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Modulation of adenosine signaling reverses 3-nitropropionic acid-induced bradykinesia and memory impairment in adult zebrafish

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ABSTRACT

Huntington's disease (HD) is a neurodegenerative disorder, characterized by motor dysfunction, psychiatric disturbance, and cognitive decline. In the early stage of HD, occurs a decrease in dopamine D_2 receptors and adenosine A_{2A} receptors ($A_{2A}R$), while in the late stage also occurs a decrease in dopamine D_1 receptors and adenosine A_1 receptors (A_1R). Adenosine exhibits neuromodulatory and neuroprotective effects in the brain and is involved in motor control and memory function. 3-Nitropropionic acid (3-NPA), a toxin derived from plants and fungi, may reproduce HD behavioral phenotypes and biochemical characteristics. This study investigated the effects of acute exposure to CPA (A_1R agonist), CGS 21680 ($A_{2A}R$ agonist), caffeine (non-selective of A_1R and $A_{2A}R$ antagonist), ZM 241385 ($A_{2A}R$ antagonist), DPCPX (A_1R antagonist), dipyridamole (inhibitor of nucleoside transporters) and EHNA (inhibitor of adenosine deaminase) in an HD pharmacological model induced by 3-NPA in adult zebrafish. CPA, CGS 21680, caffeine, ZM 241385, DPCPX, dipyridamole, and EHNA were acutely administered via i.p. in zebrafish after 3-NPA (at dose 60 mg/kg) chronic treatment. Caffeine and ZM 241385 reversed the bradykinesia induced by 3-NPA, while CGS 21680 potentiated the bradykinesia caused by 3-NPA. Moreover, CPA, caffeine, ZM 241385, DPCPX, dipyridamole, and EHNA reversed the 3-NPA-induced memory impairment. Together, these data support the hypothesis that $A_{2A}R$ antagonists have an essential role in modulating locomotor function, whereas the activation of A_1R and blockade of $A_{2A}R$ and A_1R and modulation of adenosine levels may reduce the memory impairment, which could be a potential pharmacological strategy against late-stage symptoms HD.

1. Introduction

Huntington's disease (HD) is a devastating, progressive, and fatal neurodegenerative disorder inherited in an autosomal dominant manner (Blumenstock and Dudanova, 2020). It is triggered by an expansion of a cytosine-adenine-guanine ($\geq$ 36 repeats) triplet repeat in exon 1 of the huntingtin (*HTT*) gene, located on chromosome 4 (The Huntington's Disease Collaborative Research Group, 1993; Capiluppi et al., 2020). This change leads to an expanded polyglutamine (polyQ) region in the encoded HTT protein (Capiluppi et al., 2020). As a result, the expressed HTT protein is a mutant (mHTT; Cybulska et al., 2020). The worldwide prevalence of HD is 5 in 100,000 people (Baig et al., 2016; Illarioshkin et al., 2018).

A neurobehavioral progressive triad characterizes HD with motor dysfunction, neuropsychiatric disturbance, and cognitive decline (Stahl and Feigin, 2020). The motor dysfunction is subdivided into two stages:

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in the early stage, there are abnormal involuntary movements, known as chorea, while in the late stage, the voluntary movements are impaired, causing bradykinesia, dystonia, and motor incoordination (Stahl and Feigin, 2020). The onset of the motor symptoms can occur from childhood to old age, although symptoms usually occur in adulthood (around 45 years old; Tabrizi et al., 2020). The dysfunctions can be attributed to multiple brain regions that exhibit neurodegeneration, including the cerebral cortex, thalamus, subthalamic nucleus, globus pallidus, substantia nigra, and hypothalamus. However, the hallmark of the disease is the pronounced neuronal loss in the striatum (caudate nucleus and putamen; Rubinsztein, 2002; Ramaswamy et al., 2007).

The striatum controls many behaviors, such as movement, flexibility behavior, motivation, and learning (Koch and Raymond, 2019), and contains several pathways among which are highlighted the indirect and the direct pathway (Graybiel, 2000). In the early stage of HD, the neurons of indirect pathway (acts by inhibiting voluntary movements) degenerate; it leads to a decrease in dopamine D_2 receptors (D_2R) and adenosine A_{2A} receptors ($A_{2A}R$), and thus uncontrolled voluntary movements, coinciding with chorea symptoms (Albin et al., 1989; Graybiel, 2000; Koch and Raymond, 2019). In addition, the decrease of striatal $A_{2A}R$ density is larger than the decrease of D_2R (Glass et al., 2000), probably because of an altered regulation of $A_{2A}R$ expression and stability of A_{2AR} mRNA transcript (Chiang et al., 2005) and of ENT-1-mediated altered tone of adenosine (Guitart et al., 2016). Furthermore, there is evidence that occurs an increase in $A_{2A}R$ density in striatum and hippocampus during the presymptomatic period of disease in HD animal model (Tarditi et al., 2006; Tyebji et al., 2015). In the late stage of HD, besides the damaged indirect pathway, there is the degeneration of direct pathway neurons (acts by initiating voluntary movements), a phenomenon that decreases dopamine D_1 receptors (D_1R), adenosine A_1 receptors (A_1R), and cortex stimulation. This phenomenon leads to hypokinetic symptoms typical of this stage (Albin et al., 1989; Graybiel, 2000; Koch and Raymond, 2019).

Adenosine, a purine ribonucleoside, plays a critical neuromodulatory role in the function of the corticostriatal circuit. Adenosine signaling is mediated via four G-protein coupled receptors: A_1 (inhibitory), A_{2A} (facilitatory), A_{2B} (facilitatory), and A_3 (inhibitory; Ciruela et al., 2015). Adenosine is deaminated to inosine through the action of adenosine deaminase (Latini and Pedata, 2001). Finally, adenosine levels are also regulated by either unidirectional and bidirectional transporters, called nucleoside transporters (NTs), which allow nucleosides to move between the intracellular and extracellular compartments (Fredholm et al., 2001; Ribeiro et al., 2016; Stockwell et al., 2017) or via adenosine kinase (ADK), a key enzyme of adenosine metabolism (Boison and Jarvis, 2021).

In addition, adenosine receptors are co-localized with dopamine receptors in dopamine-rich regions, allowing direct membrane interactions through the formation of heteromeric complexes and a crosstalk in intracellular signaling (Ferré et al., 2007). Specifically, in HD, $A_{2A}R$ are concentrated in the GABAergic striatopallidal neurons, together with the D_2R (Fuxe et al., 2010). Most of the evidence suggests that the A_1R-D_1R and $A_{2A}R$-D_2R act antagonistically, that is; if the $A_{2A}R$ was blocked, it is actives the D_2R; or if the $A_{2A}R$ was activated, it is blocks the D_2R (Canals et al., 2003; Ferré and Ciruela, 2019).

Some studies in HD animal models have tried to elucidate if genetic and pharmacological inactivation or activation of A_1R and $A_{2A}R$ may be effective in reducing striatal lesions and behavioral phenotype symptoms in rodents (Blum et al., 2002; Fink et al., 2004; Chou et al., 2005; Li et al., 2015; Bortolatto et al., 2017). Among the animal models of HD, the 3-nitropropionic acid (3-NPA) pharmacological model has been extensively used in HD research. 3-NPA is a mitochondrial toxin, which promotes irreversible inhibition of complex II of the respiratory chain (Brouillet et al., 1999), causing biochemical alterations (ATP decrease levels, oxidative stress, neuronal death) such as detected in post-mortem cerebrum of HD patients (Beal, 1992; Gu et al., 1996; Brouillet et al., 1998; Bortolatto et al., 2017). Animals submitted to 3-NPA injections also exhibit behavioral phenotype symptoms as observed in HD patients (Borlongan et al., 1997; Wiprich et al., 2020).

In this context, the zebrafish, a small teleost, is an interesting animal model for studying several neurodegenerative diseases (Wiprich et al., 2020; Kiper and Freeman, 2021; Razali et al., 2021) due to its high genetic and physiological similarities with mammals, and myriad advantages over other animal models (Chakraborty et al., 2018), A previous study of our research group showed that the 3-NPA chronic treatment in adult zebrafish produced bradykinesia and memory deficits in animals that resemble late-stage HD symptoms (Wiprich et al., 2020). Furthermore, previous studies have demonstrated the adenosine receptor subtypes, A_1, $A_{2A.1}$, $A_{2A.2}$, A_{2B}, and A_3 in zebrafish (Boehmer et al., 2009; Capiotti et al., 2011; Grillo et al., 2019).

Therefore, considering the role of adenosine receptors in HD pathophysiology and the relevance of zebrafish as a powerful model for neurodegenerative diseases and pharmacological studies, the investigation of adenosine signaling in zebrafish may improve our knowledge on the role of this neuromodulator in HD. Therefore, we investigate the effects of adenosine receptor agonists and antagonists as well as the modulation of adenosine levels in a pharmacological HD model induced by 3-NPA in adult zebrafish.

2. Material and methods

2.1. Animals and housing conditions

A total of 864 adult zebrafish (AB strain, 6–10 months, 0.3–0.5 g) from our breeding colony were used in equal proportions (male/female). Until the treatment, the animals were maintained in recirculating systems (Zebtec, Tecniplast, Italy) with reverse osmosis filtered water equilibrated to reach the species standard temperature (28 °C ± 2 °C), pH (7.0 to 7.5), conductivity (300–700 μS), hardness (80–300 mg/L), ammonia (< 0.02 mg/L), nitrite (< 1 mg/L), nitrate (< 50 mg/L), and chloride levels (0 mg/L). Animals were subjected to a light/dark cycle of 14/10 h, respectively. They received paramecium between 6- and 14-days post-fertilization (dpf), and after 14 dpf, they received commercial flakes (TetraMin Tropical Flake Fish®) three times a day that was supplemented with brine shrimp (Westerfield, 2000). All procedures followed the Brazilian Council of Animals Experimentation guidelines for Use of Fish in Research (CONCEA) and the Brazilian legislation (11.794/08). The sample number to behavioral analyses were based on previous studies performed for our group (Gusso et al., 2020; Wiprich et al., 2020; Petersen et al., 2021) and other research groups that used zebrafish as an animal model (Mocelin et al., 2019; Giacomini et al., 2020). All protocols were approved by the Animal Care Committee of the Pontifical Catholic University of Rio Grande do Sul (CEUA- PUCRS, protocol number 9406/2019). This study was registered in the Sistema Nacional de Gestão do Patrimônio Genético e Conhecimento Tradicional Associado - SISGEN (Protocol No. A3B073D).

2.2. Drugs

The drugs 3-nitropropionic acid, caffeine, dipyridamole, erythro-9-(2-hydroxy-3-nonyl-adenine) (EHNA), 8-cyclopentyl-1,3-dipropylxanthine (DPCPX), cyclopentyladenosine (CPA), CGS 21680 hydrochloride, and tricaine were purchased from Sigma (St. Louis, MO, USA). ZM 241385 was purchased from Tocris Bioscience (Ellisville, MO, USA).

2.3. Procedures

In all experiments, fish were randomly allocated into the experimental groups and maintained in different housing tanks for each experimental group (2 tanks per experimental group). The animals were submitted to the treatment (saline and 3-NPA) and remained in these tanks for 29 days. Each experimental condition was analyzed in animals obtained from 2 independent treatments. The animals from control and

3-NPA treated groups were randomly tested in the behavioral assays. Also, before all pharmacological interventions, behavioral tests (novel tank test and avoidance inhibitory task), and biochemical analysis the animals did not were fed.

2.4. Treatments

2.4.1. 3-Nitropropionic acid treatment

The HD phenotype in adult zebrafish was induced through the 3-nitropropionic acid (3-NPA) treatment. The animals were chosen randomly and divided into two groups considering a density of three animals per liter: control and 3-NPA-treated groups. In previous experiments, we have performed a naïve group (without any manipulation) and there were no significant locomotor changes when compared to the control (vehicle) group. For this reason, we used the vehicle group as the control group. Animals received seven intraperitoneal (i.p.) injections of 3-NPA or saline (vehicle, for the control group) every 96 h, totaling twenty-four days of treatment. All experimental subjects were previously weighted and allocated in such a way the average weight did not differ between groups. Prior to injection, the animals were anesthetized by immersion in 100 mg/L tricaine solution until they showed a lack of motor coordination and a reduced respiration rate. The animals received an i.p. injection at 60 mg/kg body weight 3-NPA or saline (vehicle-control group) in a 10-μL volume. The dose chosen was based on a previous study performed for our group, which demonstrated that the chronic treatment in adult zebrafish with 3-NPA, at three doses, 10, 20, or 60 mg/kg, decreased the distance traveled. The highest 3-NPA dose (60 mg/kg) had more pronounced effects than 10 or 20 mg/kg (Wiprich et al., 2020). Moreover, the 3-NPA at 60 mg/kg induced memory impairment (Wiprich et al., 2020), showing that 3-NPA-mediated neurotoxic alterations in zebrafish closely resemble the behavioral pathology of late-stage HD.

I.p. injections were conducted using a 3/10-mL U-100 BD Ultra-Fine™ Short Insulin Syringe with 8 mm (5/16 in) × 31 G short needle (Becton Dickinson and Company, New Jersey, USA). After the injection, the animals were placed in a tank with a highly aerated recirculation system to facilitate their recovery from anesthesia. After anesthesia recovery (approximately 5 min after tricaine exposure), the animals were returned to their home tank (a glass tank with a 5 L capacity containing a filter system). 3-NPA-treated animals were kept in the same water quality, light, and food parameters as the control group animals. During the treatment, there was a mortality of 25% for both groups (vehicle control and 3-NPA-treatment).

2.4.2. Adenosinergic drugs on 3-NPA-induced HD

To test the effects of adenosine signaling on 3-NPA-induced HD in zebrafish, the animals received a one i.p injection of adenosinergic compounds or their respective vehicle, 30 min before the beginning of each behavioral experiment on day 29 (120h) after the last injection of 3-NPA (day 25). In addition, in the next day (day 26) after the last injection of 3-NPA, the animals were weighted and allocated in such a way the average weight did not differ between groups. Caffeine (10 mg/kg), DPCPX (0.5 mg/kg), ZM 241385 (10 μg/kg), CPA (1 mg/kg), CGS 21680 (1 mg/kg), EHNA (100 μg/kg), and dipyridamole (10 mg/kg) were administered via i.p injection in a volume of 10 μL using a 3/10-mL U-100 BD Ultra- Fine™ Short Insulin Syringe with 8 mm (5/16 in) × 31 G short needle (Becton Dickinson and Company, New Jersey, USA). Caffeine, CPA, and EHNA were dissolved in saline (0.9% NaCl); CGS 2160, dipyridamole, and ZM 241385 were dissolved in 1% DMSO (dimethylsulfoxide); and DPCPX was dissolved in 3% DMSO. Drug doses and administration route were chosen based on previous studies performed for our group in zebrafish (Bortolotto et al., 2015; Siebel et al., 2015). Before drug or vehicle administration, animals were anesthetized by immersion in 100 mg/L tricaine solution until they showed a lack of motor coordination and a reduced respiration rate. After the injection, the animals were placed in a tank with a highly aerated recirculation system to facilitate their recovery from anesthesia. To behavioral analysis, different sets of animals were exposed to the novel tank test and the inhibitory avoidance task. The study design is outlined in Fig. 1.

2.5. Behavioral analysis

2.5.1. Novel tank test

The test was carried out as described previously by Gerlai et al. (2000), with modifications (Gerlai et al., 2000; Altenhofen et al., 2017; Wiprich et al., 2020). The animals were transferred from the maintenance room to the experimental room one day before the tests. The experiments were performed between 8:30 and 12:00. A total of 432 animals (n = 12 per group) were used, which were tested in experimental duplicate. The animals were placed individually in experimental tanks (30 cm long x 15 cm high x 10 cm wide) with water. After 60 s habituation, their locomotor behavior was recorded for 5 min (Altenhofen et al., 2017; Wiprich et al., 2020) for subsequent analysis with EthoVision® XT tracking software (version 11.5, Noldus) 30 frames per second. The locomotor parameters analyzed were distance traveled (m), and velocity (m/s, the ratio between distance traveled and movement). The parameter movement was considered the mobile time in which the velocity was higher than 0.6 cm/s. When the animal reached the stop velocity (<0.59 cm/s), it was considered immobile time (Tran et al., 2016; Wiprich et al., 2020).

2.5.2. Inhibitory avoidance task

The animals were transferred from the maintenance room to the experimental room one day before the tests for the inhibitory avoidance task. We performed the test (n = 11 to 12 per group) between 8:30 and 12:00 (Blank et al., 2009; Zanandrea et al., 2020). There were two sessions, training and test, with a 24-h interval between them. In each session, animals were placed individually in an experimental tank (18 cm long x 9 cm wide x 7 cm high) with water, divided by a guillotine door into two compartments of equal size: one black (right side) and one white (left side). The animal was gently placed in the white compartment (with the door closed) during the training session for 1-min habituation and environmental recognition. After this period, the divider was lifted. Once the animal crossed into the black side of the tank, the guillotine door was closed again, and two electrodes attached to an 8 V stimulator delivered a 3 ± 0.2 V AC shock pulse (intensity measured between electrodes and the center of the dark compartment) when manually activated for 5 s. The animal was gently removed from the apparatus and returned to its housing tank with only water for 24 h until the test session, which consisted of the same protocol as the training session, but without the electric shock. The latency to enter the black compartment during each session was measured in both sessions, and the expected increase in the test session was used as an index of memory retention. A 180-s ceiling was imposed on test session latency measurements.

2.6. Gene expression analysis by quantitative real-time RT-PCR (RTqPCR)

To gene expression analysis each animal was cryoeuthanized, and the whole brain was gently dissected with a scalpel blade, placed in an microbute, and immediately was frozen in liquid nitrogen. Then, was added TRIzol® Reagent in each microbute and the samples were kept at −80 °C until the analysis. Molecular analysis of gene expression of adenosine receptors subtypes (*adora1b, adora2aa, adora2ab, adora2b*) were performed following the Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE) Guidelines for RT-qPCR experiments (Bustin et al., 2009; Bustin et al., 2013). The total RNA was isolated with TRIzol® Reagent (Thermo Fisher Scientific, Waltham, Massachusetts, USA) in accordance with the manufacturer's instructions. RNA purity (Abs 260/280 nm ~2.0) and concentration were determined by NanoDrop Lite (Thermo Fisher Scientific, Waltham,

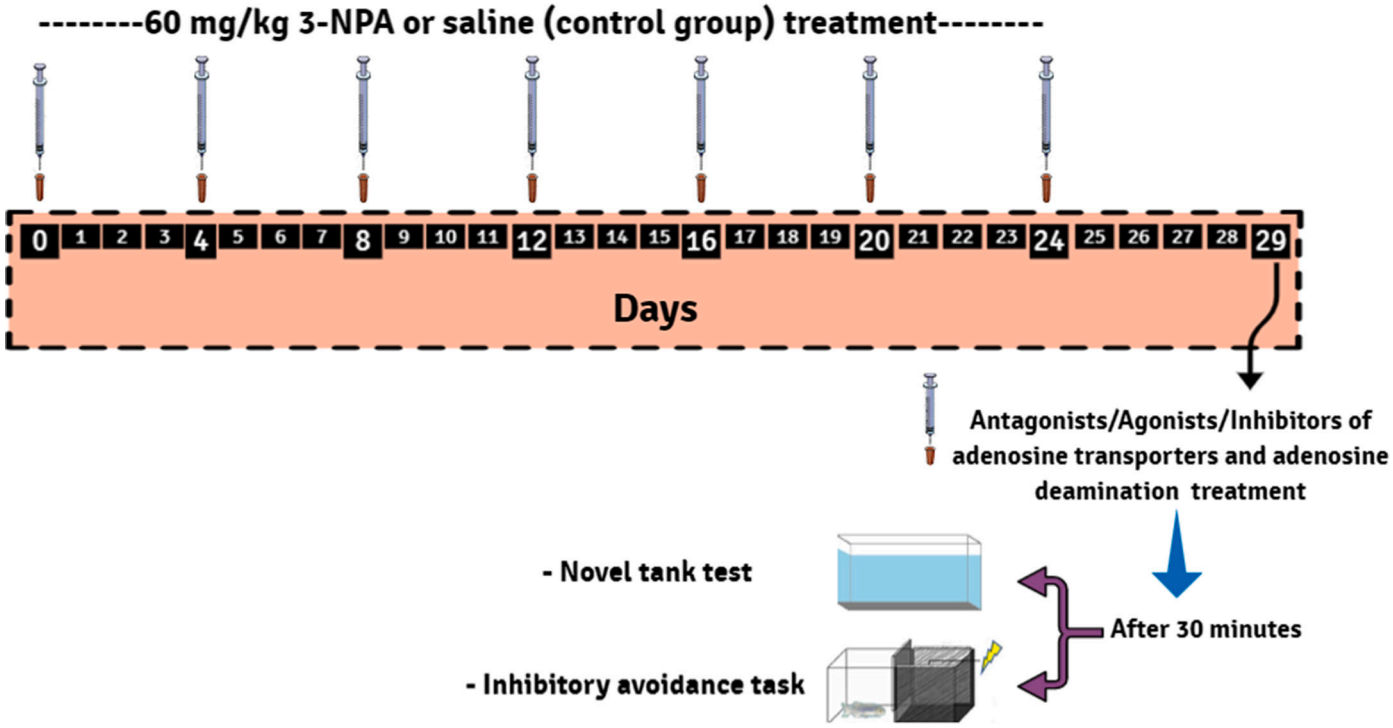

Fig. 1. Timeline of the experimental procedure. Adult zebrafish received seven intraperitoneal injections of 60 mg/kg 3-Nitropropionic acid or saline (vehicle-control group) for 24 days. One-hundred twenty hours (29 day) after the 3-NPA treatment, animals were treated with a single i.p injection of adenosine antagonists (10 mg/kg caffeine, 10 μg/kg ZM 241385, 0.5 mg/kg DPCPX) and agonists (1 mg/kg CPA, 1 mg/kg CGS 21680); and inhibitors of adenosine transporter (10 mg/kg dipyridamole) and adenosine deaminase (100 μg/kg EHNA) or their respective vehicles (saline, 1% DMSO and 3% DMSO). After 30 min of injections, behavioral analysis were performed. The behavioral analysis included a novel tank test and inhibitory avoidance task.

Massachusettes, USA) and after treated with Deoxyribonuclease I-Amplification Grade (Sigma-Aldrich Inc., St Louis, Missouri, USA) to eliminate genomic DNA contamination in accordance with the manufacturer's instruction. The cDNA was synthesized with ImProm-II™ Reverse Transcription System (Promega, Madison, Wisconsin, USA) from 1 μg of the total RNA, following the manufacturer's instruction. Quantitatie PCR was performed using SYBR® Green I (Thermo Fisher Scientific, Waltham, Massachusetts, USA) to detect double-strand cDNA synthesis on the 7500 Real-time PCR System (Applied Biosystems, Foster City, California, USA). The PCR cycling conditions were: an initial polymerase activation step for 5 min at 95 °C, 40 cycles of 15 s ate 95 °C for denaturation, 35 s at 60 °C for annealing and 15 s at 72 °C for elongation. At the end of cycling protocol, a melting-curve analysis was included and fluorescence measured from 60 to 99 °C to confirm the specificity of primers and absence of primer-dimers and showed in all cases one single peak. All real time assays were carried out in quadruplicate and, in all, cases a reverse transcriptase negative control was included by substituting the templates for DNase/RNase-free distilled water in each PCR reaction. ef1a and rpl13a were used as reference genes for normalization. The efficiency per sample was calculated using LinRegPCR version 2021.1 Software (http://LinRegPCR.nl) and the stability of the references genes, and the optimal number of reference genes according to the pairwise variation (V) were analyzed by GeNorm 3.5 Software (http://medgen.ugent.be/genorm/). Relative mRNA expression levels were determined using the $2^{-\Delta\Delta Cq}$ method (Pfaff, 2001; Bustin et al., 2013). The sequences of reverse and forward primers are demonstrated in Table 1.

2.7. Statistical analysis

The Shapiro-Wilk test was applied to evaluate the normality of the data. Novel tank test data were analyzed by two-way ANOVA followed by Bonferroni as *post-hoc* test. The non-normal data for the novel tank test were adjusted through the Log-transformation and analyzed using two-way ANOVA. Inhibitory avoidance training and test latencies for each group were compared using the Mann-Whitney *U* matched pairs test. The latencies of multiple groups were compared using Kruskal-Wallis and Mann-Whitney *U* tests.

The exclusion criteria were based as follows: Novel tank test: the fish were excluded when the software lose >10% of the total time spent in the arena; to the inhibitory avoidance task, the animals were excluded when they took longer than 60 s to enter the dark compartment in the session training.

Parametric data (novel tank test) are expressed as mean ± standard

Table 1
Primer sequences for RT-qPCR experiments included in the study.

Gene	Forward primer	Reverse primer
ef1a[1]	5′-CTGGAGGCCAGCTCAAACAT-3′	5′-ATCAAGAAGAGTAGTACCGCTAGCATTAC-3′
rpl13a[1]	5′-TCTGGAGGACTGTAAGAGGTATGC-3′	5′-AGACGCACAATCTTGAGAGCAG-3′
adora1b[2]	5′-GTTCCTCATTTACATTGCCATTCTGC-3′	5′-TGGTTGTTATCCAGTCTCTCGCTCG-3′
adora2aa[2]	5′-GCGAACTGTACGCCGAGCAGAG-3′	5′-TTATTCCCAGTGAGCGGCGACTC-3′
adora2ab[2]	5′-GGATTGGGTCATGTACCTGGCCATC-3′	5′-GCTGTTTCCAATGGCCAGCCTG-3′
adora2b[2]	5′-GTTTGTTCGCTCTCTGTTGGCTGC-3′	5′-CTAAAAGTGACTCTGAACTCCCGAATG-3′

According to [1] Tang et al. (2007), [2] Altenhofen et al. (2015).

error of the mean (SEM), while non-parametric data (inhibitory avoidance test) are expressed as interquartile range ± median. Moreover, gene expression data were analyzed by Student's *t*-test for unpaired samples and were expressed as mean ± SEM. For all comparisons, a significance level of $p < 0.05$ was considered. GraphPad Prism 8 (La Jolla, CA, USA) software was used for statistical analysis.

3. Results

3.1. Novel tank test

Our results have shown that 3-NPA treatment induced alterations in locomotor behavior. Animals treated with 3-NPA at the dose 60 mg/kg with seven i.p injections traveled less the aquarium in comparison with control animals, indicating a bradykinesia pattern induced by this drug (Fig. 2a and b; Fig. 3a and b; Figs. 4a and 6b; see Supplementary Table 1 and 2 for statistical analysis).

Furthermore, our results have shown that the selective A_1R agonist and antagonist, CPA and DPCPX, respectively, did not reverse the hypolocomotion induced by 3-NPA treatment (Figs. 2a and 3c). Surprisingly, the selective $A_{2A}R$ agonist CGS 21680 potentiated the hypolocomotor effect induced by 3-NPA (Fig. 2b, 3-NPA, $F_{(1,66)} = 130.1$; $p < 0.0001$; CGS 21680, $F_{(2,66)} = 15.99$; $p < 0.0001$; interaction, $F_{(2,66)} = 0.4485$; $p = 0.6405$). Interestingly, the non-specific adenosine receptor antagonist caffeine and the selective $A_{2A}R$ antagonist ZM 241385 were able to reverse the hypolocomotion induced by 3-NPA (Fig. 3a, 3-NPA, $F_{(1,44)} = 0.8723$; $p = 0.3554$; caffeine, $F_{(1,44)} = 0.8623$; $p = 0.3582$; interaction, $F_{(1,44)} = 25.01$; $p < 0.0001$; Fig. 3b, 3-NPA, $F_{(1,66)} = 13.84$; $p = 0.0004$; ZM 241385, $F_{(2,66)} = 0.5484$; $p = 0.5805$; interaction, $F_{(2,66)} = 25.99$; $p < 0.0001$). The 3-NPA did not alter the velocity compared to control animals. In addition, no significant difference in velocity was found in animals receiving CPA, CGS 21680, ZM 241385; or DPCPX (Supplementary Fig. 1a and b; Supplementary Fig. 2b and c). However, the non-specific adenosine receptor antagonist caffeine increased the velocity in animals treated with 3-NPA (Fig. 2a, 3-NPA, $F_{(1,44)} = 1.486$; $p = 0.2293$; caffeine, $F_{(1,44)} = 6.853$; $p = 0.0121$; interaction, $F_{(1,44)} = 3.910$; $p = 0.0543$).

To elucidate the role of nucleoside transporters and nucleoside-metabolizing enzymes, we tested an inhibitor of nucleoside transporters, dipyridamole, and the inhibitor of adenosine deaminase EHNA. Saline was used as vehicle for EHNA, and 1% DMSO was used as vehicle for dipyridamole. Neither EHNA nor dipyridamole were able to increase the distance traveled in animals treated with 3-NPA (saline; Fig. 4a and b). Moreover, there was no significant difference in velocity (Supplementary Fig. 3a and b).

CPA, caffeine, DPCPX, ZM 241385, EHNA, and dipyridamole per se reduced the distance traveled compared with control animals (saline, 1% DMSO, or 3% DMSO; Fig. 2a; Fig. 3a, b and c; Fig. 4a and b; see Supplementary Table 2 for statistical details). However, the results have shown that the CGS 21680 per se did not alter the distance traveled (Fig. 2b). In addition, only the CPA treatment decreased the velocity compared to control animals (Supplementary Fig. 1a; $p < 0.0180$).

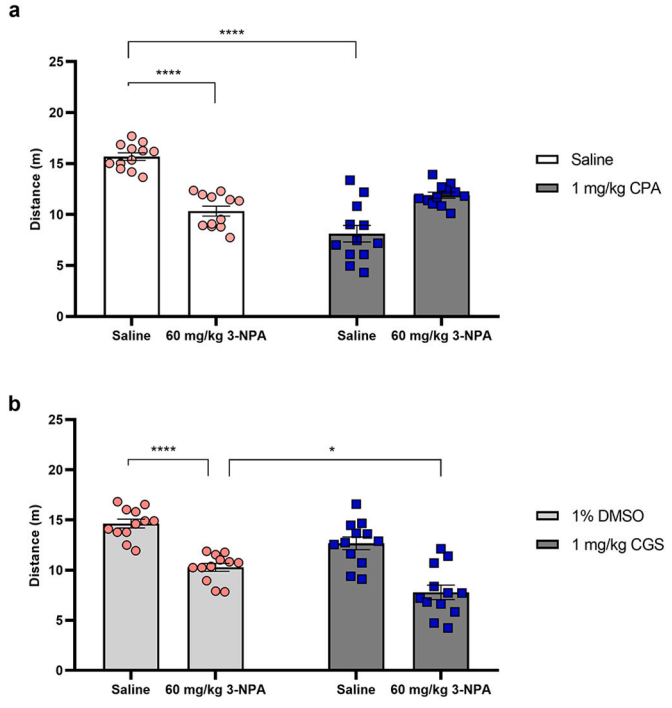

Fig. 2. Effects of adenosine agonists CPA and CGS 21680 (CGS) treatment on locomotor activity deficits induced by 3-NPA. Animals received seven i.p. injections of saline or 3-NPA for 24 days. One-hundred twenty hours (29 day) after the 3-NPA exposure, animals were treated with a single i.p. injection of (a) saline or CPA (1 mg/kg), and (b) DMSO (1%) or CGS 21680 (1 mg/kg) 30 min before the test. Data are expressed as mean ± S.E.M ($n = 12$ for each group) and were analyzed by two-way ANOVA followed by a Bonferroni post-hoc test. * $p < 0.05$, **** $p < 0.0001$.

3.2. Inhibitory avoidance task

Treatment with 3-NPA followed by antagonists, agonists, and inhibitors vehicles (on 29 days) resulted in significant differences between zebrafish training and test sessions, thus indicating an impairment in the memory of this task compared to the control groups (animals treated with saline, 1% DMSO, or 3% DMSO). Interestingly, treatment with either CPA (Fig. 5a; $p < 0.0002$), caffeine (Fig. 6a; $p < 0.0024$), ZM 241385 (Fig. 6b; $p < 0.0001$), DPCPX (Fig. 6c; $p < 0.0007$), EHNA (Fig. 7a; $p < 0.0004$), or dipyridamole (Fig. 7b; $p < 0.0015$) reversed the memory impairment induced by 3-NPA, as observed by the difference in latencies between the training and test sessions for each treatment. In contrast, there were no significant differences in the latencies between the training and test sessions with the CGS 21680 treatment, indicating that the CGS 21680 did not reverse the memory impairment induced by 3-NPA (Fig. 5b; $p = 0.0735$).

3.3. Effects of 3-NPA treatment in adenosine receptors gene expression

To investigate whether 3-NPA treatment alters adenosine receptors gene expression, we analyzed its relative mRNA expression levels of the whole brain. The analysis of the relative expression of adenosine receptors (Fig. 8) did not show significant differences in mRNA transcripts of *adora1b* ($t = 0.4265$; $p = 0.6740$), *adora2aa* ($t = 0.6284$; $p = 0.5372$), *adora2ab* ($t = 1.095$; $p = 0.2858$), and *adora2b* ($t = 0.5655$; $p = 0.5787$).

4. Discussion

In this present study, we evaluated whether the adenosine modulation would reverse the locomotor and memory deficits induced by 3-NPA. Our findings showed that 3-NPA induced a decrease in distance traveled and latency to entry into the dark zone of the shock compartment, suggesting a bradykinesia-like effect and memory impairment. The treatment with the nonselective adenosine receptor antagonist caffeine and the selective $A_{2A}R$ antagonist ZM 241385 reversed the bradykinesia and memory deficits. Our results also demonstrated that selective agonist $A_{2A}R$ CGS 21680 exacerbated the bradykinesia induced by 3-NPA. Moreover, this study also demonstrated that the A_1R agonist and antagonist, CPA and DPCPX, respectively, and either inhibitor of nucleoside-metabolizing enzyme EHNA or nucleoside transporter dipyridamole reversed the memory deficits induced by 3-NPA.

HD is a fatal neurodegenerative disorder in which the patients exhibit severe clinical symptoms such as chorea, lack of coordination, and bradykinesia (Bortolatto et al., 2017; Oosterloo et al., 2021).

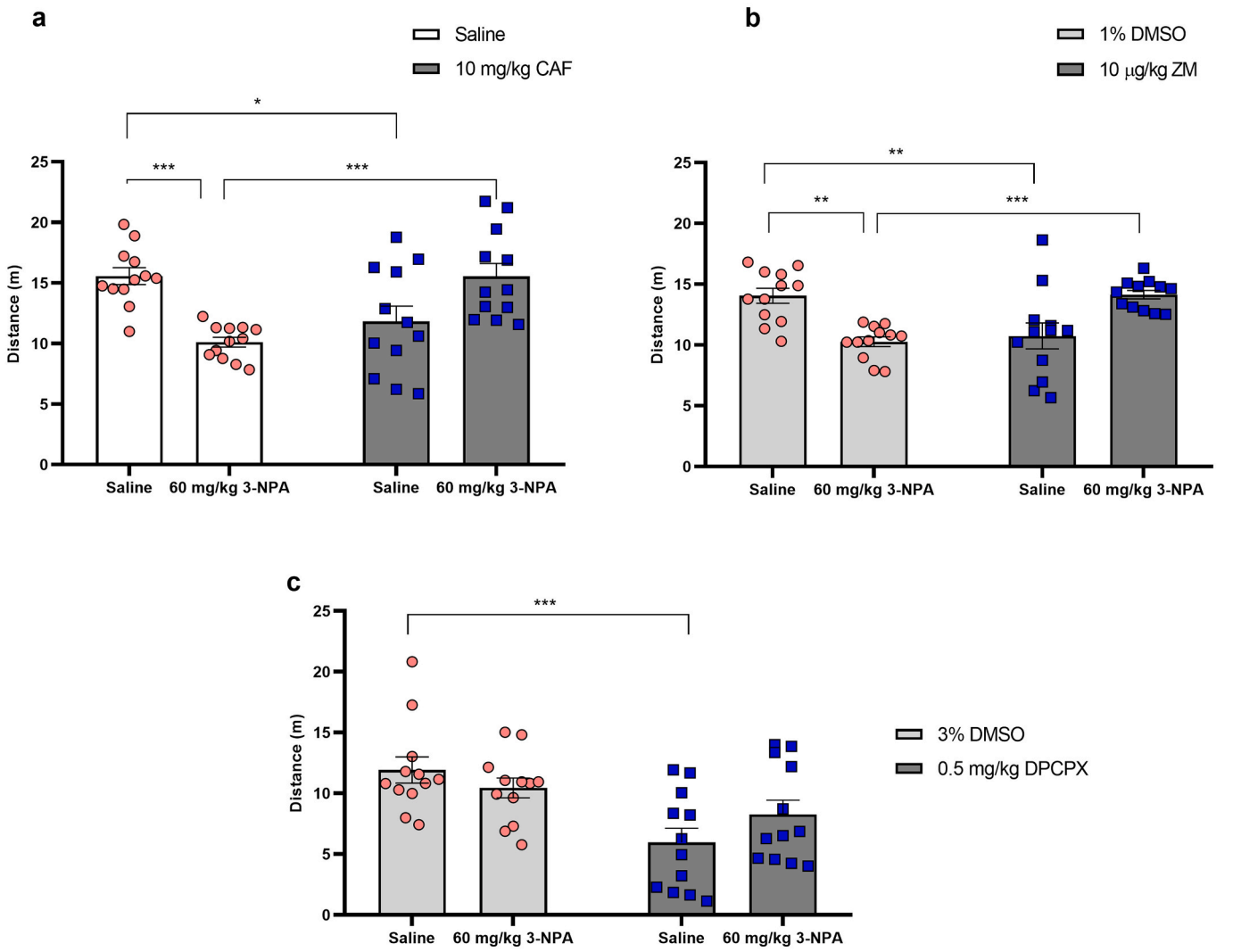

Fig. 3. Effects of adenosine antagonists caffeine (CAF), ZM 241385 (ZM), and DPCPX treatment on locomotor activity deficits induced by 3-NPA. Animals received seven i.p. injections of saline or 3-NPA for 24 days. One-hundred twenty hours (29 day) after the 3-NPA exposure animals were treated with a single i.p. injection of (a) saline or CAF (10 mg/kg), (b) DMSO (1%) or ZM 241385 (10 µg/kg), and (c) DMSO (3%) or DPCPX (0.5 mg/kg) 30 min before the test. Data are expressed as mean ± S.E.M (n = 12 for each group) and were analyzed by two-way ANOVA followed by a Bonferroni post-hoc test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Currently, there is no cure for HD, because HD patients are treated with pharmacotherapy that attenuates the symptoms. In addition, few treatments are available for symptoms attenuation, including mainly drug modulators of the dopaminergic system (McGarry et al., 2017; Claassen et al., 2019; Bashir and Jankovic, 2020). 3-NPA is known to produce behavioral changes in animals similar to those occurring in HD, thus mimic this disease and is used as a tool to develop new therapies (Túnez et al., 2010). In the present study, we showed that 3-NPA reduced the locomotion and caused memory impairment in adult zebrafish. The locomotion and memory behavioral alterations produced by 3-NPA could be justified by some reasons such as the ability of the 3-NPA to promote inhibition of SDH which decreases the mitochondrial membrane potential causing the ATP depletion, relocalization of small proteins in the cytosol, activation of caspases related to apoptosis, and a massive calcium influx that activates calcium-dependent enzymes mediating deleterious intracellular effects. Consequently, these effects induce oxidative stress promoting cell death (Brouillet et al., 2005) mainly in the striatum, cerebellum, prefrontal cortex, and hippocampus (Ahmadi et al., 2018; Torabi et al., 2020), which are similar brain regions in the zebrafish corresponding to subpallium and posterior tuberculum, cerebellum, dorsal central pallium, and dorsal lateral pallium (Wullimann, 2014; Kozol et al., 2016). Also, 3-NPA may cause neurochemical imbalance decreasing the DA levels and their metabolites (Kaur et al., 2015).

Adenosine acts as a neuromodulator on several neurotransmission systems, participating in controlling several behavioral functions, such as motor control, cognition, and emotions (Burnstock, 2015). The effects of adenosine receptor modulators drugs on motor functions and memory have been the focus of studies in several neurological diseases (Iijima et al., 2019; Paton, 2020; Janitschke et al., 2021). In the present study, the A_1R agonist CPA did not reverse the locomotor disabilities induced by 3-NPA in adult zebrafish. In contrast, the A_{2A}R agonist CGS 21680 exacerbated the hypolocomotion induced by 3-NPA. These results conflict with previous studies conducted in HD animal models using the same drugs mentioned above. In a pharmacological model of HD induced by 3-NPA, the A_1R agonist adenosine amine congener (ADAC) acute administration attenuated the dystonia caused by 3-NPA (Blum et al., 2002). The A_{2A}R agonist CGS 21680 reversed the hypolocomotion profile in R6/2 animals - the most widely used transgenic model of HD (Chou et al., 2005). On the other hand, similar results to our study regarding the CGS 21680 were found by Blum et al. (2003) that reported that 1 mg/kg CGS 21680 decreased spontaneous locomotion in animals that received 3-NPA treatment (Blum et al., 2003). Furthermore, in a transgenic rat model of HD, the CGS 21680 at doses 0.03 and 0.1 mg/kg

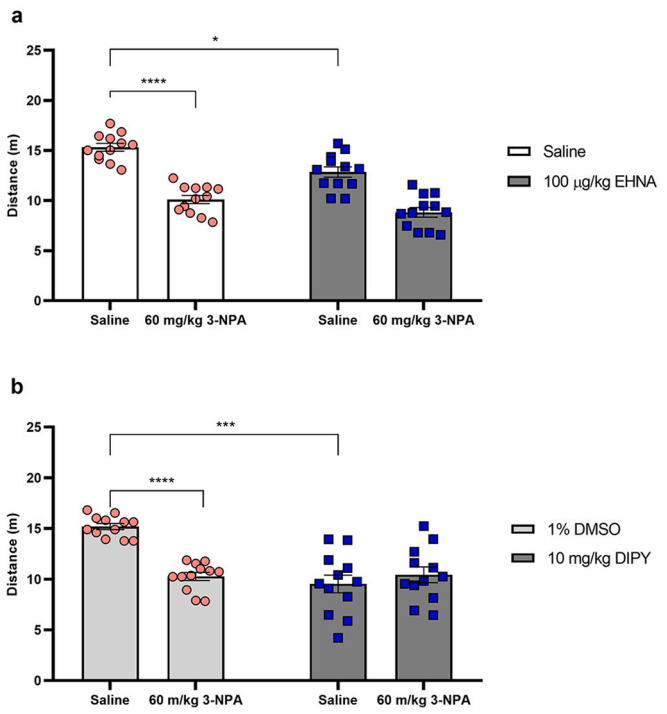

Fig. 4. Effects of the inhibitor of adenosine deaminase EHNA or nucleoside transporter dipyridamole (DIPY) treatments on locomotor activity deficits induced by 3-NPA in adult zebrafish. Animals received seven i.p. injections of saline or 3-NPA for 24 days. One-hundred twenty hours (29 day) after the 3-NPA exposure, animals were treated with a single i.p. injection of (a) saline or EHNA (100 µg/kg), and (b) DMSO (1%) or DIPY (10 mg/kg) 30 min before the test. Data are expressed as mean ± S.E.M (n = 12 for each group) and were analyzed by two-way ANOVA followed by a Bonferroni post-hoc test. * $p < 0.05$, *** $p < 0.001$, **** $p < 0.0001$.

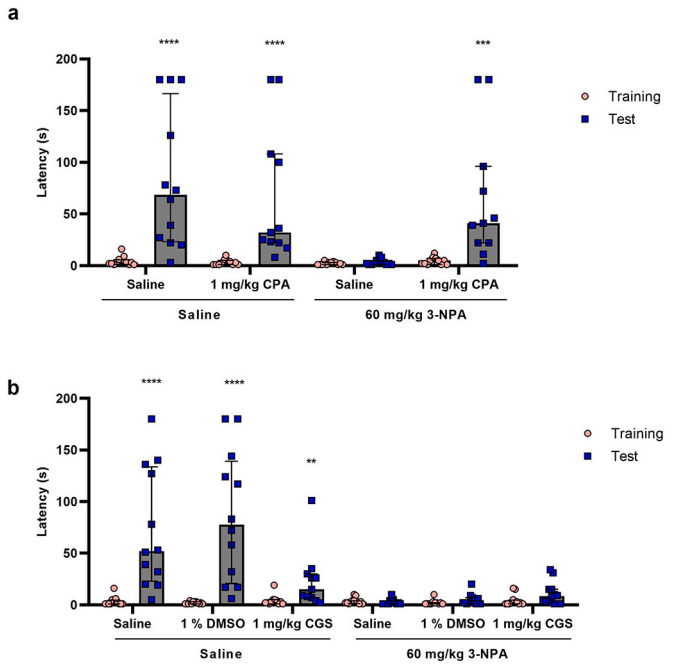

Fig. 5. Effect of adenosine receptor agonists (a) CPA or (b) CGS 21680 (CGS) treatments on the 3-NPA-induced memory impairment in the inhibitory avoidance task. Animals received seven i.p. injections of saline or 3-NPA for 24 days. One hundred twenty hours (29 day) after the 3-NPA exposure, animals were treated with a single i.p. injection of saline, 1% DMSO, CPA (1 mg/kg), or CGS 21680 (1 mg/kg) 30 min before the test. Effects of saline or 3-NPA on the latency to enter the dark compartment during training and test sessions in the inhibitory avoidance task were evaluated. Data are expressed as the median ± interquartile range ($n = 10$–12 for each group) and were analyzed individually for each group. ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$ indicate the differences between training and test sessions for each group compared by Mann-Whitney *U* matched pair test. There were no differences between training performances among all groups, as evaluated by the Kruskal-Wallis test.

produced a depressant effect on the locomotor activity compared to WT littermates (Guitart et al., 2016). These divergent results between the studies may be due to dose, treatment protocol; that is; if the agonist adenosine receptor was used after or before inducing the HD and the stage of the disease that the animals presented in the pharmacological or genetic HD model. Moreover, we hypothesized that the increase in hypolocomotion induced by CGS 21680 may be due to the $A_{2A}R/D_2R$ heterodimerization; that is, the $A_{2A}R$ activation through CGS 21680 causes blockade of D_2R decreasing the affinity of D_2R for dopamine (DA) and, consequently, depressing the locomotor function.

The pharmacological or genetic blockade of A_1R and $A_{2A}R$ antagonists also has been the focus of studies evaluating its neuroprotective effects in HD. In a pharmacological model of HD induced by quinolinic acid, the treatment with a nonselective A_1R and $A_{2A}R$ caffeine antagonist (at doses 10, 20, and 40 mg/kg) was able to attenuate both the hyperkinesia (at 7 days after the treatment) and the bradykinesia (at 14 and 21 treatment days; Mishra and Kumar, 2014). After HD induced by 3-NPA, animals treated with $A_{2A}R$ MSX-3 antagonist had an increase in spontaneous locomotion compared to control animals (without HD symptoms; Blum et al., 2003). Moreover, in rats with HD symptoms induced by 3-NPA, the treatment with SCH 58261 reversed the bradykinesia caused by 3-NPA (Bortolatto et al., 2017). On the other hand, the impact of $A_{2A}R$ genetic deletion on motor activity in R6/2 mice showed that the animals CAG120-wild type (R6/2) had a decrease in locomotor activity compared to wild type animals. In addition, the animals CAG120-A_{2AR}KO (R6/2 $A_{2A}R$ knockout) also had a decrease in locomotor activity in comparison with wild-type-A_{2AR}KO. Although, the animals CAG120-A_{2AR}KO had an increase in locomotor activity compared to CAG120-wild type, this increase was not significant (Li et al., 2015). Moreover, the blockade of $A_{2A}R$ with the SCH 58261 at 0.01 mg/kg did not prevent the impairment in motor coordination in R6/2 mice compared to WT controls (Domenici et al., 2007). Compatible with the most of previous studies, we found that the 10 mg/kg caffeine or 10 µg/kg ZM 241385 were able to reverse the bradykinesia induced by 3-NPA in adult zebrafish, while the selective antagonist A_1R DPCPX did not reverse the bradykinesia. This finding allows us to believe that the blockade of $A_{2A}R$ has a neuroprotective effect on locomotor function in the late stage of HD phenotype. In addition, we hypothesized that the neuroprotective role of $A_{2A}R$ antagonists, at low doses, could be able to block a few striatal $A_{2A}R$ located pre-synaptically on corticostriatal neurons that control glutamate release, thus inhibiting or facilitating the increase of extracellular glutamate, once the role of presynaptic $A_{2A}R$ in corticostriatal terminals has previously been shown to be the main effect of endogenously engaged $A_{2A}R$ in the control of different responses (Shen et al., 2006; Shen et al., 2013), in particular in HD models (Li et al., 2015). Moreover, the increase of the D_2R affinity for DA through of heterodimerization may promote the reversion of the locomotor suppression. In addition, another hypothesis to the reversion of locomotion by $A_{2A}R$ antagonism of is that these compounds could be reducing the size of brain lesion induced by 3-NPA, attenuating the oxidative stress and pro-inflammatory cytokines, thus promoting the improve in locomotor function (Bortolatto et al., 2017).

To better understand the role of adenosine signaling in locomotor function, we tested the effects of inhibitors of adenosine transport (dipyridamole) and adenosine deamination (EHNA) on 3-NPA-induced locomotor deficits in zebrafish. Few investigations have demonstrated any neuroprotective properties of these compounds in neurological diseases. Studies have demonstrated controversial results regarding the

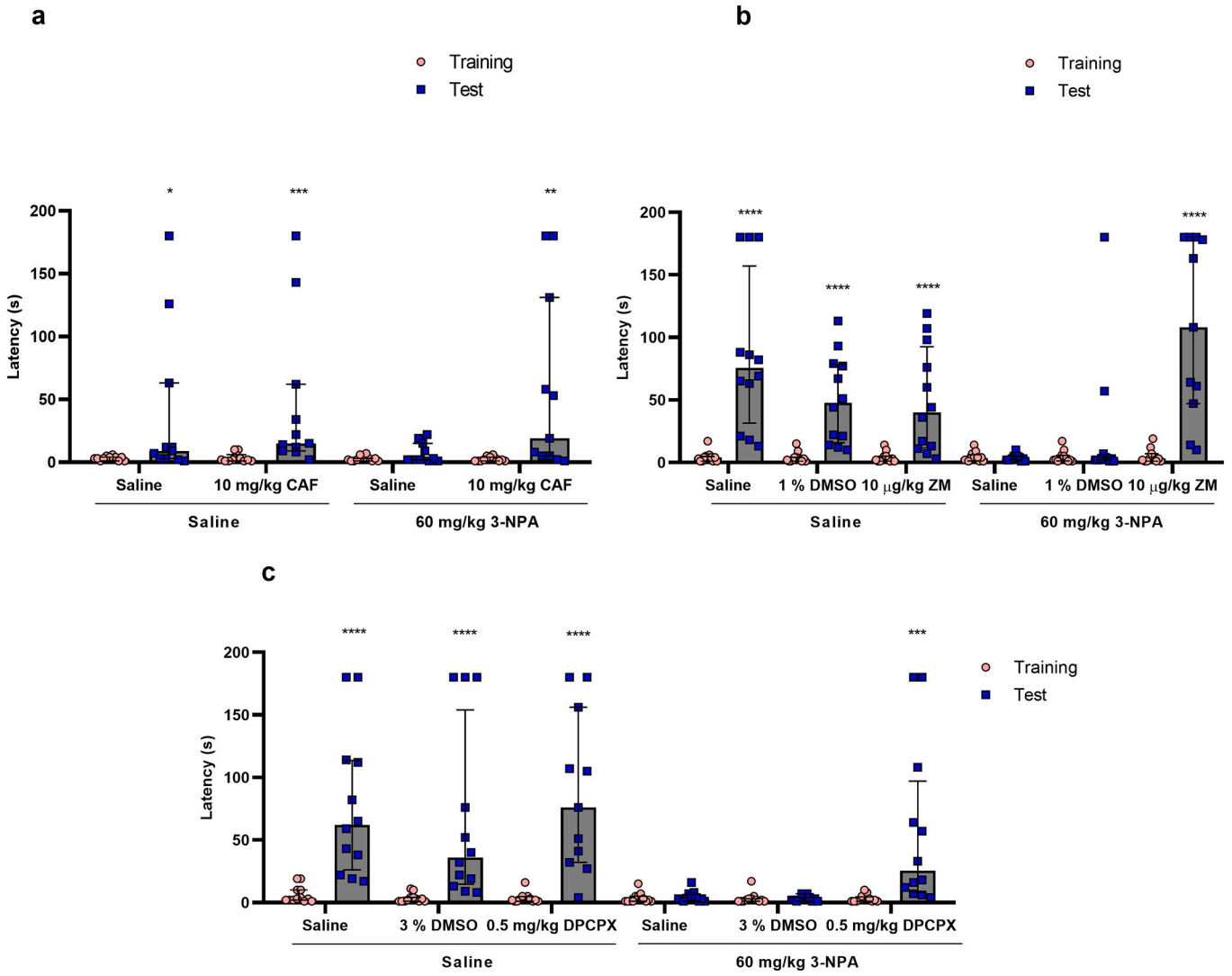

Fig. 6. Treatment with adenosine receptor antagonist (a) caffeine (CAF), (b) ZM 241385 (ZM), and (c) DPCPX reversed 3-NPA-induced memory impairment in the inhibitory avoidance task. Animals received seven i.p. injections of saline or 3-NPA for 24 days. One hundred twenty hours (29 day) after the 3-NPA exposure, animals were treated with a single i.p. injection of saline, 1% DMSO, CAF (10 mg/kg), ZM 241385 (10 μg/kg), or DPCPX (0.5 mg/kg) 30 min before the test. Effects of saline or 3-NPA on the latency to enter the dark compartment during training and test sessions in the inhibitory avoidance task were evaluated. Data are expressed as the median ± interquartile range (n = 10–12 for each group) and were analyzed individually for each group. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$ indicate the differences between training and test sessions for each group compared by Mann-Whitney *U* matched pair test. There were no differences between training performances among all groups, as evaluated by the Kruskal-Wallis test.

inhibitor of adenosine transport and adenosine deamination. The dipyridamole pretreatment produced bradykinesia in an animal model of Parkinson's disease (Tariq et al., 1998). In contrast, a study conducted by Kao et al. (2017) showed that R6/2 mice knockout for ENT1 (equilibrative nucleoside transporter type 1) did not demonstrate differences in their deterioration motor function compared with their littermate controls (R6/2 mice; Kao et al., 2017). In addition, Kao et al. (2017), using another nucleoside transporter inhibitor, JMF1907, in R6/2 mice showed that this compound counteracted the progressive deterioration in motor coordination. Moreover, the pretreatment with dipyridamole, EHNA, and other adenosine deaminase inhibitor called NBTI (S-(4-nitrobenzyl)-6-thioinosine) caused a longer latency to reach the tonic-clonic seizure in adult zebrafish (Siebel et al., 2015). Our results revealed that neither dipyridamole nor EHNA was able to reverse the bradykinesia induced by 3-NPA. The discrepancies between our work and most studies mentioned above can be attributed to the different neurological conditions, doses, and methods of dipyridamole and EHNA administration.

Another point to be discussed in our findings is the effect of A_1 agonist, A_1 and A_{2A} antagonists, and inhibitors of adenosine transport and adenosine deamination treatment per se on locomotor parameters. We observed that CPA, caffeine, DPCPX, ZM 241385, EHNA, and dipyridamole per se reduced the distance traveled compared with control animals. Studies in rodents showed that selective A_1R agonist CPA, besides A_1R and A_{2A}R antagonists like caffeine and DPCPX by itself produced a decreased spontaneous locomotor activity depending on the dose administered (Nikodijevic et al., 1990; Ferré et al., 1994; Collins et al., 2010). Furthermore, a previous study also observed that CPA alone reduced the locomotor activity in adult zebrafish (Siebel et al., 2015). On contrary, previous studies in adult zebrafish have also demonstrated that caffeine, DPCPX, ZM 241385, EHNA, and dipyridamole treatment did not alter the spontaneous locomotion (Bortolotto et al., 2015; Siebel et al., 2015). It is important to note, that in the previous studies mentioned above, zebrafish were submitted to one intraperitoneal injection with adenosinergic drugs or vehicle (saline or DMSO) after the induction of cognitive impairment or seizure model by

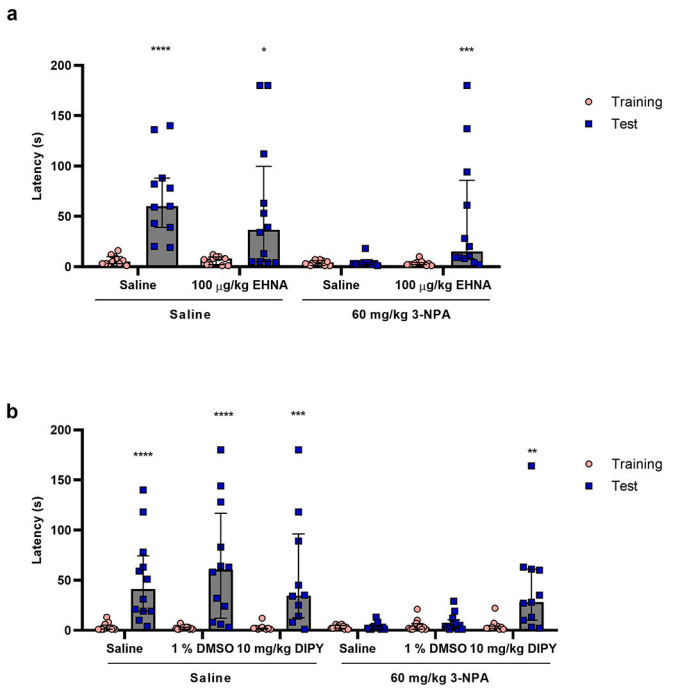

Fig. 7. Treatment with inhibitor of adenosine deaminase (a) EHNA and inhibitor nucleoside transporter of adenosine (b) Dipyridamole (DIPY) reversed 3-NPA-induced memory impairment in the inhibitory avoidance task. Animals received seven i.p. injections of saline or 3-NPA for 24 days. One hundred twenty hours (29 day) after the 3-NPA exposure, animals were treated with a single i.p. injection of saline, 1% DMSO, EHNA (100 μg/kg), or DIPY (10 mg/kg) 30 min before the test. Effects of saline or 3-NPA on the latency to enter the dark compartment during training and test sessions in the inhibitory avoidance task were evaluated. Data are expressed as the median ± interquartile range (n = 10–12 for each group) and were analyzed individually for each group. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$ indicate the differences between training and test sessions for each group compared by Mann-Whitney *U* matched pair test. There were no differences between training performances among all groups, as evaluated by the Kruskal-Wallis test.

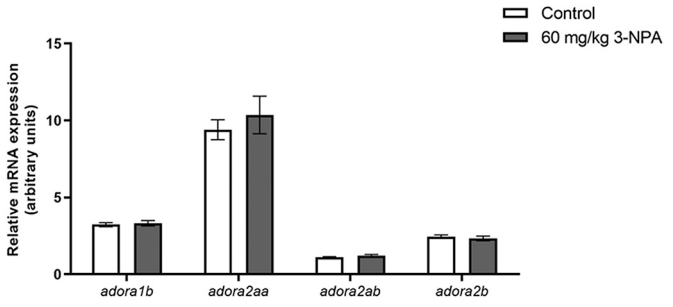

Fig. 8. Effect of treatment with 3-NPA or saline on adenosine receptor relative gene expression in adult zebrafish whole brain. Data are expressed as the mean ± S.E.M of six independent experiments (brain pool of four to six) and were analyzed by Student's *t*-test for unpaired samples.

scopolamine or immersion in PTZ, respectively (Bortolotto et al., 2015; Siebel et al., 2015). However, in the present study, the animals were submitted to one intraperitoneal injection with adenosinergic drugs with saline or DMSO as a vehicle after seven intraperitoneal injections with saline or 3-NPA, according to the HD model described (Wiprich et al., 2020). Therefore, control animals were exposed to a repeated injection protocol with saline, which could induce a more stressful condition and trigger mechanisms able to be modulated by adenosinergic compounds with impact on the animal behavior. Also, it is important to highlight that in a neurodegenerative condition, the $A_{2A}R$ antagonist treatment may be a neuroprotector once that reversed the 3-NPA effect on locomotor function. In line with our results, studies in other neurodegenerative diseases mainly Parkinson's disease have shown that the treatment with $A_{2A}R$ antagonists activates the D_2R, and consequently act on reducing cyclic adenosine monophosphate, and then decreases the protein kinase A which could result in neuroprotective effects (Kanda and Jenner, 2020; Atif et al., 2021; Shang et al., 2021).

Evidence supports a modulatory role for adenosine receptors in the control of cognition and memory (Pereira et al., 2002; Pagnussat et al., 2015). We have shown that both selective antagonists of $A_{2A}R$ and nonselective antagonists of $A_{2A}R$ and A_1R, ZM 241385, and caffeine, respectively, reversed 3-NPA-induced memory deficits in adult zebrafish, while the selective agonist of $A_{2A}R$ CGS 21680 did not reverse 3-NPA-induced cognitive decline in adult zebrafish. Other studies have also described the beneficial effects of A_{2A} antagonism on memory in animal model of neurodegenerative diseases (Chen, 2014; Woods et al., 2016; Merighi et al., 2021). The administration of $A_{2A}R$ antagonist istradefylline improvement cognitive impairment in an animal model of Parkinson's disease induced by 6-hydroxydopamine (Horita et al., 2013). Specifically, in HD, Li et al. (2015) evaluated the ability of $A_{2A}R$ inactivation to prevent cognitive deficits in two R6/2 transgenic lines of HD mice (CAG120 and CAG240) with $A_{2A}R$ knockout (KO) and their corresponding wild-type. The authors observed that genetic inactivation of $A_{2A}R$ prevented working memory deficits induced by R6/2-CAG120 at post-natal week 6 and by R6/2-CAG240 at post-natal months 2 and 3. In addition, the study investigated if the pharmacological blockade of $A_{2A}R$ with KW6002 could mimic the beneficial impact of the genetic $A_{2A}R$ inactivation on the memory deficits of R6/2-CAG240 mice at 3 months demonstrating that the KW6002 reversed the working memory deficits in R6/2 mice (Li et al., 2015). Interestingly, another study demonstrated in R6/2 mice that the combined chronic treatment with D_1R (SCH23390) plus $A_{2A}R$ (SCH58261) antagonist ameliorates cognitive dysfunction (Tyebji et al., 2015). Pagnussat et al. (2015) showed in three memory tasks, including the inhibitory avoidance task, that the $A_{2A}R$ antagonist prevented the memory deficits caused by scopolamine, while the CGS 21680 triggered memory impairment (Pagnussat et al., 2015). In addition, it was also shown that either the optogenetic activation of the $A_{2A}R$ transducing system, (Li et al., 2015) or the overexpression of $A_{2A}R$ (Temido-Ferreira et al., 2020) are sufficient to trigger memory impairment.

Our results also demonstrated that both the selective A_1R agonist CPA and antagonist DPCPX reversed 3-NPA-induced memory in adult zebrafish during the inhibitory avoidance task. To best of our knowledge, this is the first study evaluating the effects of CPA in the inhibitory avoidance task in zebrafish. Previous works demonstrated significant improvement of scopolamine-induced memory impairment using DPCPX in adult zebrafish and mice (Bortolotto et al., 2015; Pagnussat et al., 2015). Furthermore, the chronic treatment with CPA resulted in a significant memory improvement in C57BL/6 mice (Von Lubitz et al., 1993). Taken together, our results suggest that A_{2A} and A_1 antagonism and A_1 agonism can improve memory performance in an animal model of HD. Although the adenosine receptors were already identified in zebrafish through molecular studies (Capiotti et al., 2011; Boehmer et al., 2009), the affinity of adenosine receptors by their agonists and antagonists have not been reported. It is important to highlight that there is a gap of knowledge on the pharmacokinetics and pharmacodynamics of these drugs in zebrafish, which could influence the effects observed on memory. Therefore, the seemingly paradoxical effects by using both agonists and antagonists of adenosine receptors needs to be further investigated in zebrafish.

Adenosine can be released from the cytoplasm through nucleoside transports or be formed by the extracellular breakdown of adenosine triphosphate by ectonucleotidases (Boison, 2013). When adenosine levels are exacerbated, adenosine deaminase activity or the ADK enzyme

can control adenosine levels (Siebel et al., 2015; Boison and Jarvis, 2021). Despite its importance, the pharmacological modulation of adenosine production and degradation control in HD remain unclear. Previous studies demonstrated that an inhibitor of equilibrative nucleoside transporter (known as J4) prevents spatial memory cognitive decline in an Alzheimer's disease mouse model (Lee et al., 2018; Chang et al., 2021). Lastly, Bortolotto et al. (2015) showed that dipyridamole and EHNA treatment significantly improved scopolamine-induced memory impairment (Bortolotto et al., 2015). In this study, we have demonstrated that the inhibition of nucleoside transporters and adenosine deaminase activity modulates the 3-NPA-induced memory impairment in adult zebrafish. These results agree with the literature and suggest that modulation of adenosine levels via inhibition of nucleoside transporters or adenosine deamination has an essential role in memory effects in zebrafish. Regarding the control of adenosine levels by ADK, there is no previous evidence investigating this enzyme in HD.

Alteration in adenosine receptors is a feature of HD. To determine if transcriptional alterations on the adenosine receptors have occurred with the 3-NPA treatment, a RT-qPCR analysis was carried out in animals treated with 3-NPA or controls. The results showed that there was no change in the relative amount of *adoraa1, adoraa2a, adoraa2b*, and *adora2b* mRNA transcripts after 3-NPA treatment. In contrast to our data, a binding study showed that the density of A_1R was significantly reduced in the cortex and striatum in R6/2 mice compared to wild-type mice (Ferrante et al., 2014). In the same line, studies have demonstrated the relative amount of $A_{2A}R$ is reduced in the striatum of R6/2 mice, R6/1 mice and in humans with HD (Villar-Menéndez et al., 2013; Kao et al., 2017). Similarly, Cha et al. (1999) also observed a reduction in profile of $A_{2A}R$ binding in R6/2 mice (Cha et al., 1999). Another study examined whether *htt* protein length influences the ability of altering gene expression conducting mRNA profile analysis in mice that express an extended N-terminal fragment (HD46 and HD100; 964 amino acids) or full-length (YAC72; 3144 amino acids) mutant *htt* transprotein. The study showed that there were small decreases in $A_{2A}R$ mRNA in HD100 mice. However, neither HD46 nor YAC72 mice exhibited altered mRNA levels like those observed in R6/2 mice, N171-82Q mice or human HD patients (Chan et al., 2002). Therefore, our findings suggest that the effects of 3-NPA treatment occurs in a post-transcriptional or post-translational level.

One strength of this study is the assessing locomotor behavior and memory using A_1R and $A_{2A}R$ agonists and antagonists and modulators of adenosine metabolism. To our knowledge, this study is the first to report the role of five A_1R and $A_{2A}R$ modulators and two adenosine metabolism modulators in 3-NPA-induced bradykinesia and memory impairment that closely resemble the behavioral pathology of late-stage HD.

In summary, our findings indicate that the reversion of locomotor deficits induced by 3-NPA in adult zebrafish is promoted mainly through pharmacological blockade of $A_{2A}R$. In addition, there is a recovery of memory mediated through blockade of $A_{2A}R$ and A_1R, activation of A_1R, and control of adenosine levels by adenosine deaminase and nucleoside transporters. These data corroborate the hypothesis that $A_{2A}R$ is involved in the locomotor function and have a close relationship with D_2R, while the adenosine signaling is involved in memory processing in the late stage of HD. Therefore, the modulators used in the present study may be a target of therapeutic interest in HD. Future preclinical and human studies should be conducted to confirm the potential therapeutic of adenosine modulators in HD.

Ethical statement

All protocols were approved by the Institutional Animal Care Committee from Pontifícia Universidade Católica do Rio Grande do Sul (CEUA-PUCRS, permit number 9406/2019) and Comply with the guideline of the National Council for the Control of Animal Experimentation (CONCEA). This study was registered in the Sistema Nacional de Gestão do Patrimônio Genético e Conhecimento Tradicional Associado – SISGEN (Protocol No A3B073D).

CRediT authorship contribution statement

Melissa Talita Wiprich: Conceptualization, Data curation, Investigation, Methodology, Writing – original draft. **Stefani Altenhofen:** Investigation, Formal analysis. **Darlan Gusso:** Investigation. **Rafaela da Rosa Vasques:** Investigation. **Rodrigo Zanandrea:** Investigation. **Luiza Wilges Kist:** Investigation, Formal analysis. **Mauricio Reis Bogo:** Resources, Supervision. **Carla Denise Bonan:** Conceptualization, Supervision, Funding acquisition, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pnpbp.2022.110602.

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