CLIMBING RESPONSES OF FEW SPECIES OF DROSOPHILA ON EXPOSURE TO DIFFERENT ANITI EPILEPTIC DRUGS

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ABSTRACT

Feeding flies on wheat cream agar media supplemented with antiepileptic drugs are studied for adult locomotory behaviors like preferential adult geotaxis. The behavioral plasticity of climbing ability of an adult is an important event with decisive influence on their preference and capability. Drosophila sensory systems contribute to detect, localize and provide information about the availability of food and chemical features of environments. The present study revealed that the Dose dependent action of different AEDs produced a maximal effect on behaviors of Drosophila species and provides an efficient system to study genetic, neurological, and behavioral mechanisms mediating these effects. Irrespective of the species the responses are similar on exposure to higher doses with decreased activity. AED has an important role in regulating behavior through metabolism; such studies should be useful for understanding the multiple effects on behavior and health.

INTRODUCTION

Antiepileptic drugs (AEDs) have a variety of mechanisms of action which are reflected through different anticonvulsant actions and behavioral effects (Cavanna et al., 2010). Several human studies have raised concerns over AED behavioral teratogenesis. Animal studies have demonstrated that AEDs can produce cognitive deficits at dosages less than those required for anatomical teratogenesis (Fisher and Vorhees, 1992). Anatomical and behavioral teratogenesis likely differ in mechanisms since first trimester AED exposure poses the highest risk for anatomical malformations, while third trimester exposure appears to be associated with the highest risk for adverse behavioral effects (Gaily and Meador, 2007). Studies in rats have shown significant AED effects in the developing brain including apoptotic neurodegeneration (Olney et al., 2002; Bittigau et al., 2003); neurodevelopmental delay, behavioral disorders or learning disabilities as an outcome of in utero exposure to AEDs and specially VPA (Nicolai et al., 2008). The cognitive side effects of CBZ, PHT and VPA are comparable and associated with modest psychomotor slowing accompanied by decreased attention and memory (Meador, 2005). PHT is implicated in dose related decline in concentration, memory and mental speed, as well as generating anxiety, aggression, fatigue, and depression (Gillham et al., 1990). Sedation and outbursts of psychotic episodes have been described with PHT at high doses (Levinson and Devinsky, 1999). PHT produces multiple behavioral dysfunctions in rat offspring at sub teratogenic and non growth retarding doses (Adams et al., 1990).

The chronic use of VPA can impair concentration, and also reversible Parkinsonism and cognitive impairment (Nicolai et al., 2008). There is a better recognition of the behavioral phenotype in Fetal valproate syndrome (Williams et al., 2001; Dean et al., 2002). Poor concentration and hyperactivity have also been commonly reported on VPA exposure (Kini, 2006). The active metabolite carbamazepine epoxide is partly responsible for the mild cognitive and psychomotor effects attributed to CBZ (Gillham et al., 1988).

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The exposure of pregnant rats to CBZ significantly delayed skull bone development and soft tissues flattening, these structural alterations brought confrontational changes associated to the behavior parameters of the offspring (Christensen et al., 2004; Rayburn et al., 2004). A recent CNS practice guideline stated, "Behavioral and cognitive side effects need to be better evaluated and individual risks as well as group differences assessed on tests of cognition" (Hirtz et al., 2003). Behavioral side effect profiles of AEDs, both negative and positive psychotropic effects, should be considered in the choice of the optimal drug for an individual patient (Schmitz, 2006).

At the forefront of behavioral genetics research, D. melanogaster has provided important insights into the molecular, cellular and evolutionary basis of behavior (Sokolowski, 2001). Simple behavioral assays are widely applicable for studying the role of genetic and environmental factors on fly behavior on exposure to few AEDs (Sharma et al., 2010). The newly hatched adult fly will rapidly acquire characteristic behaviors of flight, chemotaxis, phototaxis, geotaxis, foraging and mating (Truman et al., 1993). In many cases the explicit circuits controlling visual (Ting and Lee, 2007), olfactory (Hallem and Carlson, 2004), mechanosensory (Kernan, 2007) and chemosensory (Stocker, 1994) inputs from the peripheral organs (eye, antennae, bristle organs and maxillary palps) have been mapped both physically and functionally. In addition, the central ‘mushroom body’ of the brain has been elucidated as a center for memory and conditioned behaviors.

Some of these well documented developmental and behavioral aspects of Drosophila make it an especially informative and adaptable model to investigate a wide variety of toxicological endpoints relevant to human biology and behavior. Flies exhibit a wide array of behaviors relevant to understanding human response to environmental challenges. These behaviors include locomotion, circadian rhythm, sleep patterns, courtship and mating, aggression, and grooming. Many of these are under the control of genetic and molecular mechanisms in Drosophila (Sokolowski, 2001; Greenspan and Dierick, 2004). Furthermore, at a physiological level the underlying neurotransmitter systems in the fly are conserved including serotonin, dopamine, GABA, glutamate and acetylcholine (Nichols, 2006).

To date, behavioral endpoints in Drosophila have been used primarily to isolate genes that specifically support a given trait rather than as a tool for screening vast numbers of chemicals (Moore et al., 1998). Locomotor activity is a complex behavior and different neural systems may influence in fly (Fleming and Copp, 1998). It can be assessed in transgenic and mutant flies through longevity assays, locomotor and climbing assays. Progressive locomotor decline can be observed in transgenic Drosophila through climbing assays (Greene et al., 2003). The duration of climbing is determined by Rapid Iterative Negative Geotaxis (RING) (startle-induced vertical climbing) as an assay for evaluating the sedative effects of AEDs (Sharma et al., 2010). A climbing or negative geotaxis assay measuring the ability of the organisms to climb up the walls of a plastic vial was used and several genotypes or drug treatments can be tested for screening experiments (Nichols et al., 2012).

Climbing ability is a frequently used assay to measure locomotor activity in D. melanogaster model of Parkinson’s disease (Todd and Staveley, 2004). The climbing response of wild-type flies remained essentially unchanged as reported by Feany and Bender (2000). The climbing response for subsequent anti-parkinson drug studies has an effective in decreasing fly locomotor function (Pendleton et al., 2000). The low levels of accumulated ethanol stimulate locomotion and high levels depress it (Heberlein et al., 2004). These behavioral assays are widely applicable for studying the role of genetic and environmental factors on fly behavior. In light of the above studies the present work determine the acute behavioral responses to commonly used conventional AEDs with respect to adult geotaxis in Drosophila species at different doses. The present study has been assessed for the dose response relationship between AEDs and their behavior in different species of Drosophila.

**MATERIALS AND METHODS**

The fly stocks, D. melanogaster, D. ananassae and D. nasuta nasuta were cultured on standard wheat cream agar medium in uncrowded culture condition at 22±1°C (rearing temperature) with a relative humidity of 70%. The progeny from these stabilized stocks treated with PHT (5, 10 and 15 mg/ml), VPA (0.2, 0.3 and 0.4 mg/ml) and CBZ (2, 4 and 8 mg/ml) were used to assess the larval pupation site preference and climbing ability (negative geotaxis) and compared to respective controls.

**Climbing ability**

Virgin females and unmated males of D. melanogaster, D. ananassae and D. nasuta nasuta were isolated, collected and aged for 5 days. All flies used in individual experiments were grown, collected and handled in parallel. Adult flies (5 days old) were aspirated, transferred to fresh food vials containing different doses of each antiepileptic drug and treated for 3 days. 20 flies were selected from treated and placed in a 100 ml glass graduated cylinder (length 25 cm and diameter 3 cm) to climb. The cylinder was sealed with parafilm at the top to prevent escape. The flies were gently knocked to the bottom of the cylinder and were allowed to climb for 30 sec. The number of flies crossing the 60 ml line (9 cm) was recorded. Four such trials were conducted for each dose of PHT, VPA and CBZ. The locomotor activity of Drosophila without drug administration i.e., control was compared to treated (modified protocol of Greene et al., 2003). The number
of flies climbed in the given time for each dose was averaged for statistical analysis.

RESULTS

Climbing assay performed on adults of Drosophila species exposed to different doses of PHT, CBZ and VPA is presented in Fig 1. The response to increase dose has resulted with decrease climbing activity of flies for all the three variable drugs of PHT, CBZ and VPA. The percentage of flies exhibiting about 50% negative geotaxis was observed at mid and high doses in all the species. At 10 and 15 mg/ml, the climbing responses were 56.25% and 55% in D. melanogaster; 57.5% and 47.5% in D. ananassae; 51.25 and 45% in D. nasuta nasuta respective with respect to PHT.

The percentage of flies exhibiting about 50% negative geotaxis was observed at high doses in D. ananassae and D. nasuta nasuta in addition to mid dose in D. nasuta nasuta on exposure to CBZ. At 8 mg/ml the climbing responses were 52.5% and 47.5% in D. ananassae and D. nasuta nasuta while it was 56.25% at 4 mg/ml in D. nasuta nasuta. While on exposure to different doses of VPA the percentage of flies exhibiting about 50% negative geotaxis was observed at mid and high doses in all the species except at 0.3 mg/ml in D. ananassae. At 0.3 and 0.4 mg/ml, the climbing responses were 53.75% and 51.25% in D. melanogaster; 56.25 and 46.25% in D. nasuta nasuta respectively while in D. ananassae (42.5%) was recorded at 0.4 mg/ml.

DISSCUSSION

The genetic analysis remains the best means to define mechanisms and to begin the process of assigning the contribution of genes to behavior. The Drosophila flies were exposed to varying doses of antiepileptic drug for three days to determine its effect on behaviors. In preclinical studies on animals, AEDs produce acute adverse effects such as sedation, ataxia, tremor, impairment of motor coordination, disturbance in locomotor activity and alterations in skeletal muscular strength.

Grip strength test is able to evaluate the acute adverse effect potential of AEDs at high (neurotoxic) doses with respect to the reduction of muscular strength (Zadroniak et al., 2009).

The animals exposed to PHT showed significant increase in locomotor activity measures. These results confirm a small but growing body of literature that demonstrates that PHT is a behavioral teratogen (Pizzi and Jersey, 1992). The observed mean values of locomotor activity were dose dependent and significantly different among different AEDs exposure on Drosophila species. Climbing activity was decreased at mid and high doses with less than 50% of the flies climbing within 30 sec while 95% of the flies climbing within 15 sec in control. Climbing rate of D. nasuta nasuta was reduced on exposure to all the three AEDs compared to D. melanogaster and D. ananassae. Interestingly, the behavioral traits observed were generally dose dependent. The nervous system, the most crucial system in the elicitation of behavior, is formed during development by networks of interacting genes and the physiological structures necessary to generate these behavior patterns. Despite the sources of complexity, the amount of research accomplished has pushed the fruit fly to the forefront of behavioral genetics research (Sokolowski, 2001).

Dose dependent action of different AEDs produced a maximal effect on behaviors of Drosophila species and provides an efficient system to study genetic, neurological, and behavioral mechanisms mediating these effects. AED has an important role in regulating behavior through metabolism; such studies should be useful for understanding the multiple effects on behavior and health.

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