Characterization of Hippocampal Synaptic Plasticity in Orchidectomized Insulin-resistant Rats

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Research Article

Keywords: Orchiectomy, Type 2 Diabetes, Flutamide, Insulin resistance, Synaptic plasticity

Posted Date: April 25th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1541755/v1

Abstract

Background

Androgen deprivation can be achieved through testosterone antagonists (chemical castration) with or without orchidectomy. There is this hypothesis that stipulates that testesterone exerts supreme effects on synaptic plasticity, however low testosterone has been linked to type- 2 diabetics mellitus, insulin resistance and Alzheimer's disease. The aim of the study was to investigate the cellular changes that occur from bilateral orchidectomy and examine the effects of androgen deprivation, orchidectomy and flutamide administration on the histoarchitectural organization of the hippocampus in male Wistar rats.

Methodology

Thirty-six (36) adult male Wistar rats were randomly divided into into six (6) groups: Control group (distilled water),orchidectomy group (bilateral orchidectomy), flutamide group (oral 10mg,20mg), orchidectomy+flutamide. Animals were sacrificed at 30 days respectively.

Results

The total plasma; testesterone, insulin levels, fasting blood glucose, and nitric oxide were assayed; the homeostasis model for insulin resistance was also calculated. Histological examinations by Hematoxylin and Eosin(H&E) and Cresyl fast violet (CFV), while immunohistochemical analysis of astrocytes were performed using Glial fibrillary acidic protein (GFAP). Results of study show that androgen deprived insulin-resistance state primarily affects fasting blood glucose and lead to increases in the level of serum insulin, glucose and nitric oxide and caused a decrease in serum testosterone levels. The hippocampal response to flutamide was unaffected by blockade of intracerebral estrogen biosynthesis. In comparison flutamide alone increased CA1 spine synapse density also whereas in combination the effects of flutamide+orchidectomy were additive rather than inhibitory.

Conclusion

Histopathological results showed that flutamide significantly restored the histological damage of rat brain in flutamide treatment+ orchidectomy.

Introduction

Neurodegeneration, testosterone deficiency syndrome and metabolic syndrome affects millions of people worldwide. There is increasing evidence from clinical and epidemiological research suggesting that testosterone deficiency, type 2 diabetes mellitus (T2DM) and Alzheimer's disease (AD); associated with dementia are linked (Yarchoan and Arnold, 2014).

Bilateral orchiectomy is surgical procedure in which both testicles are removed. The removal of both testicles is the surgical form of male castration performed as part of sex reassignment surgery (SRS) for transgender women, or as palliative treatment for advanced cases of prostate cancer (testosterone ablation therapy), as well as testicular cancer (Frey and Rebecca, 2004).

Andropause is an age-related problem, the gradual decline of testosterone levels associated with aging may be due to reduction of Leydig cell mass, testicular circulation, hypothalamic GnRH and pituitary gonadotropins (Neaves *et al..,* 2009; Winters and Troen, 2010).

Epidemiological studies have shown a direct correlation between plasma testosterone and insulin sensitivity, individuals with low testosterone levels are prone to an increased risk of type-2 diabetes mellitus, insulin resistance and Alzheimer's disease (Grossmann, 2003). Prospective studies indicated that the risk of type 2 diabetes can be reduced by 42% in men with normal testosterone levels (Alibhai *et al..,* 2010). However, clinical intervention studies reported controversial results regarding the effects that testosterone replacement therapy has on insulin resistance in aging males (Alibhai *et al..,* 2010).

Hippocampus

Damage to the hippocampus can also result from oxygen starvation (hypoxia), encephalitis, or medial temporal lobe epilepsy. People with extensive hippocampal damage may experience amnesia the inability to form or retain new memories. In rodents, the hippocampus has been studied extensively as part of the brain system responsible for spatial memory and navigation.

Synaptic Plasticity

One of the most important and fascinating properties of the mammalian brain is its plasticity; the capacity of the neural activity generated by an experience to modify neural circuit function and thereby modify subsequent thoughts, feelings, and behavior. Synaptic plasticity specifically refers to the activity-dependent modification of the strength or efficacy of synaptic transmission at pre-existing synapses, and for over a century has been proposed to play a central role in the capacity of the brain to incorporate transient experiences into persistent memory traces. Synaptic plasticity is also thought to play key roles in the early development of neural circuitry and evidence is accumulating that impairments in synaptic plasticity mechanisms contribute to several prominent neuropsychiatric disorders.

Flutamide

Flutamide, sold under the brand name Eulexin among others, is a nonsteroidal antiandrogen (NSAA) which is used primarily to treat prostate cancer. (Elks, 2014) It is also used in the treatment of androgen-dependent conditions like acne, excessive hair growth, and high androgen levels in women. It is taken by mouth, usually three times per day. (Ismaila *et al.*., 2013).

Conversely, the medication has fewer side effects and is better-tolerated in women with the most common side effect being dry skin. Diarrhea and elevated liver enzymes can occur in both sexes. Rarely, flutamide can cause liver damage, lung disease, sensitivity to light, elevated methemoglobin, elevated sulfhemoglobin, and deficient neutrophils. Numerous cases of liver failure and death have been reported, which has limited the use of flutamide. (Ismaila *et al.*., 2013). Flutamide acts as a selective antagonist of the androgen receptor (AR), competing with androgens like testosterone and dihydrotestosterone (DHT) for binding to ARs in tissues like the prostate gland. By doing so, it prevents their effects and stops them from stimulating prostate cancer cells to grow.

Methodology

Experimental Animals

The study was conducted using male Wistar rats, the animals were housed in standard polypropylene cages, and under a 12 h light/12 h dark program at an ambient temperature of 25±2 °C w. 50 adult male rats was purchased and bred with free access to feed and water, rats were randomly assigned into 6 groups, 3 for orchidectomized rats and 3 for normal rats as described below (group A-F), treatment lasted for 30 days.

Animal Groupings and Treatment

After the period of acclimatization and orchidectomy procedure, Thirty-six (36) adult male Wistar rats (with average weight of 210±5g) were randomly assigned into 6 groups labelled A,B,C,D,E, and F prior to treatment.

TABLE SHOWING GROUPING OF ANIMALS AND TREATMENTS FOR 30 DAYS.

GROUP	NO OF ANIMALS	TREATMENT FOR 30 DAYS
GROUP A	6	CONTROL ONLY
GROUP B	5	ORCHIDECTOMY
GROUP C	7	FLUTAMIDE (10MG)
GROUP D	7	FLUTAMIDE (20MG)
GROUP E	5	ORCH + FLUT (10MG)
GROUP F	5	ORCH + FLUT (20MG)

The rats were treated with flutamide for 30 days as indicated in the groupings, 14 days after the orchidectomy surgery. Rats were weighed twice a week (72 hour intervals), starting from the first day of administration using a digital weighing balance. Treatment lasted for 30 days and rats were anaesthetized and then sacrificed.

Procedure for bilateral orchidectomy

Animals were anesthetized through intraperitoneal injection of ketamine (60 mg/kg). The ventral scrotum was shaved and scrubbed with disinfectant, then a 1.5cm transverse incision was made at the midline scrotum, the testes were exteriorized through the incision, the tubules were tied with 0.4 silk sutures, and , the testicular fat were pulled out through the incision using forceps, the testes were then removed. Rats were placed on a thermal pad (at 38ºC) to keep warm until recovery from anesthesia and were subsequently treated with antibiotic orally. Rats were allowed 14 days of full recovery prior to other treatment procedures during which Flutamide treatment commenced as described above.

Ethical issue concerning the care and use of animals were obtained from the HREC of the College of Health Sciences, Osun State University and orchiectomy performed by a registered Veterinary Doctor.

Mode of administration was Oral using a cannula and 10mg of Flutamide solution was administered to Group C and Group E rats while 20mg of flutamide was admistered to Group D and Group F respectively.

Breakdown of the administration: The castrated animals in group B were given 10mg each of flutamide, castrated animals in group C were given 20mg each of flutamide solution using a cannula, intact males in group E were administered 10mg of flutamide each using a cannula and intact males in group F were administered 20mg of flutamide each using a cannula.

Mortality breakdown

12 rats died during acclimatization, 2 of the 12 rats showed the following symptoms; a bad/blind eyes, weak response to sunlight, weak/injured hind foot, 2 rats bled to death after castration and 1 rat probably died of post surgery trauma.

Animal sacrifice and tissue processing

24 hours after the last administration of experimental treatments, rats for enzymatic, histology and immunohistochemistry were then sacrificed by cervical dislocation (to eliminate the interference of ketamine induced change in biochemical redox); the thoracic cage was cut open and at least 5ml of blood was drawn from the apex of the heart for further analysis, the blood was centrifuged, 5000 rpm for 3mins to get the serum in order to deduce the glucose, insulin, and testosterone level, selected brain region Hippocampus was also excised and rinsed with distilled water and then in 10% phosphate buffer saline and stored in 4% paraformaldehyde at 4°C so as to prevent protein denaturization until futher processing. Transverse sections was made to expose the brain cortices, following which the sections will be processed to obtain paraffin wax embedded blocks for histology and antigen retrieval immunohistochemistry for Glial Fibrilar Acid Protein (GFAP).

Results And Discussion

The daily observation of the physiological status in orchidectomized and flutamide treated groups showed poor grooming in rats for the study period, some rats in this group also were less active and became hypomotile while the control groups were more active. The results of the percentage weight change and blood serum assay, insulin serum assay and glucose concentration are shown below.

Discussion

In order to achieve androgen deprivation, bilateral orchidectomy was done, some additional drugs can also be used to achieve complete androgen deficiency by blocking androgen production. This is also referred to as complete androgen deprivation (CAD). Epidemiological studies have shown a direct correlation between plasma testosterone and insulin sensitivity, and low testosterone levels are associated with an increased risk of type-2 diabetes mellitus (Grossmann, 2011). In this study, the androgen deprivation therapy (bilateral orchidectomy and flutamide treatment) causes a progressive reduction in the serum testosterone level compared with the control group, similar results were obtained by other studies whereby orchidectomy had caused 50% reduction in serum testosterone in male rats (Erben *et al.*, 2000), while testosterone replacement was able to raise the testosterone levels.

At the end of this study the plasma serum level for the orchidectomized group and flutamide group increased significantly, from this it can be deduced that orchidectomy significantly affects the production of plasma in the body. Also, the glucose levels for the orchidectomized and flutamide group increased significantly compared to the control group, this can be as a result of conservation of more glucose since orchidectomy has halted the process of reproduction which uses a significant amount of glucose, also the serum testosterone levels decreased in the orchidectomized group and flutamide group, since the organ responsible for testosterone has been removed, it will cause a reduction in its production thereby reducing the amount present in the blood stream and insulin levels in orchidectomized-insulin resistant rats, and to estimate the degree of insulin resistance, the rats also showed impaired hippocampal synaptic plasticity, this is in comparison to study carried out by (Alexis *et al.*, 2008) to show that insulin resistance impairs hippocampal synaptic plasticity, in the study, the rats were fed diet high in saturated fats and simple sugars and their water was supplemented with high fructose corn syrup, the diet was seen to increase fasting blood glucose levels and serum cholesterol and trigyceride levels, it was also found out that the diet impaired hippocampus-dependent learning, synaptic plasticity and dendritic spine density.

Also in this study, photomicroarchitecural presentation of the dentate gyrus, for the control group shows normal presentation of the dentate gyrus, characterized by well outlined pyramidal neurons. but a significant change in histology of the hippocampus was observed in the flutamide group and after orchidectomy. This shows that orchidectomy has a significant effect in the functioning of the hippocampus. Visible in the slide is the hippocampus proper formed of the Cornu Ammonis CA1 and CA2 comprised of small pyrimidial cells and also the CA3 zone formed of large pyrimidial cells. For the orchidectomized group across the various study group. It shows abnormal histology of the hippocampus. On administration of flutamide (10mg) across the various study group, the CA1 region is less visible showing abnormal histology. For the orchidectomized and flutamide group, it shows abnormal histology as the dentate gyrus is worn out and less visible. This affects the synaptic plasticity in the hippocampus.

Upon immunohistochemistry using GFAP stain, the control group shows a normal presentation with sparse astrocytes in the hippocampus, the orchidectomy group shows a deviation in the morphology of the dentate gyrus showing mild activation of astrocytes. The astrocytes in the orchidectomy and flutamide group shows increase immunopositivity characrerized by astrocyte activation. This simply shows a degradation in the synaptic plasticity in the hippocampus which will affect cognition and learning. In comparison to a study carried out by (Neil *et al.*, 2015) to test Effects of Dehydroepiandrosterone and Flutamide on Hippocampal CA1 Spine Synapse Density in Male and Female Rats, during the study ORCH males were treated with DHEA alone, DHEA flutamide, or DHEA the aromatase inhibitor letrozole. At the dose of DHEA used (1 mg/d sc), there was no significant effect on ventral prostate weight (control vehicle-injected ORCH rats 36.7 3.0 mg; DHEA injected rats 33.0 7.0 mg). However, DHEA more than doubled CA1 spine synapse density, compared with the vehicle-injected controls. Pretreatment with flutamide (5 mg/d sc) further increased the CA1 spine synapse density observed after DHEA to a level (1.207 – 0.023 synapses/ m3) higher than that of served after treatment of males with either testosterone or DHT. Pretreatment with letrozole, at a dose previously shown to abolish induction of CA1 spine synapses by DHEA in females (8), had no significant effect on the response to DHEA in ORCH males. Treatment of OVX females with DHEA increased spine synapse density by approximately 65%. As in the male, this response was further augmented by pretreatment of the females with flutamide. Flutamide alone, in OVX females, induced an increase in CA1 spine synapse density that was almost equal to the response observed with DHEA. from the previous results, DHT increased synapse density by more than 100%, compared with vehicle-injected ORCH rats. Flutamide treatment also increased CA1 synapse density but to a significantly lesser extent (66%) than DHT. Consistent with the previous results with DHEA, flutamide did not inhibit the synaptogenic effects of DHT.

Upon analysis the characterization of cellular changes occurring in the cortico-hippocampal regions in models of androgen deprived insulin-resistant adult male rats was determined. The extent of synaptic remodeling and activities of dendritic spine has been elucidated in the insulin-resistant bilaterally orchidectomized adult male rats. After immunohistochemistry. the plasma glucose, testosterone and insulin levels in orchidectomized-insulin resistant rats, and also estimated was the degree of insulin resistance.

Conclusion

Increase in the prevalence of obesity and diabetes are directly attributable to excessive caloric intake. This study suggest that elevated fasting glucose levels and hyperlipidemia are associated with neurological deficits, Orchidectomy and flutamide has also been observed to cause an increase in serum plasma level, serum glucose level which is as a result of the destruction of the normal morphology of the hippocampus, decrease in serum testosterone levels was also observed after orchidectomy and administration of flutamide, which invariably led to decreased synaptic plasticity in the hippocampus.

Declarations

Ethical Approval and Consent to participate: Ethical considerations were made in line with the guidelines on the care and use of Animals in line with the guideline for research ethics in science. Ethical approval to conduct the research was also obtained from the Health and Research Ethics committee of the College of Medicine of the Osun State University, Osogbo Nigeria. Human subjects were not used in the experiment thus there was no consent given to human subjects to participate

Consent for Publication: All the Authors are aware of the draft copy of the manuscript thus consented before sending to the Journal for review and eventual publication

Availability of supporting data: Not Applicable

Competing interests: Authors hereby declare no conflict of Interest

Funding: This was a self funded experiment; No fund was realized from any agency for this research

Authors' contributions:

1. Dr Falana B.A: Lead Author, conceived the idea and took part in every aspect of the research including the bench and laboratory work ; He was also involved in the write up.
2. Dr. Opuwari C.S: A co-Author, and a colleague; she took part in editing the manuscript
3. Akinbampe Monsurat is a junior colleague, she took part in the practical aspect of the research; from the feeding and taking care of the animals to making available all the materials needed for every step of the bench work
4. Dr. Abayomi T.A is also a Colleague; she participated also in the bench work.

Acknowledgements: Authors were grateful to Dr. Yawson E.O for technical skill during the orchidectomy and for providing Flutamide and GFAP antibodies.

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Graphs And Plates

Graphs and Plates are available in the Supplemental Files section.

Figures

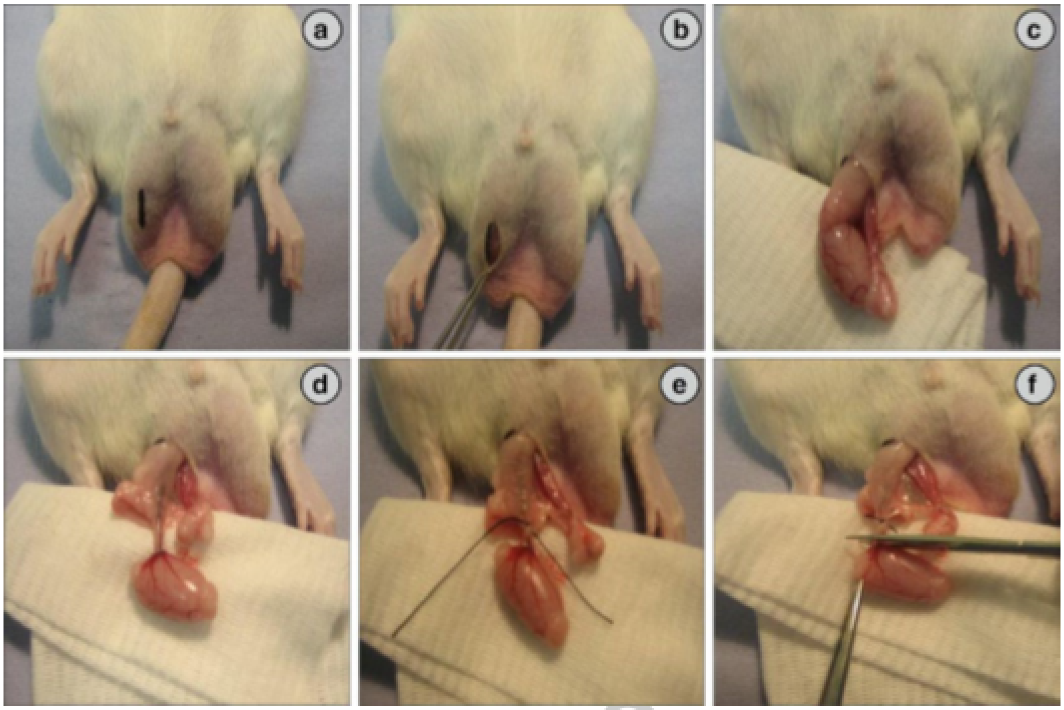

Figure 1

illustration of orchidectomy in the rat Performed by Yawson E.O & Falana B.A 2020.

Supplementary Files

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