Behavioural Brain Research 311 (2016) 368–374

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Oxytocin reversed MK-801-induced social interaction and aggression deficits in zebrafish

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HIGHLIGHTS

- MK-801 decreased social preference in the interaction test.
- MK-801 promoted significant changes of aggressive behavior in the mirror test.
- Oxytocin reversed the effects of MK-801 on social preference.
- The oxytocin receptor agonist carbetocin reversed the effects of MK-801 in both tests.
- The oxytocin receptor antagonist L-386,899 did not modulate the effects of MK-801.

ARTICLE INFO

Article history:
Received 28 February 2016
Received in revised form 27 May 2016
Accepted 27 May 2016
Available online 28 May 2016

Keywords:
Aggression
MK-801
Oxytocin
Social behavior
Zebrafish

ABSTRACT

Changes in social behavior occur in several neuropsychiatric disorders such as schizophrenia and autism. The interaction between individuals is an essential aspect and an adaptive response of several species, among them the zebrafish. Oxytocin is a neuroendocrine hormone associated with social behavior. The aim of the present study was to investigate the effects of MK-801, a non-competitive antagonist of glutamate NMDA receptors, on social interaction and aggression in zebrafish. We also examined the modulation of those effects by oxytocin, the oxytocin receptor agonist carbetocin and the oxytocin receptor antagonist L-368,899. Our results showed that MK-801 induced a decrease in the time spent in the segment closest to the conspecific school and in the time spent in the segment nearest to the mirror image, suggesting an effect on social behavior. The treatment with oxytocin after the exposure to MK-801 was able to reestablish the time spent in the segment closest to the conspecific school, as well as the time spent in the segment nearest to the mirror image. In addition, in support of the role of the oxytocin pathway in modulating those responses, we showed that the oxytocin receptor agonist carbetocin reestablished the social and aggressive behavioral deficits induced by MK-801. However, the oxytocin receptor antagonist L-368,899 was not able to reverse the behavioral changes induced by MK-801. This study supports the critical role for NMDA receptors and the oxytocinergic system in the regulation of social behavior and aggression which may be relevant for the mechanisms associated to autism and schizophrenia.

1. Introduction

Neurodevelopmental disorders comprise a group of neuropsychiatric manifestations caused by disturbances in brain development, including autism spectrum disorders [1]. Similarly, schizophrenia has also been proposed as result of alterations in neurodevelopment, usually expressing only in the adult stage [2]. The symptoms of schizophrenia can be classified into cognitive (decreased attention, memory problems), negative (anhedonia, social withdrawal) or positive (hallucinations, disorganized behavior, high levels of aggression) types [1]. The autistic patients often also show symptoms, such as deficits in social communication and interaction, repetitive patterns of behavior, restricted interests, anxiety and hyperactivity [1,3]. Historically, autism and

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http://dx.doi.org/10.1016/j.bbr.2016.05.059
0166-4328/

schizophrenia were considered to be intimately related [4,5]. Several studies indicate that these diseases share overlapping characteristics [6]. A major feature of the clinical symptoms presented by both autism and schizophrenia is the impairment in social functions [7]. Furthermore, abnormal aggression levels are observed in human patients with psychiatric disorders, such as ASD [8] and SZ [9].

Aggression is an adaptive behavior that is essential for the establishment of social hierarchies, mating, competition for food and territory [10]. In this way, a certain level of aggression can be beneficial for survival of an individual or species, as well as have a social function. Evidence shows that zebrafish exhibit a rich repertoire of aggressive behavior and the neural mechanisms can be studied for understanding the core pathogenesis of aggression [11,12].

Zebrafish is a highly social species which plays an excellent role as a model organism to study neuropathological disorders that affect the social behavior, such as autism and schizophrenia [13,14]. Social interaction is a significant and complex aspect of zebrafish behavior [15,16]; however, the mechanisms that regulate social behavior in zebrafish are not well understood. Studies suggest that fish are suitable model to evaluate social behavior and its evolution, since the structure of the brain and physiology are conserved among vertebrates [17,18]. An extensive network of nuclei that is critical for social behavior is highly conserved within vertebrates [17,19]. Furthermore, the fish are able to perform complex decisions on social context [18].

A hypo-function of ionotropic *N*-methyl-D-aspartate (NMDA) receptors is implicated in schizophrenia [20,21]. Dizocilpine (MK-801), a non-competitive antagonist of the glutamate NMDA-receptor, has been most strongly implicated in social behavior in animal models and is applied to mimic some aspects of autism and schizophrenia [22–24]. Behavioral and pharmacological actions of NMDA appear to be conserved in zebrafish [25]. Several studies have shown that MK-801 induced deficit in the social interaction parameters in rodents and zebrafish [23,26].

The role of the neuropeptide oxytocin (OT) in the different types of social behavior (including aggression) is one of the earliest discoveries in social neuroscience [27,28]. Oxytocin is a promising molecule for the treatment of psychotic symptoms in patients with several brain disorders, including schizophrenia and autism [29,30]. Modahl et al. reported that the plasma concentration of oxytocin is reduced in children with autism [31]. Hollander et al. showed that intravenous administration of oxytocin reduces repetitive behaviors and increases the understanding of the emotional meaning in individuals with ASD [32]. Studies reported the improvement of social deficits by administering oxytocin to schizophrenic patients [33,34]. In fish, oxytocin has effects on courtship and social behavior [35,36]. Braida et al. demonstrated that oxytocin and vasopressin increased social behavior and reduced the fear response to predators, indicating a neuromodulatory role in these complex behaviors in zebrafish [37].

The aim of the present study was to investigate the changes in social behavior (specifically social preferences and aggression) produced by MK-801 in zebrafish. Since oxytocin can modulate social behavior, we also examined the effects of oxytocin, oxytocin receptor agonist (carbetocin) and the oxytocin receptor antagonist (L-368,899) on the reversal of behavioral effects on the MK-801-induced changes in social behavior.

2. Materials and methods

2.1. Animals

Adult (6–8 months old) wild-type zebrafish (*Danio rerio*) with Tübingen background used in this study were obtained from our breeding stock held at the Pontifícia Universidade Católica do Rio Grande do Sul. The animals were housed in a 50 L-thermostated aquarium filled with unchlorinated water constantly aerated at a targeted temperature of 26 ± 2 °C. Fish were kept under a 14–10 h light/dark cycle photoperiod (lights turned on at 8:00 a.m.) and fed twice a day with commercial flake fish food supplemented with live brine shrimp. The protocol was approved by the Ethics Committee of the Pontifícia Universidade Católica do Rio Grande do Sul (PUCRS) under the number 13/00346-CEUA.

2.2. Materials

The drugs dizolcipine maleate (MK-801), oxytocin, carbetocin, L-368,899 and tricaine were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.3. Pharmacological treatments

The animals was individually exposed in a 300-ml beaker to 5 μM MK-801, dissolved in tank water for 15 min. The control animals were individually submitted to tank water in similar conditions. To assess the effects on the reversal of the behavioral changes induced by MK-801, we conducted the anesthesia of the animals prior to the injection, which was-obtained by immersion in a 100 mg/L tricaine solution until the animal showed lack of motor coordination and reduced respiration rate. The animals received i.p. injection with 10 ng/kg oxytocin, 10 ng/kg carbetocin, 10 ng/kg L-368,899 or saline. After the injection, the animals were placed in a separate tank with highly aerated unchlorinated tap water (26 ± 2 °C) to facilitate their recovery from anesthesia. Intraperitoneal injections were conducted using a 3/10-mL U-100 BD Ultra-Fine™ Short Insulin Syringe 8 mm (5/16 in.) × 31 G Short Needle (Becton Dickinson and Company, New Jersey, USA). The same animals were transferred to another beaker containing water, and remained there for additional 15 min. After this period, the animals were individually placed in a test tank to analyze the social behavior. The experiments were performed between 10 a.m. and 3 p.m.

2.4. Behavioral assessment

2.4.1. Social interaction

The zebrafish is a schooling fish that may exhibit preference for its conspecifics under certain circumstances. Each fish was placed in an experimental tank (30 cm × 15 cm × 10 cm, length × height × width). On one side of the experimental tank, an empty fish tank was placed; on the other side, a tank of identical size held 15 zebrafish, which were designed the “stimulus fish”. The experimental fish was allowed to acclimate to the experimental tank for a 30 s period, after which behavior was video recorded over a period of 5 min. To quantify fish preference between the “stimulus fish” side of their tank in detriment of the empty tank, the experimental fish tank was divided in four equal sections. The zones 1 and 2 of the tank correspond to the segments closer to the conspecific school and the zones 3 and 4 are considered to the segments closer to the empty tank. The amount of time the experimental fish spent on each zone was measured using ANYMaze recording software (Stoelting Co., Wood Dale, IL, USA) [38].

2.4.2. Aggression

The mirror test was used to measure aggression [38]. Each fish was placed in an experimental tank (30 cm × 15 cm × 10 cm, length × height × width). A mirror (45 cm × 38 cm) was placed at the side of the tank at an angle of 22.5° to the backwall of the tank so that the left vertical edge of the mirror touched the side of the

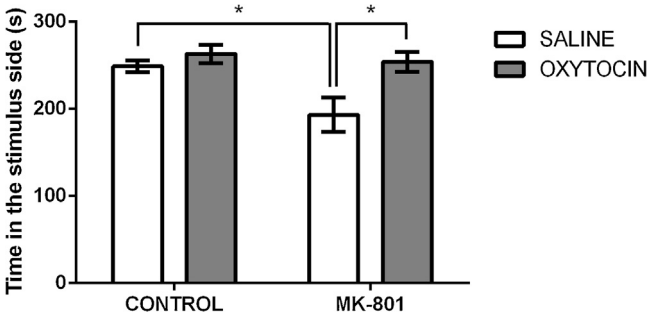

Fig. 1. Effects of oxytocin on MK-801-induced social interaction deficits in zebrafish. The data are expressed as the mean ± S.E.M. (n = 16 per group), and were analyzed by two-way ANOVA followed by Bonferroni's post-hoc test. The symbol * represents statistical difference when compared to the respective control group, P < 0.05.

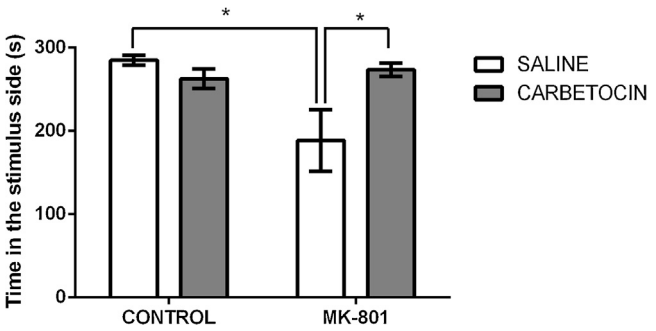

Fig. 2. Effects of carbetocin on MK-801-induced social interaction deficits in zebrafish. The data are expressed as the mean ± S.E.M. (n = 12 per group), and were analyzed by two-way ANOVA followed by Bonferroni's post-hoc test. The symbol * represents statistical difference when compared to the respective control group, P < 0.05.

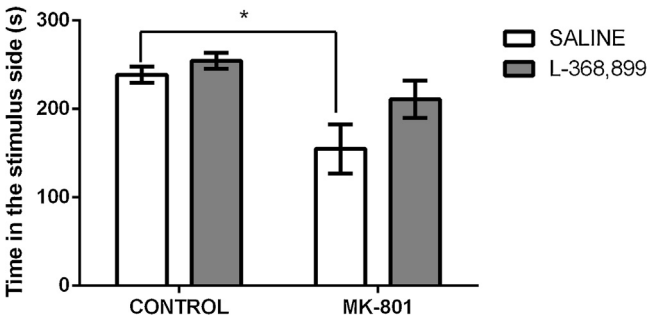

Fig. 3. Effects of L-368,899 on MK-801-induced social interaction deficits in zebrafish. The data are expressed as the mean ± S.E.M. (n = 14 per group), and were analyzed by two-way ANOVA followed by Bonferroni's post-hoc test. The symbol * represents statistical difference when compared to the respective control group, P < 0.05.

tank and the right edge was further away. Thus, when the experimental fish swam to the left side of the tank, their mirror image appeared closer to them. A test fish was added to the tank and was allowed acclimate for 60 s; the aggressive behaviors that a fish conducted toward its mirror image were subsequently recorded over a period of 5 min. The vertical lines divided the tank into four equal sections and allowed the number of entries to each section made by the fish to be counted. Entry to the leftmost segment indicated preference for proximity to the "opponent", whereas entry to the rightmost segments implied avoidance. The amount of time the experimental fish spent in each segment was measured using ANY-Maze recording software (Stoelting Co., Wood Dale, IL, USA).

2.5. Statistical analysis

The data are expressed as the mean ± S.E.M., and were analyzed by two-way analysis of variance (ANOVA) followed by Bonferroni's post hoc test. For all comparisons, the significance level was set at P < 0.05.

3. Results

3.1. Modulation with oxytocin on social interaction deficit induced by MK-801

Our results have shown that MK-801 treatment induces social interaction deficit in zebrafish. Fig. 1 shows the effect of oxytocin on MK-801-induced social interaction impairment in zebrafish. As expected, animals treated with MK-801 significantly decreased (193.1 ± 17.8 s) the time spent in the segment closest to the conspecific school compared to the control group (treated with saline and exposed to tank water) (248.8 ± 17.8 s). Animals treated with oxytocin *per se* were devoid of effects. However, when zebrafish were treated with oxytocin after MK-801 pretreatment spent a longer time (254 ± 20.9 s) in the segment closest to the conspecific school when compared to animals that were exposed to MK-801 after saline treatment (193.1 ± 17.8 s) (Fig. 1; pretreatment ($F_{(1,45)} = 4.8$, P = 0.03); treatment ($F_{(1,45)} = 6.5$, P = 0.01) and interaction ($F_{(1,45)} = 2.5$, P = 0.12)). *Post hoc* analyses indicated that the treatment with oxytocin reversed the effects of MK-801 in the social interaction test.

3.2. Effects of oxytocin receptor antagonist and agonist on social interaction response to MK-801

In order to investigate the effects of oxytocin receptor antagonist and agonist on social interaction response to 5 μM MK-801, we treated zebrafish with L-368,899 (10 ng/kg) or carbetocin (10 ng/kg), respectively, during 15 min after MK-801 exposure. Our results showed carbetocin (oxytocin receptor agonist) was able to reverse the MK-801-induced changes on the social interaction whereas the L-368,899 (oxytocin receptor antagonist) did not alter behavioral responses induced by MK-801. Animals treated with carbetocin after MK-801 treatment spent 273.4 ± 28.6 s in the segment closest to the conspecific school, while animals that were exposed to saline after MK-801 pretreatment spent 188.6 ± 28.6 s (Fig. 2). Control animals (treated with saline and exposed to tank water) and animals treated with carbetocin and exposed to tank water remained in the segment closest to the conspecific school during 285 ± 25.4 and 262.7 ± 25.4 s, respectively (Fig. 2; pretreatment ($F_{(1,39)} = 5.0$, P = 0.03); treatment ($F_{(1,39)} = 2.7$, P = 0.1) and interaction ($F_{(1,39)} = 7.9$, P = 0.008)). *Post hoc* analyses indicated that carbetocin was able to reverse the MK-801-induced changes on the social interaction. L-368,899 was not able to change behavioral responses induced by MK-801 pretreatment or when animals were pretreated with water (Fig. 3; pretreatment ($F_{(1,44)} = 12.3$, P = 0.001); treatment ($F_{(1,44)} = 3.9$, P = 0.054) and interaction ($F_{(1,44)} = 1.2$, P = 0.27)).

3.3. MK-801 induces changes in aggressive behavior

The MK-801 promoted significant changes in the aggressive behavior, which was evaluated by the time spent in the segment nearest to the mirror image. Our results demonstrated that animals treated with MK-801 remained less time in the segment nearest to the mirror (36.6 ± 5.4 s) when compared with the control group (89.1 ± 16.8 s) (P = 0.005), indicating that treatment with MK-801 induced a decrease in aggressive behavior (Fig. 4).

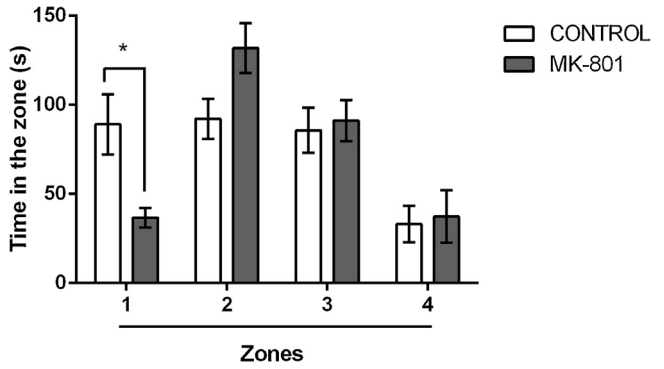

Fig. 4. Effects of MK-801-induced aggression deficits in zebrafish. The data are expressed as the mean ± S.E.M. (n = 24 per group), and were analyzed by two-way ANOVA followed by Bonferroni's post-hoc test. The symbol * represents statistical difference when compared to the respective control group, P < 0.05.

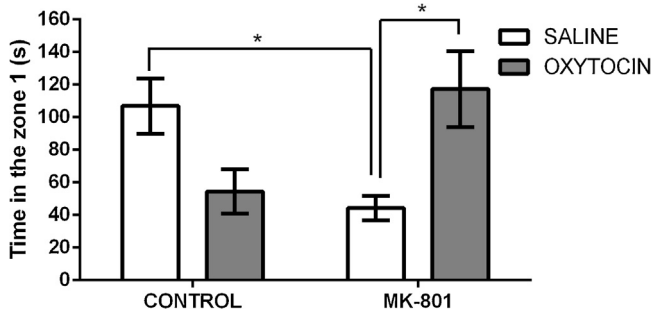

Fig. 5. Effects of oxytocin on MK-801-induced aggression deficits in zebrafish. The data are expressed as the mean ± S.E.M. (n = 15 per group), and were analyzed by two-way ANOVA followed by Bonferroni's post-hoc test. The symbol * represents statistical difference when compared to the respective control group, P < 0.05.

3.4. Modulation with oxytocin on aggressive behavior deficit induced by MK-801

The results showed that MK-801 treatment induced decrease in aggressive behavior in zebrafish. Fig. 5 demonstrates the effect of oxytocin on MK-801-induced aggressive behavior impairment in zebrafish. Animals treated with MK-801 significantly decreased (44.4 ± 22.3 s) the time spent in the segment nearest to the mirror image compared to the control group (treated with saline and exposed to tank water) (107 ± 22.3 s). Animals treated with oxytocin *per se* were devoid of effects; treated animals spent 54.4 ± 22.3 s in the segment nearest to the mirror image school, while animals that were exposed water after saline treatment spent 106.9 ± 22.3 s. However, zebrafish treated with oxytocin after MK-801 pretreatment spent 117.3 ± 22.3 s in the segment nearest to the mirror image school, while animals that were exposed to MK-801 after saline treatment spent 44.4 ± 22.3 s (Fig. 5; pretreatment ($F(1,52) = 0.0001$, $P = 0.99$); treatment ($F(1,52) = 0.42$, $P = 0.52$) and interaction ($F(1,52) = 15.7$, $P = 0.0002$)). *Post hoc* analyses indicated that the treatment with oxytocin reversed the effects of MK-801 in the aggressive behavior test.

3.5. Effects of oxytocin receptor antagonist and agonist on aggressive behavior response to MK-801

In order to investigate the effects of oxytocin receptor agonist and antagonist on aggressive behavior to 5 μM MK-801, we treated zebrafish with carbetocin (10 ng/kg) and L-368,899 (10 ng/kg), respectively, during 15 min after MK-801 exposure. Our results have shown that the oxytocin receptor agonist carbetocin was able to reverse the aggressive behavior changes induced by MK-801 pretreatment and the oxytocin receptor antagonist L-368,899 did not change behavioral responses induced by MK-801 pretreatment. Animals treated with carbetocin after MK-801 treatment spent 91.6 ± 20 s in the segment nearest to the mirror image, while animals that were exposed to saline after MK-801 pretreatment spent 35.3 ± 15 s. Animals treated with carbetocin and exposed to tank water significantly decreased (62 ± 15 s) the time in the segment nearest to the mirror image compared to the control group (127.5 ± 15 s) (Fig. 6; pretreatment ($F(1,50) = 5.9$, $P = 0.01$); treatment ($F(1,50) = 0.14$, $P = 0.7$) and interaction ($F(1,50) = 22.8$, $P = 0.0001$)). *Post hoc* analyses indicated that carbetocin is able to reverse the aggressive behavior changes induced by MK-801 pretreatment.

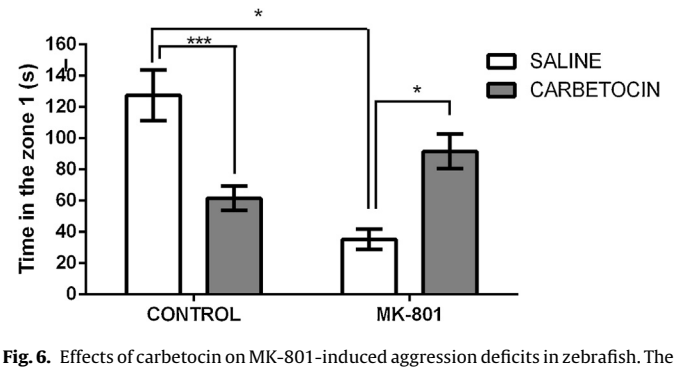

Fig. 6. Effects of carbetocin on MK-801-induced aggression deficits in zebrafish. The data are expressed as the mean ± S.E.M. (n = 14 per group), and were analyzed by two-way ANOVA followed by Bonferroni's post-hoc test. The symbol * represents statistical difference when compared to the respective control group, P < 0.05 and ***P < 0.0001.

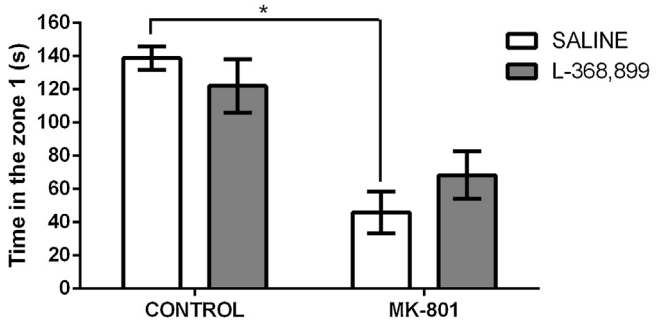

Fig. 7. Effects of L-368,899 on MK-801-induced aggression deficits in zebrafish. The data are expressed as the mean ± S.E.M. (n = 10 per group), and were analyzed by two-way ANOVA followed by Bonferroni's post-hoc test. The symbol * represents statistical difference when compared to the respective control group, P < 0.05.

The oxytocin receptor antagonist L-368,899 was not able to change behavioral responses induced by MK-801 pretreatment (Fig. 7; pretreatment ($F(1,30) = 26.1$, $P = 0.0001$); treatment ($F(1,30) = 0.04$, $P = 0.84$) and of pretreatment x treatment interaction ($F(1,30) = 1.9$, $P = 0.18$)).

4. Discussion

In this study, our results have shown that MK-801 induced a decrease in the time spent in the segment closest to the conspecific school and in the time spent in the segment nearest to the mirror image, suggesting an effect on social behavior. The treatment with oxytocin after the exposure to MK-801 showed that oxytocin was able to reestablish the time spent in the segment closest to the conspecific school, as well as the time spent in the segment nearest to the mirror image. To support the modulation of oxytocin pathway, we verified that the oxytocin receptor agonist carbetocin recovered the social and aggressive behavioral patterns.

However, the oxytocin receptor antagonist L-368,899 was not able to reverse the time spent in the segment closest to the conspecific school as well as the time spent in the segment nearest to the mirror image. Neuropsychiatric disorders, such as autism and schizophrenia, share an important feature of clinical symptoms, such as impairment in social functions [7,39]. Schizophrenia and autism are considered complex diseases with multiple factors contributing to pathogenesis [40,41]. Traditional medicines currently available are not effective, since they deal with only some of the symptoms.

MK-801 is one of the NMDA receptor antagonists, and this agent has been used for inducing schizophrenia and autism symptoms [23,42,43]. Of the three subtypes of receptors for glutamate, the NMDA receptor has been most strongly implicated in social behavior [44–46]. The effect of MK-801 on social behavior was induced in different animal models [23,47]. Several studies have shown social behavior changes due to NMDA receptor antagonists in rodents and zebrafish [23,43,47]. Moy et al. showed that MK-801 led to a loss of significant social preference measured by the time spent in each side of the chamber [43]. The neurobehavioral actions of NMDA receptor antagonism are conserved in zebrafish [25]. In zebrafish, the impact of MK-801 on social behavior has already been described. According to Maaswinkel et al., MK-801-treated zebrafish reduced social cohesion of the entire shoal [24]. Seibt et al. demonstrated that MK-801 reduced the preference of zebrafish for a stimulus group of zebrafish and Echevarria et al. reported disrupted shoaling [23,48]. Although there are studies evaluating the effect of MK-801 on social interaction, its effects on aggressive behavior are unclear. The neurobiology of aggressive behavior involves a complex network [49]. McAllister found that MK-801, among other compounds, tends to increase aggression and social behavior in mice [50]. In contrast to these previous findings and in agreement to our study, Kalinine et al. demonstrated that a single intraperitoneal dose of the MK-801 shortly before the intruder test decreased aggressive behavior in mice [51]. Our results showed that MK-801 induced deficit in social interaction and decreased aggressive behavior since animals exposed to MK-801 spent less time in the segment closest to the conspecific school as well as in the segment nearest to the mirror image.

Evidence suggests that the deregulation of oxytocinergic system may be involved in the pathophysiology of certain neuropsychiatric disorders, such as autism and schizophrenia [52,53]. The oxytocinergic system plays a crucial role in several and complex social behaviors and social interaction [54,55]. The neuropeptide oxytocin is involved in the modulation of different aspects of social behavior.

In addition to its vital function as a hormone, oxytocin also acts on an important central network as neurotransmitter peptide [56]. Most oxytocin neurons project to the posterior pituitary but some also project within the central nervous system and the endogenous central oxytocin plays a physiologically significant role in social behavior [57].

Considering that there is no standard treatment for social dysfunction and that clinical studies have identified oxytocin as a potential therapeutics, we investigated the effects of oxytocin, an oxytocin receptor agonist carbetocin and an oxytocin receptor antagonist L-368,899 in MK-801-induced changes on social interaction and aggression. Our data demonstrated that the treatment with oxytocin after the exposure to MK-801 was able to reestablish the time spent in the segment closest to the conspecific school as well as the time spent in the segment nearest to the mirror image. In addition, we verified that the oxytocin receptor agonist carbetocin reestablished the social and aggressive behavioral patterns whereas and oxytocin receptor antagonist L-368,899 was not able to reverse the decrease in the social interaction and aggression induced by MK-801. However, our results did not show any significant effect on the social preference of zebrafish exposed only to oxytocin, carbetocin, and L-368,899 in relation to control group. There are few studies assessing the effect of oxytocin receptor pharmacologically in relation to social behavior and aggression. Mooney et al. showed that oxytocin increases the social behavior and these effects were blocked by co-administration of oxytocin antagonist [58]. Similarly, Goodson et al. found that peripheral oxytocin antagonist administration decreases the time spent in close proximity both to a familiar cagemate and to a larger group of conspecifics in zebra finches [59]. Modahl et al. observed a significant correlation between oxytocin levels and social impairment in children [31]. Hollander et al. verified that oxytocin administration facilitated the processing and retention of social information in adults diagnosed with autism or Asperger's disorder [32]. Other study suggests that oxytocin may relieve the positive symptoms and reduce social and cognitive deficits in schizophrenic patients [29]. It has been demonstrated that blocking central oxytocin receptors in primates disrupts parental-like behavior and female sexual behavior [60]. Furthermore, Suraev et al. demonstrated that oxytocin, but not agonist [Thr4, Gly7]-oxytocin, significantly increased the amount of time spent close to a social stimulus in social preference test performed in late adolescence in rodents [61]. Behavioral studies employing a similar approach indicated that oxytocin treatment can increase sociability in rodents and zebrafish [37,62]. Braida et al. demonstrated that the oxytocin increased social preference in zebrafish and the antagonists dose-dependently inhibited the effect induced by the neuropeptides [37]. The lack of effect observed for oxytocin, carbetocin, or L-368,899 in social interaction in zebrafish may be due to differences in the doses, time and route of administration when compared with studies in other species. However, despite these compounds *per se* did not change the social behavior, oxytocin and carbetocin in the doses tested were able to revert the deficit in social behavior induced by MK-801, supporting a modulatory role of oxytocinergic system after the blockade of NMDA receptors.

At the present moment, there are no studies showing the effect of oxytocin in aggressive behavior in zebrafish; however, Filby et al. demonstrated that treatment with arginine vasotocin (AVT) (which is found in fishes and a key modulator of social and nonsocial behavior, similar to oxytocin) significantly reduced aggression in dominant male zebrafish [63]. Furthermore, studies demonstrated that oxytocin has also been linked to social dominance and aggression in rodents [64,65]. The potent effects of intranasal oxytocin suggest that it may be beneficial for a variety of psychiatric disorders, including schizophrenia and autism [32,66,67].

The oxytocin receptors are also highly conserved in evolution. In this way, to test whether the effects on social behavior and aggression were mediated by oxytocin receptor in zebrafish, we tested the oxytocin receptor antagonist (L-368,899) and agonist (carbetocin). Our findings indicated that the oxytocin receptor antagonist (L-368,899) was unable to reverse the effect induced by MK-801 on social interaction and aggression. On the other hand, oxytocin receptor agonist (carbetocin) reversed the effects caused by MK-801 in social behavior and aggression. Thus, we suggest that these modulatory effects may be mediated by oxytocin. Interestingly, our results demonstrated carbetocin per se was able to decrease the aggressive behavior in zebrafish while oxytocin treatment presented a trend to a similar effect, suggesting that activation of oxytocin receptors may reduce natural aggressive responses in zebrafish. In relation to aggression, Calcagnoli et al. reported an increased offensive aggression in low aggressive residents after icv administration of a selective oxytocin receptor antagonist [68]. However, Calcagnoli et al. showed that anti-aggressive and pro-social changes were entirely blocked when the binding of exogenous oxytocin to the oxytocin receptors was impeded by pretreatment with a selective oxytocin receptors antagonist [69]. Therefore, our findings demonstrated the involvement of oxytocin

and oxytocin receptor in the control of natural aggressive responses in zebrafish as well as abolishing the impaired aggression induced by MK-801.

In summary, our results support the critical role for NMDA receptors and the oxytocinergic system in the regulation of social behavior and aggression, which may be relevant for the mechanisms associated to autism and schizophrenia.

Acknowledgments

This work was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), and Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS). A.M.S and C.D.B. were recipients of fellowships from CNPq. F.F.Z and K.V.G. were the recipients of fellowships from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and FAPERGS, respectively

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