Canadian Journal of Pure and Applied Sciences
Vol. 13, No. 2, pp. 4847-4851, June 2019
Online ISSN: 1920-3853; Print ISSN: 1715-9997
Available online at www.cjpas.net

SEASONAL VARIATIONS IN HORMONAL REGULATION OF BLOOD GLUCOSE IN *BUFO REGULARIS*

*Isehunwa O. Grace and Alada AR Akinola
Endocrinology and Metabolism Unit, Department of Physiology,
College of Medicine, University of Ibadan, Ibadan, Oyo State, Nigeria

ABSTRACT

Hormones involved in the regulation of carbohydrate metabolism in amphibians have been shown to exhibit seasonal variable effects. The present study investigated the effects of adrenaline, glucagon, and cortisol on blood glucose level in the Common African toad *Bufo regularis* during three rainy and dry seasons. Adult toads of either sex weighing between 70-100g were randomly selected and used in the experimental study. Each animal was anesthetized with sodium pentobarbitone 3mg/100g body weight given intraperitoneally. The truncus arteriosus was used for blood sampling while the anterior abdominal vein was cannulated for injection of drugs. Blood sample was taken from truncus arteriosus to determine blood glucose using the modified glucose oxidase method. Adrenaline (10µg/kg) produced 70% increase in blood glucose during rainy season and 92% increase in blood glucose during dry season. Glucagon (10µg/kg) caused 102% increase in blood glucose during the rainy season and 88% increase in blood glucose during the dry season while cortisol (10µg/g) caused delayed but 50% increase in blood glucose during the rainy season and 61% increase in blood glucose during the dry season. The results of this study suggest that seasonal variation exists in the blood glucose response to adrenaline, glucagon and cortisol in the *Bufo regularis*. Therefore, seasonal changes should be taken into consideration in hormonal studies involving amphibians such as the *B. regularis*.

Key words: Blood glucose, rainy season, dry season, adrenaline, cortisol, glucagon, Common African toad.

INTRODUCTION

In an earlier study, Copeland and DeRoos (1971) reported that energy metabolism in amphibians is presumably not dependent upon closely regulated blood glucose levels. Several regulation of blood glucose still occur and most of the hormones involved in regulating mammalian carbohydrate metabolism are active in amphibians (Hanke and Neuman, 1972; Leibson and Plisetakaya, 1973).

Several studies showed that adrenaline, glucagon and insulin caused significant increases in the blood glucose of the common African toad *Bufo regularis* (Oyebola *et al*., 1998). These studies were carried out during the rainy season when ambient temperature was about 27^{o}C and the physical activity of the animal was at the utmost level. It is however not clear what the blood glucose responses to these hormones are during the dry season when the ambient temperature is about 31^{o}C and the animals are in a state of hibernation.

Earlier reports on different species of frogs showed that hormones involved in the regulation of carbohydrate metabolism exhibit seasonal variable effects (Farrar and Frye, 1977; Farrar and Frye, 1979). For instance, although adrenaline increased blood glucose levels throughout the year, its effect was doubled in fall. Glucagon caused increases in blood glucose levels in the fall and in summer, but its effect was reduced in winter (Farrar and Frye, 1977). Also, insulin favored glycogen synthesis from pyruvate in frog muscle isolated from winter *Rana pipiens* but favored pyruvate oxidation from frog muscle in summer (Farrar and Frye, 1979).

Most of the reports on seasonal variation in the hormonal influence on carbohydrate metabolism in amphibians have been on frogs. There is very little information on toads. The common African toad *Bufo regularis* is commonly found in Nigeria especially during the rainy season. However, since frogs and toads are amphibians, it may be presumed that the seasonal variations reported in frogs also exist in toads in spite of possible species difference. There has been no study in which the effects of adrenaline, glucagon and cortisol on blood glucose levels in the common African toad were compared during rainy and dry seasons. This study was therefore, designed to investigate the effects of adrenaline, glucagon and cortisol on blood glucose levels in the Common African toad *Bufo*

*Corresponding author e-mail: funmisehunwa@yahoo.com

regularis during rainy season (May –October) and dry season (November-April).

MATERIALS AND METHODS

The present study was conducted at lbadan, Southwest Nigeria, during three rainy seasons (ambient temperature: 26 - 28^{o}C) and three dry seasons (ambient temperature: 29-32^{o}C). The toads (70-100g) were randomly selected as described by Oyebola and Alada, (1992) and kept in plastic wire-gauged cage. They were denied access to insects or any food for 24 hours, but had free access to water. Each animal was then anesthetized with sodium pentobarbitone 3mg/100g body weight given intraperitoneally. The animal was secured on its back on a dissecting board.

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 drugs. Each toad was then heparinised (170units in 0.1ml amphibian saline) and allowed thirty minutes to stabilize. After stabilization period, basal blood sample (0 minute) was taken from truncus arteriosus. The animals were randomly divided into four groups.

Toads in group 1(control group) received intravenous (i.v) bolus injection of 0.7% amphibian saline (0.1ml) while toads in group 11 (adrenaline group) were given bolus injection of (5µg/kg or 10µg/kg i.v) adrenaline. Toads in groups 111 (glucagon group) and IV (cortisol group) received bolus injection of glucagon (5µg/kg or 10µg/kg i.v) and cortisol (5µg/kg or 10µg/kg i.v) respectively. Blood samples were collected at time interval of 5min, 10min, 20min, 30min, and 60min post-injection. Each drug injection was in a total volume between 0.1ml and 0.12ml given intravenously through the anterior abdominal vein cannula. Blood glucose was determined immediately blood sample was collected by modified glucose oxidase methods (Trinder, 1969). Because of the small size of the toad, animals were sampled only once in each experiment and then sacrificed.

STATISTICAL ANALYSIS

All values given are mean ± S.E.M of the variables measured. Values between two groups were compared using independent samples t- test while One-way Analysis of Variance (ANOVA) and Post Hoc Test were used to compare mean values in multiple groups. P-values at 0.05 or less were taken as statistically significant.

RESULTS

The results are shown in Tables 1-4. Values given in the tables are mean ± SEM and asterisks indicate values that are significantly different from the control values of the variable being measured.

Table 1. Effects of 0.7% amphibian saline on blood glucose levels (mg/dl) (n=10) during rainy and dry seasons.

Seasons	0min	5min	10min	20min	30min
Rainy	61.8 ± 1.4	58.3 ± 1.3	62.4 ± 1.3	57.8 ± 1.8	60.4 ± 1.6
Dry	51.5 ± 0.8	53.5 ± 0.8	55.5 ± 0.9	53.2 ± 1.1	54.7± 1.2

F-value: 123.583 (p<0.01)

Amphibian saline had no effect on blood glucose levels during the seasons, however the fasting blood glucose levels during the rainy season was significantly higher than during the dry season.

Effect of Adrenaline on Blood Glucose

Adrenaline caused immediate and significant increases in blood glucose levels during the two seasons. The two doses of adrenaline also produced different levels of hyperglycemia in a dose-dependent manner. However, adrenaline effect on blood glucose was more profound during the dry season than the rainy season when the percentage increase in blood glucose level for each season was taken into consideration. While adrenaline increased blood glucose level by as much as 90% during the dry season, the blood glucose increased by about 70% during the rainy season (Table 2).

Table 2. Effects of adrenaline on blood glucose levels (mg/dl) (n=10)

Seasons	Dose	0min	5min	10min	20min	30min	60min	% max increase
Rainy	5µg/kg	58 ±2.8	65.0±3.9*	74.6±4.0*	83.4±6.4* 97.3±4.0*	83.4±4.3*	75.8±5.5*	43
	10µg/kg	65.6±5.4	81.8±7.0*	90.1±2.9*		111.9±2.7*	98.6±3.8*	70
Dry	5µg/kg	49.1±1.3	57.7±1.6	78.5±1.7*	86.8±2.3*	91.1±2.8*	79.9±2.0*	69
	10µg/kg	50.8±4.1	63.6±4.6	81.7±6.3*	90.6±6.2*	97.8±7.1*	82.8±6.2*	92

F-value: 18.494 (p<0.01)
Mean values with asterisk in the same row are significantly different (p<0.05) from basal using post hoc comparison.

Effect of glucagon on blood glucose

Glucagon also produced an immediate dose-dependent rise in blood glucose levels during the two seasons. Although the blood glucose response to the low dose of glucagon was almost the same during the two seasons, the blood glucose response to high dose of glucagon was higher during the rainy season than during the dry season. Specifically, 10µg/kg of glucagon caused 100% increase in blood glucose during the rainy season compared with 80% increase in blood glucose during the dry season. The difference was highly significant ($p<0.01$).

Table 3. Effects of glucagon on blood glucose levels (mg/dl) (n=10).

Seasons	Dose	0min	5min	10min	20min	30min	60min	%Max Increase
Rainy	5µg/kg 10µg/kg	66.2±6.1 65.7±6.3	98.9±7.6* 131.5±5*	76.6±5.9* 122.7±6*	65.9±2.2 61.6±6.6	55.1±7.0 64.2±1.2	62.1±5.3 60.4±1.6	49 102
Dry	5µg/kg 10µg/kg	51.6±2.5 48.9±4.1	61.0±2.7 64.1±5.1*	74.6±3.5* 92.1±3.5*	69.1±2.5 80.4±6.0	65.5±2.6 73.2±5.1	59.0±2.8 41.5±2.8	44 88

F-value: 32.006 ($P<0.01$)
Mean values with asterisk in same row are significantly different ($p<0.05$) from basal using post hoc comparison

Effect of Cortisol on the Blood Glucose

Cortisol had no significant effect on blood glucose at low dose of 5µg/kg during the seasons. However, at a high dose of (10µg/kg), cortisol caused a delayed but significant increase in blood glucose levels. The increase in blood glucose levels was almost the same during the seasons.

Table 4. Effects of cortisol on blood glucose levels (mg/dl) (n=10).

Seasons	Dose	0min	5min	10min	20min	30min	60min	%Max Increase
Rainy	5µg/kg 10µg/kg	65.5±7.8 65.3±5.2	64.1±7.6 68.5±5.6	57.1±4.0 70.7±5.4	59.3±4.0 80.4±5.6*	62.9±5.3 89.6±7.0*	55.1±6.2 97.9±8.3*	0 50
Dry	5µg/kg 10µ g/kg	52.2±2.4 53.6±3.1	58.7±4.7 56.3±3.2	56.2±3.5 58.9±2.8	51.6±3.2 64.2±2.1	57.1±1.3 76.0±3.6*	48.5±2.6 86.5±3.1*	0 61

F-value: 21.948 ($p<0.01$)
Mean values with asterisk in same row are significantly different ($p<0.05$) from basal using post hoc comparison

DISCUSSION

This study confirmed that seasonal variation exists in the blood glucose of the common African toad *Bufo regularis* (Isehunwa and Alada, 2016). Similar seasonal variations have been reported in frogs and some other species of toad. Environmental temperature, availability of food and state of physical activity of the animal during different seasons are among factors that are adduced to explain the seasonal variations (Smith, 1954; Mizel, 1965; Hermansen and Jorgensen, 1969; Byrne and White, 1975).

The hyperglycemia induced by adrenaline, glucagon and cortisol is consistent with their well known actions in various animal species as well as the earlier report in toad during the rainy season (Oyebola *et al*., 1998). The observed difference in blood glucose responses to adrenaline in this study is similar to the observation in frogs, where administration of adrenaline produced seasonally variable effects on blood glucose (Farrar and Frye, 1977). The higher blood glucose response to adrenaline during the dry season as observed in the present study is most probably due to a breakdown in the rich store of liver and muscle glycogen as reported by (Isehunwa and Alada, 2016).

The difference in glycogen stores in the rainy and dry season is most probably an adaptive response of the animal to food availability and ability to move around. The difference in ambient temperature during the rainy and dry seasons is another possible reason for the observed differences in glucose response to adrenaline during the two seasons. It has been shown that temperature is an important factor in controlling glycogenolysis in frogs (Kepinov, 1947). Low temperature suppressed glycogenolysis and glycaemic response to excitement even after thyroxine treatment in frogs (Smith, 1954).

The higher response of blood glucose to glucagon during the rainy season than the dry season could also be due to seasonal variations in response to the hormone. This agrees with the report in frogs (Farrar and Frye, 1977). The ability of adrenaline and glucagon to stimulate glycogenolysis and gluconeogenesis in a variety of animal species has been recognized for many years (Illis, 1956; Sherwin, 1984; Bollyky and Greenbaum, 2007).

It is however interesting to note that adrenaline produced higher blood glucose response during the dry season while glucagon produced higher blood glucose response during the rainy season. This difference in hormonal response to blood glucose during the seasons seems to suggest that the two hormones are not acting through the same mechanisms during the two seasons. A closer look at the tissue glycogen level as reported by (Isehunwa and Alada, 2016) revealed however, a significant reduction in the liver glycogen level during the rainy season. This seems to suggest that glucagon may be acting more by breaking down the liver glycogen. Further studies looking into the roles of different receptors in the actions of the hormones may shed light on this.

The increase in blood glucose level caused by cortisol in this study also confirms its well-known hyperglycemic effect. The result agrees with the findings in frogs (Broughton and DeRoos, 1984; Hanke, 1978), toad (Isehunwa *et al*., 2013) and other animal species (Leach and Taylor, 1982; Pretty *et al*., 2009). Its hyperglycemic action is believed to be due to activation of gluconeogenesis (Baxter, 1979; Renaud and Moon, 1980; Khani and Tayek, 2001). However, the lack of effect of low dose of cortisol as observed in this study is in agreement with the observation in *Rana catesbieana* (Brougthon and DeRoos, 1984) in which large amount of corticosterone than that which is normally found in circulation of stressed amphibians was used to stimulate increase in blood glucose. Similar observations were also made in bullfrog (Laub *et al*., 1975) and fish (Vijayan and Leatherland, 1989).The slight difference in blood glucose responses observed in the present study following administration of cortisol during the two seasons may probably be due to the effect of seasonal changes.

CONCLUSION

In conclusion, this study provided ample evidence to show that seasonal variations exist in the blood glucose response to adrenaline, glucagon and cortisol in the common African toad *Bufo regularis*. The observations of this study also show that seasonal changes need to be taken into account in hormonal studies involving the Common African toad.

REFERENCES

Baxter, JD. 1979. Glucocorticoid hormone action in: Pharmacology of adrenal cortical hormones. Ed. Gill, GN. Pergamon Press, Oxford. 67-121.

Bollyky, J. and Greenbaum, CJ. 2007. The role of glucagon in postprandial hyperglycemia-The jury's Still out. J. Clin. Endocrinol. and Meta. 92(8):2879-2881.

Broughton, RE. and DeRoos, R. 1984. Temporal effects of infused corticosterone and aldosterone on plasma glucose levels in the American Bullfrog *Rana catesbeiana*. Gen. Comp. Endocrinol. 53(2):325-330.

Byrne, JJ. and white, RJ. 1975. Cyclic changes in liver and muscle glycogen, tissue, and lipid and blood glucose in a naturally occurring population of *Rana catesbeian*, Comp. Biochem. Physiol. 50A:709-715.

Copeland, PL. and DeRoos, R. 1971. Effect of mammalian insulin on plasma glucose in the mudpuppy *Necturus maculosus*. J. Exp. Zool. 178:35-43.

Farrar, ES. and Frye, BE. 1977. Seasonal variation in the effect of adrenaline and glucagon in *Rana pipiens,* Gen. Comp. Endocrinol. 33:76-81.

Farrar, ES. and Frye, BE. 1979. A comparison of adrenaline and glucagon effects on carbohydrate levels of larval and adult *Rana pipiens*. Gen. Comp. Endocrinol. 39:372-380.

Hanke, W. and Neuman, V. 1972. Carbohydrate metabolism in amphibia, Gen. Comp. Endocrinol. 3:198-208.

Hanke, W. 1978. The adrenal certex of amphibia. In "General, Comparative and clinical Endocrinology of the adrenal certex". Eds. Chester Jones, I. and Hendersion, IW. Academic press, New York, USA. 2:419-495.

Hermansen, B. and Jorgensen, CB. 1969. Blood glucose in male toads (*Bufo bufo*): Annual variation and hormonal regulation. Gen. Comp. Endocrinol. 12:313-321.

llis, S. 1956. Metabolic effects of epinephrine and related amines, Pharmacol Rev. 8:485-562.

Isehunwa, GO. and Alada, ARA. 2016. Seasonal and sex variation in carbohydrate levels of the Common African toad *Bufo regularis*. Canadian Journal of Pure and Applied Sciences. 10 (2):3847-3852.

Isehunwa, GO., Olaniyan, OT. and Alada, ARA. 2013. The role of alpha and beta adrenergic receptors in cortisol-induced hyperglycemia in the Common African toad *Bufo regularis*. Afri. J. Biotech. 12 (36):5554.

Kepinov, L. 1947. Environmental temperature and the glycogen content of the frog. Bull. Soc. Chem. Biol. 29:252.

Khani, S. and Tayek, JA. 2001. Cortisol increases gluconeogenesis in humans. Its role in the metabolic syndrome. Clin. Sci (Lond). 101:739-747.

Laub, JM., Callard, GV. and Callard, IP. 1975. The role of adrenal steriods in the negative feedback control of the amphibian adrenal gland. Gen. Comp. Endocrinol. 25:425-431.

Leach, GJ. and Taylor, MH. 1982. The effects of cortisol treatment on carbohydrate and protein metabolism in *Fundulus heteroclitus*. Gen. Comp. Endocrinol. 48:76-83.

Leibson, LG. and Plisetakaya, EM. 1973. Effect of hormones in poikilothermic vertebrates, Int. encycl. Pharm. Ther. 85:625-684.

Mizell, S, 1965. Seasonal changes in energy reserves in the common frog *Rana pipens*. Gen. Comp. Physiol. 66:251-258.

Oyebola, DDO. and Alada, ARA. 1992. High dose of histamine required for gastric acid secretion in the Common African toad *Bufo regularis*, Isr. J. Med. Sci. 28:731-735.

Oyebola, DDO., Ariwodola, JO. and Alada, ARA. 1998. Effect of glucagon, glucose, and adrenaline and insulin infusion on blood glucose level in the common African toad (*Bufo regularis*). Afri. J. Med. Med. Sci. 27:89-94.

Pretty, C., Chase, JG., Lin, J., Shaw, G., Comple, AL., Razak, N. and Parente, J. 2009. Corticosteroids and insulin resistance in the ICU. Proceedings of the 7th IFAC symposium on modelling and control in biomedical systems. Aalborg, Denmark.

Renaud, JM. and Moon, TW. 1980. Characterization of gluconeogenesis in hepatocytes isolated from the American eel, *Anguilla rostrata* LeSueur. J. Comp. Physiol. 135:115-125.

Sherwin, RS. *et al*. 1984. Effect of epinephrine on glucose metabolism in humans: Contribution of the liver. Am. J. Physiol. 247(2pt1):E157-65.

Smith, CL, 1954. The relation between seasonal hyperglycemia and thyroid activity in the frog *Rana temporaria*. J. Endocrinol. 10:184-191.

Trinder, E. 1969. Determination of blood glucose using 4 amino phenazone as oxygen acceptor. J. Chem. Pathol. 22:246-248.

Vijayan, MM. and Leatherland, JF. 1989. Cortisol-induced changes in plasma glucose, protein, and thyroid hormone levels, and liver glycogen content of coho salmon *Oncorhychus kisutch* Walbaum. Canadian Journal of Zoology. 67:2746-2750.

Accepted: May 23, 2019