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The role of purinergic and dopaminergic systems on MK-801-induced antidepressant effects in zebrafish

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ABSTRACT

Depression is a serious disease characterized by low mood, anhedonia, loss of interest in daily activities, appetite and sleep disturbances, reduced concentration, and psychomotor agitation. There is a growing interest in NMDA antagonists as a promising target for the development of new antidepressants. Considering that purinergic and dopaminergic systems are involved in depression and anxiety states, we characterized the role of these signaling pathways on MK-801-induced antidepressant effects in zebrafish. Animals treated with MK-801 at the doses of 5, 10, 15, or 20 μM during 15, 30, or 60 min spent longer time in the top area of aquariums in comparison to control group, indicating an anxiolytic/antidepressant effect induced by this drug. Animals treated with MK-801 spent longer time period at top area until 2 (5 μM MK-801) and 4 (20 μM MK-801) hours after treatment, returning to basal levels from 24 h to 7 days after exposure. Repeated MK-801 treatment did not induce cumulative effects, since animals treated daily during 7 days had the same behavioral response pattern observed since the first until the 7th day. In order to investigate the effects of adenosine A_1 and A_{2A} receptor antagonist and agonist and the influence of modulation of adenosine levels on MK-801 effects, we treated zebrafish with caffeine, DPCPX, CPA, ZM 241385, CGS 21680, AMPCP, EHNA, dipyridamole, and NBTI during 30 min before MK-801 exposure. The non-specific adenosine receptor antagonist caffeine (50 mg/kg) and the selective A_1 receptor antagonist DPCPX (15 mg/kg) prevented the behavioral changes induced by MK-801. The non-specific nucleoside transporter (NT) inhibitor dipyridamole (10 mg/kg) exacerbated the behavioral changes induced by MK-801. Dopamine receptor antagonists (sulpiride and SCH 23390) did not change the behavioral alterations induced by MK-801. Our findings demonstrated that antidepressant-like effects of MK-801 in zebrafish are mediated through adenosine A_1 receptor activation.

1. Introduction

Depression is a multifactorial disorder resulting from the interaction of genetic, epigenetic, and environmental episodes of negative mood that occur for more than two weeks (Hirschfeld, 2014). This is a serious disease, characterized by low mood, anhedonia, loss of interest in daily activities, and other symptoms. The main symptoms of depression are appetite disturbances to gain or weight loss, insomnia or hypersomnia, reduced concentration, and psychomotor agitation (Haenisch and Bonisch, 2011). Recently, the treatment of depression has used selective serotonin reuptake inhibitors (SSRIs) and serotonin–norepinephrine reuptake inhibitors (SNRI). However, these drugs have the drawbacks that they can take weeks before showing the effects of altered mood, and only 10–20% of patients show clinical effects beyond those associated with placebo (Zarate et al., 2013). Therefore, the need for rapid acting antidepressants is widely recognized. There is a growing interest in glutamate mechanisms in major depressive disorder (MDD) as a promising target for the development of new antidepressants. Studies have shown that a single intravenous infusion of ketamine, a N-methyl-D-aspartate (NMDA) receptor antagonist anesthetic agent, can alleviate depressive symptoms in patients within hours of administration (Berman et al., 2000). Zarrindast et al. (2012) also showed that the anxiolytic behavior induced by MK-801 affects locomotion, learning, and memory. The mechanism of action appears to be in part through glutamate release onto non-NMDA receptors including α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and metabotropic receptors. However, other signaling pathways such as dopaminergic, serotonergic, and mammalian target of rapamycin (mTOR) pathways seem to be involved in such effects (Dutta et al., 2015).

The purinergic system has been linked to the etiology of mood disorders via alterations in ATP and adenosine signaling as well as through inherited patterns and the neuroinflammatory hypothesis (for review,

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see Ortiz et al., 2015). It is known that adenosine signaling has a neuromodulatory role, which is involved in the regulation of important mechanisms in the CNS, such as anxiety states (Csölle et al., 2013), sleep (Huang et al., 2011; Carús-Cadavieco and de Andrés, 2012), motor function, cognition and memory (Salamone and Correa, 2009; Shen et al., 2012; Wei et al., 2011), among others. Adenosine receptors have been subdivided into four subtypes: A_1, A_{2A}, A_{2B}, and A_3. After chronic mild stress, the increase in A_1 and A_{2A} adenosine receptor binding in rat hippocampus and striatum was linked with depressive symptoms (Crema et al., 2013). Interestingly, adenosine and its metabolite inosine reduced immobility time in the forced swim test and tail suspension test in mice (Kaster et al., 2004, 2013). The A_1 receptor agonist, CHA (*N*6-cyclohexyladenosine) and A_{2A} receptor agonist DMPA (*N*6-[2-(3,5-dimethoxyphenyl)-2-(methylphenyl)ethyl]adenosine) demonstrated antidepressant effects in the forced swim test in mice (Kaster et al., 2004). Interestingly, the A_{2A} receptor antagonists preladenant and SCH 412348 had similar antidepressant effects in the tail suspension test (Hodgson et al., 2009). Extracellular adenosine levels are regulated by its production from AMP hydrolysis promoted by ecto-5′-nucleotidase (e5NT) or by its degradation into inosine by adenosine deaminase (ADA) (Bonan, 2012). Studies have shown e5NT knockout mice lack non-REM sleep responses to sleep deprivation, but no changes in anxiety-like behaviors were evident in animals (Zlomuzica et al., 2013). Adenosine can also be transported via nucleoside transporters (NTs) in bidirectional equilibrative processes controlled by chemical gradients and unidirectional concentrative processes mediated by sodium electrochemical gradients (Ipata et al., 2011; Bonan, 2012). The adenosine deaminase inhibitor EHNA (erythro-9-(2-hydroxy-3-nonyl) adenine) had antidepressant-like effects in the forced swim test (Kaster et al., 2013), which were inhibited by adenosine receptor antagonists.

The reciprocal antagonistic interaction of adenosine and dopamine receptors has been widely studied in motor function and anxiety (Coelho et al., 2014; Jenner, 2014). Dysfunction of the dopaminergic signaling is associated with several pathologies, such as Parkinson's disease, schizophrenia, depression, and attention deficit hyperactivity disorder (ADHD) (Bowton et al., 2010; Iversen and Iversen, 2007; Missale et al., 1998). The physiological actions mediated by dopamine depend on five distinct G-protein coupled receptors: D1, D2, D3, D4, and D5. Dopamine receptors can be divided into D1-like (D1, D5) and D2-like (D2, D3, D4) receptors (Beaulieu and Gainetdinov, 2011). The activation of dopamine D1-like receptors activates adenylate cyclase and increases cAMP, while the D2-like receptors exert a negative influence on the adenylate cyclase activity and decreases cAMP (Missale et al., 1998). Evidence has suggested that dysregulation of dopaminergic system is implicated in the pathogenesis of depression (Willner et al., 2005; Papakostas, 2006). An important role for dopamine D1/D5 receptor in mediating antidepressant drugs in the forced swim test (FST) (Shimazu et al., 2005) has been described whereas other studies demonstrated that dopamine D2/D3 receptor but not dopamine D1/D5

Table 1
Pretreatments with adenosinergic and dopaminergic drugs on MK-801 antidepressant effects.

Drug	Doses	Vehicle	Reference
DPCPX	15 mg/kg	DMSO 0.08%	Southam et al. (2002)
CPA	1 mg/kg	Saline	Mareš (2010)
ZM 241385	10 μg/kg	DMSO 0.08%	Mareš (2010)
CGS 21680	1 mg/kg	DMSO 0.08%	Mareš (2010)
EHNA	100 μg/kg	Saline	Southam et al. (2002)
AMPCP	100 mg/kg	Saline	Siebel et al. (2015)
Caffeine	50 mg/kg	Saline	Bortolotto et al. (2015)
Dipyridamole	10 mg/kg	DMSO 0.08%	Siebel et al. (2015)
NBTI	15 mg/kg	DMSO 0.08%	Siebel et al. (2015)
Sulpiride	50 mg/kg	Saline	Zarrindast et al. (2012)
SCH 23390	15 μg/kg	Saline	Zarrindast et al. (2012)

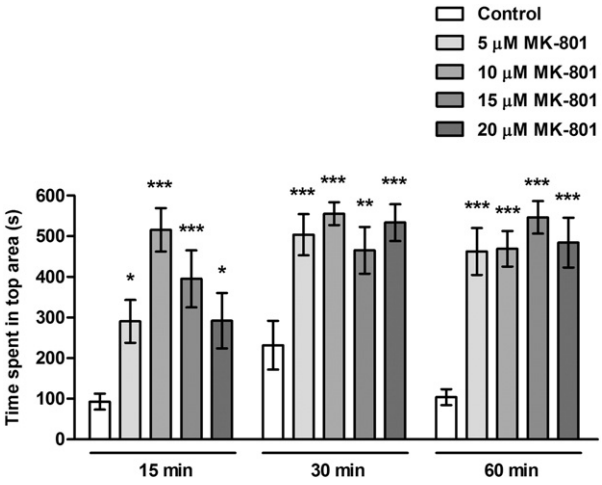

Fig. 1. Time and dose course of MK-801-induced behavioral response in zebrafish. Animals were exposed to MK-801 at 5, 10, 15, and 20 μM during 15, 30, and 60 min. MK-801-induced behavioral response was measured as time spent in top area of the tank. The data are expressed as the mean ± S.E.M. (n = 16 per group), and were analyzed by two-way ANOVA followed by Bonferroni's post-hoc test. The symbols represent statistical difference when compared to the respective vehicle control group. * $P < 0.05$, ** $P < 0.005$, *** $P < 0.0005$.

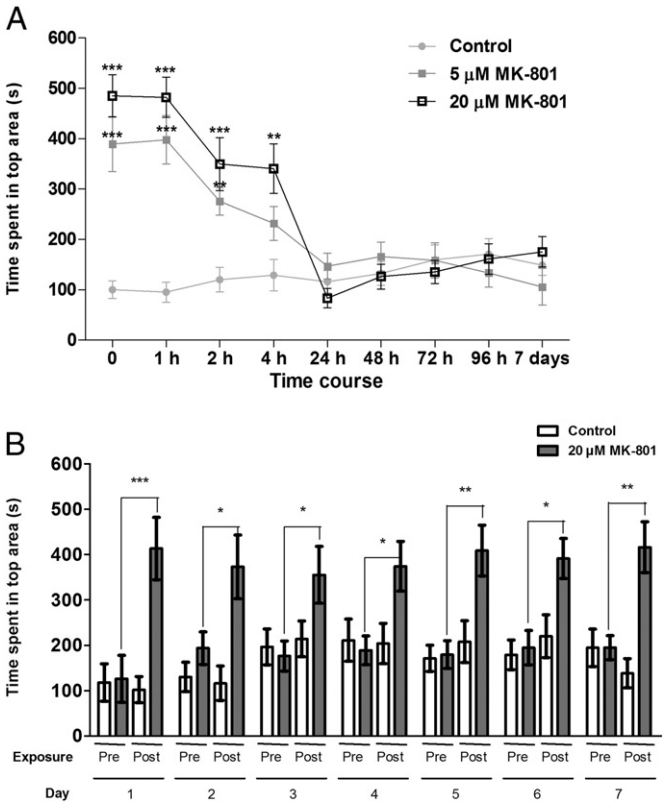

Fig. 2. MK-801-induced behavioral response in zebrafish. MK-801-induced behavioral response was measured as time spent in top area of the tank. (A) Duration of MK-801 action in zebrafish. Animals were exposed to MK-801 at 5 and 20 μM during 30 min. Behavioral parameters were analyzed in different time segments: immediately after and, 1, 2, 4, 24, 48, 72, 96 h and 7 days after treatment. (B) Effects of repeated doses of MK-801 in zebrafish. Animals were daily exposed to MK-801 at 20 μM during 30 min. Behavioral parameters were analyzed before and after MK-801 exposure. 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 symbols represent statistical difference when compared to the respective vehicle control group. * $P < 0.05$, ** $P < 0.005$, *** $P < 0.0005$.

receptor antagonist blocked the effect of antidepressant in this model (Borsini et al., 1988).

Zebrafish (*Danio rerio*) has emerged as a powerful model for behavioral studies, including aggression, anxiety, learning, memory, and shoaling and has been used as a model for studying several human diseases (Stewart et al., 2014; Bonan and Norton, 2015). Furthermore, this species has a high degree of similarity with genes from humans and rodents (Howe et al., 2013). The identification and characterization of purinergic and dopaminergic neurotransmission systems have been performed in zebrafish (Rico et al., 2011; Boehmler et al., 2004, 2007, 2009; Capiotti et al., 2011). In zebrafish, the antagonism of NMDA receptor by MK-801 increases the swimming activity and affects the preference for environment (Swain et al., 2004). In addition, MK-801 impaired memory in Y-Maze and avoidance tasks as well as induced a social interaction deficit (Cognato et al., 2012; Seibt et al., 2011). Interestingly, it is important to mention that MK-801 increases the time that the fish remained on top of the tank, indicating a possible antidepressant-like effect of MK-801 (Seibt et al., 2010).

Considering that the clinically-used antidepressant drugs are not able to provide satisfying control of depression symptoms in a large number of patients and that purinergic and dopaminergic systems are involved in depression and anxiety states, this study aims to characterize the role of signaling pathways on the MK-801-induced antidepressant-like effects.

2. Materials and methods

2.1. Animals

Adult (6–8 months old) wild-type zebrafish (*D. rerio*) 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 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 13/00371-CEUA.

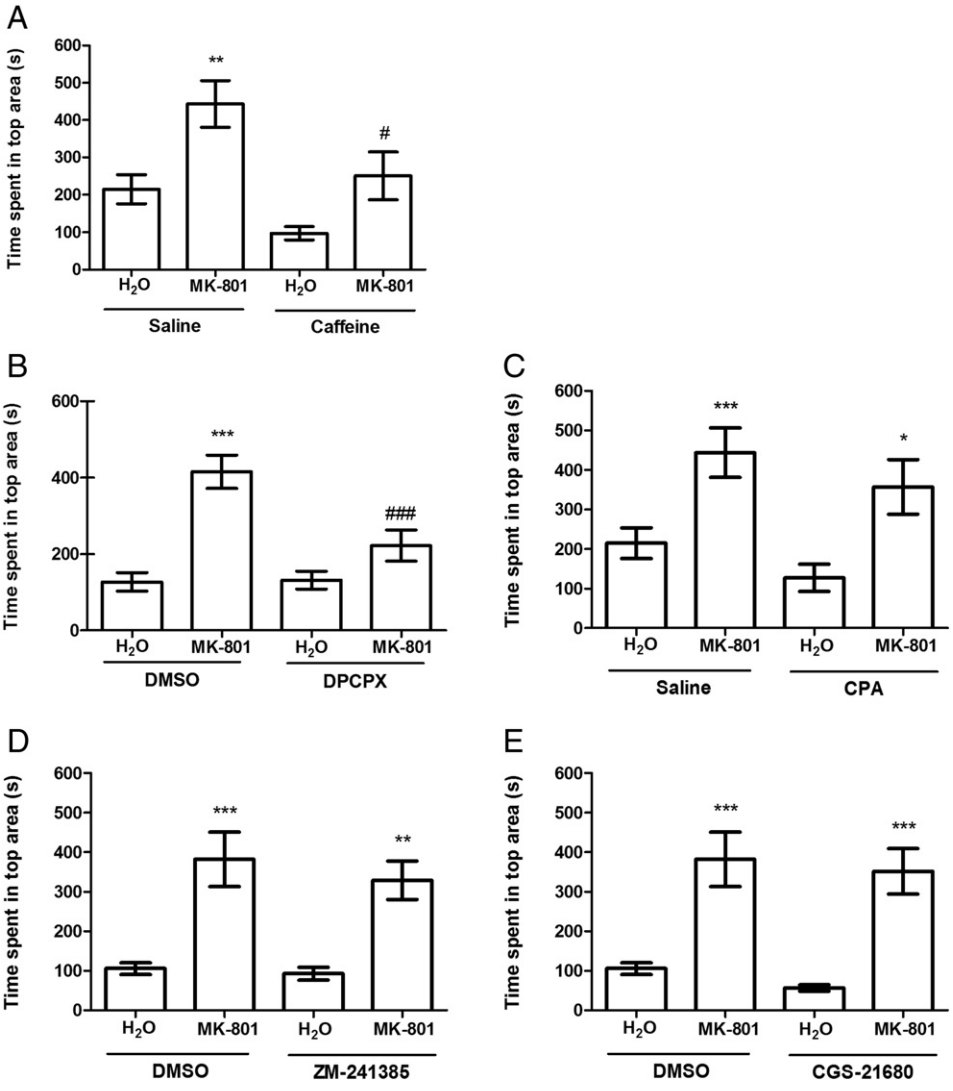

Fig. 3. Effects of caffeine (A), DPCPX (B), CPA (C), ZM 241385 (D), and CGS 21680 (E) pretreatments on MK-801-induced behavioral alterations in zebrafish. Animals were exposed to 20 μM MK-801 (during 30 min) immediately after pretreatments administration. Behavioral response was measured as time spent in top area of the tank during 10 min. The data are expressed as the mean ± S.E.M. (n = 16–26 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 vehicle control group. The symbol # represents statistical difference when compared to the respective vehicle control group. * $P < 0.05$, ** $P < 0.005$, *** $P < 0.0005$, # $P < 0.05$, ### $P < 0.0005$.

2.2. Materials

The drugs hydrogen maleate (MK-801), caffeine, dipyridamole, erythro-9-(2-hydroxy-3-nonyl-adenine) (EHNA), 8-cyclopentyl-1,3-dipropylxanthine (DPCPX), cyclopentyladenosine (CPA), *S*-(4-Nitrobenzyl)-6-thioinosine (NBTI), CGS 21680 hydrochloride hydrate, α,β-methylene adenosine 5′-diphosphate (AMPCP), sulpiride, SCH 23390 hydrochloride and tricaine were purchased from Sigma (St. Louis, MO, USA). ZM 241385 was purchased from Tocris Bioscience (Ellisville, MO, USA).

2.3. Patterning of MK-801 concentration and time treatment

In order to establish the MK-801 treatment, concentration and time treatment were determined. Zebrafish were individually exposed to MK-801 at different concentrations (5, 10, 15, or 20 μM) by their immersion in 300 mL beakers for different exposure periods: 15, 30, or 60 min. Immediately after the MK-801 exposure, animals were individually placed in an open-field tank in order to analyze the locomotor behavior. The animals behavior was registered by a video camera for 10 min and further analyzed using ANY-Maze recording software (Stoelting Co., Wood Dale, IL, USA). Control animals were placed in tank water, treated and analyzed in an identical manner.

2.4. Duration of MK-801 treatment effects

In order to determine the duration of MK-801 effects, the behavior of treated animals was analyzed along different time-segments. Zebrafish were individually exposed to 20 μM MK-801 for 30 min and had their behavior analyzed at different periods: immediately after MK-801 exposure, 1, 2, 4, 24, 48, 72, 96 h and 7 days later. The animals behavior in each time segment was registered by a video camera for 10 min and further analyzed using ANY-Maze recording software (Stoelting Co., Wood Dale, IL, USA). Control animals were placed in tank water and were treated and analyzed in an identical manner.

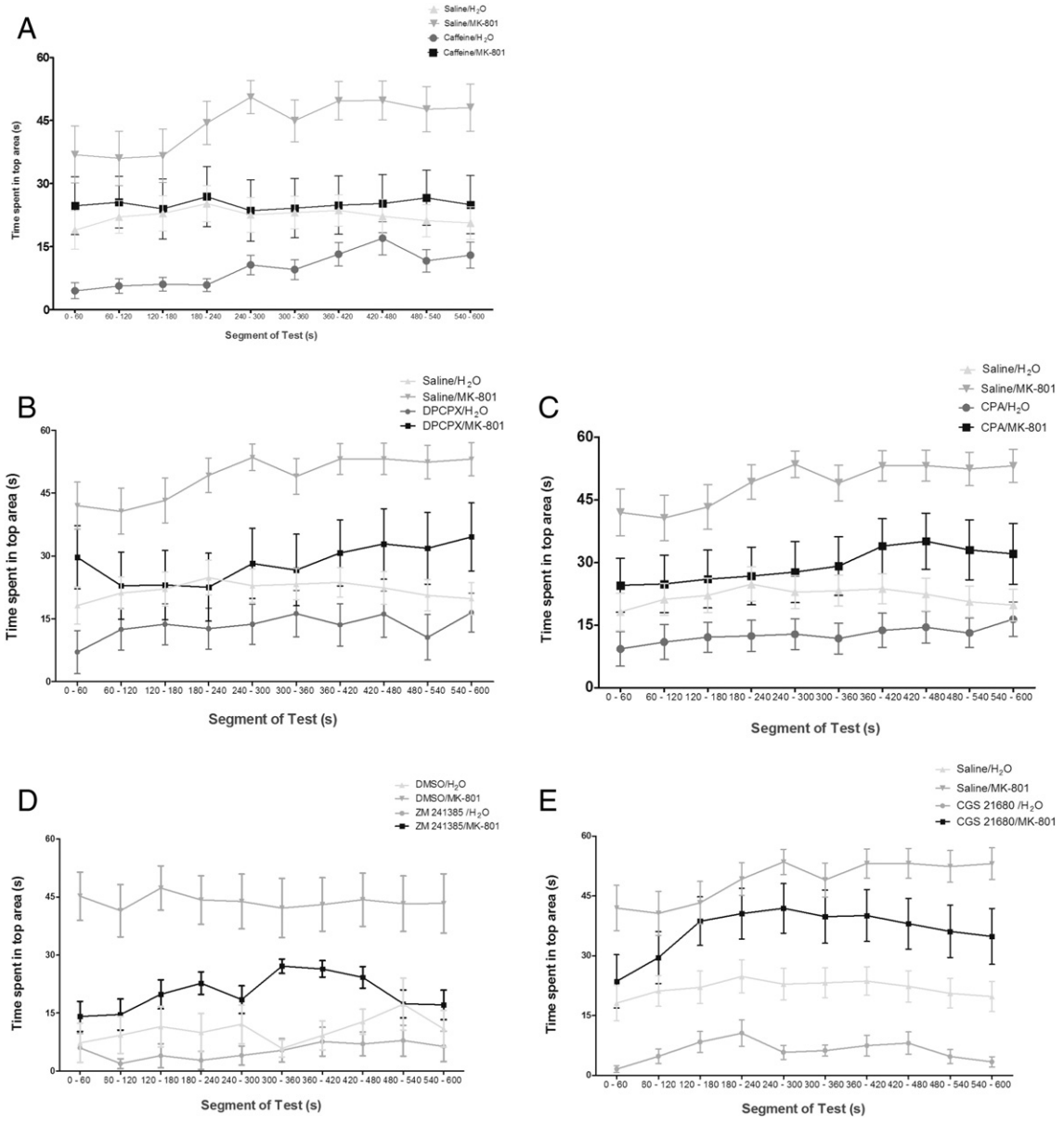

Fig. 4. Effects of caffeine (A), DPCPX (B), CPA (C), ZM 241385 (D), and CGS 21680 (E) pretreatments on MK-801-induced behavioral alterations in zebrafish. Animals were exposed to 20 μM MK-801 (during 30 min) immediately after pretreatments administration. The behavioral response was measured as time spent in top area of the tank for each 60 s. The data are expressed as the mean ± S.E.M. (n = 16–26 per group), and were analyzed by two-way ANOVA followed by Bonferroni's post-hoc test.

2.5. MK-801 cumulative effects

In order to investigate the effects of repeated dosing, zebrafish were treated with 20 μM MK-801 during 7 days. Zebrafish were daily exposed to 20 μM MK-801 or tank water (control group) during 30 min. The animals behavior was recorded during 10 min before (to verify possible changes due to adaptation to the environment) and 10 min after MK-801 exposure at each day. Control animals were placed in tank water and were treated and analyzed in an identical manner.

2.6. Pharmacological pretreatments and MK-801 treatment

To assess the effects of dopaminergic and adenosinergic signaling on MK-801 antidepressant-like effects, animals were pretreated with different adenosinergic and dopaminergic modulators. All drugs were injected intraperitoneally (i.p.) 30 min before the 20 μM MK-801 exposure. The AMPCP, caffeine, CPA, EHNA, sulpiride, SCH 23390 (diluted in saline), dipyridamole, CGS 21680 hydrochloride hydrate, ZM 241385, DPCPX, and NBTI (diluted in DMSO 0.08%) doses were selected based on preliminary studies conducted in our laboratory (Table 1). 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). Anesthesia of the animals prior to the injection was obtained by its immersion in a 100 mg/L tricaine solution until the animal showed lack of motor coordination and reduced respiration rate. 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. Thirty minutes after pretreatment injection, animals were exposed to 20 μM MK-801 during 30 min. Immediately after MK-801 exposure, animals were individually placed in an open-field apparatus (30 × 10 × 15 cm, length × width × height) in order to investigate their behavioral responses. The animals behavior was registered by a video camera for 10 min and further analyzed using ANY-Maze recording software (Stoelting Co., Wood Dale, IL, USA).

2.7. 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. Patterning of MK-801 treatment concentration and period

Our results have shown that MK-801 treatment induces alterations in exploratory behavior at different concentrations and exposure periods. All treatments induced an increase in time spent in the top area of the tanks (Fig. 1). Animals treated with MK-801 at the doses of 5, 10, 15, and 20 μM during 15, 30, and 60 min spent longer time in the top of aquariums in comparison with control animals, indicating an anxiolytic-like or antidepressant-like effect induced by this drug.

3.2. Duration and cumulative effects of MK-801 treatment

Animals were treated with MK-801 during 30 min and analyzed during 10 min at different time segments: 1, 2, 4, 24, 48, 72, 96 h and 7 days after the treatment. Animals treated with MK-801 spent longer time period at top area until 2 (5 μM MK-801) and 4 (20 μM MK-801) hours after treatment in comparison with control animals (Fig. 2A). In order to evaluate possible cumulative effects induced by MK-801

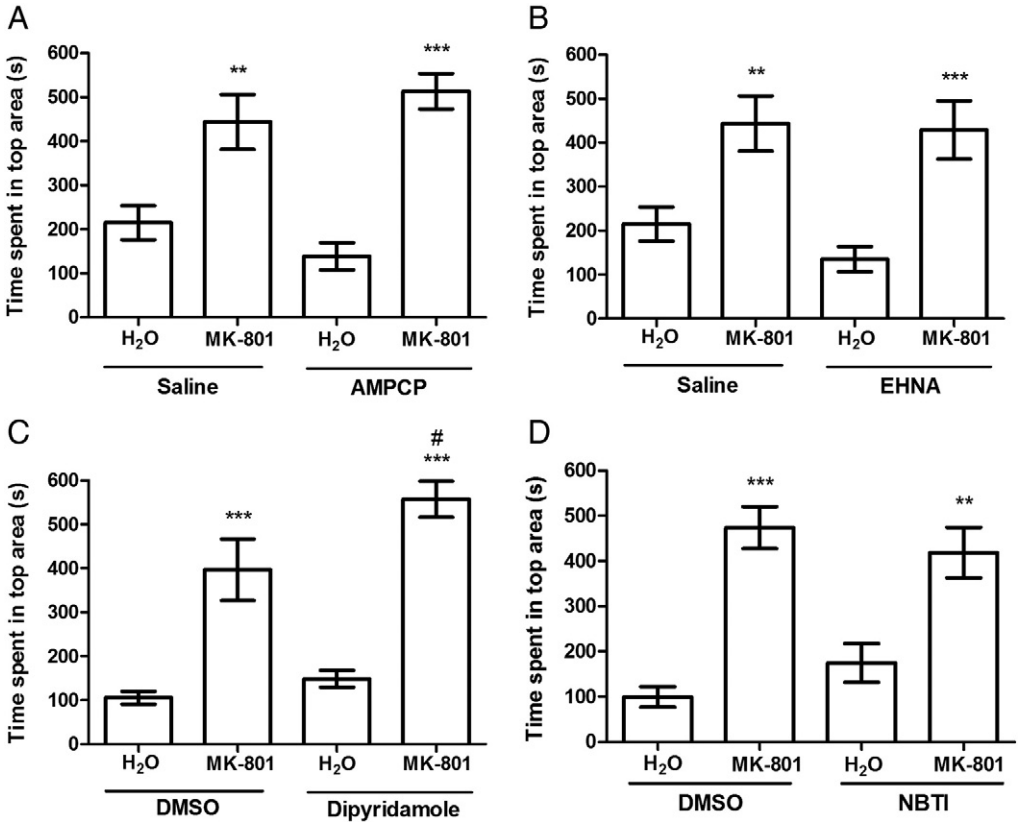

Fig. 5. Effects of AMPCP (A), EHNA (B), dipyridamole (C), and NBTI (D) pretreatments on MK-801-induced behavioral alterations in zebrafish. Animals were exposed to 20 μM MK-801 (during 30 min) immediately after pretreatments administration. The behavioral response was measured as time spent in top area of the tank during 10 min. The data are expressed as the mean ± S.E.M. (n = 11 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 vehicle control group. The symbol # represents statistical difference when compared to the respective vehicle control group. ** $P < 0.005$, *** $P < 0.0005$. # $P < 0.05$.

treatment, animals were treated with 20 μM MK-801 during 30 min once daily and had their behavior analyzed before and after each daily treatment. Our data have shown that animals treated daily with 20 μM MK-801 showed increased preference for top area in all tests. However, MK-801 repeated treatment did not induce cumulative effects, since animals treated and analyzed once per day during 7 days showed the same behavioral response pattern observed since the first until the 7th day (Fig. 2B).

3.3. Effects of adenosine A_1 and A_{2A} receptor antagonist and agonist on behavioral response to MK-801

In order to investigate the effects of A_1 and A_{2A} receptor antagonist and agonist on behavioral response to 20 μM MK-801, we treated zebrafish with caffeine, DPCPX, CPA, ZM 241385, and CGS 21680 during 30 min before MK-801 exposure. Our results have shown that the non-specific adenosine receptor antagonist caffeine (50 mg/kg) and the selective A_1 receptor antagonist DPCPX (15 mg/kg) were able to prevent the behavioral changes induced by MK-801 treatment. Animals treated with caffeine at 50 mg/kg before MK-801 treatment spent 250.6 (±255.7) s at top area, while animals that were exposed to MK-801 after saline treatment spent 443.9 (±250.2) s. Control animals (treated with saline and exposed to tank water) and animals treated with caffeine and exposed to tank water remained at top area during 215.2 (±154.8) and 96.96 (±71.5) s, respectively (Fig. 3A; F (3,64) = 8.44).

Animals treated with DPCPX at 15 mg/kg before MK-801 treatment spent 222.4 (±203.1) s at top area, while animals that were exposed to MK-801 after DMSO treatment spent 415.6 (±218.5) s. Control animals (treated with DMSO and exposed to tank water) and animals treated with DPCPX and exposed to tank water remained at top area during 127.2 (±127.1) and 131.9 (±122.2) s, respectively (Fig. 3B; F (3,106) = 16.14).

The selective adenosine A_1 agonist CPA and both the adenosine A_{2A} receptor antagonist ZM 241385 and agonist CGS 21680 were not able to change behavioral responses induced by MK-801 treatment (Fig. 3C; F (3,64) = 7.74. Fig. 3D; F (3,48) = 11.8. Fig. 3E; F (3,48) = 13.3).

Finally, we analyzed the preference for top or bottom of tanks in time segments of 30 s and observed that behavioral pattern of each group was the same in all time segments (Fig. 4). Our findings have shown that behavioral changes induced by MK-801 exposure and the different drug pretreatments were evident from the beginning until the end of analysis period.

3.4. Effects of modulation of extracellular adenosine levels on behavioral response to MK-801

In order to investigate the effects of modulation of adenosine levels on behavioral response to 20 μM MK-801, we treated zebrafish with AMPCP, EHNA, dipyridamole, and NBTI during 30 min before MK-801 exposure. Our results have shown that the adenosine reuptake inhibition by the non-specific nucleoside transporter (NT) inhibitor dipyridamole (10 mg/kg) was able to exacerbate the behavioral changes induced by MK-801 treatment.

Animals treated with dipyridamole at 10 mg/kg before MK-801 treatment spent 557.4 (±129.9) s at top area, while animals that were exposed to MK-801 after DMSO treatment spent 397 (±221.2) s. Control animals (treated with DMSO and exposed to tank water) and animals treated with dipyridamole and exposed to tank water remained at top area during 105.7 (±51.3) and 148.9 (±66.5) s, respectively (Fig. 5C; F (3,44) = 29.35).

The inhibition of nucleotide hydrolysis by the ecto-5′-nucleotidase inhibitor AMPCP, the inhibition of adenosine deaminase by the ADA inhibitor EHNA and the adenosine reuptake inhibition by the inhibitor of equilibrative nucleoside transporters (ENTs) NBTI were not able to

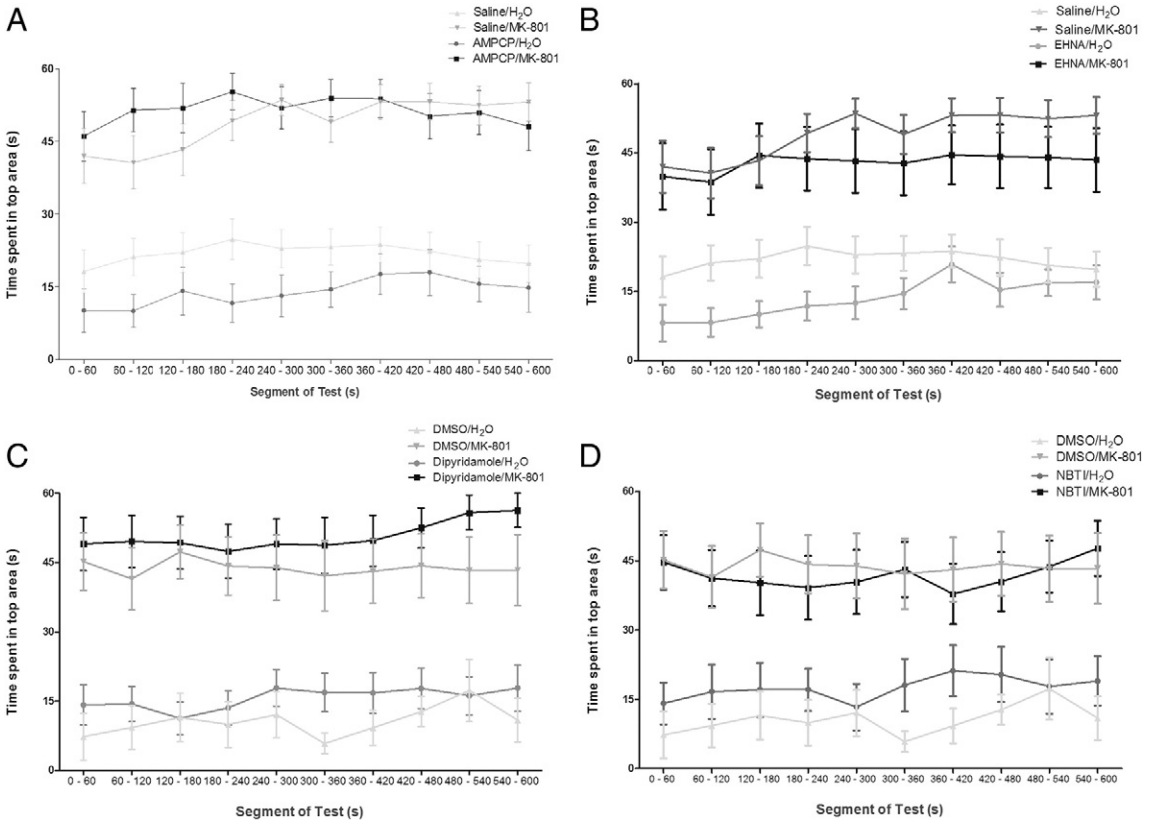

Fig. 6. Effects of AMPCP (A), EHNA (B), Dipyridamole (C), and NBTI (D) pretreatments on MK-801-induced behavioral alterations in zebrafish. Animals were exposed to 20 μM MK-801 (during 30 min) immediately after pretreatments administration. The behavioral response was measured as time spent in top area of the tank for each 60 s. The data are expressed as the mean ± S.E.M. (n = 11 per group), and were analyzed by two-way ANOVA followed by Bonferroni's post-hoc test.

change MK-801 behavioral responses (Fig. 5A; F (3, 60) = 14.35, Fig. 5B; F (3,64) = 9.18, Fig. 5D; F (3,60) = 17.56).

In addition, our results have shown that behavioral changes induced by MK-801 exposure and the different drug pretreatments were evident from the beginning until the end of analyses period. We analyzed the preference for top or bottom of tanks in time segments of 30 s and observed that behavioral pattern of each group was the same in all time segments (Fig. 6).

3.5. Effects of modulation of dopamine receptor antagonists on behavioral response to MK-801

In order to investigate the effects of dopamine receptors on behavioral response to 20 μM MK-801, we treated zebrafish with dopamine D2/D3 receptor antagonist sulpiride (50 mg/kg) and dopamine D1-like receptor antagonist SCH 23390 (15 μg/kg). There was an increase in the time spent in the top area after the pretreatment with 15 μg/kg SCH 23390, but not with sulpiride, when compared to the vehicle control ($P < 0.05$). However, these dopamine receptor antagonists were not able to alter the behavioral responses induced by MK-801 exposure (Fig. 7A; F (3,64) = 6.47, Fig. 7B; F (3,64) = 6.97). Our studies have shown that the different treatments induced a similar behavioral pattern in all time segments analyzed (Fig. 7C, D).

4. Discussion

In this study, our findings showed that MK-801 induced a preference for the top area of the tank, suggesting an antidepressant-like effect. The pretreatment with the non-selective adenosine receptor antagonist caffeine and with the selective adenosine A_1 receptor antagonist DPCPX prevented the preference for the top of the tank. However, our results demonstrated that non-specific nucleoside transporter (NT) inhibitor dipyridamole exacerbated the behavioral response induced by MK-801.

Depression is a psychiatric disorder that affects millions of people worldwide and is estimated that will be the second largest cause of death by the year 2020. Depressed patients present increased risk of cardiovascular events and suicide (Fabbri et al., 2013; Zarate et al., 2013). In addition, currently available traditional medicines (SSRI and SNRI) are not absolutely effective, in view of the fact that depressed patients treated with these drugs often have relapses and persistent symptoms (Ates-Alagoz and Adejare, 2013).

Different studies have shown that neurological and psychiatric disorders, such as Alzheimer's disease, epilepsy, chronic pain, schizophrenia, Parkinson's disease, anxiety and major depressive disorders are developed from disturbances in NMDA signaling (Ates-Alagoz and Adejare, 2013; Kaster et al., 2012). NMDA receptor antagonists induced antidepressant-like effects in different animal models (Ates-Alagoz and Adejare, 2013). MK-801 is a noncompetitive NMDA receptor antagonist and its antidepressant properties have been evaluated through different behavioral studies (Ates-Alagoz and Adejare, 2013). Evidence has shown that MK-801 provoked anxiolytic effects, which induced changes on mobility, learning, and memory (Zarrindast et al., 2012). Seibt et al. (2010) observed that 20 μM MK-801 exposure during 30 and 60 min promoted anxiolytic effects in zebrafish. Therefore, it appears that NMDA receptor antagonists may be useful for the development of a new generation of improved treatments for depression. In agreement, our findings have shown that MK-801 treatment induces antidepressant-like effects, in view of the fact that animals treated with MK-801 spent longer period on the top area of the tank.

To determine the range of concentrations of MK-801 and the time treatment able to promote the antidepressant-like effect expected, concentration and time courses were carried out. The results demonstrated

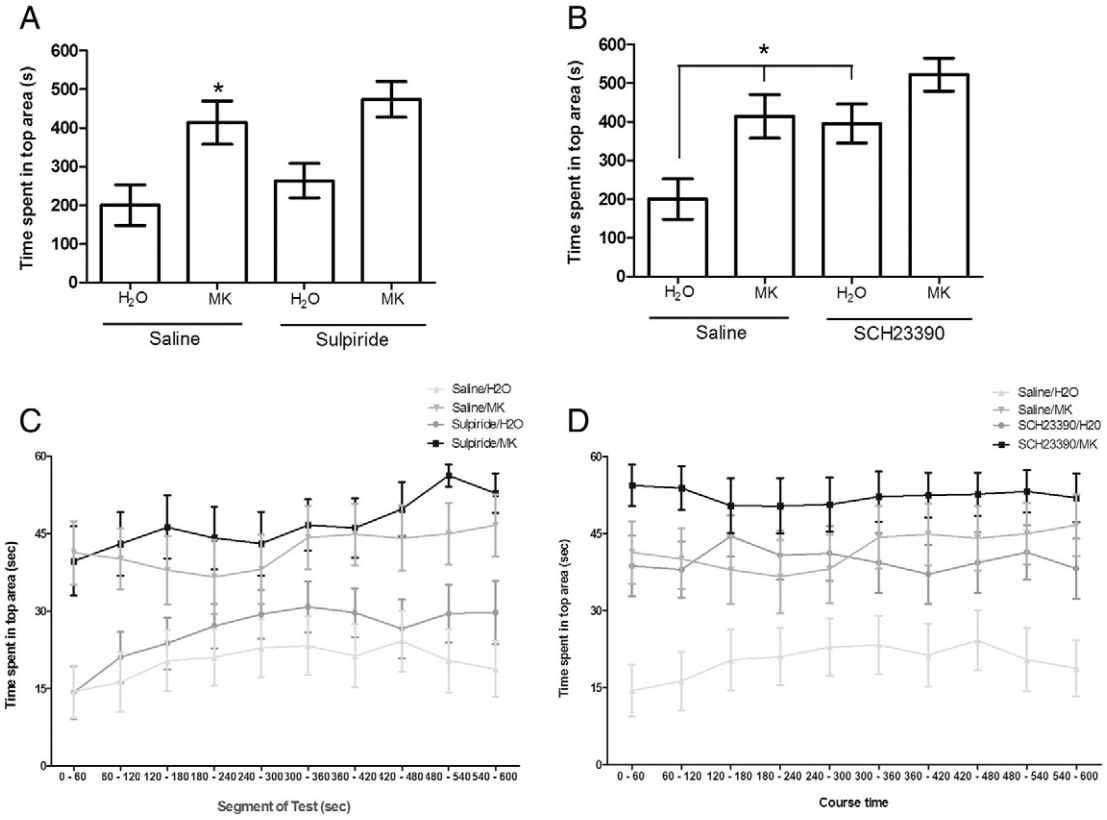

Fig. 7. Effects of sulpiride (A, C), and SCH 23390 (B, D) pretreatments on MK-801-induced behavioral alterations in zebrafish. Animals were exposed to 20 μM MK-801 (during 30 min) immediately after pretreatments administration. The behavioral response was measured as time spent in top area of the tank during 10 min (A, B) and for each 60 s (C, D). 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 (SCH 23390 + H_2O and saline + MK-801) when compared to the respective vehicle control group (saline + H_2O). * $P < 0.05$.

that all tested concentrations (5, 10, 15, and 20 μM) in the different exposure times (15, 30, and 60 min) showed significant behavioral differences in relation to control group. These data are consistent with the data presented in previous studies which demonstrate that acute exposure to MK-801 induces anxiolytic behavior, increased movement, and preference for the top area of the aquarium (Zarrindast et al., 2012; Seibt et al., 2010).

In order to analyze the duration of the antidepressant effect of MK-801, animals were exposed for 30 min to the lowest (5 μM) and highest (20 μM) concentrations tested in the previous experiment. It has been observed that animals exposed to 5 μM MK-801 for 30 min spent longer period in the top of the tank up to 2 h after exposure. However, the animals exposed to 20 μM MK-801 presented behavioral alterations until 4 h after exposure and returned to control levels from 24 h to 7 days later the exposure. Our results have also shown that this effect is not changed by repeated exposure. Animals exposed to 20 μM MK-801 during 30 min once time daily during 7 consecutive days showed behavioral response pattern that was similar from the first until the 7th day. The treated and control groups were analyzed before each daily exposure in order to exclude possible behavioral changes induced by an adaptive behavior to the environment. The results showed that animals remained on top of the aquarium only immediately after exposure, showing behavioral pattern similar to control group on the subsequent day. Therefore, these results suggest that MK-801 presents a rapid and not cumulative antidepressant-like effect. The acute effect observed is in agreement with previous studies that investigated other noncompetitive NMDA receptor antagonists. Duman (2014) reports that NMDA antagonists block the tonic firing of GABAergic interneurons, reinforcing the idea that glutamate burst promote results from disinhibition of glutamate terminals (Homayoun and Moghaddam, 2007). Therefore, studies indicate that agents that increase glutamate release or act directly on postsynaptic AMPA receptors may also have rapid-acting antidepressant effects (Maeng et al., 2008).

Adenosine gained attention by modulation of dopaminergic and glutamatergic transmission in mood disorders (Hirota and Kishi, 2013). Studies show that adenosine A_1 receptors decreased glutamate release attenuating the motor impulsivity (Marek, 2012). Considering that adenosine signaling modulates normal and pathological behaviors and is involved in mood and motivation, we investigated the influence of adenosine receptors and the modulation of adenosine levels on antidepressant effects induced by MK-801. We analyzed the influence of the adenosine receptor antagonist caffeine, A_1 receptor antagonist DPCPX, A_1 receptor agonist CPA, A_{2A} receptor antagonist ZM 241385, and A_{2A} receptor agonist CGS 21680. In addition, the modulation of adenosine levels was investigated through pretreatment with the ecto-5′ nucleotidase inhibitor AMPCP, ADA inhibitor EHNA, non-specific NT inhibitor dipyridamole and equilibrative NT inhibitor NBTI. Our data showed that pretreatment with the nonspecific adenosine antagonist caffeine inhibited MK-801-induced antidepressant-like effects. Caffeine stimulates motor activity, modulates the sleep onset and quality, and improves attention and memory (Cunha and Agostinho, 2010; Rivera-Oliver and Díaz-Ríos, 2014). The pharmacological action of caffeine is primarily through a non-selective adenosine receptor antagonism with A_1 and A_{2A} receptors as preferential targets. Evidence indicates that caffeine enhances the release of GABA by inactivating the A_1 receptor (Ferreira et al., 2014). Da Silva et al. (2005) demonstrated that blockade of A_1 receptor by caffeine reduced the locomotor effect induced by MK-801 by decreasing the release of GABA. Our results showed that DPCPX, a selective A_1 receptor antagonist, promoted a similar effect to that observed to caffeine, preventing MK-801 antidepressant effects. In addition, our data demonstrate that there is no involvement of A_{2A} receptors in the antidepressant effect of MK-801. Therefore, our results indicate that MK-801 effects are dependent on activation of A_1 adenosine receptors, which could reduce GABAergic neurotransmission and contribute to MK-801 antidepressant-like effects.

The modulation of extracellular adenosine levels is promoted by the action of ecto-5′-nucleotidase and adenosine deaminase, enzymes responsible for production and degradation of adenosine, respectively. Other important mechanism to control extracellular adenosine levels involves the action of NTs. Dipyridamole inhibits adenosine uptake, increasing the bioavailability of adenosine in the synaptic cleft and potentiating its effect (Kulkarni and Mehta, 1985). Lobato et al. (2008) demonstrated that dipyridamole potentiates the antidepressant effect induced by zinc. In agreement, we observed that the non-specific NT inhibitor dipyridamole exacerbates MK-801-induced antidepressant-like effects, possibly due to an increase in extracellular adenosine levels promoted by NT inhibition, potentiating its activity.

The interaction of glutamatergic and dopaminergic pathways is involved in the modulation of anxiety-related behaviors. Zarrindast et al. (2012) showed MK-801 administration induced anxiolytic-like behaviors, which were potentiated by dopamine D1 receptor antagonist (SCH 23390) and blocked by dopamine D2 receptor antagonist (sulpiride). These authors did not observe changes in locomotor activity and defecation, indicating that the applied doses do not seem to affect anxiety-related behaviors. Some studies have shown that the increase in locomotor activity as induced by NMDA receptor antagonists may be linked to the increased synthesis, release, and metabolism of dopamine in various brain regions. Although D1 receptor antagonist SCH 23390 followed by water exposure induced an increase on the time spent in the top area, the pretreatment with D1 and D2/D3 receptor antagonists did not alter the behavioral responses induced by MK-801. These data demonstrated that the antidepressant effect induced by MK-801 in zebrafish is not directly related to the dopaminergic system.

In summary, our results demonstrated that antidepressant effects of MK-801 in zebrafish are promoted mainly through adenosine A_1 receptor activation. In addition, our findings indicate that the A_{2A} receptors, nucleoside-metabolizing enzymes, and dopaminergic signaling are not directly involved in the antidepressant effect induced by MK-801 in zebrafish.

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References

Ates-Alagoz, Z., Adejare, A., 2013. NMDA receptor antagonists for treatment of depression. Pharmaceuticals 6 (4), 480–499. http://dx.doi.org/10.3390/ph6040480.

Beaulieu, J.M., Gainetdinov, R.R., 2011. The physiology, signaling, and pharmacology of dopamine receptors. Pharmacol Rev 63, 182–217.

Berman, R.M., Cappiello, A., Anand, A., Oren, D.A., Heninger, G.R., Charney, D.S., et al., 2000. Antidepressant effects of ketamine in depressed patients. Biol Psychiatry 47 (4), 351–354.

Boehmler, W., Obrecht-Pflumio, S., Canfield, V., Thisse, C., Thisse, B., Levenson, R., 2004. Evolution and expression of D2 and D3 dopamine receptor genes in zebrafish. Dev Dyn 230, 481–493.

Boehmler, W., Carr, T., Thisse, C., Thisse, B., Canfield, V.A., Levendon, R., 2007. D4 dopamine receptor genes of zebrafish and effects of the antipsychotic clozapine on larval swimming behavior. Genes Brain Behav 6, 155–166.

Boehmler, W., Petko, J., Woll, M., Frey, C., Thisse, B., Thisse, C., et al., 2009. Identification of zebrafish A_2 adenosine receptors and expression in developing embryos. Gene Expr Patterns 9 (3), 144–151.

Bonan, C.D., 2012. Ectonucleotidases and nucleotide/nucleoside transporters as pharmacological targets for neurological disorders. CNS Neurol Disord Drug Targets 11, 739–750.

Bonan, C.D., Norton, W.H., 2015. The utility of zebrafish as a model for behavioural genetics. Curr Opin Behav Sci 2, 34–38.

Borsini, F., Lecci, A., Mancinelli, A., D'Aranno, V., Meli, A., 1988. Stimulation of dopamine D-2 but not D-1 receptors reduces immobility time of rats in the forced swimming test: implication for antidepressant activity. Eur J Pharmacol 148 (3), 301–307.

Bortolotto, J.W., Melo, G.M., Cognato Gde, P., Vianna, M.R., Bonan, C.D., 2015. Modulation of adenosine signaling prevents scopolamine- induced cognitive impairment in

zebrafish. Neurobiol Learn Mem. 118, 113–119. http://dx.doi.org/10.1016/j.nlm.2014.11.016.
Bowton, E., Saunders, C., Erreger, K., Sakrikar, D., Matthies, H.J., Sen, N., et al., 2010. Dysregulation of dopamine transporters via dopamine D2 autoreceptors triggers anomalous dopamine efflux associated with attention-deficit hyperactivity disorder. J Neurosci 30, 6048–6057.
Capiotti, K.M., Menezes, F.P., Nazario, L.R., Pohlmann, J.B., de Oliveira, G.M., Fazenda, L., et al., 2011. Early exposure to caffeine affects gene expression of adenosine receptors, DARPP-32 and BDNF without affecting sensibility and morphology of developing zebrafish (*Danio rerio*). Neurotoxicol Teratol 33, 680–685.
Carús-Cadavieco, M., de Andrés, I., 2012. Adenosina y control homeostático del sueño. Acciones en estructuras diana de los circuitos de vigilia y sueño. Rev Neurol 55, 413–420.
Coelho, J.E., Alves, P., Canas, P.M., Valadas, J.S., Shmidt, T., Batalha, V.L., et al., 2014. Overexpression of adenosine A2A receptors in rats: effects on depression, locomotion, and anxiety. Front Psychiatry 5, 67. http://dx.doi.org/10.3389/fpsyt.2014.00067.
Cognato, G.P., Bortolotto, J.W., Blazina, A.R., Christoff, R.R., Lara, D.R., Vianna, M.R., et al., 2012. Y-Maze memory task in zebrafish (*Danio rerio*): the role of glutamatergic and cholinergic systems on the acquisition and consolidation periods. Neurobiol Learn Mem 98, 321–328.
Crema, L.M., Pettenuzzo, L.F., Schlabitz, M., Diehl, L., Hoppe, J., Mestriner, R., et al., 2013. The effect of unpredictable chronic mild stress on depressive-like behavior and on hippocampal A1 and striatal A2A adenosine receptors. Physiol Behav 109, 1–7. http://dx.doi.org/10.1016/j.physbeh.2012.11.001.
Csölle, C., Baranyi, M., Zsilla, G., Kittel, A., Gölöncsér, F., Illes, P., et al., 2013. Neurochemical changes in the mouse hippocampus underlying the antidepressant effect of genetic deletion of P2X7 receptors. PLoS One 8 (6), e66547.
Cunha, R.A., Agostinho, P.M., 2010. Chronic caffeine consumption prevents memory disturbance in different animal models of memory decline. J Alzheimers Dis 20 (Suppl. 1), S95–S116. http://dx.doi.org/10.3233/JAD-2010-1408.
Da Silva, R.S., Hoffman, A., de Souza, D.O., Lara, D.R., Bonan, C.D., 2005. Maternal caffeine intake impairs MK-801-induced hyperlocomotion in young rats. Eur J Pharmacol 509 (2–3), 155–159. http://dx.doi.org/10.1016/j.ejphar.2005.01.001.
Duman, R.S., 2014. Pathophysiology of depression and innovative treatments: remodeling glutamatergic synaptic connections. Dialogues Clin Neurosci 16 (1), 11–27.
Dutta, A., McKie, S., Deakin, J.F., 2015. Ketamine and other potential glutamate antidepressants. Psychiatry Res 225 (1–2), 1–13.
Fabbri, C., Di Girolamo, G., Serretti, A., 2013. Pharmacogenetics of antidepressant drugs:an update after almost 20 years of research. Am J Med Genet B Neuropsychiatr Genet 162B (6), 487–520. http://dx.doi.org/10.1002/ajmg.b.32184.
Ferreira, D.P., Stutz, B., de Mello, F.G., Reis, R. a M., Kubrusly, R.C.C., 2014. Caffeine potentiates the release of GABA mediated by NMDA receptor activation: involvement of A1 adenosine receptors. Neuroscience 281C, 208–215. http://dx.doi.org/10.1016/j.neuroscience.2014.09.060.
Haenisch, B., Bonisch, 2011. Depression and antidepressants: insights from knockout of dopamine, serotonin or noradrenaline re-uptake transporters. Pharmacol Ther 1290, 52–368.
Hirota, T., Kishi, T., 2013. Adenosine hypothesis in schizophrenia and bipolar disorder: a systematic review and meta-analysis of randomized controlled trial of adjuvant purinergic modulators. Schizophr Res 149 (1–3), 88–95. http://dx.doi.org/10.1016/j.schres.2013.06.038.
Hirschfeld, R.M., 2014. Differential diagnosis of bipolar disorder and major depressive disorder. J Affect Disord 169S1, S12–S16 [Dec].
Hodgson, R.A., Bertorelli, R., Varty, G.B., Lachowicz, J.E., Forlani, A., Fredduzzi, S., et al., 2009. Characterization of the potent and highly selective A_{2A} receptor antagonists preladenant and SCH 412348 [7-[2-[4–2,4-difluorophenyl]-1-piperazinyl]ethyl]-2-(2-furanyl)-7H-pyrazolo[4,3-e][1,2,4]triazolo[1,5-c]pyrimidin-5-amine] in rodent models of movement disorders and depression. J Pharmacol Exp Ther 330 (1), 294–303.
Homayoun, H., Moghaddam, B., 2007. NMDA receptor hypofunction produces opposite effects on prefrontal cortex interneurons and pyramidal neurons. J Neurosci 27 (43), 11496–11500.
Howe, K., Clark, M.D., Torroja, C.F., Torrance, J., Berthelot, C., Muffato, M., et al., 2013. The zebrafish reference genome sequence and its relationship to the human genome. Nature 496 (7446), 498–503.
Huang, Z.L., Urade, Y., Hayaishi, O., 2011. The role of adenosine in the regulation of sleep. Curr Top Med Chem 11 (8), 1047–1057.
Ipata, P.L., Camici, M., Micheli, V., Tozz, M.G., 2011. Metabolic network of nucleosides in the brain. Curr Top Med Chem 11, 909–922.
Iversen, S.D., Iversen, L.L., 2007. Dopamine: 50 years in perspective. Trends Neurosci 30, 188–193.
Jenner, P., 2014. An overview of adenosine A2A receptor antagonists in Parkinson's disease. Int Rev Neurobiol 119, 71–86. http://dx.doi.org/10.1016/B978-0-12-801022-8.00003-9.
Kaster, M.P., Rosa, A.O., Rosso, M.M., Goulart, E.C., Santos, A.R., Rodrigues, A.L., 2004. Adenosine administration produces an antidepressant-like effect in mice: evidence for the involvement of A1 and A2A receptors. Neurosci Lett 355 (1–2), 21–24.
Kaster, M.P., Machado, D.G., Santos, A.R.S., Rodrigues, A.L., 2012. Involvement of NMDA receptors in the antidepressant-like action of adenosine. Pharmacol Rep 64, 706.713.
Kaster, M.P., Budni, J., Gazal, M., Cunha, M.P., Santos, A.R., Rodrigues, A.L., 2013. The antidepressant-like effect of inosine in the FST is associated with both adenosine A1 and A2A receptors. Purinergic Signal 9 (3), 481–486. http://dx.doi.org/10.1007/s11302-013-9361-8.
Kulkarni, S.K., Mehta, A.K., 1985. Purine nucleoside-mediated immobility in mice: reversal by antidepressants. Psychopharmacology (Berl) 85 (4), 460–463.
Lobato, K.R., Binfaré, R.W., Budni, J., Rosa, A.O., Santos, A.R.S., Rodrigues, A.L.S., 2008. Involvement of the adenosine A1 and A2A receptors in the antidepressant-like effect of zinc in the forced swimming test. Prog Neuropsychopharmacol Biol Psychiatry 32 (4), 994–999. http://dx.doi.org/10.1016/j.pnpbp.2008.01.012.
Maeng, S., Zarate Jr., C.A., Du, J., Schloesser, R.J., McCammon, J., Chen, G., et al., 2008. Cellular mechanisms underlying the antidepressant effects of ketamine: role of alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptors. Biol Psychiatry 63 (4), 349–352.
Marek, G.J., 2012. Activation of adenosine$_1$ receptors induces antidepressant-like, antiimpulsive effects on differential reinforcement of low-rate 72-s behavior in rats. J Pharmacol Exp Ther 341 (2), 564–570. http://dx.doi.org/10.1124/jpet.112.191718.
Mareš, P., 2010. Anticonvulsant action of 2-chloroadenosine against pentetrazol-induced seizures in immature rats is due to activation of A1 adenosine receptors. J Neural Transm 117 (11), 1269–1277. http://dx.doi.org/10.1007/s00702-010-0465-9.
Missale, C., Nash, S.R., Robinson, S.W., Jaber, M., Caron, M.G., 1998. Dopamine receptors: from structure to function. Physiol Rev 78, 189–225.
Ortiz, R., Ulrich, H., Zarate Jr., C.A., Machado-Vieira, R., 2015. Purinergic system dysfunction in mood disorders: a key target for developing improved therapeutics. Prog Neuropsychopharmacol Biol Psychiatry 57C, 117–131.
Papakostas, G.I., 2006. Dopaminergic-based pharmacotherapies for depression. Eur Neuropsychopharmacol 16 (6), 391–402.
Rico, E.P., Rosemberg, D.B., Seibt, K.J., Capiotti, K.M., Da Silva, R.S., Bonan, C.D., 2011. Zebrafish neurotransmitter systems as potential pharmacological and toxicological targets. Neurotoxicol Teratol 33, 608–617.
Rivera-Oliver, M., Díaz-Ríos, M., 2014. Using caffeine and other adenosine receptor antagonists and agonists as therapeutic tools against neurodegenerative diseases: a review. Life Sci 101 (1–2), 1–9. http://dx.doi.org/10.1016/j.lfs.2014.01.083.
Salamone, J.D., Correa, M., 2009. Dopamine/adenosine interactions involved in effort-related aspects of food motivation. Appetite 53 (3), 422–425.
Seibt, K.J., Oliveira, R. da L., Zimmermann, F.F., Capiotti, K.M., Bogo, M.R., Ghisleni, G., Bonan, C.D., 2010. Antipsychotic drugs prevent the motor hyperactivity induced by psychotomimetic MK-801 in zebrafish (*Danio rerio*). Behav Brain Res 214, 417–422.
Seibt, K.J., Piato, A.L., da Luz Oliveira, R., Capiotti, K.M., Vianna, M.R., Bonan, C.D., 2011. Antipsychotic drugs reverse MK-801-induced cognitive and social interaction deficits in zebrafish (*Danio rerio*). Behav Brain Res 224 (1), 135–139.
Shen, H.Y., Singer, P., Lytle, N., Wei, C.J., Lan, J.Q., Williams-Karnesky, R.L., et al., 2012. Adenosine augmentation ameliorates psychotic and cognitive endophenotypes of schizophrenia. J Clin Invest 122, 2567–2577.
Shimazu, S., Minami, A., Kusumoto, H., Yoneda, F., 2005. Antidepressant-like effects of selegiline in the forced swim test. Eur Neuropsychopharmacol 15 (5), 563–571.
Siebel, A.M., Menezes, F.P., Capiotti, K.M., Kist, K.W., Schaefer, I.C., Frantz, J.Z., et al., 2015. Role of adenosine signaling on pentylenetetrazole-induced seizures in zebrafish. Zebrafish 12 (2), 127–136.
Southam, E., Stratton, S.C., Sargent, R.S., Brackenborough, K.T., Duffy, C., Hagan, R.M., et al., 2002. Broad spectrum anticonvulsant activity of BW534U87: possible role of an adenosine-dependent mechanism. Pharmacol Biochem Behav 74 (1), 111–118.
Stewart, A.M., Braubach, O., Spitsbergen, J., Gerlai, R., Kalueff, A.V., 2014. Zebrafish models for translational neuroscience research: from tank to bedside. Trends Neurosci 37 (5), 264–278. http://dx.doi.org/10.1016/j.tins.2014.02.011.
Swain, H.A., Sigstad, C., Scalzo, F.M., 2004. Effects of dizocilpine (MK-801) on circling behavior, swimming activity, and place preference in zebrafish (*Danio rerio*). Neurotoxicol Teratol 26 (6), 725–729.
Wei, C.J., Singer, P., Coelho, J., Boison, D., Feldon, J., Yee, B.K., et al., 2011. Selective inactivation of adenosine A(2A) receptors in striatal neurons enhances working memory and reversal learning. Learn Mem 18, 459–474.
Willner, P., Hale, A.S., Argyropoulos, S., 2005. Dopaminergic mechanism of antidepressant action in depressed patients. J Affect Disord 86 (1), 37–45.
Zarate, C., Duman, R.S., Liu, G., Sartori, S., Quiroz, J., Murck, H., 2013. New paradigms for treatment-resistant depression. Ann N Y Acad Sci 1292 (1), 21–23.
Zarrindast, M.R., Nasehi, M., Pournaghshband, M., Yekta, B.G., 2012. Dopaminergic system in CA1 modulates MK-801 induced anxiolytic-like responses. Pharmacol Biochem Behav 103, 102–110.
Zlomuzica, A., Burghoff, S., Schrader, J., Dere, E., 2013. Superior working memory and behavioural habituation but diminished psychomotor coordination in mice lacking the ecto-5′-nucleotidase (CD73) gene. Purinergic Signal 9 (2), 175–182. http://dx.doi.org/10.1007/s11302-012-9344-1.