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Research Article

Neurobehavioral Alterations Induced in Diabetes Are Attenuated by Beta-adrenergic Blockade

Ibironke G.F and Asifat O.S

Department of Physiology College of Medicine, University of Ibadan Nigeria

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Abstract

The aim of this study was to the evaluate the participation of beta adrenergic receptors in anxiety- like disorders which is a psychiatric condition reported in diabetic patients. Neurobehavioral activities were estimated in control mice using the open field, hole-board, elevated plus maze and the light / dark board tests. In another group, the Albino wistar rats were rendered diabetic by a single intraperitoneal (i.p) injection of alloxan (120mg/kg) and neurobevioral deficits estimated with the above named tests .Propranolol (40mg/kg) , a non-specific beta-adrenergic receptor antagonist was administered (i.p) to the third group before the induction of diabetes after which the neurobehavioral tests were carried out. Diabetes resulted in a significant ($p<0.05$) reduction in total locomotion, rearing and grooming frequencies in the open- field test and the time spent in the open arm in the elevated plus maze test. The time spent in the light arena of the light/ dark board test was also significantly ($p<0.05$) reduced .However, pre-treatment with propranolol significantly ($p<0.05$) reversed these observation as shown by increases in total locomotion ,rearing and grooming frequencies and the time spent including the number of entries into the open arm of the elevated plus maze ,the time spent in the light arena of the light and dark box test also increased . These observations are compatible with an anxiolytic state as opposed to the state of anxiety induced diabetes mellitus. In conclusion, these results suggest the participation of the adrenergic receptors in the anxiety-like behavior characteristic of diabetes in diabetic mice.

Keywords: *Diabetes, Neurobehavioral, Anxiety and Beta-blockade*

INTRODUCTION

The effects of diabetes mellitus on the central nervous system (CNS) results in cognitive dysfunction and cerebrovascular disease. The brain was not usually to be a target of chronic diabetic complications , however , substantial evidence suggest that diabetes mellitus causes brain damage (Mooradian,1988). Peripheral neuropathy has being the primary neuroscience focus of diabetes research in the past, but of recent chronic diabetes was found to affect the CNS in several ways, for example ,it was found to cause cognitive dysfunction (Jacobson et al,2007), dementia (Ott et al, 1996), deficits in white matter microstructure (Kodl et al, 2008) , hippocampal neurogenesis (Lobnig et al , 2006) and alterations in gray matter density (Musen et al,2006). The adrenergic system is also known to affect many neurobehavioral activities including reconsolidation of appetitive learning (Amy et ,al 2008) ,reversal of neurobehavioral and neurochemical alterations in STZ induced diabetic rats (Jagre et al ,2014) and improvement of spatial memory (Bjorkland et al,(2001).

So far, the role of the adrenergic system in diabetic alterations has been given little attention, hence this study focused on the adrenergic modulation of CNS changes in diabetes with emphasis on neurobehavior and specifically on anxiety.

MATERIALS AND METHODS

Male Swiss Albino mice (25-35g) obtained from the pre-clinical animal house, College of Medicine, University of Ibadan, Nigeria were used for the body. They were kept at room temperature under standard laboratory conditions with a 12-h light – dark cycle and fed with mouse cubes (Ladokun feeds Nig Ltd ,Ibadan) and water ad libitum

Drugs and chemicals

The following drugs and chemicals were used: propranolol hydrochloride (Research Biomedicals Inc, Natick , M . A) and alloxan (Sigma Aldrich,USA).

Induction of diabetes

Diabetes was induced by a single dose of alloxan (120mg/kg) injectected intraperitoneally after a 24 -hour fast .Mice with blood glucose levels above 185mg/dl were considered diabetic and were used for further tests .

Behavioral Assays

Elevated plus Maze Test: The anxiety status of the animal was assessed using the above named test (Pellow et al ,1986) .The elevated plus maze consists of two open arms (30cm×5cm) and two closed arms (30cm×5cm×15cm) that extended from a central platform (5cm×5cm) . The entire maze was elevated 40cm above the floor .During the first

Author for Corresponding : +2348058826713
E-mail: gibironk@yahoo.com

5min of the free exploration , the number of entries and the time spent in the open and closed arms were recorded . An entry was defined as the point the animal places all the four paws on to the arm .The maze floor was constructed from black Plexiglas and walls from clear Plexiglas .

Hole–board test: Anxiety levels were also evaluated in male mice by using a hole-board apparatus (35cm×35cm×15cm) .Its walls were made from of clear Plexiglas and the arena was constructed from black Plexiglas and divided into 16 equal squares with 16 holes (diameter 3.5cm) .The equipment was elevated 56cm above the floor . Each animal was placed on the central square of the arena and the number of head dips was recorded for 5min. An increase in the number of head dips reveals a positive anxiolytic -like effect (File and Pellow ,1985) .

Light-dark exploration test: The apparatus consists of a Plexiglas box with two compartments (20×20cm each), one of which was illuminated with white light, while the other remained dark. Each animal was placed at the junction of the light/dark areas ,facing the illuminated compartment . The time spent as well as the number of entries into the light and dark spaces were recorded for 5min (Young,1991)

Open-field test: The open-field box is a rectangular area composed of a hard floor measuring 36×36×26cm and made of a white painted wood. The was divided was divided by permanent read markings into 16 equal squares at the bottom. Each mouse was introduced singly into one corner of the field and the total locomotion (number of units entered with all paws) ,rearing frequency (number of times the animal stood on its hind limbs or with its four limbs against the walls of the observation box and grooming frequency (number of body cleaning with paws , picking of the body and pubs with mouth and face washing activities) withing each each 10min interval were recorded .

After each of these assays, the arena was cleaned with 70 percent alcohol to eliminate olfactory bias and the area allowed to dry before introducing a fresh animal.

Statistical analysis

Results were expressed as mean ± SEM . The behavioral data were analysed using the student´s t- test . A value of $p<0.05$ was regarded as significant

RESULTS

Open-field test.

The results showed that diabetes significantly ($p<0.05$) reduced the total locomotion (fig 1), rearing (fig 2) and the grooming (fig 3) frequencies compared with the control values .Pre- treatment with propranolol significantly ($p<0.05$) increased these values obtained in diabetic mice towards control values .

Elevated plus-maze test.

In this test, diabetes resulted in a significant ($p<0.05$) reduction in the number of entries into (fig 4) and the time spent (fig 5) in the open arm of the elevated plus maze apparatus. Pre-treatment with propranolol however significantly ($p<0.05$) reversed these observations

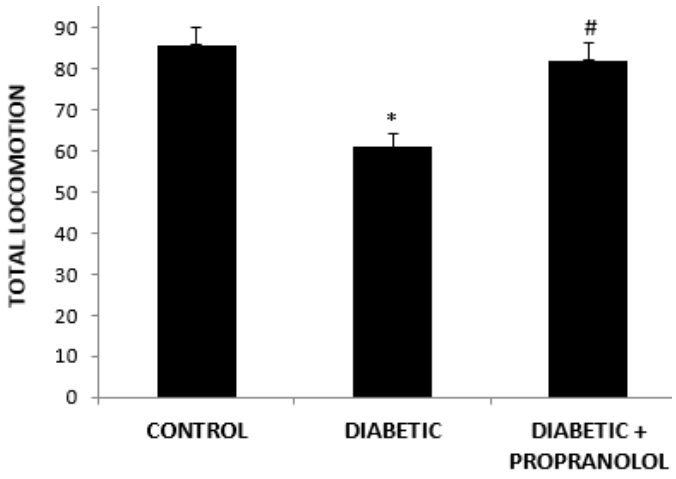

Figure 1:
Effect of propranolol pre –treatment on total locomotion in diabetic mice in the open field test
Values are expressed as means ± Standard Error of Mean (SEM), n = 6*P < 0.05, (Control vs Diabetic) #P < 0.05 (Diabetic vs Diabetic + Propranolol)

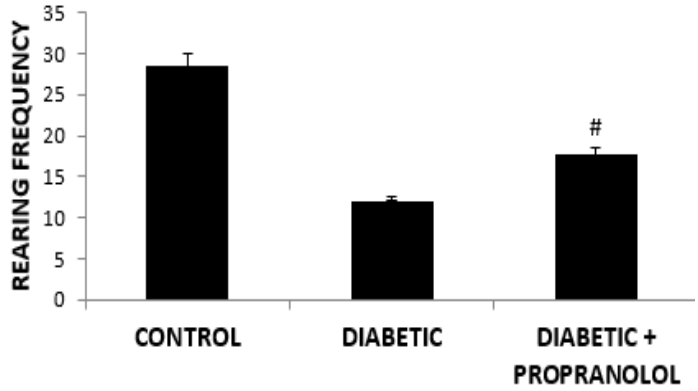

Figure 2:
Effect of propranolol pre-treatment on rearing frequency in diabetic mice in the open field test.
Values are expressed as means ± Standard Error of Mean (SEM), n = 6*P < 0.05, (Control vs Diabetic) #P < 0.05, (Diabetic vs Diabetic + Propranolol)

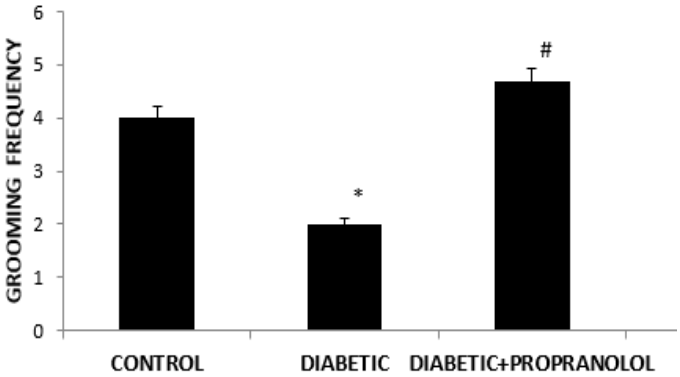

Figure 3:
Effect of propranolol pre-treatment on grooming frequency in diabetic mice in the open field test
Values are expressed as means ± Standard Error of Mean (SEM), n = 6*P < 0.05, (Control vs Diabetic)
#P < 0.05, (Diabetic vs Diabetic + Propranolol)

Light/dark board test

Diabetes significantly ($p<0.05$) reduced the number of entries (fig 6) and the time spent (Fig7) in the light arena of the light /dark box apparatus. A reversal was obtained on pre-treatment with propranolol.

Hole board test

The number of head dips in diabetic mice were significantly ($p<0.05$) reduced compared with the control. Propranolol pre-treatment significantly increased the head dip in the diabetic rat towards control values (fig 8).

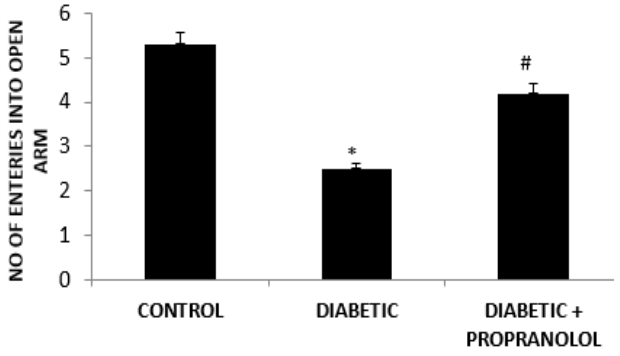

Figure 4:
Effect Of Propranolol Pre-Treatment On The Number Of Entries Into The Open Arms In Diabetic Mice in The Elevated Plus Maze Test.Values are means ± Standard Error of Mean
*P < 0.05, (Control vs Diabetic) #P < 0.05, (Diabetic vs Diabetic + Propranolol)

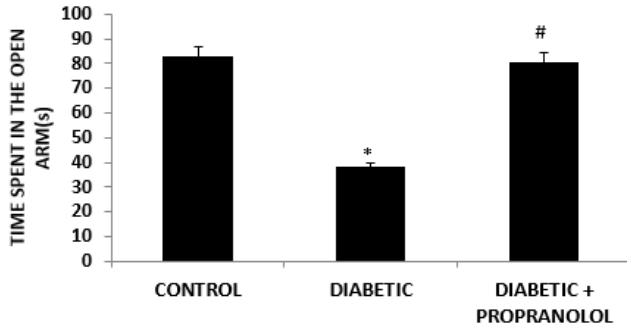

Figure 5:
Effect of Propranolol Pre-Treatment on the Time Spent in The Open Arms In Diabetic Mice In The Elevated Plus Maze Test.
Values are expressed as means ± Standard Error of Mean, n = 6
*P < 0.05, (Control vs Diabetic) #P < 0.05, (Diabetic vs Diabetic + Propranolol)

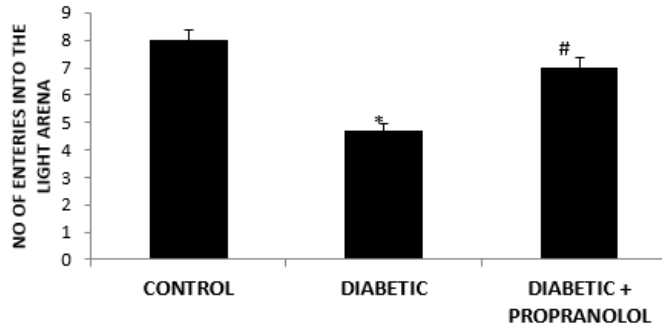

Figure 6:
Effect of propranolol pre-treatment on the number of entries into the light arena in diabetic mice in the light/dark box test.
Values are expressed an means ± Standard Error of Mean, n = 6
*P < 0.05, (Control vs Diabetic) #P < 0.05, (Diabetic vs Diabetic + Propranolol)

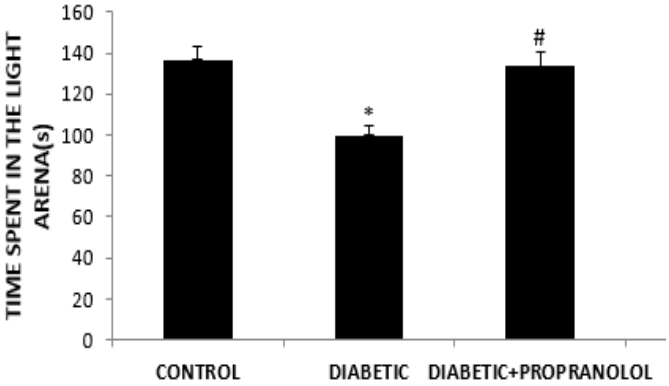

Figure 7:
Effects of propranolol pre-treatment on the time spent in the light arena in diabetic mice in the light/dark box arena test.
Values are expressed as means ± Standard Error of Mean, n = 6
*P < 0.05, (Control vs Diabetes) #P < 0.05, (Diabetic vs Diabetic + Propranolol)

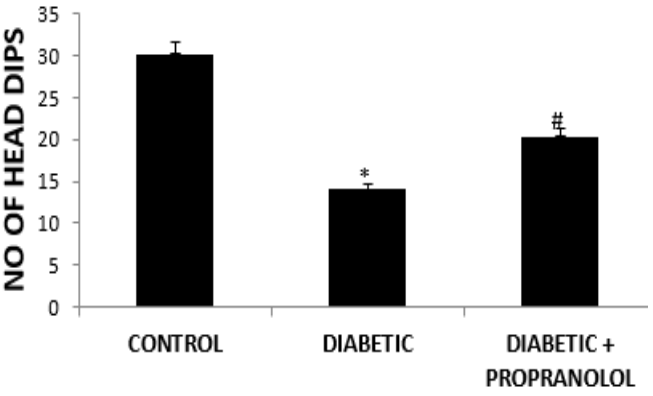

Figure 8:
Effect of propranolol pre-treatment on the number of head dips in diabetic mice in the hole board test.Values are expressed as means ± Standard Error of Mean, n = 6*P < 0.05, (Control vs Diabetic) #P < 0.05, (Diabetic vs Diabetic + Propranolol)

DISCUSSION

Given that the prostaglandin system is critically involved in nociceptive processing and sleep-wake regulation, this study attempted to look at the serum level of PGE_2 following sleep deprivation. The hyperalgesia caused by chronic constriction injury was accompanied by increased serum PGE_2 in the ligated group. This can be explained by the fact that PGE_2 is a one of the principal mediator of inflammation and thus, promote the development of inflammatory sign including pain (Haack *et al.*, 2009). PGE_2 is able to sensitize the nociceptive system through binding to Enzyme-Prostaglandin receptors located on peripheral terminals of primary sensory neurons resulting in an increased sensitivity to noxious stimuli and also changes neuronal excitability and synaptic dis-inhibition in the spinal cord which manifest in hyperalgesia and this has been suggested to play a role in the development of spontaneous pain (Vanegas and Schaible, 2001).

Our previous finding of anti-nociceptive effect of REM sleep deprivation on neuropathic pain (Under review) was further elucidated by this present study which showed a corresponding decrease in the serum level of PGE_2 in the test (ligated sleep-deprived) group. This may be said to be the basis for reduction in pain perception observed in the test group

since PGE_2 is a nociceptive mediator. Though, the molecular mechanism responsible for this decrease in PGE_2 was not part of the scope of this study, future studies can look into this. Also, very few reports have shown the relationship between sleep deprivation, PGE_2 and pain perception, and this result is contrary to the study of Haack *et al.,* (2009) which reported an increase in urinary level of PGE_2 following sleep deprivation in which they opined that the loss of inhibitory pain control mediated by PGE_2 probably accounted for most of the chronic pain symptoms, including the occurrence of spontaneous pain. Based on our search, we could not access any report on the link between REM sleep-deprivation, serum level of PGE_2 and neuropathic pain except for the report of Haack *et al.,* (2009) which is contrary to our finding. We propose that the probable reason for the difference may be attached to medium of assessing the PGE_2 i.e., urinary versus serum.

Studies have linked sciatic nerve ligation which is a stressor to an imbalance in reactive oxygen species (ROS) and anti-oxidant enzymes (Senoglu *et al.,* 2009). Free radicals have been found to induce tissue injury and pain in neuropathic pain models (Muthuraman *et al.,* 2008). This was also confirmed by the present study in which CCI of the sciatic nerve caused oxidative stress (generation of free radicals) which was expressed by an increase serum in MDA and a reduction in serum SOD of the ligated group. Following the sciatic nerve ligation of the test group, one would have expected that sleep deprivation which has been confirmed a stressor, to cause a further increase in oxidative stress with a corresponding increase in products of tissue peroxidation and decrease in anti-oxidant enzymes. In contrast, sleep deprivation produced a decrease in serum level of MDA (a product of tissue peroxidation) with an insignificant reduction in serum SOD level (an antioxidant enzyme) meaning that there was no release of free radicals and thus, the increase in pain threshold (decreased pain perception).

Many studies have reported existing relationship between mediators of inflammatory disorder and markers of oxidative stress as a complex one. The inflammatory process is accompanied by oxidative stress. Pro-inflammatory cytokines and growth factors stimulate release of reactive oxygen species (ROS) which act as signalling mediators for a variety of signal transduction pathways and gene expression. The transcription factors that have been implicated in many inflammatory responses are the nuclear factor-kappa B (NF-kB) and activator protein-1 (AP-1). Both NF-kB and AP-1 are sensitive to many different oxidative stress stimuli and some findings suggest that they may mediate cytokine and adhesion molecules expression (Vlahopoulos *et al.,* 1999). NF-kB when activated induces the expression of cytokines and adhesion molecules in a positive feedback loop. Moreover, this pathway is also redox-dependent and is activated by oxidative stress. Therefore, it can be postulated that antioxidant defence capacity may affect inflammatory response. For example, increased antioxidants in blood could, by inhibiting NF-kB, lead to a decrease in intercellular adhesion molecule-1 (ICAM-1), and interleukin-8 (IL-8) which are inflammation markers. For instance, in chronic obstructive pulmonary disease (COPD) which is accompanied by inflammation (both airway and systemic) and by oxidative stress, the pathogenic mechanisms explaining this association are multi-factorial and involve an intricate interplay between inflammatory and oxidative processes. On one hand, ROS activates NF-kB and other redox-sensitive transcription factors such as AP-1, which cause an increased gene expression of both pro-inflammatory cytokines and protective enzymes. On the other hand, these cytokines play an important role as activators of neutrophils and as chemo attractants, which will, in their turn, determine the inflammatory mediators released from neutrophils (Mac-Nee 2000; Sadowskaa *et al.,* 2005).

Another probable factor that may explain the observed decrease in MDA level could be that the multiple platform method used in sleep depriving these animals reduced stress compared to the single platform method. The multiple platform method has been reported to reduce stress as it allows for movement of animals and interaction with cohorts thus reducing stress that might have resulted from social isolation and restricted movement (Machado *et al.,* 2004).

In conclusion, Induction of neuropathic pain by chronic constriction injury of the sciatic nerve followed by REM sleep deprivation caused a decrease in serum levels of PGE_2, Malondialdehyde, and no significant effect on Super oxide dismutase. We therefore propose that REM sleep deprivation alters prostaglandin and anti-oxidant enzymes in rats induced with neuropathic pain.

DISCUSSION

The results of this study showed that diabetes caused neurobehavioral alterations that manifested in an increased state of anxiety that was reversed by reversed by beta -blockade . Anxiety is a state of cognitive and behavioral preparedness that an organism mobilizes in response to a future or distant potential threat , in its pathological form , anxiety is a maladaptive state that impairs the ability of an organism to respond optimally to its environment (Leonardo and Hen ,2008) . Anxiety states in this study have been estimated using the elevated plus maze ,the open-field , the light and dark board and the hole board tests . Our observation of a reduction in the number of entries into the open arm of the elevated plus maze , reduction in the number of head dips in the hole board test and reduced total locomotion in the open -field test are all indices of anxiety in the diabetic rat .

The elevated plus maze is frequently used for the study of anxiety related behaviors in rodents (Torres and Escarabajal,2002) . An anxious animal Torres and Escarabajal,2002) . An anxious animal will naturally prefer dark and confined spaces as observed in this study by a reduction in the number of entries into the open arm of the elevated plus maze and increased time spent in the dark arena of the light/dark box test.

A short time spent /reduction in the number of entries into the open arm is considered an index of anxiety (Pellow et al ,1985 ; Elliot et l ,2004) thus confirming the anxiogenic nature of diabetes in this study . Our observations in this study buttressed the prescription of propranolol as it may help in patients with situational or performance anxiety , but it must be used with caution in patients on insulin as it may mask the signs and symptoms of hypoglycemia by blocking the action of epinephrine and norepinephrine on β1 and β2 adrenergic receptors .

In conclusion ,these results illustrate the protective effect β adrenergic blockade on diabetes induced neurobehavioral alterations in rats

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