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Afr. J. Biomed. Res. Vol. 25 (January, 2022); 83 - 87

Research Article

Effects of Exogenously Administered Cortisol on Lipolysis in the Common African Toad, *Bufo regularis*

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ABSTRACT

The role of cortisol on lipolysis in amphibians is not known. This study was designed to investigate the effects of cortisol on lipolysis in the common African toad, *Bufo regularis*. Adult toads were collected and used for the study. The animals were fasted 24h and anaesthetized by sodium thiopentone 50mg/kg intraperitoneally. Blood was collected from truncus arteriosus for estimation of blood glucose and blood free fatty acids levels. Cortisol caused significant increase in blood free fatty acids and glucose levels in the common African toad. Pretreatment with prazosin 0.2mg/kg produced significant reduction in blood free fatty acids but caused increase in blood glucose levels. Propranolol 0.5mg/kg pretreatment caused significant increase in blood free fatty acids and significant reduction in glucose levels. The combination of both blockers abolished the cortisol-induced hyperglycemia and caused significant reduction in blood free fatty acids. The results of this study confirmed that cortisol caused lipolysis from toad adipose tissue. Thus, cortisol administration caused increase in blood free fatty levels and induced hyperglycemia. The alpha-adrenergic receptors are involved in the release of free fatty acids in the common African toad *Bufo regularis*.

Keywords: *Cortisol, Free fatty acids, hyperglycemia, prazosin, propranolol, common African toad*

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Received: February 2021; Corrected version accepted: September 2021

INTRODUCTION

Free fatty acids (FFA) are the dominant oxidative fuel for all major tissues during fasting except the brain which depends largely on glucose derived mainly from gluconeogenesis (Chen *et al.*, 1999). Lipoprotein lipase (LPL) hydrolyses triglycerides of chylomicrons and very low-density lipoproteins (VLDL). The LPL activity determines triglyceride concentration and causes release of free fatty acid into tissues that utilize them (Eckel, 1989; Fielding and Frayn, 1998). The role of FFA in gluconeogenesis is species specific. For instance, free fatty acids stimulate gluconeogenesis in rats but inhibits in dogs, cats, and guinea pigs (Corredor *et al.*, 1969; Arinze and Hanson, 1973; Jomain-Baun and Hanson, 1975). There is discrepancy in results in humans. For instance, the studies of (Clore *et al.*, 1991; Puhakainen *et al.*, 1993) reported that FFA stimulated gluconeogenesis while Frye et al (1996) reported FFA inhibited gluconeogenesis.

Cortisol increases fuel substrates through mobilization of glucose (Rizza *et al.*, 1982; Dinneen *et al.*, 1993), free fatty acids (Djurhuus *et al.*, 2002) and amino acids (Horber and Haymond, 1990; Berneis *et al.*, 1997) from endogenous stores. Chronic exposure to cortisol is associated with impaired metabolism and insulin action which results in hyperglycemia and dyslipidemia. Cortisol induces insulin resistance (Rizza *et al.*, 1982; Dinneen *et al.*, 1993). It has been reported that cortisol contributes to the metabolic syndrome and cardiovascular disease (Andrews and Walker, 1999; Darmon *et al.*, 2006). There are many but conflicting reports on the effects of cortisol on lipolysis. For instance, in vitro studies in adipocytes showed that cortisol increased (Djurhuus *et al.*, 2002; Xu *et al.*, 2009), unchanged (Lee *et al.*, 2012), and decreased (Ottosson *et al.*, 2000) adipose tissue lipolysis. The effects of cortisol on lipolysis varies with species, the specific adipose tissue depot (Lee *et al.*, 2008), concentration and duration of exposure and effects of other hormones in the medium (Geer *et al.*, 2014; Stimson *et al.*, 2017) thus accounting for the conflicting reports. In vivo studies in humans have shown that cortisol acutely increased whole-body lipolysis (Divertie *et al.*, 1991; Djurhuus *et al.*, 2002, 2004). However, recent study shows that acute supraphysiological concentration of cortisol and in the presence of insulin and adrenaline is required to cause lipolysis in human subcutaneous adipose tissue and not visceral adipose tissue (Stimson *et al.*, 2017). Adrenocotropic hormone (ACTH) and catecholamines have been reported to cause release of free fatty acids from rat adipose tissue while addition of phentolamine an alpha-adrenergic blocking agent inhibited the release of free fatty acid (Schote and Page, 1960). Cortisol and other glucocorticoids have been documented to be secreted by amphibians in different stress conditions (Narayan et al., 2013; Jones et al., 2016; Fonner et al., 2017;

Gabor et al., 2018) and several non-invasive methods of cortisol sampling in amphibians have been developed (Santymire et al., 2018; Forsburg et al., 2019). There is paucity of information on the effect of cortisol on lipolysis in amphibians. Therefore, it is important to know the metabolic response of amphibian species to cortisol in view of the earlier reported differences in responses of mammals to cortisol. This study was designed to investigate the effects of cortisol on blood free fatty acids and glucose levels in the Common African toad *Bufo regularis*. Furthermore, the role of adrenergic receptors on the effects of cortisol on lipolysis was investigated.

MATERIALS AND METHODS

Animals: One hundred adult toads (70-100g) were used for the study. The toads were collected randomly from around ponds and banks of slow-moving streams around University of Ibadan, Ibadan, Oyo state, South-western Nigeria.

Experimental procedure: The toads were fasted 24h and anaesthetized with sodium thiopentone 50mg/kg intraperitoneally. The truncus arterious was dissected free from surrounding tissue for blood sample collection while the anterior abdominal vein was cannulated for drug injection. Fluidity of blood was maintained by heparin injection. Each toad was heparinised (170 units/0.1ml) and allowed 30mins to stabilize. After stabilization, toads in group 1 (control) received 0.7% amphibian saline intravenously (i.v) through anterior abdominal vein cannula while toads in group 11 (untreated) received cortisol (50µg/kg i.v). Toads in groups 111, 1V, and V were pretreated with propranolol 0.5mg/kg i.v, prazosin 0.2mg/kg i.v, combined propranolol 0.5mg/kg i.v and prazosin 0.2mg/kg i.v respectively 30mins before given cortisol 50µg/kg i.v. Each drug injection was in a volume between 0.1ml and 0.12ml given intravenously.

Biochemical analysis: Blood samples were collected at 0mim, 30min, 60min, and 90min post-injection period for estimation of blood glucose and blood free fatty acid levels. Blood glucose was estimated by modified glucose oxidase method (Trinder, 1969). Blood free fatty acids was measured using modified colorimetric determination of free fatty acids in biological fluids (Itaya and Ui, 1965). Owing to 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. Differences between two groups were compared using student t-test while one-way analysis of variance (ANOVA) was used to compare mean values in multiple groups. $P < 0.05$ was taken as statistically significant

RESULTS

The results of the experiments are shown in Figure 1, and Tables 1 and 2. Injection of 0.7% amphibian saline had no effect on blood glucose and blood free fatty acids levels. However, injection of cortisol 50µg/kg caused significant increase in blood glucose and free fatty acids levels (Figure 1). Pretreatment of toads with propranolol blocked the increase in glucose levels caused by cortisol injection and caused rise in blood free fatty acids compared with untreated toads (Tables 1 and 2). In toads pretreated with prazosin, cortisol injection did not produce any significant effect on blood glucose levels but caused significant reduction in blood free fatty acids (Tables 1and 2) compared with untreated animals. Combined pretreatments with propranolol and prazosin abolished the increase in blood glucose levels caused by cortisol injection and caused reduction in blood free fatty acids (Tables 1 and 2).

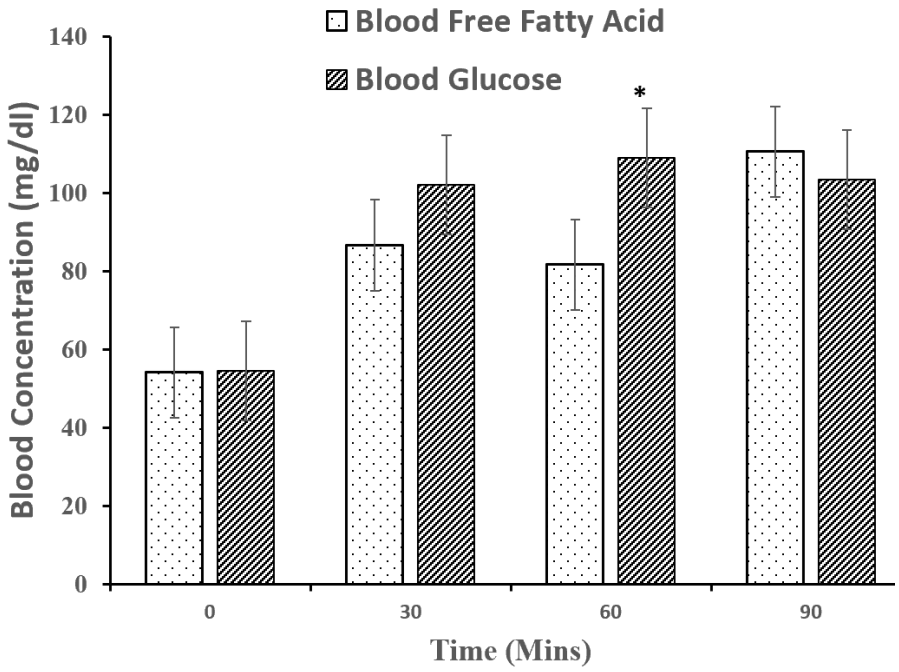

Figure 1
Effect of cortisol (50 µg/kg) on blood free fatty acids and blood glucose levels in the common African toad *bufo regularis*

Table 1:
Comparison of Blood Glucose of Control, Cortisol, and Pretreated groups (Prazosin and Propranolol groups)

Treatment	**Blood glucose (mg/dl)**			
Time	0min	30min	60min	90min
Amphibian saline 0.7% (control)	65.2±1.4	53 ±5.4	64±5.5	55.2±3.2
Cortisol 50µg/kg	54.4 ± 1.3	*102 ± 4.6	*109 ± 4.6	*103.4 ± 2.9
Propranolol 0.5mg/kg + cortisol 50µg/kg	64.2 ± 6.9	76.8 ± 3.4	88 ± 2.7	47 ± 9.3
Prazosin 0.2mg/kg + cortisol 50µg/kg	53.6 ± 7.7	94.2 ± 3.9	100.6 ± 3.4	83.6 ± 5.1
Prazosin 0.2mg/kg + propranolol 0.5mg/kg + cortisol 50µg/kg	56.8 ± 6.1	**69.8 ± 6.2	**59.4 ± 3.4	**58.8 ± 8.1

**P< 0.01 significant increase compared with basal 0 min and control*
*****P< 0.01 significant decrease compared with cortisol group***

Table 2:
Comparison of Blood Free Fatty Acids of Control, Cortisol, and Pretreated groups (Prazosin and Propranolol groups)

	Blood free fatty acids (mg/dl)			
Time Treatment	0min	30mins	60mins	90mins
Amphibian saline 0.7% control	70.0± 2.0	66.2± 6.5	58.6 ± 5.6	67.7±9.8
Cortisol 50µg/kg	54.8 ± 3.4	86.6 ± 8.4*	*81.2 ± 3.4	*110.5 ± 4.6
Propranolol 0.5mg/kg + cortisol 50µg/kg	54.5 ± 3.3	157.9 ± 10.9**	**137.3 ± 10.1	**121.2 ± 4.0
Prazosin 0.2mg/kg + cortisol 50µg/kg	52.5 ± 3.4	31.9 ± 11.1***	71.2 ± 6.7	78.5 ± 6.6
Prazosin (0.2mg/kg) + propranolol (0.5mg/kg) + cortisol (50µg/kg) (0.5mg/kg) + cortisol (50µg/kg)	56.2 ± 2.1	51.8 ± 1.0	92.3 ± 6.6	80.5 ± 6.2

**P< 0.05 significant increase compared with basal 0min; **P< 0.01 significant increase compared with cortisol group*
****P< 0.01significant decrease compared with cortisol group*

Cortisol 50µg/kg caused significant increase in blood glucose levels 30mins, 60mins, and 90mins post-injection period. Pretreatment with propranolol 0.5mg/kg blocked the increase in blood glucose caused by 50µg/kg cortisol injection. In prazosin pretreated toads, cortisol injection (50µg/kg) produced no significant difference in blood glucose levels compared with cortisol group

Combined pretreatment with propranolol 0.5mg/kg and prazosin 0.2mg/kg abolished the increase in blood glucose level caused by cortisol injection (50µg/kg).

Cortisol injection 50µg/kg caused significant increase in blood free fatty acids compared with basal (0min). Pretreatment with propranolol 0.5mg/kg caused significant increases in blood free fatty acids compared with cortisol group. Prazosin 0.2mg/kg pretreatment caused reduction in blood free fatty acids compared with cortisol group. Combined pretreatment with propranolol 0.5mg/kg and prazosin 0.2mg/kg caused reduction in blood free fatty acids compared with cortisol group.

DISCUSSION

Cortisol injection caused increase in blood free fatty acids levels in the toads. This is consistent with studies in humans (Samra *et al.,* 1998; Djurhuus *et al.,* 2002, 2004., Campbell *et al.,* 2011; Stimson *et al.,* 2017) and rats (Schote and Page, 1960). Cortisol promotes lipolysis by mobilizing free fatty acids from adipose tissue and enhanced hepatic gluconeogenesis (Grav Holt *et al.,* 2002; Geer *et al.,* 2014). Studies in humans (Ottosson *et al.,* 1994; Stimson *et al.,* 2017) and rats (Xu *et al.,* 2009; Campell *et al.,* 2011) showed that cortisol regulates lipolytic pathway through increase in transcription of key lipases enzymes, adipose triglyceride lipase and hormone sensitive lipase. The observation of the present study in which cortisol injection caused increase in blood free fatty acids confirmed its lipolytic activity in the toad *bufo regularis* and may be due to increase in transcription of lipases enzymes (Stimson *et al.,* 2017). The lipoprotein lipase activity hydrolyses triglycerides and releases free fatty acids to tissues that utilize them. (Zechner, 1997; Fielding an Frayn, 1998, Kovar *et al*., 2004). It has been reported that adrenocorticotropic hormone (ACTH) injection caused mobilization of free fatty acids from rat adipocytes (Schote and Page, 1960).

The increase in glucose level following cortisol injection confirms its hyperglycemic effect (Broughton and Deroos, 1984; Leach and Taylor, 1982; Pretty *et al.,* 2009; Isehunwa *et al.,* 2013). Cortisol causes muscle protein breakdown, lipolysis of adipose tissue, hepatic gluconeogenesis, impairs glucose uptake in muscle thereby increase circulating glucose levels in mammals (Geer *et al.,* 2014). The rise in glucose level and concomitant increase in free fatty acids level observed in the present study suggests that cortisol injection most probably suppressed secretion and action of insulin. As a result of the insulin release suppression, there was decrease in peripheral glucose uptake and increase in circulating glucose levels (Dinneen *et al.,* 1993; Macfarlane *et al.,* 2008). It has also been shown that an increase in plasma free fatty acids inhibits insulin-stimulated glucose uptake in man (Ferrannini *et al.,* 1983). Thus, the findings of the present study revealed that under stress cortisol administration caused increase in blood free fatty acids and induced hyperglycemia. The decrease in glucose levels observed in toads pretreated with propranolol seems to confirm that the beta adrenergic

receptors are involved in cortisol-induced hyperglycemia in the toad *bufo regularis* (Isehunwa *et al.,* 2013) while the significant rise in blood FFA in propranolol pretreated toads is most probably an indication that the beta adrenergic receptors may not be involved in the release of FFA from toad adipose tissue. During beta-blockade, there was unopposed alpha stimulation which could result in activated lipoprotein lipase enzyme causing increase in the level of blood FFA. Therefore, pretreatment of toads with propranolol did not block the release of free fatty acids hence the significant rise in blood FFA compared with untreated toads. This observation contrasts the findings in humans by Imura et al (1971) which reported that beta adrenergic blockage inhibited fat mobilization whereas alpha blockage stimulated fat mobilization from adipose tissue.

In toads pretreated with prazosin, an alpha blocking agent, there was significant reduction in blood FFA suggesting that the alpha-adrenergic receptors are probably involved in the release of blood FFA from the adipose tissue. As a result, pretreatment with prazosin blocked release of FFA from adipose tissue. This is consistent with the findings in rats (Schote and Page, 1960) which reported that prazosin blocked the increase in blood free fatty acids caused by ACTH. The reduction in blood FFA in toads pretreated with prazosin in the present study could be that prazosin inhibited lipoprotein lipase enzyme activity with subsequent reduction in blood FFA. Lipoprotein lipase activity hydrolyses triglycerides and releases free fatty acids to tissues that utilize them. (Zechner, 1997; Fielding an Frayn, 1998; Kovar *et al*., 2004). The findings of the present study contrast the observations in humans (Day *et al.,* 1982; Shaw *et al.,* 1978) that prazosin caused reduction in triglycerides while propranolol increased triglycerides levels. Combined pretreatment with both blockers abolished the increase in glucose levels caused by cortisol and resulted in reduction of free fatty acid levels compared with untreated animals. This observation seems to suggest that the beta and alpha- adrenergic receptors are involved cortisol-induced hyperglycemia and increased release of free fatty acids in the toads. The findings of this study revealed that cortisol caused lipolysis and induced hyperglycemia in the toads.

In conclusion, this study showed that administration of cortisol caused lipolysis and induced hyperglycemia in the common African toad *bufo regularis*. The results also suggest that the alpha-adrenergic receptors are most probably involved in cortisol-induced increase in blood free fatty acid levels whereas the beta-adrenergic receptors are involved in cortisol hyperglycemia in the common African toad.

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