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Antipsychotics produce locomotor impairment in larval zebrafish

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Abstract

Zebrafish has been a favored vertebrate genetic model organism for studying developmental processes. It also holds a great potential for understanding the genetic basis of behavior and associated behavioral disorders. Despite such potential, their use in the study of behavior is greatly under-explored. It is well known that multiple classes of drugs used to treat psychiatric diseases produce extrapyramidal side (EPS) effects and consequent movement disorders in humans. The underlying molecular causes of these drug-induced movement disorders are poorly understood. Here we report that zebrafish treated with the antipsychotics fluphenazine and haloperidol (both of which can induce severe EPS in humans) develop movement defects. In contrast, another antipsychotic olanzapine, which produces mild to little EPS in humans, leads to minimal movement defects in zebrafish. These results establish a rapid assay system in which the effects of EPS-inducing agents can be assessed. Thus, future genetic screening in zebrafish shall identify genes and pathways that elucidate drug-induced movement disorder in human as well as provide insights into the brain control of locomotor activity. Future chemical screening in zebrafish may act as a preclinical test for the EPS effect of certain drugs, as well as a test used to researching drugs made to counteract the effects of EPS.

Keywords: Drug-induced movement disorders; Extrapyramidal side effects; Antipsychotics; Dopamine; Locomotor behavior; Zebrafish; Genetics; Model organism

1. Introduction

The execution of any complex behavior in an animal inevitably requires regulation of the locomotor centers in the brain. The importance of the brain in regulating locomotor activity is well highlighted by the fact that destruction of brain dopaminergic (DA) neurons causes Parkinson's disease, the most common movement disorder characterized by bradykinesia, tremor, rigidity, and postural imbalance [16]. The projection of DA neurons to the striatum is important for regulating motor output. How this pathway is regulated and how it regulates movement are largely unclear. This is in part due to the fact that locomotor activity can be complicated by many factors such as muscle integrity, making it difficult to find the brain underpinnings through directly assessing movement in a genetically tractable animal [15].

It has been well established that both antipsychotics and antidepressants can have extrapyramidal side effects (EPS) leading to movement disorders in individuals who are treated with these medications [6,12]. These antipsychotics include haloperidol and fluphenazine, both of which are dopaminergic antagonists that primarily inhibit D2-family of receptors; the anti-depressants include the selective serotonin-reuptake inhibitors (SSRI) [18]. The EPS side effects of these drugs usually develop within 1 month of the initiation of the offending medication in approximately 60% patients and in approximately 90% within 3 months [20]. The likely risk factors include prior history of movement defects, age, gender, and genetically determined differences in drug metabolism and possibly drug action [20]. It is important to point out that the development of these drug-induced movement disorders is not restricted to aged individuals and young people are equally susceptible; in addition, the conditions are generally reversible once the medications are removed, suggesting that the drugs produce an interference with neuron function rather than killing the neurons.

Similar to our understanding of brain control of locomotor activity, the molecular and cellular mechanisms underlying

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drug-induced movement disorders are largely unclear. On-target toxicity to undesired neural circuitry (e.g. blockade of striatal dopamine D2 receptors) has been proposed [17]. A recent study suggests that the alternatively spliced dopamine D2S and D2L receptors may differentially contribute to the actions of antipsychotic and psychotic agents in mice [21]. Alternatively, the EPS side effects may be due to off-target toxicity to yet unknown molecular receptors. Association of dopamine D3 receptor gene variants with neuroleptic-induced akathisia has been reported [5]. Taken together, genetic factors are implicated in mediating EPS effects of these antipsychotic agents, but the molecular identity of the majority of these factors remains to be determined.

If EPS-causing antipsychotics or anti-depressants can induce movement defects in a genetically tractable animal, subsequent forward genetic analyses will allow the identification of genes and pathways that mediate locomotor impairment by anti-psychotics. These analyses will also shed important light on the brain control of locomotor activity: for instance, they provide a means to identify mutants that move normally in the presence of the drug, thus circumventing the lack-of-specificity problem encountered when screening directly for movement-defective mutants. With these goals in mind, we set out to determine whether movement defects can be induced with antipsychotics in the zebrafish *Danio rerio*. Being a vertebrate, zebrafish share extensive similarity to humans in cellular structure, organ physiology, and genetic blueprint, and have been used to model human cancer and cardiovascular disorders [4,9,19]. Neurotox-in-induced Parkinson's disease models have also been described in zebrafish [1,3,13]. Most water-soluble compounds directly administered in the tank water can be easily taken up inside zebrafish, making it feasible to carry out large-scale screening [8]. The early development of zebrafish is rapid: in merely five-day post-fertilization, zebrafish already possess many patterns of behavior including free swimming, food hunting, and escape from predators [7]. Their nervous system is also well developed; for example, the brain DA neurons can be detected as early as 24 h post-fertilization [10].

Here we report that upon treatment with the antipsychotics fluphenazine and haloperidol that induce severe EPS in humans, larval zebrafish displayed movement defects that include both reduced swimming speed and erratic movements. In contrast, treatment with olanzapine that has mild to minimal EPS effect in humans only mildly reduced larval swimming speed and caused little erratic movements. These findings, together with the amenability of zebrafish to large-scale genetic and chemical screening, shall permit identification of genes and pathways involved in drug-induced movement disorders and the brain control of locomotor activity, as well as surveying a broad array of chemical compounds to identify those devoid of EPS.

2. Methods

2.1. Zebrafish maintenance and husbandry

Zebrafish were housed in a fish facility at the University of California, San Francisco. ~30 pairs of healthy adult zebrafish were used for the study. For mating, a pair of adult zebrafish was placed in a breeding cage overnight. The fertilized eggs were then placed in petri dishes filled with egg water (0.2 g Instant Ocean salts, 0.12 g $CaSO_4$/l water). Instant Ocean salts was purchased from Aquatic Ecosystems, Apopka, FL, USA. Eggs were kept at 28 °C. Procedures for the study of zebrafish conform to recommended UCSF Animal Use guidelines.

2.2. Drug treatment

Two sets of experiments were undertaken to investigate the effects of antipsychotics on larval zebrafish: A) Healthy thirteen- or fourteen-day old larval zebrafish (also known as fry) derived from the laboratory strain (AB) were used. We used 10 larvae in a group because less variability in basal locomotor activity was observed in this setting, as previously reported [14]. Groups of 10 larval zebrafish were placed in the following solutions: 1) Fry water (100 g Instant Ocean/1 L distilled H_2O) only (Control). 2) Different concentration of fluphenazine ranging from 0.1 to 50 μM (Sigma) dissolved in fry water for 40–45 h. 3) L-dopa dissolved in fry water (31.6 mM) (Sigma) for 24 h. 4) After exposure to fluphenazine for 40–45 h, larval zebrafish were transferred to fry water for 24 h. 5) After exposure to fluphenazine for 40–45 h, larval zebrafish were transferred to L-dopa (31.6 mM) for 24 h. Drug concentrations were determined base on knowledge of doses used in humans as well as our experimental trials: the blood concentration of fluphenazine in treated human patients is ~4.5 μM, and the doses of other two antipsychotics are in similar range to fluphenazine [2,11]; the daily dose of L-dopa compared to fluphenazine is ~60:1 in humans [11]. A minimum of 10 groups was examined for each treatment condition. B) To test whether responses to antipsychotics can be observed in 7-day old larval zebrafish after shorter exposure time (for the purpose of high throughput screening), 7-day old fry were exposed to the following solutions: 1) Fry water only (control). 2) 0.01% DMSO only (control, used to dissolve haloperidol and olanzapine). 3) Fluphenazine dissolved in the fry water. 4) Haloperidol dissolved in 0.01% DMSO and fry water. 5) Olanzapine dissolved in 0.01% DMSO and fry water. The treatment time in this experiment was 2 h. A minimum of 20 groups was examined for each treatment condition.

2.3. Measuring locomotor activity and erratic movements in zebrafish

Quantitative determination of the locomotor activity of larval zebrafish was as previously described [3]. Briefly, they were placed in a view tray on top of the light necessary for successful video recording, allowed to habituate for 5 min, and their locomotor activity was recorded for 5 min using a digital video camera linked to a computer. The data were analyzed with Digital Imaging Analysis Software (DIAS) (Solltech, Ohio), and followed with Microsoft Excel.