Research report

Effects of piracetam on behavior and memory in adult zebrafish

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Abstract

Piracetam, a derivative of γ-aminobutyric acid, exerts memory-enhancing and mild anxiolytic effects in human and rodent studies. To examine the drug's behavioral profile further, we assessed its effects on behavioral and endocrine (cortisol) responses of adult zebrafish (Danio rerio) – a novel model species rapidly gaining popularity in neurobehavioral research. Overall, acute piracetam did not affect zebrafish novel tank and light–dark box behavior at mild doses (25–400 mg/L), but produced nonspecific behavioral inhibition at 700 mg/L. No effects on cortisol levels or inter-/intra-session habituation in the novel tank test were observed for acute or chronic mild non-sedative dose of 200 mg/L. In contrast, fish exposed to chronic piracetam at this dose performed significantly better in the cued learning plus-maze test. This observation parallels clinical and rodent literature on the behavioral profile of piracetam, supporting the utility of zebrafish paradigms for testing nootropic agents.

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1. Introduction

Piracetam is a cyclic derivative of γ-aminobutyric acid (GABA). Since its discovery in the 1960s, it has been widely used in humans [38,39,43,47,55] and rodents [4,5,36] as a memory-enhancing (nootropic) agent. With low toxicity and few side effects, piracetam is effective in treating dementia and cognitive impairment [40,47], stroke [39] and ischemia [38]. Piracetam modulates neuroplasticity, neuroprotection and brain metabolism [53], has anticonvulsant effects [14], as well as reduces symptoms of clinical depression, anxiety and alcohol withdrawal [11,27].

Piracetam has also been extensively tested in various rodent models. In addition to nootropic activity [21,32], animal anxiety-like behavior has been found to be sensitive to this drug. For example, acute piracetam reduces anxiety in rat social interaction [16] and in rabbit conflict tests [46]. Similarly, mild chronic doses of piracetam reduce rat anxiety in the open field, elevated plus-maze, foot shock-induced fighting [4] and Vogel’s conflict tests [29].

Despite numerous clinical and experimental studies, the mechanisms of piracetam’s action remain poorly understood [32,52]. The drug is known to modulate membrane fluidity, which may affect GABA receptor binding, and neurotransmitter release [10]. Another proposed mechanism of piracetam’s action is at the benzodiazepine site of the GABA receptor, since flumazenil inhibits its effects [30]. In addition to targeting GABA receptors, piracetam can also interact with glutamate receptors, suggesting another potential mechanism for its nootropic action [27].

A more comprehensive understanding of piracetam psychopharmacology requires further studies, utilizing novel approaches and new model species, in addition to humans and rodents. Although piracetam has been studied in several fish species (modulating their vestibular and feeding behavior [7,20,26]), its effects on fish anxiety and memory are currently unclear. As adult zebrafish (Danio rerio) are becoming popular screens for various psychotropic drugs [12,54], the behavioral effects of piracetam have not been tested in this model.

Given the sensitivity of zebrafish anxiety and cognition to various pharmacological manipulations [8,54], these fish may represent a promising novel model to study the effects of piracetam and similar psychotropic compounds. Additionally, zebrafish possess all major nuclei, neurotransmitters and receptors, allowing for translation of their behavioral and physiological modulation by nootropic compounds [1,34,35]. Our study focused on testing this possibility in a battery of zebrafish tests, also expanding the range of model species to examine the behavioral effects of piracetam. Furthermore, since zebrafish display robust endocrine (cortisol) responses to various stressors [8,12], we also examined their endocrine responses to acute and chronic piracetam treatment.

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2. Methods

2.1. Animals and housing

A total of 336 adult (3–5 month-old) wild type short-fin zebrafish (1:1 male:female ratio) were obtained from a local commercial distributor (50 Fathoms, Metairie, LA). All fish were given at least 10 days to acclimate to the laboratory environment and were housed in groups of 15–20 fish per 40-L tank. The tanks were filled with filtered (facility) water maintained at 25–27°C. Illumination was provided by ceiling-mounted fluorescent light tubes on a 12-h cycle. Fish were fed Tetramin Tropical Flakes (Tetra USA, Blacksburg, VA). All fish used in this study were experimentally naïve. Following behavioral testing, the animals were euthanized in 500 mg/L Tricaine (Sigma–Aldrich, USA), and immediately dissected on ice for further analysis. This study was performed in full compliance with Institutional and National guidelines on animal experimentation.

2.2. Pharmacological manipulations and cortisol assay

Acute treatment was performed by immersing zebrafish for 20 or 90 min in a 3-L plastic beaker containing piracetam (100, 400, or 700 mg/L) prior to testing. Piracetam was also administered chronically by adding 200 mg/L piracetam to the home tank water for 7 or 8 days. The drug concentration was maintained by daily changing water and re-administration of this dose. The doses and treatment times were based on our pilot experiments with a wide range (25–1000 mg/L and 5–90 min, respectively), as well as on previously published studies using piracetam in rodents [4,25] and fish [7,20,26]. Behavioral testing in the novel tank was performed in all conditions.

2.3. Behavioral testing

2.3.1. Novel tank and light–dark anxiety tests

Behavioral testing was performed between 12.00 and 16.00. In all experiments, testing was performed in a tank containing standard facility water, adjusted to the holding room temperature. Zebrafish were placed individually in a novel tank test, representing a 1.5-L trapezoidal tank (15 height × 28 top × 23 bottom × 7 width cm; Aquatic Habitats, Apopka, FL) maximally filled with water. Novel tanks rested on a level, stable surface and were divided into two equal virtual horizontal portions, marked by a dividing line on the outside walls [54]. Zebrafish behavior was manually recorded by two trained observers (inter-rater reliability >0.85) for 6-min (standard novel tank test) or 30 min (extended novel tank test), scoring the latency to reach the upper portion of the tank (s), time spent in the upper portion of the tank (s), number of transitions (entries) to the upper portion of the tank, number of erratic movements, number of freezing bouts and freezing duration (s). Erratic movements were defined as sharp spontaneous changes in direction or velocity and repeated rapid darting behaviors. Freezing was defined as a total absence of movement, except for the gills and eyes, for 2 s or longer. Reduced exploration (longer latency to reach the top, fewer entries to the top, longer freezing) or elevated erratic movements in this test typically represent anxiety in zebrafish [54].

The light–dark test consisted of a rectangular tank, modified from the mouse light/dark box (15 height × 30 length × 16 width cm), and maximally filled with aquarium water [25]. The box rested on a level, stable surface and was divided into two equal vertical portions, demarcated by black and white coloration. Endpoints were recorded and scored over a 6-min period by two observers (inter-rater reliability >0.85) using USB LifeCam webcams (Microsoft, Redmond, WA) set 45 cm above the center of the light–dark box. Observers and laptops were located at least 2 ft away from the light–dark box to reduce possible confounding effects. Behavioral endpoints included latency to cross into the white half, time and number of transitions (entries) to the white. Reduced exploration (longer latency and fewer entries) of the white in this test reflects high anxiety states [54].

2.3.2. Analysis of novel tank habituation

The zebrafish novel tank test has previously been established as a sensitive model of intra-/inter-trial habituation, reflecting their short-term or long-term spatial memory phenotypes, respectively [54]. To apply this approach here, zebrafish novel tank behaviors (recorded as described above in 6-min trials) were analyzed for their per-minute distribution, and then compared as the first vs. last minute for each behavioral endpoint (similar to traditional animal habituation assays [49]). To further assess habituation to novelty over a longer duration of time, 30-min novel tank trials were then conducted in another cohort of naive zebrafish (n=23 per group).

Habituation responses were then assessed in a similar manner. Finally, in a separate cohort of experimentally naïve zebrafish (n=15 per group), inter-trial habituation was analyzed using daily 6-min trials for 7 days, comparing the day 1 scores with those of subsequent days 2–7.

2.3.3. Plus-maze test

To assess zebrafish memory, we used a cued-learning plus-maze test, which consisted of a transparent, four-armed, plus-shaped maze with each individual arm (10 cm × 10 cm in height and width, 530 cm length) and a central square.
Experiment 1: behavioral effects of acute piracetam (100–700 mg/L for 20-min) on adult zebrafish tested in a 6-min novel tank test (n = 12 per group; *P < 0.05 vs. controls, Tukey’s test for significant ANOVA data).

3. Results

Experiment 1 aimed to determine the active dose range of acute piracetam by testing mild doses (100–700 mg/L) of this drug. In the novel tank test, there was significant dose effect for time spent in top (*F_{3,47} = 2.9, P < 0.05), average entry duration (*F_{3,47} = 3.6, P < 0.05), and a trend for freezing bouts (*F_{3,47} = 2.5, P = 0.08), but not for the latency to enter the top, number of top entries, or erratic movements (*F_{3,47} = 0.2–1.2, NS). While acute administration of 100 and 400 mg/L did not affect zebrasfish activity, the highest dose of piracetam (700 mg/kg) significantly inhibited their novel tank swimming (Fig. 2).

Intra-trial habituation in this 6-min test was not improved by lower doses (data not shown) but significantly inhibited with the highest dose tested (Fig. 2). To allow the drug more time to exert its behavioral effects, we extended the pretreatment time to 90 min in a separate experiment, but again failed to detect behavioral effects the two mild doses elicited (data not shown). To further explore the possibility of behavioral effects of piracetam in mild non-sedative doses, the drug (100 and 400 mg/L administered acutely for 20 min) was examined using the 6-min light–dark box test. Again, no overt behavioral differences were observed for acute piracetam in this test (data not shown).

In Experiment 2, chronic piracetam administration (200 mg/L for 7 days) did not evoke sedation or anxiolysis in the novel tank (Fig. 3A and B), albeit showing a trend towards mild anxiolysis in the light–dark test (Fig. 3C). Inter- or intra-trial habituation in the 6-min novel-tank test was not improved by chronic piracetam treatment used here (data not shown).

In contrast, the cued learning plus maze test (Experiment 3) showed that chronic piracetam (200 mg/L) exerts a robust nootropic effect on zebrasfish, significantly increasing the number of target arm entries and time spent in the target arm. There was also a trend towards shorter latencies to enter the target arm of the maze for drug-treated animals (Fig. 4). Interestingly, overall locomotion
Fig. 3. Experiment 2: behavioral effects of chronic piracetam (200 mg/L for 7 days; \(n=20–23\) per group) on adult zebrafish tested in the 6-min novel tank test (A – day 7; B – days 1–3 vs. 5–7) and light–dark box (C; day 8). \(\#P=0.05–0.1\) (trend) vs. control, U-test.

Fig. 4. Experiment 3: Memory-enhancing (nootropic) effects of chronic piracetam (200 mg/L for 7 days; \(n=15\) per group) on adult zebrafish tested in a 6-min cued learning plus-maze test (see Section 2 for details). \(*P<0.05\), \(**P<0.005\), \(\#P=0.05–0.1\) (trend) vs. control, U-test.

was higher in the piracetam-treated cohort as entries to each arm were increased (e.g., target, empty, and total arm entries; see Fig. 4), consistent with elevated mobility observed in rodents for this drug [4]. Finally, no effects of acute or chronic piracetam were observed for whole-body cortisol levels in all three experiments (data not shown).

4. Discussion

While the precise mechanisms and sites of action of piracetam remain poorly understood, previous studies suggest that it indirectly modulates neurotransmission and neuroplasticity [9,28,37,44]. Piracetam also increases the number of postsynaptic
receptors [52], modulates the GABA-ergic [33] and glutamater-
gic [27] systems, and has been suggested to exert anxiolytic and
nootropic effects via several different mechanisms [45].

Despite the growing popularity of fish paradigms in neuro-
science research [12,54], piracetam has not been extensively tested
in these models. Our study is the first report on the behavioral
effects of piracetam in adult zebrafish, as the only other published
study utilized larvae, reporting increased acoustic startle habituation
[3].

Overall, testing acute doses of piracetam in our study failed to
produce immediate anxiolytic or habituation-enhancing effects.
However, the high dose of 700 mg/L produced clear sedation and
non-specifically impaired habituation (Fig. 2) without affect-
ing cortisol levels. This observation seems to contradict earlier
reports in mice (e.g., [15,19,31]) on piracetam as a potential anxi-
olytic agent without sedative effects, but with corticoid-reducing
activity.

One explanation for this discrepancy is that zebrafish and
rodents may have different species-specific responses to pirac-
etam. Notably, this is not the first report of psychotropic drugs
exerting somewhat dissimilar profiles in zebrafish compared to
rodents. For example, the benzodiazepine agents chloridiazepoxide
[2] and diazepam (own unpublished observations) did not
produce anxiolysis in zebrafish over a broad dose range, but
drove sedation. Therefore, our present piracetam data (Figs. 2–4)
seem to be in line with this notion. In contrast, chronic piracetam
treatment exerted robust nootropic effects on zebrafish in the cued-
learning plus-maze test, strikingly paralleling its nootropic profile
in rodents [4] and humans [27]. Moreover, increased number of
total arm entries in this experiment suggests that some mild acti-
vation of exploratory locomotion (together with a similar trend in
the light–dark box) may be a part of behavioral action of chronic
piracetam.

The lack of behavioral effects of acute or chronic piracetam on
zebrafish novel tank test habituation was interesting, and merits
further studies. Given the memory-enhancing effects of piracetam
in humans and rodents [4,27,47], it was logical to expect improved
habituation in piracetam-treated fish. However, it is important
to consider the manner in which zebrafish habituate to novel
environments. Unlike rodents, which gradually reduce exploratory
locomotion over time, zebrafish increase their swimming during
habituation [54]. While this zebrafish phenotype may reflect a
shift from exploration to normal locomotion (as they habituate),
it makes it difficult to dissect the two factors by observing zebrafish
behavior in novelty-based tests. On one hand, zebrafish paradigms
may be more sensitive to habituation-impairing experimental
manipulations (e.g., [54]). At the same time, non-specific behav-
ioral inhibition (such as observed here for 700 mg/L piracetam)
may resemble habituation deficits, and a special attention should be
paid to both habituation and overall activity levels, to avoid mis-
interpretation of data. For example, this situation is common in
rodent studies [22,23], and researchers should be aware of this gen-
eral problem with habituation assays. While these limitations may
not preclude extensive testing of drug effects on zebrafish mem-
ory, knowing species-specific behavioral phenotypes may help to
better dissect and interpret the observed responses in such stud-
ies. Given increasing activity during habituation in zebrafish, this
also suggests that some novelty-based aquatic models may be less
sensitive to pro-habitation effects due to ceiling effect, thereby
requiring more specialized memory tests, such as the cued learning
paradigm used here to detect nootropic effects of piracetam (Fig. 3).

The lack of overt effects of piracetam on cortisol levels in our
study was also unexpected, given the drug's ability to reduce
the levels of corticoids reported in rodent literature [19,31].
However, this phenotype may be related to species differences in
central regulation of stress neuroendocrine axes. For exam-
ple, the human and rodent hypothalamo-pituitary-adrenal (HPA)
axis is regulated by multiple neurotransmitters, including the
GABA-ergic system thought to be modulated by piracetam [30].
In contrast, the hypothalamo-pituitary-interrenal (HPi) axis, the
zebrafish homolog of HPA, is most tightly controlled by the central
serotonergic system [50,51], which may not represent the primary
target of piracetam, and therefore remain unaffected here. The lack
of robust anxiolytic effects of piracetam in our zebrafish study is
also consistent with unaltered cortisol levels reported here.

Overall, our study shows that piracetam exerts specific behav-
ioral effects on adult zebrafish, depending on the dose, paradigm
and duration of treatment. This is generally in line with previ-
ously published reports on the effects of piracetam in various
rodent models [4], especially its nootropic action [46]. Clearly,
future studies are needed to dissect the effects of various doses
of piracetam on zebrafish anxiety, memory and motor activity.
This research, utilizing diverse and novel models such as the
zebrafish, will foster a better understanding of the complex actions
of this agent, eventually leading to more effective treatments for
various cognitive and affective brain disorders. The sensitivity of
some zebrafish memory-related behaviors to piracetam sup-
ports their utility in developing novel screens for compounds with
potential nootropic properties. Finally, the use of other zebrafish
strains (e.g., high-anxiety leopard zebrafish) as well as various
mutant and transgenic zebrafish in this paradigm may enable fur-
ther characterization of genetic and physiological mechanisms
involved in learning and memory, as well as in fish sensitivity to
piracetam and related compounds.

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