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Female ovarian hormone- Estriol's proconvulsant effect with kainic acid in Drosophila system model

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Abstract

The effect of estriol would be evaluated for its effect on Drosophila fly model. Seizures in drosophila and man have several similarities and the utility of the fruit fly as a genetic model system for studying human seizures disorder and seizure susceptibility has clearly been demonstrated (Glasscock et al., 2005). Drosophila fly of 3-4 days old unmated fly were used. Drosophila model, optimized for screening of drugs dissolved in DMSO was used. Thirty flies were housed in each treatment vial. Three to four days old unmated male flies was treated with 8 mg/ml KA containing media for required time duration. A regimen for drosophila fly model where it was treated with kainic acid and resulted in increases as well as decrease of climbing speed for adult Drosophila fly. As higher organism has more colossal complexity thats why drosophila fly model is favored. This model is convenient to use and it is very relevant too.

Key-words: Estriol, seizures, drosophila fly model, climbing speed, kainic acid

Introduction

Seizures's association with estrogens are problematic to define due to complexity and heterogeneity of seizures. Contradictory data makes it more interesting subject regarding association of estrogen and epileptic seizures. General perception regarding estrogen's on seizures was till now that it increases neuronal excitability and mediates proconvulsant effects (Veliskiova,2006, Edwards et, al.,1999, Nicoletti et ,al.,1985) but estrogen has also exhibited anticonvulsant effect, this fact is supported by various animal and clinical data Veliskiova,2007, Reibel et al.,2000, Kalkbrenner and Standley, 2003, Tominaga et al.,2003). Estrogen is present in three biologically active form i.e estrone (E1), estradiol (E2) and estriol (E3). Most investigated form of estrogen is estradiol as it is present in post menopausal women.

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Earlier studies of estradiol administration in rodents have revealed proconvulsant effects (Nicoletti et al., 1985). Estrogens applied to cortex could increase seizures (Marcus et al., 1966). In addition to this estradiol was shown to facilitate PTZ induced kindling (Lu et al., 2006), amygdala-kindled seizures (Buterbaugh, 1989) and kainic acid- induced seizures (Woolley, 2000) in adult female rats. Clinically also, it exacerbates seizures in women with epilepsy (Reddy, 2009).

Numerous similarities have been observe between seizures in drosophila fly and man (Glasscock et al.,2005, Hekmat-Scafe et al., Fergestad et al., 2006, Stilwell et al., 2006, Baraban, 2007,Song et al., 2007), this drosophila fly model is also useful in screening of anticonvulsant drug screening and drug target identification (Kuebler and Tanouye, 2002,Reynolds et al.,2004,Tan 2004, Hekmat-Scafe 2005). As higher organism has endless complexity, drosophila fly model is getting preference being simple organism (Joyce and Palsson, 2006). Inherent complexity of mammalian brain however does not render the available rodent epilepsy models as amenable to systems modeling (Gorter et al.,2006), because of the above reason we have selected drosophila fly model for understanding the mechanism underlying epileptogenesis. Seizures like activity in drosophila adults is known to be associated with loss of motor coordination and altered locomotor activities (Wang et al., 2004).

Material and Methods

Animals

Drosophila melanogaster wild type Oregon-R strain was selected for the study. They were maintained at 24+1°C, 60% relative humidity and standard 12 hours light (9 AM to 9PM) and 12 hours dark cycle was maintained. The standard fly medium was used and it consisted of agar-agar, maize powder, dried yeast, nipagin and brown sugar. Standard methods of fly handling and manipulation were followed. Stringency required in behavioral studies was strictly adhered to at various levels, conditions of housing, light intensity. 3-4 days old unmated male flies were used and 30 flies were housed in each treatment vial. Institutional Animal Ethics Committee (IAEC) of Pinnacle Biomedical Research Institute (PBRI), Bhopal (Reg. No. 1824/PO/ERe/S/15/ CPCSEA) had approved the study and study approval reference number is PBRI/IAEC/PN-16026.

Drugs

Kainic acid, estriol and clomiphene citrate were purchased from Sigma, USA and diazepam was procured from Ranbaxy-Sun Pharma, India. Estriol was dissolved in dimethylsulfoxide (DMSO) and administered in doses of 0.005 and 0.01 mg/kg i.p. DMSO was given to control group as a vehicle. Clomiphene was dissolved in distilled water and administered intraperitoneally at dose of 0.9 mg/kg. Diazepam was taken as standard and given at 3 mg/kg.

EXPERIMENTAL DESIGN

Drosophila fly model methodology

Drosophila model, optimized for screening of drugs dissolved in DMSO, was used. Routine fly cultures was maintained at 24 $\pm$ 1ºC, and 12 hrs light (9 AM to 9 PM) and 12 hrs dark cycle (Mohammad et al. 2009). Three to four days old unmated male flies was treated with 8 mg/ml KA containing media for required time duration. Thirty flies was housed in each treatment vial. After completion of 7 days, flies was placed in a 96-well plate and the movie was captured using a camera and the locomotor activity analyzed (Noldus Instruments). We took a glass column having 2 cm of internal diameter and 30 cm of length. Every fly was introduced for 90 seconds, dot/comma methods of measurement was applied, for purpose of monitoring locomotor activity of fly where the dot key depicts the moving fly and comma key depicts the resting fly. For calculation of climbing speed , the formula is s=h/t where s= climbing speed, h=height climmbed in cm, t= activity (seconds).

Table 1: Effect of various treatments on Drosophila fly model:

Following treatment groups was analyzed for loco motor behaviour change:

Groups	Treatment
1	Normal media containing 0.4% DMSO, 2 days, followed by normal media, 5 days
2	Normal media containing 8 mg/ml kainic acid and 0.4% DMSO, 2 days, followed by normal media containing 8 mg/ml kainic acid, 5 days
3	Normal media containing 8 mg/ml kainic acid and 0.4% DMSO in which the drug (Estriol, 5mM) was dissolved at 10 mM final concentration, 2 days, followed by normal media containing 8 mg/ml kainic acid, 5 days.
4	Normal media containing 8 mg/ml kainic acid and 0.4% DMSO in which the drug (Estriol, 10mM) was dissolved at 10 mM final concentration, 2 days, followed by normal media containing 8 mg/ml kainic acid, 5 days.
5	Normal media containing 8 mg/ml kainic acid and 0.4% DMSO, in which the drug (diazapam) was dissolved at 10 mM final concentration, 2 days, followed by normal media containing 8 mg/ml kainic acid, 5 days.

Results and Discussion

We observed through direct and indirect testing whether KA could produce effect in drosophila adults fly , although convulsions was not produced but flies treated with KA for 12 hours exhibited hyperactive movements. A decreased in "total directionality" was observed in KA treated flies compared to control (normal food). Flies treated with kainic acid took a serpent nous path, 16 mg/ml was the LC_{50} dose (lethal data not presented). We substituted half the dose i.e 8 mg/ml for development and evaluating a chronic model.

We did not witnessed seizure like behavior in flies treated with 8 mg/ml, we followed a regimen by (Mohammad F et al., 2009) . They have developed a regimen where 7 days of chronic KA treatment followed by 7 days of absent KA treatment, it causes a decrease and increase in climbing speed in flies.

Due to immense complexity of higher organisms, approaches are currently focused on simpler organism (Joyce AR and Palsson , 2006, Chintapalli et al., 2007), thats why we included drosophila fly model in the study as the seizure in drosophila and man have several similarities and the utility of the fruit fly as a model system for studying human seizure disorders and seizure-susceptibility has clearly been established (Glasscock et al.,2005, Hekmat-Scafe et al., Fergestad et al., 2006, Stilwell et al., 2006, Baraban, 2007,Song et al., 2007).

Conclusion

As higher organism has more colossal complexity thats why drosophila fly model is favored. This model is convenient to use and it is very relevant too.

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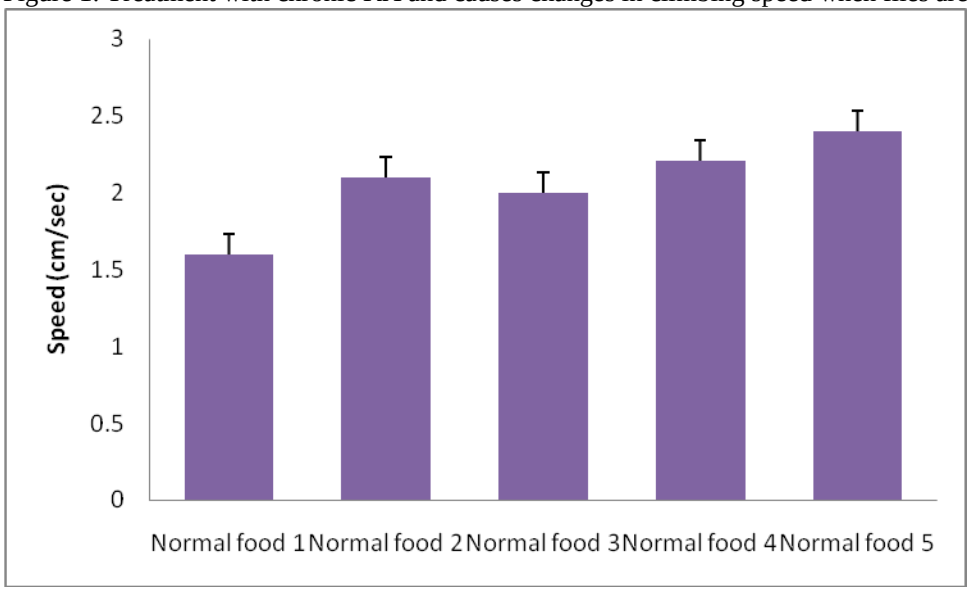
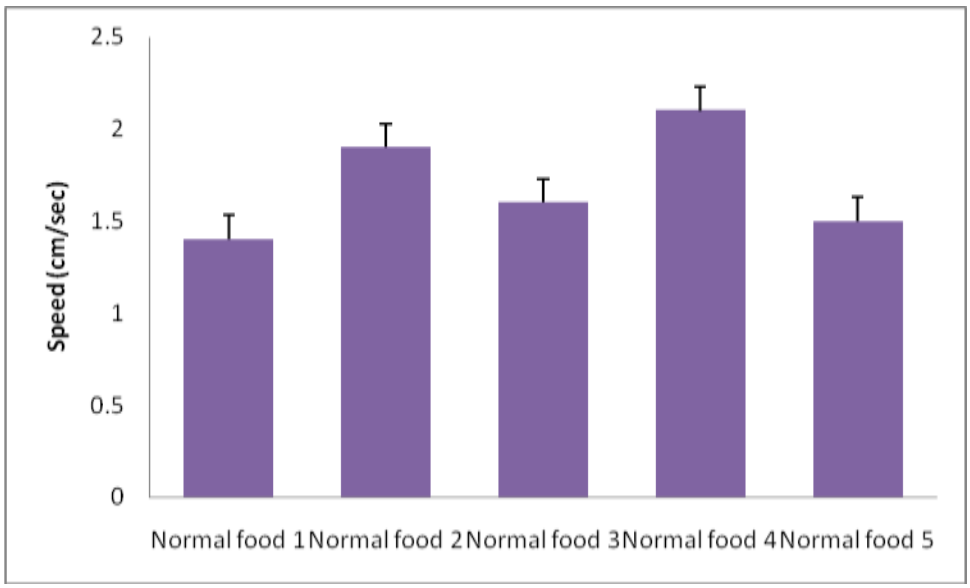
Figure 1: Treatment with chronic KA and causes changes in climbing speed when flies are treated with normal food.



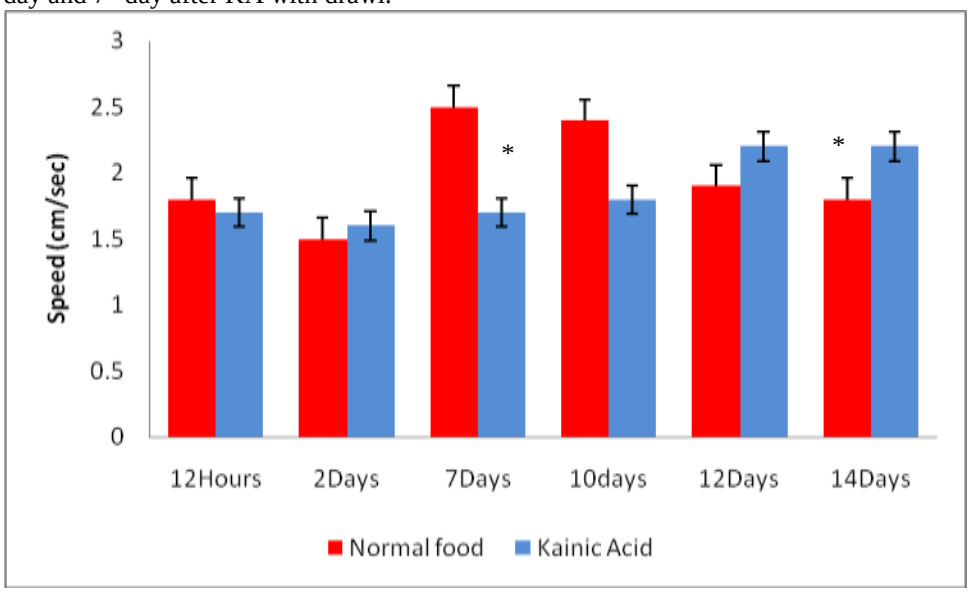
All the values were expressed as mean ± SEM. A treatment with kainic acid for flies treated with normal food.

Figure 2: Absence of KA causes changes in climbing speed when flies are treated with normal food



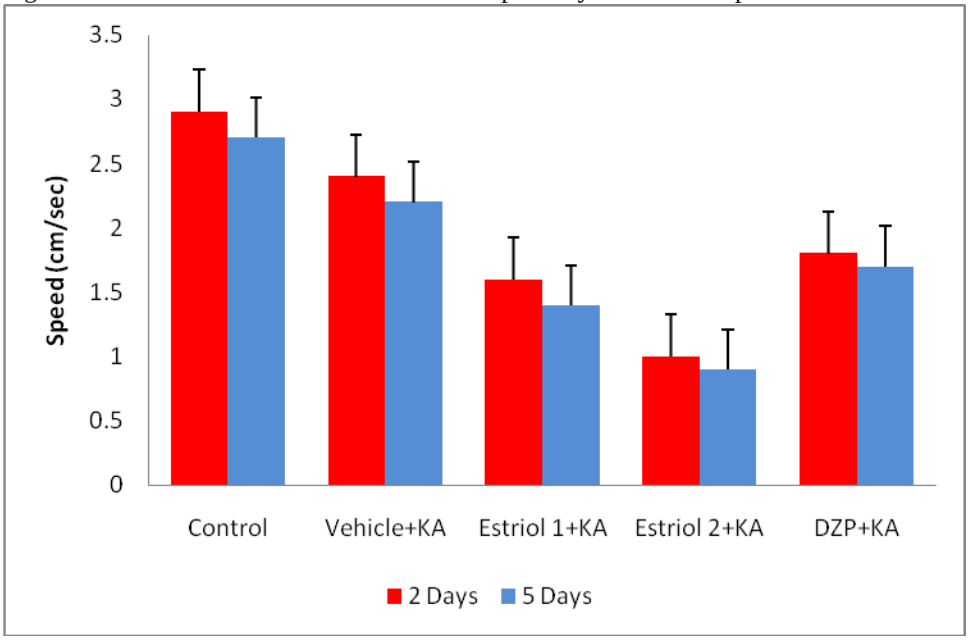
All the values were expressed as mean ± SEM.

Figure 3: Climbing speed was recorded on 12 hours, 2nd day and 7th day after KA treatment and then on 3rd day, 5th day and 7th day after KA with drawl.



All the values were expressed as mean ± SEM. Climbing speed was recorded on 12 hours, 2nd day and 7th day after KA treatment and then on 3rd day, 5th day and 7th day after KA with drawl. For control climbing assay to examine consistency among normal food flies housed in different vials. For stats we applied ANOVA. * indicates significant difference between control (normal food) and KA treatment. *p* values are provided over asterisks.

Figure 4: Effect of various treatments on drosophila fly model on respective time.



All the values were expressed as mean ± SEM. VEH: Vehicle (Dimethyl Sulfoxide); EST: Estriol; CLO: Clomiphene; DZP: Diazepam; KA: Kainic acid; KA (25 mg/kg) was administered once every 2 days for 5 weeks while EST 1 (0.005 mg/kg) and EST 2 (0.01 mg/ kg) and CLO (0.9 mg/kg) were administered daily.

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