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Research Report

Distinct models of induced hyperactivity in zebrafish larvae

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ABSTRACT

The analysis of behavioural hyperactivity can provide insights into how perturbations in normal activity may be linked to the altered function of the nervous system and possibly the symptoms of disease. As a small vertebrate zebrafish have numerous experimental advantages that are making them a powerful model for these types of studies. While the majority of behavioural studies have focused on adult zebrafish, it has become apparent that larvae can also display complex stereotypical patterns of behaviour. Here we have used three compounds (pentylenetetrazole (PTZ), aconitine and 4-aminopyridine) that have different neuronal targets (GABA, sodium and potassium channels), to induce distinct patterns of hyperactivity in larvae. Our studies have revealed that each compound produces a number of distinct concentration-dependent activity patterns. This work has shown for the first time that at sub-convulsive concentrations, PTZ can reverse the normal behavioural response to alternating periods of light and dark in zebrafish larvae. It also appears that both PTZ and 4-aminopyridine produce distinct changes in the normal startle response patterns immediately following light/dark transitions that may be the result of an elevation in stress/anxiety. Aconitine produces a general elevation in activity that eliminates the normal response to light and dark. In addition to differences in the patterns of behaviour each compound also produces a unique pattern of *c-fos* (an immediate early gene) expression in the brain. While more work is required to make direct links between region specific neuronal activity and individual behaviours, these models provide a framework with which to study and compare mechanistically different types of inducible behaviours.

1. Introduction

The zebrafish has emerged as an excellent model system for studying mechanisms of altered behaviour (Baraban et al., 2005; Blaser and Gerlai, 2006; Burgess and Granato, 2007; Irons et al., 2010; Li et al., 2009; Steenbergen et al., 2010; Winter et al., 2008) and for screening potential therapeutics (Chakraborty et al., 2009; Goldsmith, 2004; Irons et al., 2010; Kokel and Peterson, 2008; Kokel et al., 2010; Rihel et al., 2010; Seibt et al., 2010; Zon and Peterson, 2010). In addition to high fecundity and low rearing costs, the stereotypical patterns of behaviour and conserved drug responses have made the zebrafish a creditable alternative to traditional mammalian models. Neuroactive compounds such as ketamine, dizocilpine, phencyclidine, kainic acid, caffeine, pentylenetretrazole (PTZ), cocaine and picrotoxin (Berghmans et al., 2007; Kim et

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al., 2010; Seibt et al., 2010; Wong et al., 2010; Zakhary et al., 2011) have been used to induce behavioural hyperactivity that appears to mimic some of the symptoms of disease states, such as schizophrenia, epilepsy and anxiety. These models have primarily assessed altered patterns of behaviour in adult zebrafish, while the majority of larval studies have been used to evaluate seizure activity induced by high levels of neuroactive compounds (Burgess and Granato, 2007; Kim et al., 2010).

Recent work has shown that larvae can display complex, stereotypical patterns of behaviour and the analysis of these behaviours can provide valuable information regarding the therapeutic potential and target specificity of new compounds (Kokel et al., 2010; Rihel et al., 2010). Similar to adult studies, induced hyperactivity in larvae may also produce phenotypes that could be used to mirror symptoms of disease. While the adult models of hyperactivity have suggested links to disease states, these associations are based solely on the interpretation of the induced patterns of behaviour and correlations with mammalian behaviours, with little information regarding mechanism. In order to make stronger links between these types of models and the symptoms of a disease more work is required to define the regions of the zebrafish brain that are activated and determine if these regions are comprised of the same types of neurons and are regulated in the same fashion (i.e. neurotransmitters) as those linked to specific disease states.

Here we have used zebrafish larvae to conduct a detailed analysis of the patterns of behaviour produced by neuroactive compounds that are known to act on different targets. Three compounds, one well-characterized and two with undefined phenotypic effects in zebrafish larvae were selected because of the ability of the compound to elevate neuronal activity. We have shown that PTZ, which has primarily been used as a convulsant in zebrafish (Baraban et al., 2005, 2007; Berghmans et al., 2007; Kim et al., 2010; Lee et al., 2010), along with aconitine and 4-aminopyridine (4-AP) can induce complex changes in behaviour. Each compound induces a distinct concentration-dependent behavioural profile in zebrafish larvae that becomes obvious once the behaviour is analyzed using multiple parameters. An initial analysis of *c-fos* expression has revealed that each compound produces a distinct, regionalized, pattern of elevated neuronal activity. Being able to link specific behaviours with the activation of specific regions of the brain and ultimately with specific neuronal subtypes will allow us to begin to understand how these distinct types of behaviours are regulated.

The abbreviations used are: 4-AP, 4-aminopyridine; PTZ, pentylenetretrazole; *c-fos*, FBJ murine osteosarcoma viral oncogene homolog; LIGHT/DARK, light/dark; hpf, hours post fertilization; dpf, days post fertilization; qPCR, quantitative polymerase chain reaction; EF1α, Elongation factor 1alpha; SSC, Saline–Sodium Citrate; NBT-BCIP, nitro-blue tetrazolium and 5-bromo-4-chloro-3'-indolyphosphate; AP, alkaline phosphatase; ISH, *in situ* hybridization; HPI, hypothalamic-pituitary-interrenal; Pa, pallium; Sp, subpallium, Po, preoptic; Cb, cerebellum; VT, ventral thalamus; PT, posterior tuberculum; Hy, hypothalamus; OB, olfactory bulb; forebrain, Fb; Te, tegmentum; Hb, hindbrain.

2. Results

2.1. *Characterization of altered light/dark response patterns*

The effect of each compound on larval activity was initially evaluated across a broad concentration range in order to establish a biologically relevant range beginning at a concentration that produced no measurable change in activity to the highest level that was not toxic. Following this initial visual assessment larval activity (defined as distance traveled in cm) was measured in a 96-well plate with the ViewPoint ZebraLab tracking software. In order to evaluate sensory responsiveness larvae were treated for 30 min with an individual compound and then presented with four 10-minute periods of light then dark (Irons et al., 2010) (Fig. 1). The concentration range was further narrowed following the initial response profile to between a level that produced a detectable change in activity in either the light or dark phase and a level that resulted in a drop in activity over a 2-hour period, but did not lead to lethality. Following this initial assessment the concentrations that produced unique phenotypic patterns were selected to highlight the differences between each compound.

The γ-Aminobutyric acid (GABA) antagonist PTZ has been one of the most widely studied excitatory compounds and has been used in the past to evaluate the effects of anticonvulsants on zebrafish (Baraban et al., 2005; Berghmans et al., 2007). Previous studies have evaluated increased larval activity generated by PTZ at concentrations between 5 and 30 mM {Baraban, 2005 #2;Berghmans, 2007 #37}. In the current study, we initially evaluated the activity produced by PTZ using a ¼ log dilution series between 500 μM and 25 mM. There was no significant change in activity at 500 μM and at concentrations above 10 mM larvae displayed sporadic tremors followed by lateral recumbency similar to the profile previously described as a transition from a phase II to phase III seizure state (Baraban et al., 2005). Between 1 and 10 mM PTZ produced a concentration-dependent profile that appears to consist of two distinct components, a gradual reversal in the normal light/dark response profile followed by an overall increase in activity at higher concentrations.

Beginning at 1 mM there was a reduction in activity during the dark phase, leading to a significantly lower activity level than controls by the 3rd dark cycle (asterisks Fig. 1A; *t*-test, $p<0.05$). Incremental increases in PTZ (of 1 mM) produced a profile in which the activity during the dark phase was further decreased and the light cycle activity began to increase. At levels of PTZ above 2.5 mM (Fig. 1A) the light phase activity became larger than the dark phase activity. As the concentration of PTZ was increased further, the reversal of the normal light/dark response became more prominent leading to what appeared to be a complete reversal of the light/dark response pattern at 5 mM. At concentrations above 5 mM this pattern of activity was retained, however the overall activity level for both the light and dark phases continued to increase until 10 mM, at which time the response to changes in light/dark was all but eliminated. This concentration-dependent profile has revealed a phenotypic change in activity induced by subconvulsive levels of PTZ that has not been previously reported for zebrafish.