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Progesterone Improve Streptozotocin-Induced Prefrontal Nissl Substance Deficit

Opeyemi Adebola A[1]*, Oluwole Busayo A[2] and Afolayan S[3]

[1]Department of Anatomy, Benjamin S Carson School of Medicine, Babcock University, Nigeria

[2]Professor of Anatomy at the Department of Anatomy, College of Health Sciences, University of Ilorin, Nigeria

[3]Department of Anatomy, College of Health Sciences, University of Lagos, Nigeria

***Corresponding author:** Opeyemi Adebola A, Department of Anatomy, Benjamin S Carson School of Medicine, Babcock University, Ilisan-Remo, Ogun State, Nigeria

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Abstract

Background: Nissl bodies are essential to the well-being of a neuron; decrease in the Nissl bodies of a neuron indicates neural degeneration, neurodegenerative diseases cause neural cells to lose both the functional and sensory abilities. This study investigate the activities of progesterone on Nissl substance of the prefrontal cortex cells in Adult Male Wistar Rats acutely exposed to a streptozotocin.

Materials and Methods: Forty eight (48) Rattus novergicus, weighing 220±30g were randomly selected into six groups. Group 1 (0.2ml Normal saline for 14 days), Group 2 (low dose of 4mg/kg of progesterone for 7 days), Group 3 (8mg/kg of progesterone for 7 days), Group 4 (double dose of 30mg/kg of streptozotocin only), Group 5 (double dose of 30mg/kg of streptozotocin start followed by 4mg/kg of progesterone for 7 days), Group 6 (double dose of 30mg/kg of streptozotocin start followed 8mg/kg of progesterone for 7 days). The administration was intra-peritoneal and all animals were euthanized using 20mg/kg of intramuscular ketamine, cardially perfused with 4% paraformaldehyde, the brains and prefrontal cortex was removed for biochemical and histological analysis.

Results: Between three to seven days Streptozotocin administration caused degeneration of beta cells, in rats Groups 4, 5, and 6 shows induced-diabetic symptoms via tests. Histological structure of the prefrontal cortex tissue of the rats' brain sampling indicates intoxication with streptozotocin cause chromatolysis in the brain sampling.

Conclusion: Progesterone proved to regulate the activities of stress markers and play protective role in some parts of the prefrontal cortex Nissl substance of the Rattus novergicus.

Keywords: Streptozotocin; Progesterone, Prefrontal; Nissl substances

Introduction

Streptozotocin (STZ) has been implicated to cause diabetes mellitus type in rats experiment for decades [1-3]. STZ injection impairs cognitive function by occurrence of dysfunction of glucose metabolism in the brain, suppressing the activation of essential enzymes to produce insulin resistance, and it selectively reducing the autophosphorylation process of the insulin receptor [4]. Decreased glucose and energy metabolism has been reported in STZ and it affects the dendritic morphology in the limbic structures, such as prefrontal cortex, occipital cortex, and hippocampus [5,2]. Diabetes mellitus result into wide range of damages to organs and systems in the body [2]. STZ causes the death of pancreatic β-cells by alkylation of DNA resulting in reduced synthesis and release of insulin, and in the fragmentation of DNA through production of reactive oxygen species [6]. Diabetes induced by STZ is associated with polydipsia and loss in body weight [6]. Complications related to diabetes mellitus are associated with oxidative stress induced by the generation of free radicals [7]. Free radicals result in the consumption of antioxidant defenses leading to disruption of cellular functions and oxidative damage to membranes and enhance susceptibility to lipid peroxidation, increased generation of Reactive Oxygen Species (ROS) [8].

STZ treatment in the brain of rats have been associated with an increase tau hyperphosphorylation and neuroinflammation, a disturbance of brain insulin signalling, reduced synaptic plasticity and amyloid β peptides [9]. Sub-diabetogenic doses of STZ induced cognitive and brain cholinergic deficits [10], oxidative stress as well as decrement in brain glucose/energy metabolism [11], and insulin resistant brain state [12]. STZ disturbs multiple metabolic and cell signalling pathways in the brain [13]. Cognitive dysfunction and dementia have been proven to be common complications of DM [14]. Phenotypes associated with obesity and alterations on insulin homeostasis are at increased risk for developing cognitive decline and dementia [14]. Both types 1 and 2 diabetes are risk factors for decreased performance in several neuropsychological functions, chronic hyperglycemia and hyperinsulinemia primarily stimulates the formation of Advanced Glucose End products (AGEs), which leads to an overproduction of ROS [14].

Progesterone (PRO) possesses antioxidant properties that are involved in the scavenge ring of ROS in cancer cells and increasing Superoxide Dismutase (SOD) activity in human endometrial stromal cells [15]. Progesterone induced antioxidant genes expression in

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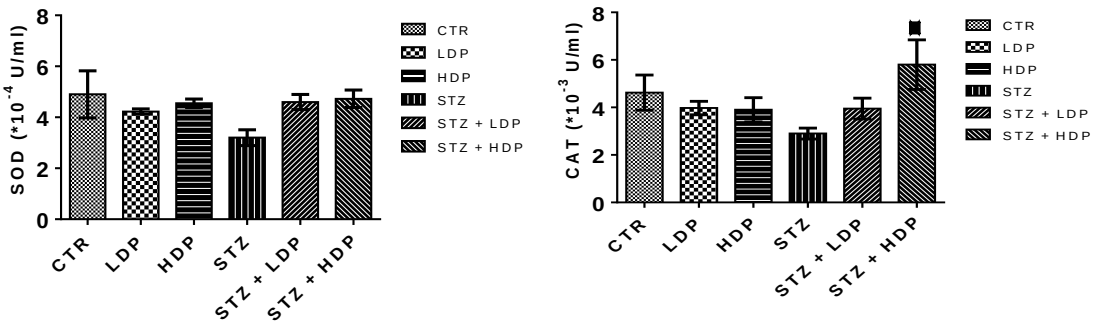

Figure 1: Graph showing superoxide dismutase and catalase levels across all groups. μ- P<0.05 statistical significant difference compared to animals exposed to streptozotocin (group 4). CRT (Control), LDP (Lower Dose Progesterone), HDP (Higher Dose Progesterone), STZ (Streptozotocin), STZ+LDP (Streptozotocin + Lower Dose Progesterone), STZ+HDP (Streptozotocin + Higher Dose Progesterone).

cardiomyocytes cells and exert antioxidant and ant-apoptotic effects [16,17]. Progesterone ability to reduce the cerebral edema associated with traumatic brain damage first became apparent when we observed that males had significantly more edema than females after cortical contusion [17]. Progesterone-treated rats also showed less neuronal degeneration twenty one "21" days after injury in the medial dorsal thalamic nucleus, a structure that has reciprocal connections with the contused area [17]. Progesterone reduced tau hyperphosphorylation when administered both alone and in combination with estrogen [18]. However, estrogen and progesterone administered independently and interactively regulate AD-like neuropathology and suggest that an optimized hormone therapy may be useful in reducing the risk of AD [18].

The Prefrontal Cortex (PFC), which covers the most frontal part of the mammalian contains Brodmann areas 9, 10, 11, 12, 46, and 47 [19]. The basic activity of this brain region is considered to be the orchestration of thoughts and actions in accordance with internal goals [19]. The PFC has also been associated with other functions like planning complex cognitive behavior, personality expression, decision making, and moderating social behavior [13].

Materials and Methodology

The animals (n=48) male Wistar rats of two weeks (2wks) old which was sourced from at the Department of Zoology, University of Ilorin. They are reared and acclimatized for four months (16wks) in the experimental facility of the Faculty Of Basic Medical Science Animal House, University of Ilorin, for 12-hour light: 12-hour dark cycle with room temperature of 30˚C. All rats received standard laboratory animal's chow and water ad-libitum during the whole period of experiment. The wistar rats are groups into six (6) with eight (8) rats per cage of a group, Water was provided as libitum, and animals were fed irradiated rodent diet (Diet 2919 Teklad Global 35% Protein Rodent Diet) there diet was selected preferentially over standard chow because of its slightly higher energy density compared with that of conventional mouse diet (10.3 kcal/g). This higher energy level is advantageous in support of the diabetic state. Studies using these rats for assessment of islet cell preparations were approved by the University of Ilorin Institutional Animal Care and Use Committee, conducted in compliance with the Animal Welfare Act, and adhered to principles stated in the Guide for Care and Use of Laboratory Animals of the University of Ilorin Animal Ethics Committee in-line with the National Institute of Health (NIH) guidelines on the use of animals in experiment research.

The rats were inspected daily for signs of pain or distress, including changes in respiration, appetite, urine output, excessive thirst, dehydration, activity, weight loss exceeding 10% of the initial value, unkempt appearance, abnormal posture, and twitching or trembling. The measurement of body weight and observation of body condition acted as a marker of appetite. The measurement of skin turgor and observation of cage bedding for urine output acted as a marker of thirst. Rats in group (STZ, STZ+LDP & STZ+HDP) were injected intraperitoneally with a two low dose of 30 mg/kg b.w streptozotocin which was given along with oil vehicle of citrate buffer solution of 0.1ml of 4.5pH and after 24 hours Progesterone (Progesterone injection B.P 25mg/ml) therapy was initiated at a dose subjected to in the groups. The dose and frequency of Progesterone administration range, low dose of 4 mg/kg/b.w/day for Group 2 and 5 (LDP & STZ+LDP) and high dose of 8 mg/kg/b.w/day for Group 3 and 6 (HDP & STZ+HDP), while Group 1 and 4 (CTR & STZ) received none. The administration of Progesterone is for seven [7] days.

At the end of the experiment, the animals were anaesthetized with intermuscular injection of 20mg/kg of ketamine, skin excised and transcardially perfused with saline and subsequently with 4% paraformaldehyde in 0.1M phosphate. The brains were removed and the prefrontal cortex was excised from the brain and put in appropriate fixative for subsequent histological analysis and biochemical assay.

Prefrontal Catalase activity was estimated [20]. SOD activity was determined by the method [21] and total prefrontal protein by the Biuret method [22] using kits from Randox Laboratories Limited (Antrim, UK). Prefrontal lobes of rats' brains were fixed in 4% paraformaldehyde and processed for paraffin embedding. Paraffin sections (5μ) were stained with cresyl fast violet [23] to study the morphology of the prefrontal neurons.

Data collected were analysed using Microsoft Excel and one-way analysis of variance (ANOVA) followed by Tukey's (HSD) multiple comparison test with the aid of SPSS V20. Data were presented as means±SEM (standard error of mean) P value less than 0.05 (p<0.05) was considered statistically significant.

Results

Superoxide dismutase and catalase level reduced in animals exposed to streptozotocin (group 4), though not statistically significant (P>0.0.05) Compared To Control (CTR) and animals given different doses of progesterone (groups LDP and HDP); but the SOD and CAT level increased in animals exposed to streptozotocin and treated with progesterone (groups STZ+LDP and STZ+HDP). The level of Catalase (CAT) increased significantly (μ- P<0.05) in animals treated with high dose of progesterone compared to animals exposed to streptozotocin. CTR-control; LDP- low dose progesterone; HDP- high dose progesterone; STZ- single injection of streptozotocin; STZ + LDP- streptozotocin followed by low dose progesterone; STZ + HDP- streptozotocin followed by high dose progesterone (Figure 1).

The white arrows points to the location of normal neuron, which indicates the presence of Nissl bodies in the cytoplasm of the pyramidal cells of prefrontal cortex of adult male Wistar rats while the yellow arrows points to the location of abnormal normal neuron showing dispersed and loss of Nissl bodies in the cytoplasm of the neuron, this outcome suggests process of Chromatolysis which is a precursor of apoptosis. Streptozotocin has deleterious effect on the pyramidal cells of the prefrontal cortex of adult male Wistar rats (Figure 2).

Discussion

The level of superoxide dismutase remained the same in the prefrontal cortex of rats that collected progesterone alone, Superoxide dismutase and catalase level reduced in animals exposed to Streptozotocin Alone (STZ), though not statistically significant (P>0.0.05) Compared To Control (CTR) and animals given different doses of progesterone (groups LDP and HDP); the SOD and CAT level increased in animals exposed to streptozotocin and treated with progesterone (groups STZ+LDP and STZ+HDP). The level of Catalase (CAT) increased significantly (μ- P<0.05) in animals treated with high dose of progesterone compared to animals exposed to streptozotocin. This result suggests that progesterone was able to regulate the activities of superoxide dismutase. Progesterone might have an ameliorative effect in the group five (STZ+LDP), but in group six (STZ+HDP) it serves a more protective role, in the prefrontal cortex of adult male Wistar rats corresponding with [24,10].

Nissl bodies are essential to the well-being of a neuron; a decrease in the number of Nissl bodies in a neuron indicates neural degeneration [25]. Nissl bodies are granular bodies found in the cytoplasm of a cell which comprises of rough endoplasmic reticulum and rosettes of free ribosome and are sites of protein synthesis. Protein synthesis (also termed mRNA translation) is a key step in the expression of a cell's genetic information, in which the information contained within the coding region of the mRNA is used to direct the synthesis of the new protein, a process that is catalysed by the ribosome [26].

The rats that collected normal saline alone (CTR) showed that most of the neurons in the pyramidal layer prefrontal cortex have intact cyto-architecture and densely stained Nissl bodies. The rats that received a double doses of Streptozotocin alone (STZ) revealed disintegrated and dispersed Nissl bodies in the cytoplasm of most neurons in the pyramidal layers of the cortex. The rats that collected progesterone alone in (LDP) and (HDP) revealed that most of the neurons in the pyramidal layer of the cortex have intact cyto-architecture and densely stained Nissl bodies. The rats that received double doses of Streptozotocin thereafter progesterone revealed (STZ+LDP) and (STZ+HDP) that some of the neurons found in the pyramidal layers still possess Nissl bodies in their cytoplasm. This can be said to be the influence of progesterone ameliorating on the toxic effect of Streptozotocin on the pyramidal cells. Progesterone effect on some of these neurons showed presence of normal cytoplasm and densely stained Nissl bodies.

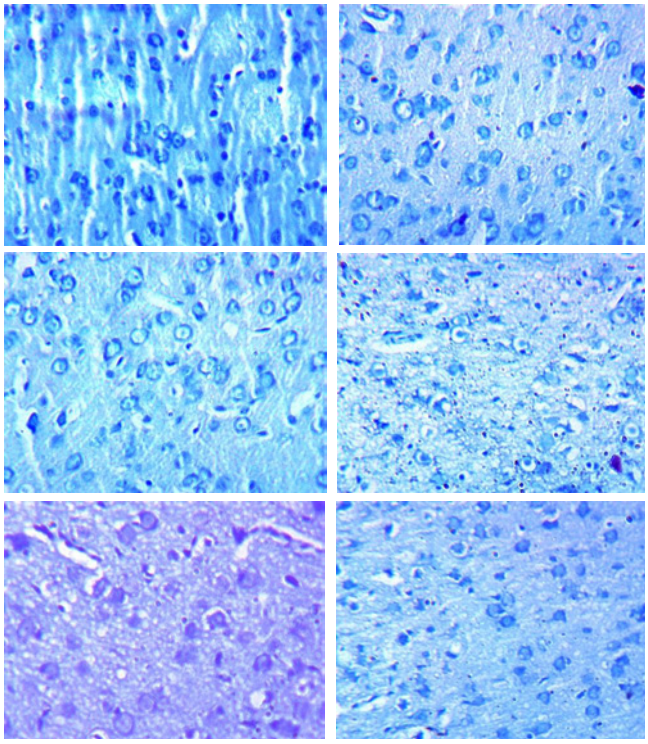

Figure 2: Photomicrograph showing the pyramidal cells of the Granular Layer (GL) of the prefrontal cortex of adult male Wistar rats (Toluidine Blue stain x 400). CRT (Control), LDP (Lower Dose Progesterone), HDP (Higher Dose Progesterone), STZ (Streptozotocin), STZ+LDP (Streptozotocin + Lower Dose Progesterone), STZ+HDP (Streptozotocin + Higher Dose Progesterone).

The degenerative changes (i.e Chromatolysis which is the disintegration of Nissl bodies) observed (STZ, STZ+LDP and STZ+HDP) in this study is as a result of exposure to Streptozotocin correlates with a study by [27,28] suggested chromalytic changes in the prefrontal cortex of rats as a result of STZ exposure also the work of [29] suggested that intoxication can cause chromatolysis in rat's brain.

Progesterone in (Figure 2) (STZ+LDP and STZ+HDP) showed the ability to repair damage neurons in this study by reviving the lost Nissl bodies of the neurons and also protecting some of the Nissl bodies from Streptozotocin injury which is supported by [29,30].

Conclusion

In conclusion this study show progesterone amelioration on the toxic effect of Streptozotocin on the prefrontal cortex neurons pyramidal cells and Nissl substance by normalization of metallic

function in diabetic rats treated with progesterone due to stimulation effect on insulin secretion from pancreatic insulin level.

The area of limitation is based on the percentage and volume ratio of the progesterone given, in which I will call for more work on.

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Conflict of interest: No conflict of interest.

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