Investigation of ketamine pharmacological modulation of aggressive behavior in adult zebrafish

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Introduction

Ketamine is a glutamate N-methyl-d-aspartate (NMDA) receptor antagonist used as an anesthetic agent [1]. However, in recent years, studies have reported the antidepressant potential of ketamine, which produces fast and robust effect on depressive patients resistant to conventional treatment [2].

Pharmacological parameters of ketamine for use as an antidepressant agent as optimal dose, route of administration and effects of chronic treatment are not yet fully characterized [3]. In general, some NDMA receptor antagonists can cause severe deficiencies and behavioral disorders [4]. In some reported cases, higher doses of ketamine administered for a prolonged period were responsible for inducing various side effects [5].

Aggressiveness is a common behavioral disorder observed after administration of many psychoactive substances. This behavior may be a side effect of administration of ketamine since it consists of a behavior which is highly regulated by glutamatergic neurotransmission [4, 6]. The effects of ketamine on aggressive states are not well characterized and there are studies evaluating this behavior in rats [7, 8], mice [4] and humans [9]. In this sense, the zebrafish (Danio rerio) is an alternative animal model which has a several advantages that enables the characterization of behavioral effects psychoactive substances. The aim of this study is to evaluate the effects of acute exposition to ketamine on aggressiveness in adult zebrafish using the Mirror Biting Test.

Material and Methods

Animals and housing

A total of 149 adult wild type short-fin zebrafish (6 to 8 months / 3.6 - 4.0 cm long/ all genders) were obtained from a local commercial distributor and housed in groups of 30 fish in a 30 L tank. The system water quality was subjected to mechanical, biological and chemical filtration (using...
The illumination of the house tank was performed with a fluorescent light lamp turned on at 08:00 h and off at 18:00 h according to the standards of zebrafish care [10]. The zebrafish’s feeding was carried out twice a day with an automatic feeder. The experiments were approved by Ethics Committee on Animal Use (CEUA) of University of Sao Paulo (Protocol 14.1.750.53.6).

Pharmacological manipulations

The method of exposure was based on immersion of zebrafish in an aqueous solution containing ketamine. Zebrafish were exposed to ketamine or drug-free water for 20 minutes in a 1000 mL tank and then subjected to behavioral assays. The experimental groups were exposed to concentrations of 5, 20, 40 or 60 mg L⁻¹ of ketamine, selected according to other published studies [11-13]. The ketamine used in this study was the injectable medication containing ketamine hydrochloride (Dopalen®, Ceva).

Mirror Biting Test

The Mirror Biting Test was performed in a rectangular tank (20 height x 30 length x 15 cm width) containing an internal mirror in one of its side walls. After the exposure time, zebrafish was individually removed from exposure tank and inserted in the test tank, in the opposite side of the mirror. The test was recorded for 5 minutes with a digital camera positioned frontally to the tank [14]. All tests were conducted between 8h and 12h. The results were analyzed and manually quantified after by a trained observer. The quantified behavioral parameters were the duration of contact with the mirror and the latency to first contact.

Statistical analysis

The validation of behavioral data was conducted using analysis of variance (ANOVA) followed by Tukey's post-hoc test for statistically significant results with OriginPro 8® software (OriginLab Co.). All data is presented as mean ± SEM.

Results and Discussion

The latency to the first mirror contact was significantly changed with acute exposition to ketamine (F(4, 144) = 4.44205, p < 0.05). Similarly, the mirror biting duration was also significantly modified by ketamine (F(4, 144) = 3.55128, p < 0.05). According to the Tukey test for multiple comparisons, we can observe an interesting effect in the experimental groups in the two assessed and quantified parameters (Figure 1). The dose of 40 mg L⁻¹ caused a significant reduction in latency relative to the control and exposed to 60 mg.L⁻¹ groups. In addition, the concentration of 40 mg.L⁻¹ increased the duration of contact with the mirror significantly from lower doses, without a significant variation compared to the control group.

The mirror-induced aggression (MIA) is a well-established and simple to record paradigm that can be used to quantify aggressive behavior [14-17]. Fish attacks their image reflected in a mirror because they consider it as if it was an intruder [18]. In the Mirror Biting Test, aggressive behaviors are associated with the time spent on attacks against the fish’s mirror image [16]. Although the mirror-induced aggression is a behavioral endpoint suitable for measuring aggressive behavior in fish, a limitation of this
test is that it does not measure all aspects of an aggressive interaction with other fish\[17].

In this test, reduced latency to first contact with the mirror and increased mirror biting duration indicates increased aggressiveness \[14, 16\]. Although it is observed an increase in the mirror biting duration for exposed groups at doses of 5, 40 and 60 mg L\(^{-1}\), this increase is not significant compared to the control group, according to analysis of variance. Statistically, our behavioral results showed that ketamine does not induce aggressive behavior in zebrafish.

Our findings suggest that ketamine does not appear to exert any effect on the behaviors evaluated by Mirror Biting Test, not increasing nor reducing aggressive behaviors, at subanesthetic doses. Riehl et al. (2011) observed that ketamine, at the doses of 20 mg L\(^{-1}\) and 40 mg L\(^{-1}\), decreased levels of whole-body cortisol in relation to non-exposed fish. In addition, Newman et al. (2012) observed that acute exposure to ketamine followed water self-administration in mice reduced the number of attack bites frequency and ketamine did not interact with ethanol on enhancing aggressive behavior. In another study, we observed that ketamine also increased hyperactivity in adult zebrafish at the doses of 40 and 60 mg L\(^{-1}\), which can explain the higher values for mirror biting duration as a hyperactivity consequence \[13\].

Dopamine and serotonin are involved in the modulation of aggressive behavior \[19\]. Increases in dopamine levels are associated with increased aggressive behaviors \[20\]. In contrast, higher levels of serotonin reduce states of aggressiveness \[20\]. The role of ketamine in modulation of aggressive behavior is probably associated with the regulation of the neurotransmitters levels such as dopamine and serotonin. In this sense, a biochemical and molecular investigation is needed to investigate it would be expected that ketamine acts on aggressiveness states in adult zebrafish.

Conclusions

Ketamine is a promising antidepressant agent. However, the possible adverse effects induced by ketamine must be studied in detail. In this context, aggression is one these adverse effects reported after ketamine administration in subanesthetic doses. Our results not suggest that ketamine administered in doses subanesthetic modulates aggressive behaviors in adult zebrafish. However, more behavioral parameters and biochemical and molecular studies should be evaluated to fully elucidate the mechanism of action of ketamine on regulation of states of aggressiveness.

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Author Contributions

EGC performed behavioral tests and statistical analysis and wrote the manuscript. BSM coordinated the study and wrote and revised the manuscript. All authors read and approved the final version of manuscript.

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