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The role of alpha and beta adrenergic receptors in cortisol-induced hyperglycaemia in the common African toad (*Bufo regularis*)

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The role of adrenergic receptors in cortisol-induced hyperglycaemia is not well known. The present study investigates the effects of adrenergic receptor blockers in cortisol-induced hyperglycaemia in the common African toad (*Bufo regularis*). Each toad was fasted and anesthetized with sodium pentobarbitone (3 mg/100 g i.p). The animals (control) received intravenous (i.v) injection of 0.7% amphibian saline while animals (untreated) were given cortisol (20 µg/kg). In pre-treatment groups, animals received prazosin (0.2 mg/kg i.v), propranolol 0.5 mg/kg or combination of prazosin (0.2 mg/kg i.v) and propranolol (0.5 mg/kg i.v) before i.v injection of cortisol (20 µg/kg). Thereafter, blood samples were collected for estimation of blood glucose level using the modified glucose oxidase method. Cortisol caused significant increase in blood glucose level from 44.4±3.8 to 71.7±9.7 mg/dl. Pre-treatment of the toads with propranolol (0.5 mg/kg i.v) caused significant reduction (p≤ 0.01) in cortisol-induced hyperglycaemia while pre-treatment with prazosin (0.2 mg/kg i.v) produced no significant effect on hyperglycaemia induced by cortisol. The combination of both prazosin and propranolol completely abolished the effects of cortisol on blood glucose level. The results suggest that cortisol-induced hyperglycaemia in the toad (*B. regularis*) is mediated probably by both the α- and β-adrenergic receptors with the beta adrenergic receptors playing dominant role.

Key words: Cortisol, hyperglycaemia, prazosin, propranolol, amphibian saline, common African toad.

INTRODUCTION

Cortisol is a well known hyperglycaemic agent. The hyperglycaemic effect has been reported in frogs (Hanke, 1974, 1978; Broughton and Deroos, 1984) and other animal species (Chan and Woo, 1978a; Leach and Taylor, 1982; Pretty et al., 2009). Its hyperglycaemic action is due to activation of gluconeogenesis (Baxter, 1979; Renaud and Moon, 1980; Khani and Tayek, 2001). The hyperglycaemic response to cortisol involves metabolic actions such as glucose release from the liver as a product of glycogenolysis, increase in gluconeo-genesis and decrease in peripheral glucose utilisation. The relative contribution of each of these effects differ in different species and under different nutritional conditions (Hanke,1978).The hyperglycaemia develops slowly, suggesting that protein catabolism and enzyme activation are necessary before glucose release. Corticosteroids interact with the sympathoadrenal system to enhance the actions of catecholamines and affect glucose metabolism and insulin sensitivity through a signaling pathway involving or intersecting with catecholamines and the sympathetic nervous system (Pretty et al., 2009). Previous studies in rats show that adrenal glucocorticoids facilitated beta receptors function *in vivo* and that adrenalectomy resulted in loss of responsiveness to

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to catecholamines (Davies et al., 1981; Davies and Lefkowtiz, 1984; Taylor and Hancox, 2000).The study of Yang and Zhang (2004) shows that corticosteroid hormones have permissive effect to the catecholamine through glucocorticoid receptors. Adrenergic receptors transmit adrenaline and noradrenaline signals between cells (Gilsbach and Hein, 2008) and are important mediators of physiologic responses to endogenous catecholamines (Gilsbach and Hein, 2012). Previous studies show also that there is interaction between the hormones adrenaline and cortisol in their physiologic actions (Peter, 2011; George et al., 2013). However, while the receptors mediating the hyperglycaemic response to other hormones like adrenaline and other sympathomimetics amines have been studied, the role of adrenergic receptors in cortisol-induced hyperglycaemia in amphibians has not received much attention. The aim of the present study was to find out the possible role of alpha and beta adrenergic receptors in cortisol-induced hyperglycaemic in the common African toad (*Bufo regularis*).

MATERIALS AND METHODS

Experiments were carried out on 240 adult toads (*B. regularis*) of both sexes weighing between 70-100 g. The toads were obtained from the banks of slow-moving streams, around ponds and wet bushes. The collection process is that of randomly picking the toads as one finds them during the night search. Hence selection of the animal is unbiased. Each animal was fasted 24 h before the start of the experiment and anaesthesized with sodium pentobarbitone 3 mg/100 g 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 collection. The anterior abdominal vein was cannulated for drug injection. Each toad was heparinised (170 units/0.1 ml) and allowed 30 min stabilization. After stabilization period, basal blood collection (0 min) was made from the truncus arteriosus.

The animals were randomly divided into five groups (1-V) of 48 toads per group. Toads in group I (control) received intravenous (i.v) injection of 0.7 % amphibian saline while toads in group II (untreated) received cortisol (20 µg/kg i.v). Toads in groups III, IV, and V were pre-treated with prazosin (0.2 mg/kg i.v), propranolol (0.5 mg/kg i.v), or combination prazosin (0. mg/kg i.v) and propranolol (0.5 mg/kg i.v), respectively, 30 min before injection of cortisol (20 µg/kg i.v). In each animal, 0.05 ml per sample was drawn directly from the truncus arteriousus for glucose determination. Blood samples were collected at time interval of 0, 5, 10, 20, 30, 60, and 90 min, post-injection. Each drug injection was in a total volume between 0.1 and 0.12 ml given intravenously through the anterior abdominal vein cannula. Blood glucose was determined immediately using modified glucose oxidase method of Trinder (1969). Because of the small size of the toad, animals were sampled only once in each experiment and then sacrificed.

Determination of liver and muscle glycogen

To determine the glycogen content of *B. regularis*, six toads were collected and used per group. After surgical procedure and 30 min stabilization period, each animal was given 0.7% amphibian saline (control group) through anterior abdominal vein cannula. For the cortisol group, each animal received 20 µg/kg cortisol injection. In pre-treatment groups, animals received either prazosin (0.2 mg/kg i.v), (propranolol 0.5 mg/kg i.v) or combination of prazosin (0.2 mg/kg i.v) and propranolol (0.5 mg/kg i.v) before i.v injection of cortisol (20µg/kg). Thereafter, the whole liver and gastrocnemius muscle of each animal were removed quickly, 60 min post injection under anaesthesia, cleared of adherent tissues and blood was blotted away using blotting paper. They were weighed separately using an electronic weighing balance, model DT 1000 England. Immediately, one gram each of the liver and muscle of the toad was removed separately and the glycogen content determined using anthrone reagents method (Seifter et al., 1950; Jermyn, 1975).

Isolation and purification of glycogen

1 g of the liver and 1 g of the muscle each were placed in individual pre-heated Erlnmeyer flasks containing 10 ml of 30% KOH solution. The liver and muscle were digested separately by heating the flasks for 20 min in a steam bath with occasional shaking until the tissues dissolved. The solution was allowed to cool. Then, 4 ml of the aliquot from each of the flasks was taken and placed in a 15 ml centrifuge tube each. 5 ml of 95% ethanol was added to each sample, mixed and centrifuged for 5 min; it was then decanted and drained for 5 min. The glycogen precipitated from each sample was dissolved in 0.5 ml distilled H_2O separately and mixed thoroughly. This was reprecipitated with 5 ml of 95% ethanol and recovered by centrifugation. The centrifugation was repeated four times until a white precipitate was obtained. The final glycogen precipitate was dissolved in 2 ml of distilled H_2O. 0.5 ml aliquot was taken from the unknown glycogen solution obtained from above. Then, 0.5 ml of concentrated HCl, followed by 0.5 ml formic acid (88%) and 4 ml of anthrone reagent were added in a step wise manner. The anthrone reagent was added slowly and mixed thoroughly. 0.5 ml of distilled water was treated as above and used as a blank. Several dilutions of the glycogen standard (0.2 mg/ml) were prepared. The dilutions used were 0.1, 0.2, 0.3, 0.4 ml of standard glycogen solution with enough distilled water to make 0.5 ml. These dilutions of glycogen standard were then treated as above. A standard curve was prepared from this.

All the tubes containing the solutions were heated in boiling water for ten minutes and allowed to cool. A portion of the contents from each tube was poured into a cuvette, bubbles were allowed to disperse and the absorbance was read. The absorbance was read at 630 nm against the blank. Calculation of glycogen was done using Equation 1:

$$\text{Mg glycogen/100g fresh liver} = \text{Mg glycogen/ml} \times \frac{10}{4} \times \frac{2}{0.5} \times \frac{100}{\text{Total fresh liver weight}}$$

Statistical analysis

All values given are mean ±S.E.M of the variables measured. Values 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.

RESULTS AND DISCUSSION

Effects of 0.7% saline and cortisol on blood glucose and glycogen content (liver and muscle)

Infusion of 0.7% amphibian saline had no effect on blood glucose level (Figures 1 and 2). The mean fasting glucose level in the toad, *B. regularis*, was 44.4±3.8 mg/dl. Infusion of cortisol 20 µg/kg caused significant

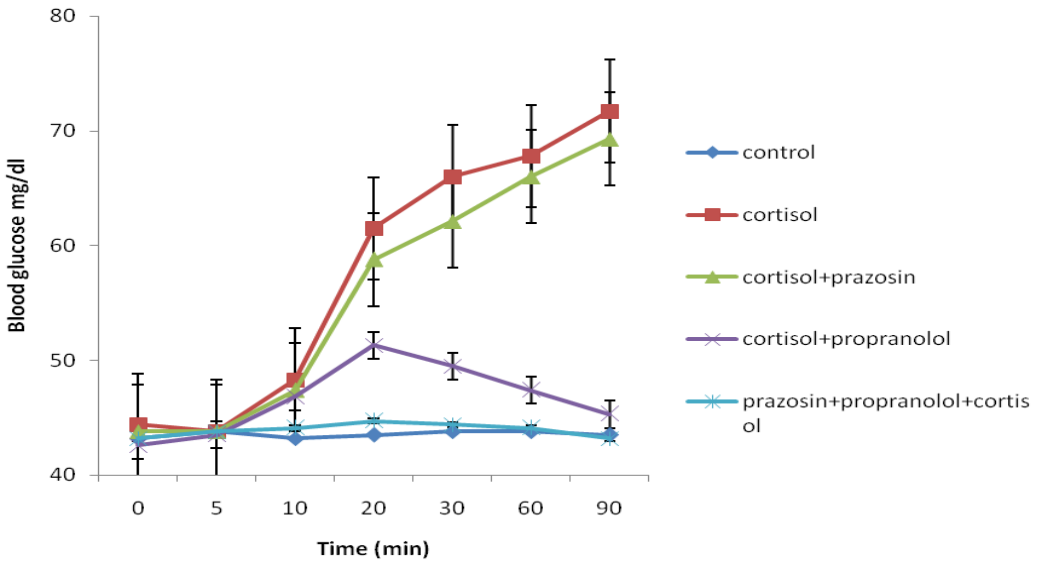

Figure 1. Effects of 0.7% amphibian saline cortisol (20 µg/kg) in untreated toads and in prazosin treated (0.2 mg/kg), propranolol treated (0.5 mg/kg), and both prazosin (0.2 mg/kg) and propranolol (0.5 mg/kg) on blood glucose levels of the treated groups. The points are mean±S.E.M. of seven determinations.

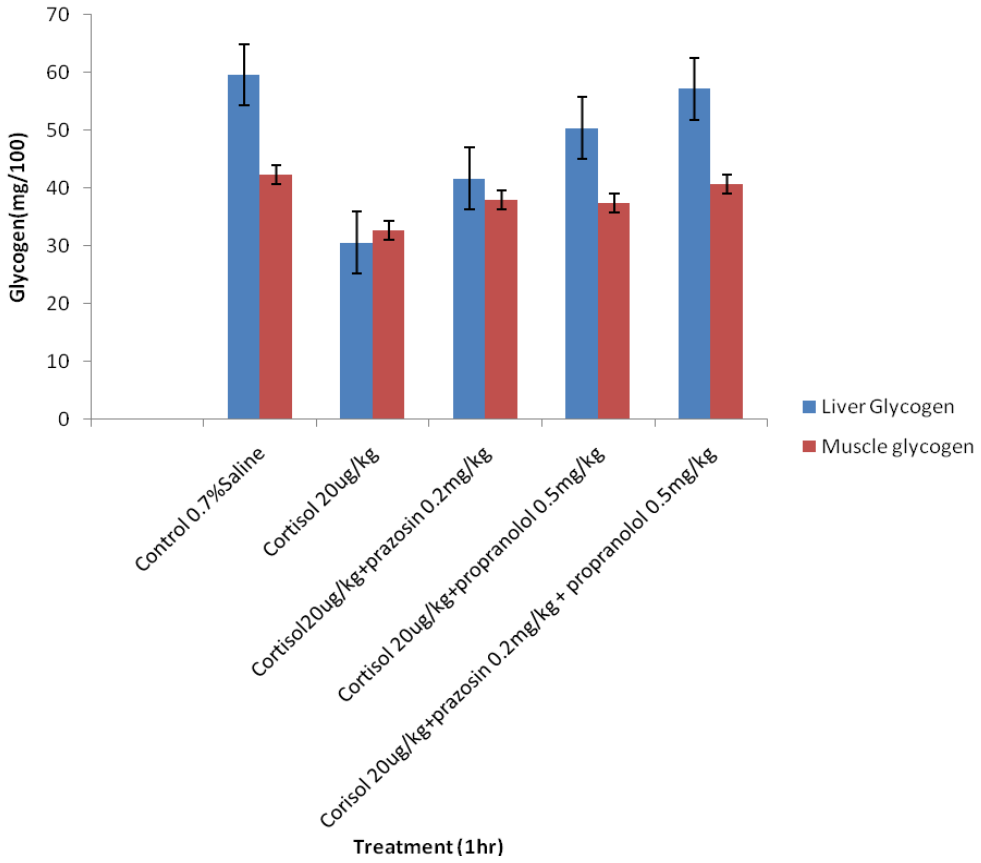

Figure 2. Graph of liver and muscle glycogen content (Mg glycogen/100 g fresh liver and muscle (mean±S.E.M) in toads infused with 0.7% amphibian saline, cortisol, and pre-treated with prazosin and propranolol.

increase in blood glucose level from a mean basal value of 44.4±3.8 mg/dl to maximum value of 71.7±9.7 mg/dl 90 min post injection and decrease in glycogen content of liver and muscle when compared with the control (Figures 1 and 2). Cortisol-induced hyperglycaemia was delayed and became significant 20 min post-injection period (Figure 1) and hyperglycaemia was progressive throughout the post- injection period.

Effects of pre-treatment with prazosin (0.2 mg/kg) and propranolol (0.5 mg/kg)

These are shown in Figure 1. The hyperglycaemic response to cortisol infusion was completely abolished by propranolol but prazosin did not produce any significant effect in cortisol-induced hyperglycaemia when compared with the untreated toads (Figure 1). A combination of both blockers completely abolished cortisol- induced hyperglycaemia in *B. regularis* when compared with the untreated toads (Figure 1). Figure 2 shows the effect of adrenergic blockers on liver and muscle glycogen. Pre-treatment of the toads with prazosin caused significant reduction in liver glycogen while pre-treatment with propranolol produced no significant reduction in liver and muscle glycogen level. The combination of both prazosin and propranolol caused non-significant reduction in liver and muscle glycogen (Figure 2).

The rise in blood glucose following cortisol injection in this study confirms its hyperglycaemic effect. The results agree with findings in frogs (Hanke, 1974, 1978; Broughton and DeRoos, 1984; Tavoni et al., 2013) and other animal species (Chan and Woo, 1978a; Khani and Tayek, 2001). The significantly higher levels of blood glucose following cortisol injection in the toad are probably due to an enhanced liver gluconeogenesis. Cortisol has been reported to exert its hyperglycaemic effect through activation of gluconeogenesis (Baxter, 1979; Renauid and Moon, 1980; Resmini et al., 2009). Al-Nagdy et al. (1995) reported increased levels of blood lactate and pyruvate following cortisone injection. In the present study, the stimulated liver gluconeogenesis may account for the decrease in liver and muscle glycogen following cortisol injection and compared with the control. Therefore, it is not unlikely that both the hepatic and muscle glycogen must have contributed some amount of glucose under the influence of cortisol. The results of the present study in which cortisol caused reduction in liver and muscle glycogen agrees with the findings in fishes (Foster and Moon, 1986; Vijayan and Leatherland, 1989) and in rats (Tavoni et al., 2013). Cortisol, a major glucocorticoid exerts its hyperglycaemic effect through activation of gluconeogenesis (Baxter, 1979; Renaud and Moon, 1980; Khani and Tayek, 2001). Since 0.7% amphibian saline injection had no effect on blood glucose, the hyperglycaemic effect of cortisol could not be ascribed to the stress of the injections.

In the prazosin pre-treated animals, the non-significant reduction in cortisol-induced hyperglycaemia seems to suggest that the alpha adrenergic receptors may not play a significant role in the production of hyperglycaemic response to cortisol in the toad. The significant reduction of liver glycogen by prazosin also, is an indication of the non significant involvement of alpha adrenoceptors in cortisol hyperglycaemia. The present findings contrast the report in man, that alpha adrenergic blockade by phentolamine infusion suppressed plasma adrenocorticotropic hormone (ACTH) and cortisol level while propranolol caused no significant change in plasma ACTH and cortisol although it enhanced plasma ACTH response to insulin induced hypoglycaemia (Nakai et al; 1973; Al-Damluji, 1988). Pretreatment with propranolol significantly reduced the rise in blood glucose response to cortisol injection. Since propranolol is a beta-blocker, it seems likely that the ability of cortisol to increase blood glucose in toads is mediated through the beta adrenergic receptors. Also, the non significant reduction in the liver and muscle glycogen in toads by propranolol further confirms that the beta adrenergic receptors are involved in the cortisol hyperglycaemia. The result is consistent with the study in rats in which glucocorticoids enhanced β-receptors mediated responses (Davies and Lefkowtig, 1984; Taylor and Hancox, 2000). Cortisol has been reported to exert its effects through the beta adrenergic receptors (George et al., 2013).The combination of both prazosin and propranolol completely abolished the rise in blood glucose following cortisol injection in the toad in the present study. Since prazosin alone did not cause a significant reduction in cortisol hyperglycaemia, the complete abolition of cortisol-induced hyperglycaemia by both prazosin and propranolol shows that prazosin potentiated the effect of propranolol on cortisol-induced hyperglycaemia. In conclusion, the results of the present study show that both the alpha and beta adrenergic receptors are involved in the cortisol-induced hyperglycaemia in the common African toad, *B. regularis*. However, the beta adrenergic receptors played a dominant role than the alpha adrenergic receptors in cortisol hyperglycaemia in the toad.

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