Experimental Research

ANNALS OF MEDICINE & SURGERY

OPEN

Improvement of autistic-like behaviors in adult rats prenatally exposed to valproic acid through early suppression of orexin receptor

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Introduction: Autism spectrum disorder (ASD) is a disabling psychiatric disease characterized by impairments in communication and social skills. The pathophysiology of autism is complex and not fully known. Considering the incidence of sleep disorders in individuals with ASD and the important role of orexin in sleep, it is possible to hypothesize that an alteration of the orexinergic system could be implicated in the pathogenesis of autism symptoms. The present study was conducted to investigate the effect of suvorexant [dual orexin receptor antagonists (DORAs)] on autism-like behavior in prenatally valproic acid (VPA)-exposed rats].

Methods: Wistar female rats were administered VPA [600 mg/kg, intraperitoneally (i.p.)] or normal saline (10 ml/kg, i.p.; vehicle control) on gestational day 12.5. Thirty-two male offspring were divided into four groups: Control, VPA, Suvorexant + VPA, and VPA + Risperidone. The pups were given suvorexant [20 ml/kg, by mouth/orally (p.o.)] or risperidone (1 ml/kg, p.o.) daily from postnatal day (PND) (40–54). The offspring were tested for repetitive behaviors and cognitive ability with a Y-maze task on PND 55, and social interaction was assessed by play behavior in the open field on PND 56. And anxiety with using the three-chamber social assay on PND 56.

Results: In the Y-maze apparatus, spontaneous alteration significantly decreased in the prenatal VPA-treated rats compared to control rats showing autistic-like behavior, and 2-week suvorexant increased the alternation, indicating the beneficial effect of suvorexant. Prenatal treatment with VPA, impaired play behavior (sniffing, grooming, and darting), and increased anxiety-related behavior. Suvorexant treatment attenuated the problems in male offspring's social behavior.

Conclusion: Our results showed that suvorexant improved ASD-associated behaviors in the VPA-treated rats, and the orexinergic system may be associated with the pathogenesis of autism symptoms.

Keywords: autism, DORA, orexin, valproic acid-induced autism

Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by deficits in social communication, stereotyped behaviors, and a reduced range of interests and activities. Autism is among the most mysterious disorders of child development, with a significant increase during the last two decades[1]. The pathology of autism is complex and not fully known. Many neuroregulatory systems are involved in determining ASD; however, how these complex systems interact and cause the onset of the symptoms of autism remains unclear[2]. In recent years, researchers have shown an increasing interest in orexin, which seems to be involved in the neurochemistry of neuropsychiatry[3]. Orexin, also known as hypocretin, is a hypothalamic neuropeptide that plays an important role in the regulation of metabolism, appetite, stress, excitement, sleep regulation, and cognition[4]. Orexin imbalance appears to be related to numerous neurological disorders including depression, addiction, anxiety, and schizophrenia[3]. One of the most common complaints among children with autism is sleep problems[5]. The

HIGHLIGHTS

- The present study was conducted to investigate the effect of suvorexant (dual orexin receptor antagonists (DORAs) on autism-like behavior in prenatally valproic acid (VPA)-exposed rats).
- In the Y-maze apparatus, spontaneous alteration significantly decreased in the prenatal VPA-treated rats compared to control rats showing autistic-like behavior, and 2-week suvorexant increased the alternation indicated benefit effect of suvorexant. Prenatal treatment with VPA, impaired play behavior (sniffing, grooming, and darting), and increased anxiety-related behavior. Suvorexant treatment attenuated the problems in male offspring's social behavior.
- Our results showed that suvorexant improved ASD-associated behaviors in the VPA-treated rats, and the orexinergic system may be associated with the pathogenesis of autism symptoms.

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Annals of Medicine & Surgery (2024) 86:166–171

Received 2 March 2023; Accepted 28 April 2023

Published online 22 November 2023

http://dx.doi.org/10.1097/MS9.0000000000000788

consequence of disrupted sleep is an increase in the severity of repetitive behaviors and social and communication difficulties[6]. Since the orexin system is essential in maintaining sleep–wake cycles, it is possible that a deficiency of the orexin system will lead to sleep disorders in ASD[7]. Considering the incidence of sleep disorders in individuals with ASD, it is possible to hypothesize that an alteration of the orexinergic system could be implicated in the pathogenesis of autism symptoms. In recent research, plasma levels of orexin A and oxytocin were higher in autistic patients than in the healthy population[8]. This finding supported the contribution of oxytocinergic mechanisms in ASD. Since the activities of the orexin system and the dopaminergic system are potently influenced by each other, it is reasonable to predict that the activity of the orexin system may play a role in autism[9]. Currently, the usage of orexin receptor antagonists in treating neuropsychiatric disorders is proposed[10]. Suvorexant is a dual orexin receptor antagonist (DORA) highly selectively blocks orexin receptor types 1 and 2[11]. In clinical trials, suvorexant antagonists have reported improved quality of sleep with few side effects[12]. Moreover, it has been reported that orexin antagonists are effective at blocking addiction-related behaviors[1,13]. Recent preclinical studies indicate that orexin-1 receptor antagonist has an effect on stress responses such as anxiety-like behavior[14]. In human studies, a polymorphism in the Orx-1 receptor gene has been associated with major mood disturbance, and increased orexin peptide levels were correlated with social interaction[15]. Since orexins and autism display a reciprocal interaction in some physiological activities and have critical effects on sleeping and social behavior, we decided to investigate the effect of suvorexant as a dual orexin A and B receptor antagonist on autistic-like behaviors.

Materials and methods

Animals

Adult (3–4 months) male and female Albino Wistar rats (issued from the animal house of Golestan University of medical science) were allowed to mate overnight. Rats were allowed free access to water and a standard laboratory diet in a controlled environment (23 ± 2°C temperature and 45–65% humidity) and in artificially lighted rooms with 12 h light/dark cycle lights on at 7:00 AM. All the experiments were performed in accordance with the guidelines for laboratory animal use and care set by the Animal Ethics Committee of Golestan University with ethical code (IR.DU.REC.1401.008).

The work has been reported in line with the ARRIVE statement[16]. After mating sperm-positive vaginal smear was taken to indicate the first day of pregnancy [gestational day 1 (GD1)]. Only the male pups were used in the study[17], and approximately three to five male pups were born from each dam. They were randomly assigned to four groups ($n = 8$).

Group I was control rats whose mothers were subjected to saline on GD 12.5, group II was valproic acid (VPA)-exposed rats which received a single subcutaneously dose of 600 mg/kg VPA 12.5 days after conception. Group III was VPA + Suvorexant rats treated prenatally with VPA and postnatally with suvorexant, as described above. Group IV was VPA + Risperidone rats treated prenatally with VPA and postnatally with risperidone. Risperidone or suvorexant with doses 1 and 20 mg/kg, were administered using a stainless-steel oral gavage needle for 2 weeks from postnatal day (PND) 40 to PND 54. Mothers were kept with their litters until weaning on PND 21. Behavioral procedures were performed on PND 55–56, and all the tested animals were at a range of 120–150 g.

Play behavior test

Based on the results, the impairment of social interaction is an essential clinical manifestation of autism[18]. The play behavior test was used to evaluate impaired social interaction. In order to enhance social interactions, the animals were housed separately the night before the experiment. Two animals from the same group but different litters and cages (VPA vs. VPA, Control vs. Con) were placed into an acrylic plastic circular cage under dim light for 15 min on PND 55. Pairs were tested randomly for groups, and the paired rats did not show a significant difference in body weight of more than 15 g. The following parameters were recorded for 10 min as indicators of social engagement[19]: pinning frequency (the frequency of play behavior, i.e. the number of times the resident rat laid on its back and showed his belly to the intruder), darting frequency (the number of times the resident moved rapidly toward, in parallel, or away from the intruder), and the time (seconds) spent sniffing the intruder and self-grooming as stereotype behaviors.

Repetitive behavior in Y-maze test

The Y-maze spontaneous alternation test was used to evaluate repetitive/restricted behavior at PND 56[8]. Each rat was placed at the end of one arm of the Y-maze and allowed to move freely throughout the maze. Five sessions were allocated to each rat. For each session, the first choice of the rat (whether the rat first entered the left or right arm) was evaluated. The parameter analyzed was the percentage (%) of alternation between the right and left arms, which was continuously assessed concerning the arm visited in the previous session. This model is based on rats' natural proclivity, alternating between the visited goal arms in each trial and a series of successive trials[20]. Thus, a higher percentage of alternation between the arms was considered normal rat behavior, whereas minor alternation indicated cognitive inflexibility and repetitive behavior. For statistical analysis, the data were transformed into scores: 0 = no alternations, repeatedly visiting the same arm for all five sessions, 1 = one alternation, 2 = two alternations, 3 = three alternations, and 4 = four alternations, constantly alternating between the visited arms for all five sessions.

Assessment of social interaction

The social interaction was investigated using a three-chamber social interaction testing apparatus (57 cm × 36 cm × 30 cm)[21]. Briefly, the testing method consisted of three sessions. The first 5-min session was the habituation phase for treated rats. In the following second 10-min session of the sociability phase, a stranger rat was randomly placed in any side chamber called as Stranger1 chamber while another chamber was called as empty chamber. In the last 10-min session of the social preference phase, a novel rat was placed in the previously referred to empty chamber and called as Stranger2 chamber. The Stranger1 chamber was referred as the familiar chamber in this phase. The treated rat was free to explore the chambers in all sessions. The time spent in both side chambers by the treated rat was noted.

Result

Effect on repetitive behavior

In the Y-maze apparatus, spontaneous alteration significantly decreased in the prenatal VPA-treated rats compared to control rats ($P < 0.001$), suggesting repetitive behavior in the prenatal VPA-treated rats. Nonetheless, the postnatal administration of risperidone reversed the rate of spontaneous alternation behavior after the treatment of VPA-induced diminution.

Similarly, 2-week treatment with suvorexant significantly increased the spontaneous alteration ($P < 0.01$) compared to the prenatal VPA-treated rats, as shown in Figure 1. However, suvorexant treatment did not modify spontaneous alternation compared to the control and risperidone ($P > 0.05$).

Effects on the social interaction

Social investigation (sniffing, dart, pining, grooming) is considered an important factor in the social interaction test of rats[22]. Prenatal VPA treatment impaired the social behavior of offspring. On the frequency of pinning (Fig. 2A), prenatal VPA treatment decreased the frequency of pinning compared to the control ($P < 0.001$). Suvorexant treatment in the VPA group increased the pinning frequency compared to the group exposed only to VPA ($P < 0.001$). However, the number of pinning remained lower than that of the control group ($P < 0.001$). After 14 days of treatment, risperidone was observed to improve pinning frequency compared to the VPA group ($P < 0.01$). However, no significant difference was denoted between the suvorexant and risperidone groups ($P > 0.05$).

Statistical analysis showed significant differences in the frequency of darts (Fig. 2B). Prenatal VPA treatment decreased the frequency of darts compared to the control group ($P < 0.001$), Suvorexant treatment increased the number of darts; however, the number of darts remained lower compared to the control group treatment (Fig. 2B) ($P < 0.001$). Further, risperidone + VPA-treated rats significantly increased the number of darts. Moreover, a one-way ANOVA conducted on the main effect of the prenatal VPA treatment indicated that the VPA-exposed animals significantly increased the number of grooming (Fig. 2C) compared to the control group ($P < 0.001$). The excessive grooming behavior in the VPA-treated group was significantly corrected by suvorexant and risperidone ($P < 0.001$); sniffing (Fig. 2D) decreased both by VPA exposure ($P < 0.001$). However, VPA + suvorexant rats had more sniffing time than the VPA-treated animals ($P < 0.001$).

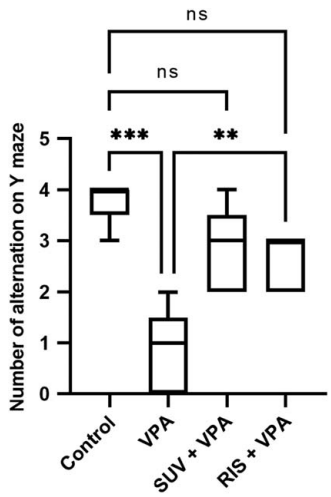

Figure 1. Effects of prenatal VPA and suvorexant and risperidone treatments on Y-maze spontaneous alternation in male rat offspring ($n = 8$ for all groups); $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$. ns, not significant; VPA, valproic acid.

Effect on sociability and social novelty preference

In the first session of the social preference test, control rats spent significantly more time in the compartment with Stranger1 compared to the compartment with an empty cup (Fig. 3), indicating normal sociability, social motivation, and affiliation. Prenatal VPA-treated rats exhibited less time in the cup containing Stranger1 ($^{*}P < 0.001$) and more time spent in the empty chamber ($^{*}P < 0.001$) as compared to control rats. This indicates the low social preference in prenatal VPA-treated rats. Suvorexant treatment in the VPA group increased the time spent with strangers compared to the group exposed only to VPA ($P < 0.001$). However, this time remained lower than that of the control group ($P < 0.05$). Further, risperidone + VPA-treated rats significantly increased the time spent in the Stranger1 cup as compared to the VPA group ($P < 0.001$).

In the second session of the social preference test, control rats spent more time with the newly encountered rat (Stranger2) ($P < 0.001$), indicating intact social memory and predilection for novel experiences. Unlike prenatal VPA-treated rats, they did not show a preference for the chamber containing a newly introduced mouse (Stranger2) over a chamber containing a now familiar mouse (Stranger1) in session II compared to control rats ($P < 0.001$). Indifferent behavior of VPA rats in this test is indicative of decreased social motivation and novelty. Suvorexant and risperidone treatment in the VPA group increased the time spent in Stranger2 cups compared to the group exposed only to VPA ($P < 0.001$).

Discussion

Findings from this study showed that the prenatal exposure of rats to VPA reduced social interaction (decreased preference for unfamiliar rats in sociability tests, respectively, in three-chamber apparatus), spontaneous alternation (increased repetitive patterns of behavior or memory in Y-maze task), and play behavior (decrease sniffing time, dart number, pinning frequency). This obviously indicated that prenatal exposure to VPA-induced social dysfunction that is consistent with the characteristic of autism[23,24]. Postnatal treatment with suvorexant (orexin antagonist) and risperidone (antipsychotic drugs) improved the behavior deficits in VPA-treated rats. Prenatal exposure to VPA increases the risk of autism, possibly through its ability to change gene expression via epigenetic remodeling by inhibition of histone deacetylase (HDAC) activity[25]. The exposure of rats to VPA impaired sociability, evidenced in reduced sociability index, observed as a preference to spend more time in an empty chamber than the chamber containing novel rats, as well as social novelty deficit is shown in the tendency of VPA rats to spend more time with a previously encountered rat than a novel rat. Repetitive behavior is considered to be a major symptom in autistic patients, which has often been attributed to anxiety, fear, and defective social behaviors[26] and might be a consequence of communication problems[27]. In this study, prenatal exposure of rats to VPA reduced spontaneous alternation behavior indicative of

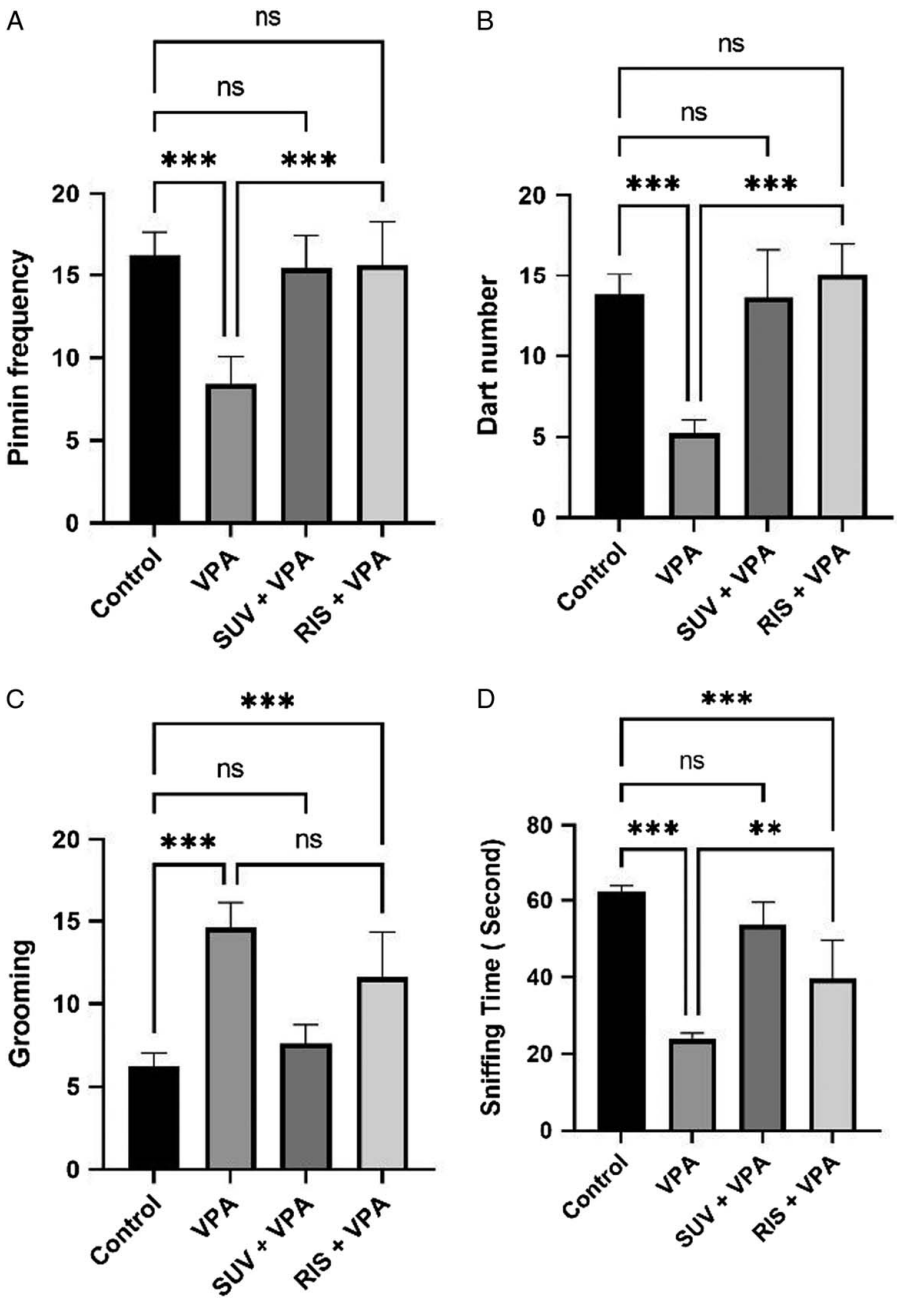

Figure 2. Effects of prenatal VPA treatment on pinning number (A), dart number (B), grooming (C) self grooming (D) and time spent sniffing the intruder, as Stereotype Behaviors in male rat offspring (n=8 for all groups); $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{*}P < 0.001$. The data are shown as the mean ± SEM. ns, not significant; RIS, risperidone; SUV, suvorexant; VPA, valproic acid.

increased repetitive behavior in the Y-maze task, which was ameliorated by postnatal administration of suvorexant and risperidone. Similarly, in a study conducted by Heidari *et al.*[28], risperidone decreased the repetitive behavior in Y-maze. In another research by Hara *et al.*[29], behavioral tests indicated that sniffing duration in VPA-exposed mice significantly decreased, and treatment with risperidone significantly alleviated social interaction by increasing the sniffing duration. These results show a correlation between orexin-B and orexin-A and the severity of ASD and support the involvement of orexinergic mechanisms in ASD[8]. Their finding fits in the context of current research in the field, which places dysregulations of the orexinergic at the core of social interaction deficits and stereotyped behavior in patients with ASDs. In addition, orexins can influence the process of attention and decision-making. In 2005, Lambe *et al.*[30] showed for the first time that administration of orexin-B significantly improved the performance of rats in attention task. Moreover, antagonist of OX1 receptors impaired the behavior of rats performing sustained attention tasks[31].

Some data suggest that orexin neurons receive abundant input from the limbic system, which suggests that orexins might regulate physiological responses to emotional and stressful stimuli[4]. However, in another report, the almorexant (DORA) decreased

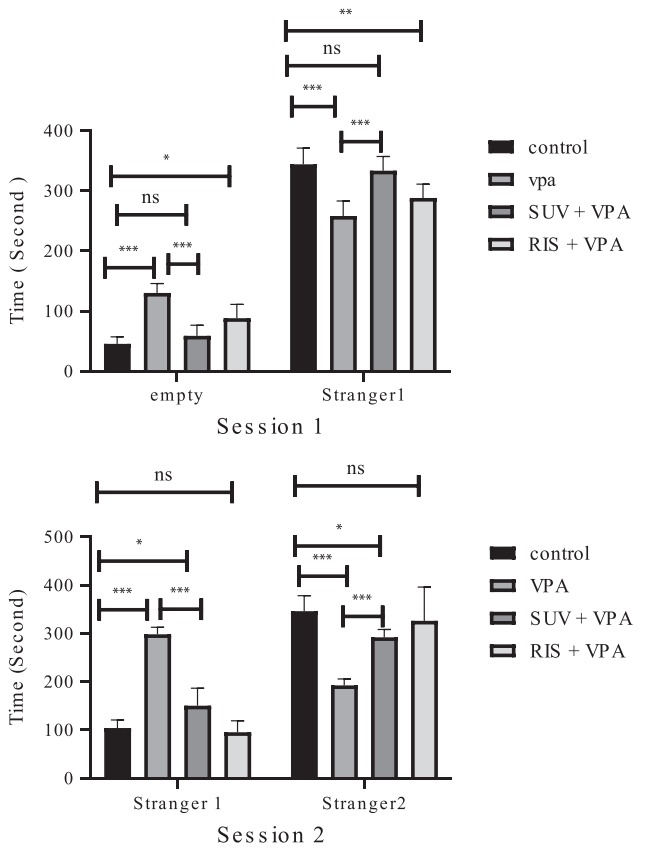

Figure 3. Session I: Social affiliation and sociability. The mean length of time (±SEM) in the chamber with the stranger (stranger side) compared to the opposite chamber (empty side). Session II: Social memory and novelty. The mean duration of time (±SEM) in the chamber with the unfamiliar mouse from the sociability phase (Stranger1) and in the opposite chamber with a new, unfamiliar rat (Stranger2) ($n=8$ for all groups); $^{*}P<0.05$, $^{**}P<0.01$, and $^{***}P<0.001$. The data are shown as the mean ± SEM. ns, not significant; RIS, risperidone; SUV, suvorexant; VPA, valproic acid.

the fear response to a conditioned fear cue[32]. A decrease in attention can ameliorate stereotyped behavior in Y-maze and also cause a decrease in social behaviors. In recent human studies, a polymorphism in the Orx-1 receptor gene has been associated with major mood disorders, and increased orexin peptide levels were correlated with positive emotions and social interaction[33]. Orexin neurons also project to other brain regions related to arousal and play a role in maintaining the activities of these neurons[34,35]. More importantly, the prefrontal cortex also widely receives direct orexinergic projections, and these fibers are essential in maintaining arousal and higher cognition functions[36,37]. The involvement of the frontal lobe in the neurobiology of ASD has long been documented in the literature[38]. Thus, the orexin system directly or indirectly interacts with other cortical neurons to play a role in cognition, which is important for accomplishing the neurobiology of autism.

Conclusion

In conclusion, our results provided experimental evidence that suvorexant can prevent autistic-like behavior in the VPA rat model of ASD. Such beneficial effects of suvorexant may support the involvement of orexinergic mechanisms in ASD.

Ethical approval

All the experiments were performed in accordance with the guidelines for laboratory animal use and care set by the Animal Ethics Committee of Damghan University with the ethical code (IR.DU.REC.1401.008). The work has been reported in line with the ARRIVE statement.

Consent

Not applicable.

Sources of funding

There is no funding for the present study.

Author contribution

F.P. and H.S.: study design and concept and drafting; I.G. and H. S.: literature search and performing the study.

Conflicts of interest disclosure

The authors declare no conflicts of interest.

Research registration unique identifying number (UIN)

Not applicable.

Guarantor

Hamid Sepehri.

Data availability statement

Available upon reasonable request.

Provenance and peer review

Not commissioned, externally peer-reviewed.

Acknowledgements

This paper is taken from a part of the first author's MSc thesis.

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