

*Full Length Research Paper*

# **Sex differences in the neuroprotective effect of insulin against chemically-induced convulsions in mice**

**Susanna Adeola Adebayo<sup>1\*</sup>, Oluwole Isaac Adeyemi<sup>1</sup>, Adegbenga Rotimi Owolabi<sup>2</sup> and Moses Atanda Akanmu<sup>1</sup>**

<sup>1</sup>Department of Pharmacology, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria.

<sup>2</sup>Department of Pharmacology and Medical Therapeutics, College of Health Sciences, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria.

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**Insulin, an important regulator of peripheral metabolism, has been reported to interact with many neurotransmitter systems including those associated with convulsion. The effect of insulin against pentylenetetrazole and strychnine-induced convulsions in mice, as well as possible sex differences, were evaluated in this study. Mice of both sexes weighing between 20 and 25 g were administered insulin intraperitoneally at doses of 1, 2, 4 and 8 IU/kg. Each mouse received a convulsive dose of pentylenetetrazole (100 mg/kg, i.p.) or strychnine (2 mg/kg, i.p.) and was observed for the onset of convulsions and occurrence of death. Against pentylenetetrazole-induced convulsions, all the doses of insulin used significantly ( $p < 0.05$ ) prolonged the onset of convulsions and significantly delayed the time of death in male mice when compared with control. However, in female mice, only insulin 8 IU/kg significantly prolonged the onset of convulsions, while insulin 4 IU/kg significantly delayed the time of death. Against strychnine-induced convulsions, insulin at the doses of 2 and 4 IU/kg significantly ( $p < 0.05$ ) prolonged the onset of convulsions in male mice relative to control, while 8 IU/kg insulin significantly prolonged the time of death in male mice compared to control. However, none of the doses of insulin administered to female mice were effective against strychnine-induced convulsions. These results show that insulin produced sex-related protective effects against chemically-induced convulsions in mice.**

**Keywords:** Insulin, convulsion, pentylenetetrazole, strychnine, male mice, female mice.

## **INTRODUCTION**

Insulin, a polypeptide hormone produced by the  $\beta$ -cells of the Islets of Langerhans in the pancreas, interacts with its receptors in peripheral tissues like liver, fat and muscle to

stimulate the uptake of glucose, fatty acids and amino acids leading to their storage as glycogen, fats and proteins respectively. The central nervous system (CNS)

\*Corresponding author. E-mail: saojox@gmail.com.

was previously thought to be insulin insensitive, but the discovery of insulin and its receptors in the CNS in 1967 and 1978 respectively, radically changed this view (Wada et al., 2005; Huang et al., 2010). Since then, there has been extensive research into the activities of insulin and its receptors in the brain and spinal cord.

Insulin receptors in the CNS and those in peripheral tissues share similar structural and functional characteristics. The insulin receptor, a member of the tyrosine kinase receptor family, is a big trans-membrane glycoprotein formed from two 135,000 Da  $\alpha$  subunits and two 95,000 Da  $\beta$  subunits linked by disulphide bonds to form a  $\beta$  -  $\alpha$  -  $\alpha$  -  $\beta$  heterotetramer (Bedse et al., 2015). When insulin binds to its receptors in the CNS, diverse neuronal effects are produced. Insulin and its receptors in the CNS have been found to play various roles far beyond their traditional peripheral effects. They are now known to play important roles in the growth, structure and function of neurons, and promote cognitive functions such as learning and memory, while impaired insulin receptor signaling has been linked to the pathogenesis of neurodegenerative diseases like Alzheimer's and Parkinson's diseases (Chiu and Cline, 2010; Bedse et al., 2015; Neth and Craft, 2017). Previous works have also reported the neuromodulatory actions of insulin in the CNS by regulating receptor density and affinity at the membrane surface and tyrosine phosphorylation of receptor subunits (Wan et al., 1997; Caraiscos et al., 2007; Ferrario and Regan, 2018). Insulin receptor signaling has thus been implicated in the regulation of synaptic neurotransmission, playing an important role in synaptic plasticity, learning and memory.

Convulsions or seizures result from disturbances in both excitatory and inhibitory neurotransmitter systems, including the glutamatergic and gabaergic systems (Koutroumanidou et al., 2013). Insulin receptor signaling has been reported to modulate the gabaergic, NMDA and glycinergic systems, among other neurotransmitter systems (Caraiscos et al., 2007; Neth and Craft, 2017; Trujeque-Ramos et al., 2018). Therefore, this study investigated the effect of insulin on convulsions induced by the administration of pentylenetetrazole and strychnine in mice. Due to the reported existence of sex differences in some metabolic effects of insulin (Woods et al., 2003; Clegg et al., 2006; Hallschmid et al., 2012), possible sex-related differences in the effect of insulin on convulsions were explored by the use of both male and female mice.

## MATERIALS AND METHODS

### Animals

Adult mice of both sexes (Vom strain, National Veterinary Research Institute, Jos, Nigeria) weighing between 20.0 and 25.0 g were used in this study. They were inbred and maintained under natural

daylight/night condition at the Animal House of Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife. Females were kept separately from males to prevent mating. All animals were fed with standard feeds (Pfizer Feed Plc, Lagos, Nigeria) and had access to food and water *ad libitum*. The experimental protocols followed the internationally accepted principles for laboratory animal use and care (EEC directive of 1986; 86/609/EEC; Ethical clearance certificate number for the use of animals: PHP11/12/H/2764).

### Drugs

Insulin (Actrapid®: Novo Nordisk, Bagsvaard, Denmark), pentylenetetrazole (Sigma Chemicals, St. Louis, USA), strychnine (Sigma-Aldrich, Switzerland), phenobarbital (Evans Medical, London, United Kingdom) and diazepam (Roche, Basel, Switzerland), dextrose 50% solution (Unique Pharmaceuticals, Sango-Ota, Nigeria) were used. Fresh solutions of appropriate concentrations of the drugs were prepared just before the test for each series of experiment. Drugs were administered to the animals intraperitoneally at a volume of 1 ml/100 g body weight.

### Evaluation of pentylenetetrazole-induced convulsions

Six groups of mice ( $n = 16$ ; males = 8, females = 8) were administered normal saline 10 ml/kg (i.p.), diazepam 2 mg/kg (i.p.) and insulin 1, 2, 4 and 8 IU/kg (i.p.) respectively. The mice administered normal saline or diazepam received a subsequent dose of normal saline 10 ml/kg (i.p.), while the mice that were administered insulin received a subsequent dose of dextrose solution 3 g/kg (i.p.) in order to offset the hypoglycemic effect due to insulin (Uysal et al., 1996; Siegel et al., 2014). Twenty minutes after the administration of normal saline, and insulin, and 30 minutes after diazepam administration, each mouse received a convulsive dose of pentylenetetrazole 100 mg/kg (i.p.). The onset of convulsions (in seconds) and time of death (in seconds), within a period of 1 and 24 h respectively, were recorded as previously described (Hamad et al., 2014).

### Evaluation of strychnine-induced convulsions

Six groups of mice ( $n = 16$ ; males = 8, females = 8) received normal saline 10 ml/kg (i.p.), phenobarbital 30 mg/kg (i.p.) and insulin 1, 2, 4 and 8 IU/kg (i.p.) respectively. The mice administered normal saline or phenobarbital received a subsequent dose of normal saline 10 ml/kg (i.p.), while the mice that were administered insulin received a subsequent dose of dextrose solution 3 g/kg (i.p.) in order to offset the hypoglycemic effect due to insulin (Uysal et al., 1996; Siegel et al., 2014). 20 min after the administration of normal saline and insulin, and 30 min after phenobarbital administration, each mouse received a convulsive dose of strychnine 2 mg/kg (i.p.). The onset of convulsions (in seconds) and time of death (in seconds), within a period of 1 and 24 h respectively, were recorded as previously described (Salahdeen and Yemitan, 2006; Hamad et al., 2014).

### Statistical analysis

All data was analyzed by one-way analysis of variance (ANOVA), and Student – Newman – Keuls post-hoc test was carried out to determine a significant effect. Analyses were undertaken using the Primer of Biostatistics software (Version 3.01, 1992) and GraphPad Prism software (Version 5.01, 2007). Results were expressed as

**Table 1.** Effect of insulin administration on the onset of pentylenetetrazole-induced convulsions in male and female mice.

SEX	Group 1 (NS +NS)	Group 2 (DZP+NS)	Group 3 (1 IU+DEX)	Group 4 (2 IU + DEX)	Group 5 (4 IU+ DEX)	Group 6 (8 IU+DEX)
Males	62.71±5.83	>>3600.00*	117.30±8.76*	94.38±9.70*	103.70±11.46*	96.63±6.25*
Females	58.88±4.64	>>3600.00*	90.75±13.33	85.25±5.15	89.00±11.47	105.50±20.46*

NS: normal saline 10 ml/kg; DZP: Diazepam 2 mg/kg; 1 IU, 2 IU, 4 IU and 8 IU: Insulin dose (IU/Kg); DEX: Dextrose solution 3 g/kg. \*  $p < 0.05$ .

**Table 2.** Effect of insulin administration on the time of death following pentylenetetrazole-induced convulsions in male and female mice.

SEX	Group 1 (NS +NS)	Group 2 (DZP+NS)	Group 3 (1 IU+DEX)	Group 4 (2 IU + DEX)	Group 5 (4 IU+ DEX)	Group 6 (8 IU+DEX)
Males	304.20±56.77	>>86400.00*	591.60±122.80*	621.90±89.59*	725.30±64.26*	549.80±71.19*
Females	284.60±46.90	>>86400.00*	536.40±77.36	389.00±77.61	604.30±75.29*	481.80±71.08

NS: normal saline 10 ml/kg; DZP: Diazepam 2 mg/kg; 1 IU, 2 IU, 4 IU and 8 IU: Insulin dose (IU/Kg); DEX: Dextrose solution 3 g/kg. \*  $p < 0.05$ .

**Table 3.** Effect of insulin administration on the onset of strychnine-induced convulsions in male and female mice.

SEX	Group 1 (NS +NS)	Group 2 (PNB+NS)	Group 3 (1 IU+DEX)	Group 4 (2 IU + DEX)	Group 5 (4 IU+ DEX)	Group 6 (8 IU+DEX)
Males	113.30±8.10	203.30±30.51*	130.50±7.56	181.30±26.55*	233.90±13.41*	168.30±7.32
Females	176.10±19.92	200.90±16.78	143.80±10.84	179.50±13.02	193.80±13.36	178.40±17.13

NS: normal saline 10 ml/kg; PNB: Phenobarbital 30 mg/kg; 1 IU, 2 IU, 4 IU and 8 IU: Insulin dose (IU/Kg); DEX: Dextrose solution 3 g/kg. \*  $p < 0.05$ .

mean ± standard error of mean (S.E.M.).  $p < 0.05$  was taken as significant difference from control.

## RESULTS

### Effect of insulin on the onset of convulsions following pentylenetetrazole administration in male and female mice

In both male and female mice, diazepam completely prevented the occurrence of convulsions after pentylenetetrazole administration. In male mice, all the doses of insulin used in this study significantly delayed the onset of convulsions when compared with normal saline. However, only insulin 8 IU/kg significantly delayed the onset of convulsions in females as described in Table 1.

### Effect of insulin on the time of death following pentylenetetrazole administration in male and female mice

There was complete prevention of death in the diazepam-treated groups in both male and female mice. Insulin at all the doses used significantly prolonged the time of

death in male mice when compared with normal saline, whereas in female mice only insulin 4 IU/kg significantly prolonged the time of death as shown in Table 2.

### Effect of insulin on the onset of convulsions following strychnine administration in male and female mice

The administration of phenobarbital significantly delayed the onset of convulsions in male mice following the administration of strychnine when compared with normal saline, but it did not in females. Insulin doses of 2 and 4 IU/kg significantly delayed the onset of convulsions in males, whereas no insulin dose significantly affected the female mice as presented in Table 3.

### Effect of insulin on the time of death following strychnine administration in male and female mice

Phenobarbital administration significantly prolonged the time of death in both male and female mice compared with normal saline. While insulin 8 IU/kg prolonged the time of death significantly in male mice, none of the insulin doses had a significant effect on the time of death

**Table 4.** Effect of insulin administration on time of death following strychnine-induced convulsions in male and female mice.

SEX	Group 1 (NS +NS)	Group 2 (PNB+NS)	Group 3 (1 IU+DEX)	Group 4 (2 IU + DEX)	Group 5 (4 IU+ DEX)	Group 6 (8 IU+DEX)
Males	263.00±45.04	>>86400.00*	167.50±6.13	223.70±43.56	290.50±24.80	462.00±33.34*
Females	244.60±28.69	513.70±91.80*	201.60±14.03	194.70±11.42	221.10±8.50	230.70±28.36 <sup>†</sup>

NS: normal saline 10 ml/kg; PNB: Phenobarbital 30 mg/kg; 1 IU, 2 IU, 4 IU and 8 IU: Insulin dose (IU/Kg); DEX: Dextrose solution 3 g/kg. \*  $p < 0.05$ .

in female mice as shown in Table 4.

## DISCUSSION

The effect of insulin on chemically-induced convulsions in male and female mice was determined using pentylenetetrazole and strychnine-induced convulsions in this study. Diazepam, a known benzodiazepine receptor agonist, at the dose of 2 mg/kg (i.p.) completely abolished seizures induced by pentylenetetrazole (100 mg/kg, i.p.) in both male and female mice, and prevented mortality within 24 h. Diazepam produces anticonvulsant effect by promoting gabaergic synaptic inhibition in the brain. It binds to its site on the GABA<sub>A</sub> receptor and increases the frequency with which the chloride ion channel of the receptor opens, thereby increasing the efficiency of GABA (Nutt and Malizia, 2001). Although insulin did not prevent the incidence of convulsions or mortality due to pentylenetetrazole administration in this study, the results obtained are suggestive of a sex-related difference in the protective effect of insulin against pentylenetetrazole-induced convulsions. Previous studies have reported a differential sensitivity to insulin in male and female rats which was due to the effect of gonadal hormones (Clegg et al., 2006; Hallschmid et al., 2012). The sex difference observed in insulin's effect in this study could be attributed to hormonal differences between male and female mice. Insulin has been reported to upregulate GABA<sub>A</sub> receptors on the post-synaptic membrane through receptor recruitment thereby increasing GABA-mediated inhibition in CNS (Wan et al., 1997; Trujeque-Ramos et al., 2018). This could account for the protective effect that insulin produced against pentylenetetrazole-induced convulsion reported in this study.

Phenobarbital, a GABA<sub>A</sub> receptor agonist, enhances the effect of GABA by increasing the duration of chloride ion channel opening (Löscher and Rogawski, 2012). In this study, phenobarbital at the dose of 30 mg/kg (i.p.) delayed the occurrence of seizures and prevented mortality due to strychnine-induced convulsion in male mice but not in female mice. Strychnine produces convulsions by binding to glycinergic receptors in the CNS (Maher et al., 2014). There is no report of a sex

difference in the anticonvulsant effect of phenobarbital against strychnine-induced convulsion in previous studies. Insulin at all the doses used in this study did not prevent the incidence of convulsions or mortality resulting from strychnine-induced convulsions in male and female mice, but it conferred some protection against strychnine-induced convulsion in male mice. Insulin has been reported to increase the potency of glycine on its receptors in the CNS (Caraiscos et al., 2007; Yan-Do and MacDonald, 2017). The protective effect of insulin against strychnine-induced convulsion in male mice suggests that insulin facilitated glycine-mediated inhibition in the CNS. However, the doses of insulin that produced protective effects against strychnine-induced convulsions in male mice were not effective in females. Therefore, the effects of insulin as well as phenobarbital against strychnine-induced convulsions in male and female mice revealed sex differences in this study.

## CONCLUSION

The results obtained from this study showed that insulin produced protective effects against chemically-induced convulsion in mice, probably via facilitation of the gabaergic and glycinergic inhibitory neurotransmitter systems. This effect of insulin was sex-related as female mice were not protected against pentylenetetrazole and strychnine-induced convulsions at certain doses of insulin that protected male mice in this study. Therefore, the results of this work demonstrated sex differences in the response of mice to varying doses of insulin used against chemically-induced convulsions. Further studies are required to elucidate the mechanisms mediating these sex differences.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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## REFERENCES

- Bedse G, Di Domenico F, Serviddio G, Cassano T (2015). Aberrant insulin signaling in Alzheimer's disease: current knowledge. *Frontiers in Neuroscience*. 9:204. doi: 10.3389/fnins.2015.00204
- Caraiscos VB, Bonin RB, Newell JG, Czerwinska E, Macdonald JF, Orser BA (2007). Insulin increases the potency of glycine at ionotropic glycine receptors. *Molecular Pharmacology* 71:1277-1287.
- Chiu S, Cline HT (2010). Insulin receptor signaling in the development of neuronal structure and function. *Neural Development* 5:7.
- Clegg DJ, Brown LM, Woods SC, Benoit SC (2006). Gonadal hormones determine sensitivity to central insulin and leptin. *Diabetes* 55:978-987.
- Ferrario CR, Reagan LP (2018). Insulin-mediated synaptic plasticity in the CNS: Anatomical, functional and temporal contexts. *Neuropharmacology* 136:182-191.
- Hallschmid M, Higgs S, Thienel M, Ott V, Lehnert H (2012). Postprandial administration of intranasal insulin intensifies satiety and reduces intake of palatable snacks in women. *Diabetes* 61:782-789.
- Hamad MN, Sulaiman AA, Numan IT, Abdul-Rasak SA (2014). Study of anticonvulsant effect of ethyl acetate fraction of *Matricaria recutita* extract in mice. *International Journal of Pharmaceutical Sciences* 6(4):224-227.
- Huang CC, You JL, Lee CC, Hsu KS (2010). The role of insulin signaling in synaptic plasticity and cognitive function. *Chang Gung Medical Journal* 33:115-125.
- Koutroumanidou E, Kimbaris A, Kortsaris A, Bezirtoglou E, Polissiou M, Charalabopoulos K, Pagonopoulou O (2013). Increased seizure latency and decreased severity of pentylenetetrazol-induced seizures in mice after essential oil administration. *Epilepsy Research and Treatment* 2013:532-657.
- Löscher W, Rogawski MA (2012). How theories evolved concerning the mechanism of action of barbiturates. *Epilepsia* 53(8):12-25.
- Maher A, Radwan R, Breiting H (2014). *In vivo* protection against strychnine toxicity in mice by the glycine receptor agonist ivermectin. *BioMed research international* 2014:640790
- Neth BJ, Craft S (2017). Insulin Resistance and Alzheimer's Disease: Bioenergetic Linkages. *Frontiers in Aging Neuroscience* 9:345.
- Nutt DJ, Malizia AL (2001). New insights into the role of GABA<sub>A</sub>-benzodiazepine receptor in psychiatric disorder. *British Journal of Psychiatry* 179:390-396.
- Salahdeen HM, Yemitan OO (2006). Neuropharmacological effects of aqueous leaf extract of *Bryophyllum pinnatum* in mice. *African Journal of BioMedical Research* 9:101-107.
- Siegel PH, Lee Y, Pikov V (2014). Millimeter-wave non-invasive monitoring of glucose in anesthetized rats. 39th International Conference on Infrared, Millimeter, and Terahertz waves (IRMMW-THz), pp. 1-2.
- Trujeque-Ramos S, Castillo-Rolón D, Galarraga E, Tapia D, Arenas-López G, Mihaiescu S, Hernández-López S (2018). Insulin Regulates GABA<sub>A</sub> Receptor-Mediated Tonic Currents in the Prefrontal Cortex. *Frontiers in Neuroscience* 12:345.
- Uysal H, Kuli P, Caglar S, Inan LE, Akarsu ES, Palaoglu O, Ahyan IH (1996). Anti-seizure activity of insulin: insulin inhibits pentylenetetrazole, penicillin and kainic acid-induced seizures in rats. *Epilepsy Research* 25:185-190.
- Wada A, Yooko H, Yanagita T, Koyabashi H (2005). New twist on neuronal insulin signaling in health, disease and therapeutics. *Journal of Pharmacological Sciences* 99:128-143.
- Wan Q, Xiong ZG, Man HY, Ackerley CA, Braunton J, Lu WY, Becker LE, MacDoland JF, Wang YT (1997). Recruitment of GABA (A) receptors to post-synaptic domains by insulin. *Nature* 388:686-690.
- Woods SC, Seeley RJ, Baskin DG, Schwartz MW (2003). Insulin and the blood-brain barrier. *Current Pharmaceutical Design* 9:795-800.
- Yan-Do R, MacDonald PE (2017). Impaired "Glycine"-mia in Type 2 Diabetes and potential mechanisms contributing to glucose homeostasis. *Endocrinology* 15(5):1064-1073.