rats

Figure 3 represents the changes in GABA concentrations in the homogenates of nucleus accumbens (N.Ac.) of the control, CMS, CMS+mirtazapine- 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 mirtazapine.

Discussion

In the present study, 3-weeks single daily dose of mirtazapine induced a statistically significant increase in sucrose consumption by albino rats exposed to 6-weeks of CMS-induced anhedonia that simulates human depression. It also reduced the immobility time in the FST as a screening test of antidepressants. Additionally, it increased GABA and reduced glutamate concentrations of the homogenates of nucleus accumbens (N.Ac.) of the tested albino rats.



Mirtazapine is a widely used antidepressant and the aim of this study was to further investigate its effect on glutamate and GABA contents in N.Ac. of brains of rats. Thus, their levels were assessed after 3 weeks of mirtazapine treatment in a model of depressive symptoms induced by stress exposure. Its ip administration was found to produce reduction of the duration of immobility in both tests [4]. The effect of repeated administration of imipramine or mirtazapine, two antidepressant drugs with different mechanisms of action, was studied on foot shock stress-induced increase in the extracellular concentration of norepinephrine in the prefrontal cortex of freely moving rats. Two-weeks administration of either imipramine or mirtazapine reduced anxiety and depression in such rats exposed to footshock stress by reduction in stress-induced augmentation in cortical norepinephrine output without its reduction in limbic system during exposure to this kind of stress [4].

GABA is thought to play a role in improvement of depressed mood as well as norepinephrine and serotonin. A clinical study showed that there was a low concentration of GABA in plasma and cerebrospinal fluid (CSF) of individuals with major depression [9]. In addition to that, low GABA concentration, measured by proton ((1)H) magnetic resonance spectroscopy (MRS) study, has also been found in the occipital cortex of depressed subjects and when these patients were treated with SSRI, results revealed a normalization of the low GABA concentration, suggesting a role of GABA in the mechanism of antidepressant action [9]. However, the application of serotonin or norepinephrine induced a large enhancement of the amplitude and frequency of spontaneous inhibitory post-synaptic currents (sIPSC) resulting in increase GABA release [16]. This is based on the fact that GABA release is dependent on this sIPSC in the prefrontal pyramidal neurons of the rat brain [17]. Venlafaxine treatment as an example of SNRI antidepressant drug was associated with increase GABA level in the prefrontal cortex of cocaine-dependent subjects. This level was low in these subjects before venlafaxine treatment. This result was associated with decrease cocaine self-administration [18]. Mirtazapine is thought to be suitable for the treatment of tinnitus due to its proposed ability to increase the availability of serotonin and thereby to increase GABA activity which is an inhibitory neurotransmitter in the auditory system [19].

The result of the present study is supported by the theory of oxidative stress as a pathophysiological mechanism that referred to the toxic effects of free radical metabolic by-products. These free radicals play complementary roles in cellular signaling, physiological immunological responses and mitosis.

Oxidative stress prevents the reparation of oxidative damages by blocking of any structural repair and replacement mechanisms by either their overproduction or deficiencies in antioxidant defense of each cell. The cellular damage may range from cellular structural damage and mitotic arrest, to apoptosis and cell necrosis, depending on the level of oxidative stress severity. These damaging mechanisms were reported in the pathogenesis of psychiatric disorders. The brain is extremely vulnerable to oxidative damage for several reasons. These include its high oxygen utilization with the production of huge amounts of free radical by-products. Additionally, its mechanisms of antioxidant defenses together with its lipid-rich constitution that help oxidation of many of its constituents result in a great reduction in its significant neurotransmitters that are necessary for the proper function of higher brain centers. Additionally, the brain is also susceptible to perpetual damage from oxidative cellular injury or necrosis, under the effect of neurotoxins including the released excitatory amines (mainly glutamate) and the activated inflammatory response with the production of many inflammatory mediators as tumor necrosis factor and interleukins. Psychiatric diseases, involving depressed mood, could result from this intrinsic oxidative vulnerability of the brain with the strong evidence for neurodegenerative changes associated with these psychiatric disorders [20].

Efficacy of mirtazapine was proved when used in a clinical trial for treating both the depressive symptoms and excessive alcohol use of comorbid major depressive disorder and alcohol dependence. The results pointed to a great decrease in both depressive symptoms and level of drinking. Fast clinical improvements were observed after starting mirtazapine, as well, with a rapid onset of clinical response. The recommendation of this trial was to do double-blind studies to clarify further efficacy of mirtazapine in depressed patients with alcohol abuse. The improvement could be related to a possible reduction in oxidative damage induced by this comorbidity. This will encourage scientists to measure oxidative markers and to study GABAergic receptors by magnetic resonance imaging of such patients [21].

In conclusion, from a neurochemical and behavioral points of view, the present study pointed to a modulating role of mirtazapine on 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.

Disclosure

The author reports no conflicts of interest in this work.

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