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RESEARCH ARTICLE

THE ROLE OF ADRENERGIC RECEPTORS IN THE ACTION OF ADRENALINE ON PLASMA LACTATE, GLUCOSE, LIVER, AND SKELETAL MUSCLE GLYCOGEN IN THE COMMON AFRICAN TOAD BUFO REGULARIS

Isehunwa Grace Olufunmilayo, Shittu Sheu Tijani, Oghenekaro Ekuahorovwe Joel, Dibie Chioma Prudent, and Alada AbdulRasak Akinola
Department of Physiology, College of Medicine, University of Ibadan, Ibadan.

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Abstract

In mammals, the roles of liver and skeletal muscle glycogen in adrenaline-induced hyperglycaemia are well known. However, in amphibians, there is limited information on the relative contribution of liver and skeletal muscle glycogen in adrenaline-induced hyperglycaemia. This study investigated the effects of adrenaline on blood glucose, plasma lactate, liverand skeletal muscle glycogen and the role of adrenergic receptors in the common African toad, bufo regularis. One hundred and twenty-five adult common African toads (70-100g) of both sexes were randomly collected and used in the study. Blood samples were collected from truncus arteriosus to estimate glucose and lactate levels. Blood glucose was determined immediately by modified glucose oxidase method. Plasma lactate was determined by modification of the Barker-Summerson method. Liver and gastrocnemius muscle glycogen were determined using anthrone reagents method. Adrenaline caused significant increase in blood glucose, lactate levels and significant reduction in liver and muscle glycogen. When toads were pre-treated with propranolol or prazosin, adrenaline's reduction in liver and muscle glycogen was significantly reduced while the increase in blood glucose and lactate levels was prevented. Combination of both blockers abolished the increase in blood glucose, lactate levels and blocked reduction in liver and muscle glycogen produced by adrenaline. The results of this study showed that adrenalinecaused liver and skeletal muscle glycogen breakdown, increase in plasma lactate levels which resulted in hyperglycaemia in the common African toad bufo regularis. The study also showed that both alpha- and beta-adrenergic receptors are involved in mediating the effects of adrenaline on blood glucose, plasma lactate, liver, and muscle glycogenolysis and gluconeogenesis.

Introduction: -

Adrenaline has been reported to cause hepatic glucose production by stimulating glycogenolysis and gluconeogenesis and causes inhibition of glucose disposal by insulin-dependent tissues (Sherwin, 1984, Sherwin and Sacca, 1984, Dibe et al, 2020). Adrenaline stimulates glycogen breakdown in skeletal muscles, increases glycogen

Corresponding Author: - g.isehunwa@gmail.com
Address**: -** Department of Physiology, College of Medicine, University of Ibadan, Ibadan.

phosphorylation and decreases glycogen synthase activity (Toole and Cohen, 2007, Jensen et al, 2008). Adrenaline also stimulates secretion of glucagon from alpha cells and inhibits secretion of insulin from beta cells of the pancreas (Hamilton et al, 2018, Han and Bonen, 1998, Peterhoff et al, 2003). In the skeletal muscle, adrenaline has been shown to increase plasma lactate concentration, caused reduction in glycogen content and blocks insulin-mediated glycogen synthase activation (Jensen et al, 2011).

The skeletal muscle glycogen is an important energy substrate in exercise and major store for ingested carbohydrate (Jensen and Richter, 2012). The glycogen content in skeletal muscle is limited but contributes to regulation of blood glucose (Jensen et al, 2011). The liver is the main organ involved in the endogenous production of glucose through glycogenolysis and gluconeogenesis.

Adrenaline-induced hyperglycaemia is an integrated response which includes liver and muscle glycogenolysis, increased gluconeogenesis, decreased peripheral glucose utilization, inhibition of insulin and stimulation of glucagon secretion (Oyebola et al, 2011). However, the contribution of each component differs in different species and under different nutritional status (Al-Jibouri et al, 1980). Previous studies in humans and rats (Rizza, 1980, Al-Jibouri et al, 1980, Moratinos et al, 1986, Oyebola and Alada, 1993) have shown that activation of alpha, beta, or both alpha- and beta-adrenergic receptors are involved in adrenaline-induced hyperglycemia. There is limited information on the role of adrenergic receptors in the action of adrenaline on plasma lactate, liverand muscle glycogen in amphibians. This study investigated the effects of adrenaline on blood glucose, plasma lactate, liver and skeletal muscle glycogen and the role of adrenergic receptors in the common African toad bufo regularis.

Methods:-

The study was carried out according to the guidelines of the University of Ibadan Animal Care and Use Research Ethics Committee (UI-ACUREC). One hundred and twenty-five adult common African toads (70-100g) of both sexes were randomly collected at night from banks of slow-moving streams and around ponds within the University of Ibadan, Ibadan, Southwest, Nigeria. The toads were brought into the laboratory after capture and kept inside plastic wire-gauged cage. They were denied access to insects or any food for 24h but had free access to water. The cage was kept in the dark room till the following day. After fasting for 24h, each animal was anaesthetized with sodium thiopentone 50mg/kg intraperitoneally. Each animal was then secured on its back on a dissecting board and the thorax was opened. The truncus arteriosus was dissected free from surrounding connective tissue and used for blood sample collection. The anterior abdominal vein was cannulated for injection of drug. After surgery, each animal was heparinized (170 units /0.1ml) and allowed to stabilize for 30 mins. After stabilization, animals in group I (control) received 0.7% amphibian saline while animals in group II (untreated) were given adrenaline 40µg/kg intravenously through anterior abdominal vein cannula. Animals in groups III, IV and V were pre-treated with prazosin 0.2mg/kg, propranolol 0.5mg/kg, combined prazosin 0.2mg/kg and propranolol 0.5mg/kg respectively 30mins before given adrenaline 40µg/kg intravenously through the anterior abdominal vein cannula. Blood samples were taken at 0 min, 5 mins, 10 mins, 30 mins and 60 mins post-injection time for blood glucose estimation and lactate determination. Each drug injection was in a volume between 0.1ml and 0.12ml given intravenously. Owing to small size of the animal, each animal was sampled once and then sacrificed.

Blood glucose level was determined immediately by modified glucose oxidase method (Trinder, 1969). Blood samples for lactate determination were collected into Eppendorf bottles containing Sodium Fluoride and Potassium Oxalate. Plasma lactate was determined by modification of the Barker-Summerson method for determination of lactic acid (Pryce, 1969). The whole liver and gastrocnemius muscle of each animal were removed quickly 30 min post- injection time under anaesthesia and weighed immediately. Thereafter, 1g of liver and muscle were excised separately and the glycogen content was determined by modified anthrone reagents method (Seifter et al, 1950; Jermyn, 1975).

Statistical Analysis

All values given are mean ± S.E.M of the variables measured. Values between two groups were compared using One-way and two- way analysis of variance (ANOVA) was used to compare mean values in multiple groups.

Results:-

The results are shown inTable 1 and Figures 1 to 3.

Effect of adrenaline injection

Adrenaline injection caused significant reduction in liver glycogen (figure 1) and muscle glycogen (figure 2) levels 30 min. post injection time compared with control group. Injection of adrenaline also caused significant increasein blood glucose (Table 1) and lactate levels (figure 3) compared with the control toads.

Effects of pre-treatments with alpha-adrenergic and beta-adrenergic blockers

Pretreatment with prazosin 0.2mg/kg or propranolol 0.5mg/kg 30mins before adrenaline injection caused significant reduction in the depletion of liver and muscle glycogen (figures 1 and 2) and prevented the rise in blood glucose (table 1) and lactate levels (figure 3).

Effects of pretreatment with combined alpha- and Beta-adrenergic blockers

Pretreatment with combined propranolol 0.5mg/kg and prazosin 0.2mg/kg 30 mins before adrenaline injection blocked completely depletion in liver (figure 1) and muscle glycogen (figure 2) while adrenaline-induced increase in blood glucose and lactate was abolished by both alpha- and beta- adrenergic blockers table 1 and figure 3.

	Blood Glucose (mg/dl)				
Time (Mins)	0	5	10	30	60
Control (0.7% amphibian saline)	61.8 ± 16.5	62.8 ± 6.4	71.8 ± 9.4	60.2 ± 12.6	58.4 ± 8.8
Adrenaline (40µg/kg)	62.6 ± 19.2	#152.8 ± 31.6	#133.2 ± 25.5	#104.8± 16.5	79.6 ± 17.5
Adrenaline (40µg/kg) + prazosin (0.2mg/kg)	61.8 ± 5.5	**95.4 ± 13.0	**82.2 ± 9.2	*75 ± 8.4	63.6 ± 11.0
Adrenaline (40µg/kg) + Propranolol(0.5mg/kg)	62.6 ± 13.9	***52.6 ± 6.9	**65.4 ± 10.1	*61.6 ± 10.3	54.6 ± 18.1
Adrenaline (40µg/kg) + Prazosin (0.2mg/kg) + propranolol(0.5mg/kg)	70.4 ± 12.2	*70 ± 14.8	**55.4 ± 6.9	***40 ± 7.1	64.6 ± 6.6

Table 1:- Effects of Adrenaline (40µg/kg) injection on blood glucose levels (mg/dl) in untreated (adrenaline only), prazosin, propranolol, and combined prazosin and propranolol pretreated toads. n=5 toads for each timed collection.

The points are mean ± S.E.M (n=5), $^{*}P< 0.05$ significantly different from control group (Amphibian saline), $^{\#}P< 0.05$, $^{\#\#}$ p< 0.01, $^{\#\#\#}$ p< 0.001 significantly different from untreated group (Adrenaline only).

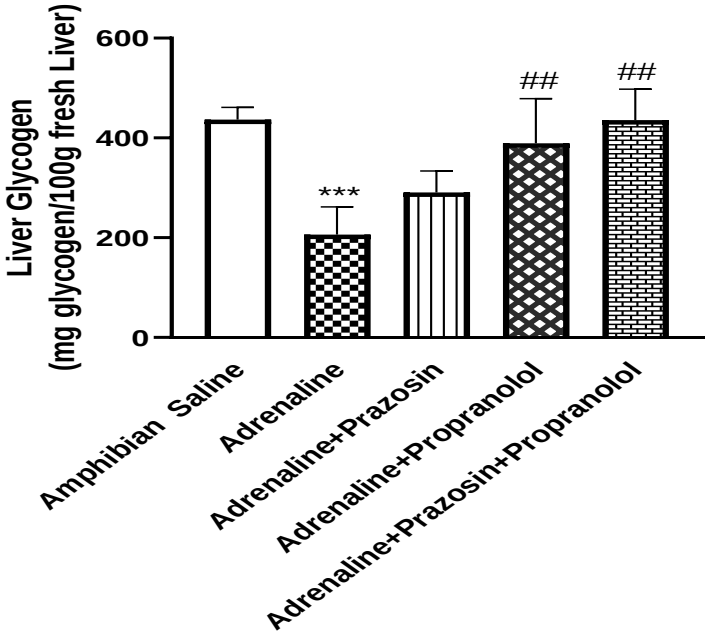

Figure 1:- Effects of adrenaline 40µg/kg on liver glycogen in untreated (adrenaline only) toads, in prazosin (0.2mg/kg), propranolol (0.5mg/kg),and combined prazosin (0.2mg/kg) and propranolol(0.5mg/kg) treated toads.

The points are mean ± S.E.M (n=5), ***P< 0.001 significantly different from control group (Amphibian saline), ##P< 0.01 and ##P< 0.001 significantly different from untreated group (Adrenaline only).

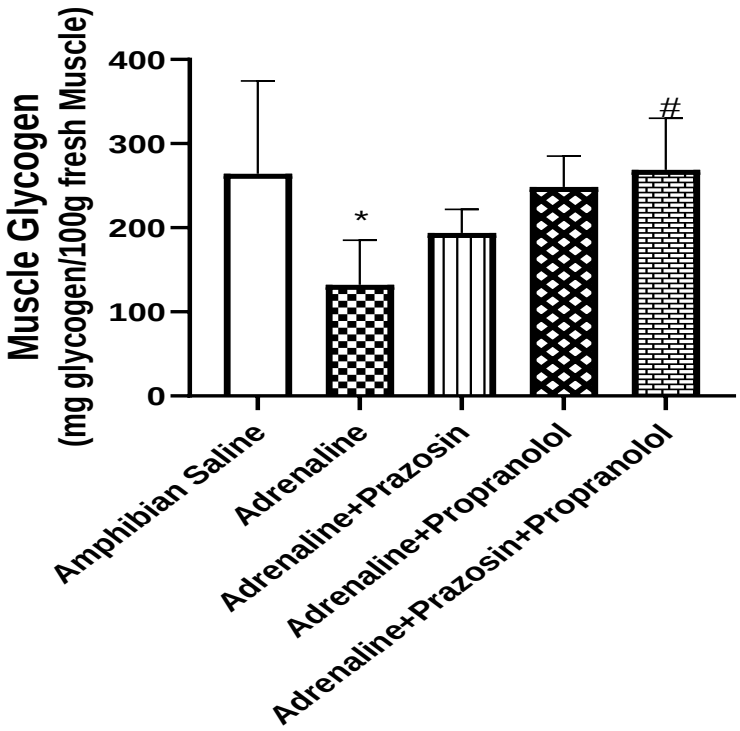

Figure 2:- Effects of adrenaline 40µg/kg on gastrocnemius muscle glycogen in untreated (adrenaline only) toads and in prazosin (0.2mg/kg),propranolol (0.5mg/kg),and combined prazosin (0.2mg/kg) and propranolol(0.5mg/kg) treated toads.

The points are mean ± S.E.M (n=5), *P< 0.05 significantly different from control group (Amphibian saline), #P< 0.05 significantly different from untreated group (Adrenaline only).

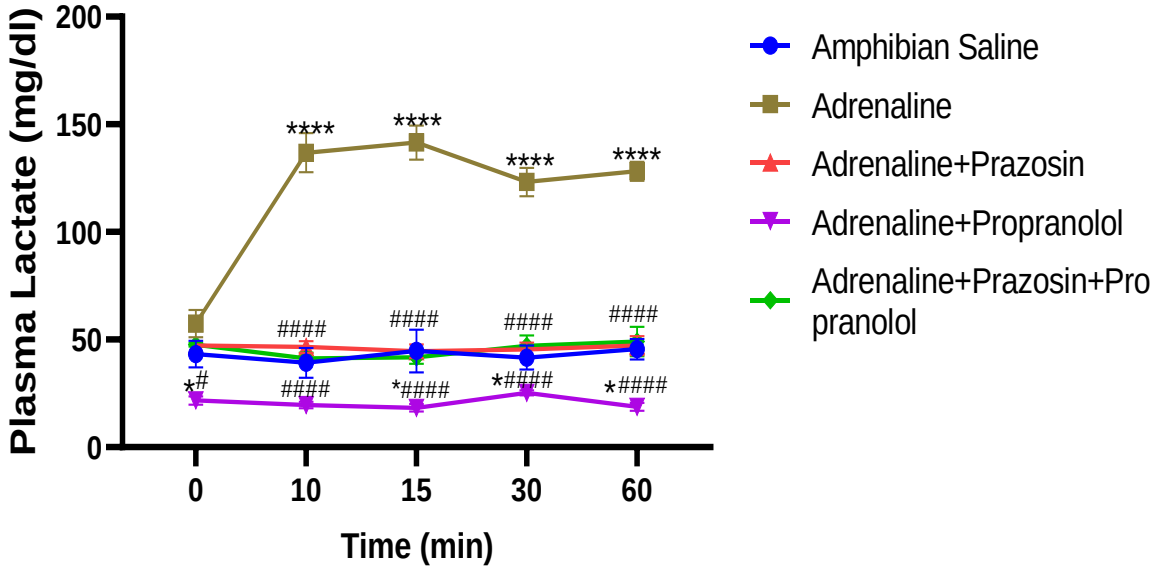

Figure 3:- Effects of Adrenaline (40µg/kg) injection on plasma lactate levels (mg/dl) in untreated (adrenaline only), prazosin, propranolol, and combined prazosin and propranolol pretreated toads.

The points are mean ± S.E.M (n=5), $^{*}P< 0.05$, $^{****}P< 0.001$ significantly different from control group (Amphibian saline), $^{\#\#\#\#}P< 0.001$ significantly different from untreated group (Adrenaline only).

Discussion:-

The result of this study confirms the hyperglycemic effect of adrenaline in the common African toad. This is consistent with previous studies in mammals (Rizza et al, 1980; Sherwin and Sacca, 1984; Oyebola and Alada, 1993; Oyebola et al, 2011; Jensen et al, 2011, Dibe et al, 2020), and some amphibians (Farrar and Frye, 1977, 1979b, Herman, 1977, Oyebola et al, 1998). The findings of the present study in which adrenaline caused significant reduction in both the liver and gastrocnemius muscle glycogen seem to suggest that liver and skeletal muscle glycogen may have contributed to adrenaline hyperglycemia probably through glycogenolysis and gluconeogenesis. This agrees with studies in mammals (Moratinos et al, 1986; Nolte et al, 1994; Dufour et al, 2009; Watt et al, 2001; Kolnes et al, 2015; Dibe et al; 2020). However, the results of this study contrast the findings of (Chesley et al, 1995; Kjaer et al, 2000) which reported that adrenaline had no effect on glycogenolysis in exercising humans. Adrenaline has been reported to cause hepatic glucose production through increases in cyclic AMP and activation of protein Kinase A (Erraji-Benchckroun et al, 2005, Pierce et al, 2002) and phosphorylase activity leading to liver and skeletal muscle glycogen breakdown (Johanns et al, 2016).

The observation of the present study in which adrenaline caused significant increase in plasma lactate levels agrees is consistent with studies in mammals (Al-Jibouri et al, 1980; Watt et al, 2001; Gjedsted et al, 2011; Jensen, et al, 2011). The increase in blood lactate levels may be as a result of skeletal muscle glycogen breakdown. Plasma lactate is produced through breakdown of muscle glycogen (Orngreen et al, 2015) and is a preferred substrate for gluconeogenesis in the liver (Sacca et al, 1983). The results of this study revealed that like humans and other mammals, adrenaline stimulated liver and skeletal muscle glycogenolysis and gluconeogenesis to induce hyperglycemia in the common African toad. Liver glycogen contributes directly to the release of glucose into the blood stream whereas skeletal muscle glycogen is unable to produce glucose directly due to lack of glucose 6-phosphatase enzyme. Skeletal muscle glycogen is broken down to lactate, transported to the liver and through gluconeogenesis contributes to blood glucose (via the Cori cycle) (Jensen et al, 2011). This may explain the reduction in gastrocnemius muscle glycogen produced by adrenaline injection. However, physiological increase in plasma adrenaline level has been reported not to produce significant effect on muscle glycogenolysis (Laurent et al, 1998), but caused reduction in hepatic glycogen and increase in glucose levels (Dufour et al, 2009).

Pre-treatment of toads with propranolol or prazosin greatly reduced the depletion in liver and muscle glycogen caused by adrenaline while preventing the rise in blood glucose and lactate levels. The combination of both adrenergic blockers completely blocked depletion in liver and muscle glycogen and the increase in blood glucose and lactate levels thus suggesting the involvement of the adrenergic receptors inadrenaline-induced increase in blood glucose and lactate levels. This finding agrees with the study in cats (Al-Jibouri et al, 1980) which reported blockage of increase in blood glucose and lactate levels by propranolol and phentolamine.

Conclusion:-

The study showed that adrenaline induced hyperglycemia in the common African toad. The hyperglycemic effect of adrenaline could have been the result of liver and skeletal muscle glycogenolysis and gluconeogenesis. Both alpha- and beta- adrenergic receptors are involved in the increase in blood glucose, liver and skeletal glycogenolysis and gluconeogenesis in the common African toad.

Declarations

Ethics approval and consent to participate

The study was carried out according to the guidelines of the University of Ibadan Animal Care and Use Research Ethics Committee (UI-ACUREC)

Consent for publication

Not applicable.

Availability of data and material

Not Applicable.

Competing interests
The authors declare that they have no competing interests.

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Authors' Contributions
GO designed the experiments and involved in writing the manuscript. EJ and CP carried out the experiments and analysis of data. ST was involved in the analysis of data. ARA did overall project supervision and manuscript editing. All authors read and approved the final manuscript.

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Department of Physiology, Faculty of Basic Medical Sciences, University of Ibadan, Nigeria

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