The effect of citalopram and risperidone on aggressive behavior of Siamese fighting fish (Betta splendens)

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Abstract
Since of aggression, many fish can't keep with each other, aggressive fish may hurt other fish for various reasons. This makes a lot of lesions. The present study was conducted to determine the effect of citalopram a selective serotonin reuptake inhibitors and an antipsychotic drug on aggressive behavior of siamese fighting fish (Betta splendens). In separate experiments, we assessed the acute citalopram (2.5, 5, 10, 20 mg/L) and risperidone (20, 30, 40, 50 mg/L) treatments on aggressive behavior using a resident-intruder design. The results showed that risperidone couldn’t reduce aggressive behavior in any doses. But all doses of citalopram reduced fish aggression in various times. The onset time of citalopram at different doses of 2.5, 5, 10 and 20 mg/L was appeared respectively after 20 h, 14 h, 6 h and 3 h.

Keywords: citalopram, risperidone, aggressive behavior, Siamese fighting fish (Betta splendens).

Introduction
Many ornamental fish can't keep with each other because of their aggressive and territorial behavior and they may hurt the other fish, this makes limitations in keeping various fish in a time. The Siamese fighting fish is an Anabantid native to Southeast Asia. Typical fighting fish habitats in Thailand are quiet fresh water ponds with muddy bottoms or flooded rice paddies (Gordon and Axelrod., 1968). These fish are naturally aggressive and will attack intruders (most often males vs. males) who venture too close to their territories (Jaroensutasinee & Jaroensutasinee., 2004). Male Siamese fighting fish are highly territorial with aggressive interactions containing a strong signaling component. In addition to directly aggressive behaviors such as biting, males display by spreading their fins, extending gill covers and tail beating (Simpson., 1968). Fighting usually involves physical damage and can result in death (Ichihashi et al., 2004).

The number of hormonal and neurochemical inductors of aggression is quite numerous. According to the literature, serotonergic, androgenic, noradrenergic, dopaminergic, GABAergic, opioidergic mechanisms are involved in the formation of aggressive behavior (Chichinadze et al., 2011).

Changes in concentrations of different microelements (e.g., magnesium (Henrotte et al., 1997), lipids (Olson et al., 2008), and other organic and inorganic compounds (Baron & Richardson.,1994) are also considered to contribute to aggressive behavior. Dopamine (DA) and serotonin have been implicated in the regulation of aggressive behavior. The relationship between brain serotonergic systems, social status, and aggression has been established through a large number of correlatives and experimental. Serotonin is an important neurotransmitter in the regulation of social interactions in many animals. Correlative studies in numerous vertebrate species, including fishes, indicate that aggressive males have lower relative serotonergic activity than less aggressive males (Perreault et al., 2003). In the last decade, SSRIs such as fluoxetine and sertraline proved to be effective tools for chronically elevating serotonin activity and are now routinely used to study the role of serotonin in aggressive behavior (e.g., Fuller., 1996; Larson and Summers., 2001). Correlative studies in fish have demonstrated the common vertebrate pattern of elevated serotonergic activity in less aggressive individuals (Winberg et al., 1992; Winberg et al., 1993; Winberg and Winberg., 1997). Also numerous clinical studies...
document how atypical antipsychotics such as risperidone successfully reduce aggressive behavior (Miczek et al., 2002).

Dopaminergic transmission has been demonstrated to be essential for the expression of aggressive behavior. The antiaggressive effects of neuroleptics (typical as well as atypical) are well known (Rodriguez et al., 1998). Several studies support an association between the dopaminergic system and aggression (Ferrari et al. 2003; Retz et al. 2003; Hoglund et al., 2005). An ideal subject to study aggression in animals is the Siamese fighting fish (Ichihashi et al., 2004). Thus, this study used the Siamese fighting fish as a model species to test the hypothesis that acute concentrations of citalopram and risperidone can reduce the aggressive behavior of aggressive. This study has investigated the effect of citalopram a selective serotonin reuptake inhibitors and risperidone an antipsychotic drug on aggressive behavior of Siamese fighting fish (Betta splendens).

Materials and methods
We conducted this experiment during February and March 2011. Water temperature was maintained at 25–26 °C, and artificial light was provided for 12 h per day. The fish were fed dry Betta fish food daily. The males were visually isolated from each other when not taking part in trials. Citalopram is a selective serotonin re-uptake inhibitor (SSRI). Citalopram is one of the few agents with no clinically relevant interaction with another receptor or enzyme system. Citalopram is a very potent and highly selective inhibitor of neuronal serotonin (5-hydroxytryptamine, or 5-HT) reuptake (Hyttel et al., 1995). Risperidone is a benzisoxazole derivative with a relatively potent D dopamine antagonist and a highly potent 5-HT₂ antagonist (Meltzer et al., 1994). In vitro and in vivo studies, it has shown that risperidone differs from other typical and atypical antipsychotics by its more pronounced predominance of 5-HT₂a versus D₂ receptor occupancy and its more gradual occupancy of D₂ receptors (Rodriguez et al., 1998).

For this study, adult male fish were obtained from a local wholesaler. All experimental males had a similar body size (standard length: 32.38± 11 mm). Fish were maintained individually in small plastic containers of aged and oxygenated tap water. The drugs were mixed in distilled water to make the solutions of citalopram and risperidone.

The fish in the control group were housed in only fresh dechlorinated water for the entirety of the experiment. When behavior was to be tested 24 hours before starting the experiment each fish was taken out of its personal container and placed in a large tank with two liters fresh dechlorinated water to acclimation and forming territorial behavior. There were two partition in the tank(a glass partition and a plastic partition to prevent visual contact and fighting experimental fish with the intruder) then add the drug into the tank and after 3 hours of time (as for the pretest) in the test tank remove the middle partition and let to experimental fish to see the intruder fish. The fish reactions were recorded with the help of an ethogram (See Table 1).

Behavior was scored by two ways: at the first case we score behavior of fish by zero or one. Each twenty second period where the fish exhibited an aggressive act was scored as a one. All twenty second periods where the fish did not exhibit an aggressive act was scored as a zero. In second case we score behavior of fish by zero to four, in this case the behavior of dependent that aggressive measure gives various scores, the less aggressive act were zero and the more aggressive acts were 4. See Table 1 for a sample log sheet with twenty second intervals noted. Each fish was allowed five minutes in the tank with the intruder fish and then replaced in its personal container (McCurdy, 2006).

Statistical analysis
Data analysis performed with SPSS. All results were expressed as mean ± SD and a p-level <0.05 was considered statistically significant.

Results
Males treated with citalopram were significantly less aggressive overall towards the intruder than control group. The average number of aggressive behavior (according to score mode 1) were significantly lower (p-value<0.05, One-way ANOVA, Duncan’s post hoc test) for citalopram-treated males (Fig 1). Figure 2 shows the average number of aggressive acts per test session for each group of citalopram exposed fish (according to Table 1).

Risperidone shows some differences with control groups though did not reduced average number of aggressive behavior in all of doses and cases. There was no significant difference in average number of aggressive acts (Fig.3) between control and risperidone-exposed fish treatments (p>0.05) and no significant difference in aggressive behaviors (fig.4).

Onset and duration times for effects of citalopram
In a separate study we survey the onset time and the time of remaining the effect of citalopram in the fish as below (table 2).

Table 1—Ethogram used to score aggressive acts

<table>
<thead>
<tr>
<th>Mode 1</th>
<th>Mode 2</th>
<th>Act</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>Bottom Rest</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>Hover- stationary, fins not spread</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>Breathing- gulping air at surface</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>Swimming slowly, fins not spread</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>Leaves- swims away from mirror</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>Gill Spreading</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>Shaking body, fins and gill spread</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>5-shaped movements</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>Fin Spread with body Horizontal</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>Attack with contact</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>Attack without contact</td>
</tr>
</tbody>
</table>

The times when the fish restarted to show aggressive behavior noted by an average for all fish (Table.3).
Fig. 1: Average number of aggressive behavior in different doses of citalopram. Data are express as mean±SD.

Fig. 2: Average number of aggressive behavior in different doses of citalopram. Data are express as mean±SD.

Fig. 3: Average number of aggressive behavior in different doses of risperidone. Data are express as mean±SD.

Fig. 4: Average number of aggressive behavior in different doses of risperidone. Data are express as mean±SD.

Table 2 - Onset time for different dose of citalopram

<table>
<thead>
<tr>
<th>Dose</th>
<th>Time (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cit 20 mg/L</td>
<td>3</td>
</tr>
<tr>
<td>Cit 10 mg/L</td>
<td>6</td>
</tr>
<tr>
<td>Cit 5 mg/L</td>
<td>14</td>
</tr>
<tr>
<td>Cit 2.5 mg/L</td>
<td>20</td>
</tr>
</tbody>
</table>

Table 3 - The duration time for the effects of citalopram

<table>
<thead>
<tr>
<th>Dose</th>
<th>Time (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cit 20 mg/L</td>
<td>45.8</td>
</tr>
<tr>
<td>Cit 10 mg/L</td>
<td>44.9</td>
</tr>
<tr>
<td>Cit 5 mg/L</td>
<td>13</td>
</tr>
<tr>
<td>Cit 2.5 mg/L</td>
<td>12.2</td>
</tr>
</tbody>
</table>

Discussion

Pharmacological strategies of increasing 5-HT levels, such as the use of 5-HT precursors, 5-HT reuptake inhibitors, in addition to 5-HT1A and 5-HT1B receptor agonists are able to reduce aggressive behavior in animals (Chichinadze et al., 2011). It is well known that acute or chronic application of 5-HT or serotonergic drugs inhibit aggression in a wide range of vertebrates, including: fish, rainbow trout, coral reef fish, electric fish, the lizard (Anolis carolinensis), pigeons, sparrows, hamsters, mice, rats, dogs, and humans (Summers & Winberg., 2006). These results were in agreement with previous Perreault et al. (2003) and Parsons (2005) studies. In this study all doses of citalopram significantly decreased aggression in Siamese fighting fish and confirm the role of serotonergic system in aggressive behavior of fishes. The dopaminergic system plays an active role in the modulation of aggressive behaviors. In animal studies, hyperactivity in the dopamine systems associated with increases in impulsive aggression (Seo et al., 2008). Studies on aggressive behaviors in rodents showed that elevated dopamine levels have been continuously observed before, during, and following aggressive fights (Hadfield, 1983; Miczek et al., 1994; Tidey & Miczek, 1996).

Dopamine levels manipulated pharmacologically have been shown to increase or decrease aggressive behavior (Seo et al., 2008). Atypical antipsychotic agents, such as risperidone (Rocca et al., 2002), clozapine and (Chengappa et al., 1999), have also been found to be effective in treating impulsive aggression. In this investigation risperidone did not affect the aggression in all groups, it may be for low absorbent of risperidone via fish,
also it cannot be excluded that the exposure time applied in the present study (24 h) is too short for risperidone to elicit an effect. Eventually can conclude in Siamese fighting fish central serotonergic system is more effective than dopaminergic system in aggressive behavior.

Ethical Note

Males were never allowed physical contact with one another and we observed no injuries from attempted contact through the aquarium walls. We did not require any licenses to carry out this study.

References


Source of support: Nil; Conflict of interest: None declared