Full Length Research Paper

The role of adrenergic receptors in nicotine-induced hyperglycemia in the common African toad (*Bufo regularis*)

Isehunwa, G. O.[1]*, Adewunmi, G. O.[1], Alada, A. R. A[1] and Olaniyan, O. T.[2]

[1]Department of Physiology, University of Ibadan, Ibadan, Nigeria.
[2]Department of Physiology, Bingham University, Karu, Nasarawa, Nigeria.

The role of adrenergic receptors in nicotine-induced hyperglycaemia has not been well studied in amphibians. Thus, this study investigates the effects of alpha and beta adrenergic receptor blockers in nicotine-induced hyperglycaemia in the common African toad *Bufo regularis*. Toads fasted for 24 h were anaesthetized with sodium pentobarbitone (3 mg/100 g body weight) intraperitoneally (i.p) and given intravenous (i.v) injection of 0.7% amphibian saline, or nicotine (50 µg/kg), nicotine(50 µg/kg i.v) 30 min after pretreatment with prazosin (0.2 mg/kg i.v), propranolol (0.5 mg/kg i.v) or combination of both prazosin (0.2 mg/kg i.v) and propranolol (0.5 mg/kg, i.v). Thereafter, blood samples were also collected from truncus arteriosus for estimation of blood glucose level using the modified glucose oxidase method. Nicotine caused significant increase (P<0.01) in the levels of blood glucose in the common African toad. Pre-treatment of the toads with prazosin (0.2 mg/kg i.v) or propranolol (0.5 mg/kg, i.v) significantly (p < 0.01) reduced the hyperglycaemia induced by nicotine (50 µg/kg i.v). However, the combination of prazosin (0.2 mg/kg i.v) and propranolol (0.5 mg/kg, i.v) abolished the hyperglycaemic effect of nicotine (50 µg/kg i.v). The above results on glucose metabolism suggests involvement of both alpha and beta adrenoceptors in nicotine-induced hyperglycaemia in common African toad *B. regularis*.

Key words: Nicotine, hyperglycaemia, prazosin, propranolol, common African toad *Bufo regularis*.

INTRODUCTION

Nicotine, the main psychoactive and addictive compound in tobacco (Benowitz, 1988), is a low molecular weight alkaloid found in cigarettes and through insecticide inhalation (Hosseini, 2011). Its consumption alters cardiovascular, neural, and endocrine functions through its effects on the central and peripheral nervous system (Benowitz, 1988; Matta et al., 2007; Hosseini, 2011).

Another important role of nicotine is the ability to induce hyperglycaemia in animals such as dogs, rats and rabbits (Grayson and Oyebola, 1985; Oyebola and Alada, 1993; Oyebola et al., 2009). Nicotine acts indirectly on blood glucose levels by stimulating adrenaline release from the

adrenal glands that in turns triggers the glycaemic response via acetylcholine nicotinic receptors (Tsuijimoto et al., 1965; Milton, 1966). In particular, nicotine rapidly increases blood sugar levels by mobilizing glucose release from hepatic and muscle glycogen stores through adrenergic actions (Molimard, 2013). The hyperglycaemic response to adrenaline is mediated by both alpha and beta adrenoceptors (Al-Jibouri et al, 1980).

Previous studies showed occurrence of modulation in alpha and beta adrenoceptors during nicotine-induced hyperglycaemia (Grayson and Oyebola, 1985; Oyebola et al., 2009). However, there are conflicting reports on receptor-types involved in the hyperglycaemic response to nicotine action. For instance, both alpha and beta adrenoceptors are modulated in dogs (Grayson and Oyebola, 1985) and rabbits (Oyebola et al., 2009) during nicotine-induced hyperglycaemia; whereas in rats, only beta-adrenoceptors are functional (Oyebola and Alada, 1993). Therefore, our study aimed at investigating the role of the alpha and beta adrenergic receptors in nicotine-induced hyperglycaemia in the common African toad (*Bufo regularis*).

The levels of blood glucose in the test and control groups were measured using modified glucose oxidase method (Trinder, 1969). Finally, our studies confirm the involvement of both apha and beta adrenergic receptors in nicotine-triggered glucose metabolism.

MATERIALS AND METHODS

Laboratory animals

Adult male and female toads (n=240) weighing between 70-100 g were studied. The toads were obtained from the banks of slow-moving streams, around ponds and wet bushes. The collection process, which took place at night, was done randomly to prevent bias.

Experimental procedures

Toads were fasted for 24 h before treatment. They were anaesthe-sized by intraperitoneal (i.p) injection of sodium pentobarbitone (3 mg/100 g body weight). Blood was collected from truncus arteriosus after careful dissection to remove connective tissues surrounding it. The anterior abdominal vein was cannulated for drug injection. Thereafter, each toad was heparinised (170 units/0.1 ml) to main-tain fluidity of blood and allowed 30 min to stabilize. After stabiliza-tion period, basal blood collection (this represented 0 min) was made from the truncus arteriosus.

Control and treatment groups

The animals were randomly divided into five groups (I to V) of 48 toads per group. Toads in control group I were injected intrave-nously (i.v) with 0.7 % amphibian saline; whereas, those in group II (untreated) were given 50 µg/kg i.v nicotine injections. Group III, IV, and V were pre-treated with prazosin 0.2 mg/kg i.v, 0.5 mg/kg i.v propranolol or combination of both 0.2 mg/kg i.v. prazosin and 0.5mg/kg i.v propranolol, respectively. After 30 min, group 111-V were injected with a dose of nicotine (50 µg/kg i.v).

Each drug injection was in a total volume between 0.1 and 0.12 ml

given intravenously through the anterior abdominal vein cannula. In each animal, blood sample (0.05 ml per sample) was drawn directly from the truncus arteriousus for blood glucose determination. Blood samples were collected after 0, 5, 10, 20, 30, 60 and 90 min post-drug injection. Blood glucose levels were determined following a modified glucose oxidase method by Trinder (1969). Owing to the small size of the toad, animals were sampled only once in each experiment and then sacrificed.

Determination of liver and muscle glycogen

In order to determine the glycogen content of *B. regularis*, six toads were analysed in each group. Following the surgical procedure and thirty minutes for the animal to stabilize, each toad was given 0.7 % amphibian saline or 50 µg/kg nicotine through anterior abdominal vein cannula. The whole liver and gastrocnemius muscle of each anaesthetized toad were quickly removed sixty minutes post injection period. These tissues were weighed using an electronic weighing balance (Coledo DT 1000 England). Thereafter, 1 g of liver and muscle were excised separately and the glycogen content determined using anthrone reagents method (Seifter et al., 1950; Jermyn, 1975).

Purification and quantitation of glycogen

One-gram each of liver and muscle were placed in individual pre-heated Erlnmeyer flasks containing 10 ml of 30% potassium hydroxide 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 was transferred into a sterile 15 ml centrifuge tube. Subsequently, 5 ml of 95% ethanol was added to each sample, mixed and centrifuged at 3500 rpm for 5 min; it was then decanted and drained for 5 min. Precipitated glycogen from each sample was dissolved in 0.5 ml distilled water. The tube contents were re-precipitated with 5 ml of 95% ethanol and recovered through centrifugation. Centrifugation was repeated four times until a white precipitate was obtained. The final glycogen precipitate was dissolved in 2 ml of distilled water. Then, 0.5 ml aliquot was taken from the unknown glycogen solution obtained above. The next step involved step-wise addition of 0.5ml of concentrated HCl, 0.5 ml formic acid (88%) and 4 ml of anthrone reagent. Similarly, 0.5 ml of distilled water (used as a blank) was treated following above steps.

Several dilutions of the glycogen standard (0.2 mg/ml) were prepared. The dilutions used are 0.1, 0.2, 0.3, 0.4 ml of standard glycogen solution with distilled water to make a total volume of 0.5 ml. These dilutions of glycogen standard were then treated and used to generate a standard curve.

All the tubes containing the solutions were heated in boiling water for ten minutes and allowed to cool. Content from each tube was poured into a cuvette, followed by measurement of absorbance at 630 nm against the blank. Glycogen content was calculated from the following Equation 1:

$$\text{Mg glycogen/100 g fresh liver} = \text{Mg glycogen/ml} \times \frac{10}{4} \times \frac{2}{0.5} \times \frac{100}{Total\ fresh\ liver\ weight} \quad (1)$$

Statistical analysis

All values provided represent mean ± standard error of mean (S.E.M) of the variables measured. Differences between two groups were compared using student t test whereas one-way analysis of variance (ANOVA) was employed in comparison between mean values in multiple groups. $P \leq 0.05$ were taken as statistically significant.

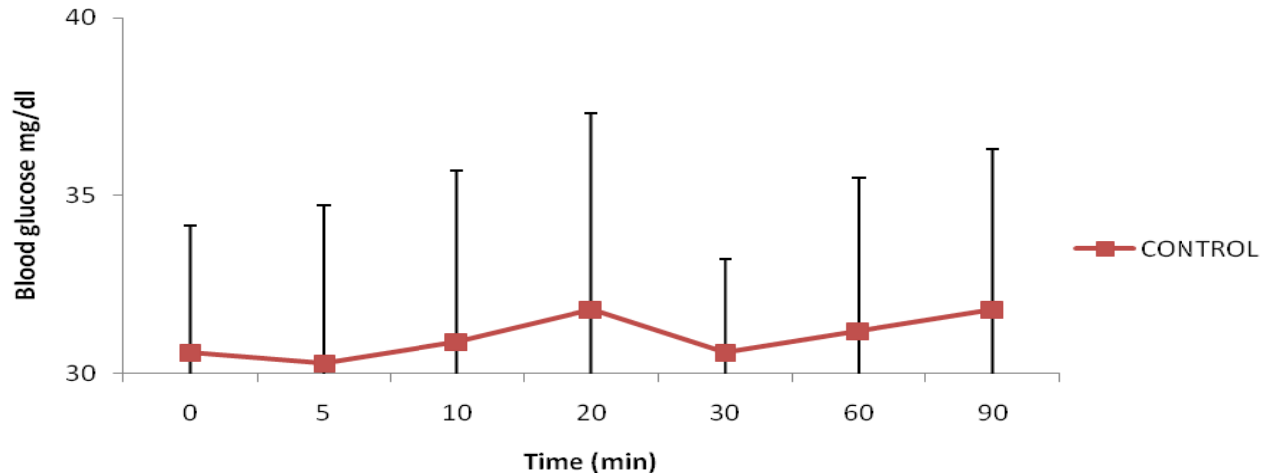**Figure 1.** The effects of 0.7% amphibian saline on blood glucose levels. Amphibian saline produced no significant effect (p≥0.05) on blood glucose level. The points represent Mean ± S.E.M. N=7 toads for each timed observation.

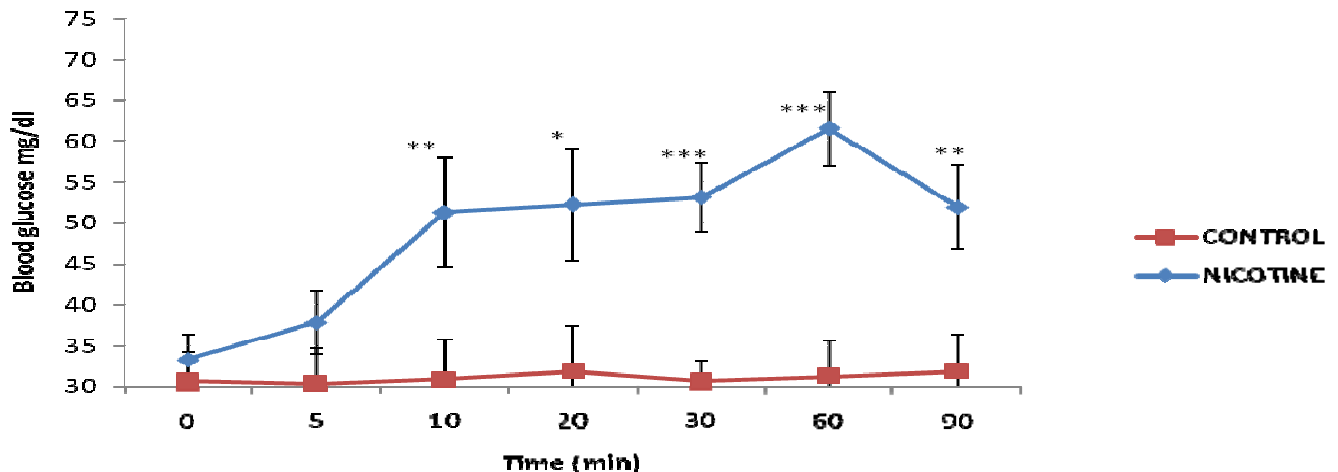**Figure 2.** The effects of nicotine (50 µg/kg) on blood glucose levels (untreated toads). Note the hyperglycaemic effects of nicotine which became significant (p <0.05) 10 min post-injection period. Nicotine (50 µg/kg) caused blood glucose to increase from basal value (33.3±2.97 mg/dl) to maximal value of (61.5±4.52 mg/dl***) 60 min post-injection. The points are mean ±S.E.M. Asterisk (*) indicate values that are significantly different (*P≤ 0.05), (**p≤0.01) and (***p≤0.001) from the control. (n=7 toads for each timed observation).

RESULTS

The results are shown in Figures 1 to 6 and Table 1. All values given are mean ± S.E.M of the variables measured. Toads in group 1 were not pre-treated with any adrenoceptor blocker and are referred to as the untreated animals.

Effect of 0.7% amphibian saline and nicotine injection on blood glucose, liver and muscle glycogen levels

Infusion of 0.7% amphibian saline had no effect on blood glucose level (Figure 1). However the mean fasting glucose level in the toad *B. regularis* was 33.3±3.0

mg/dl. Injection of nicotine 50 µg/kg caused a significant increase in blood glucose level from mean basal value of 33.3±3.0 mg/dl to a maximum value of 61.5±4.5 mg/dl 60 min post injection (Figure 2). The hyperglycaemic effect of nicotine became significant 10 min post-injection and rose progressively till 60 min post-injection period. Injection of nicotine 50 µg/kg caused significant reduction in liver and muscle glycogen content compared with the control group.

Effect of nicotine during prazosin and propranolol pre-treatment

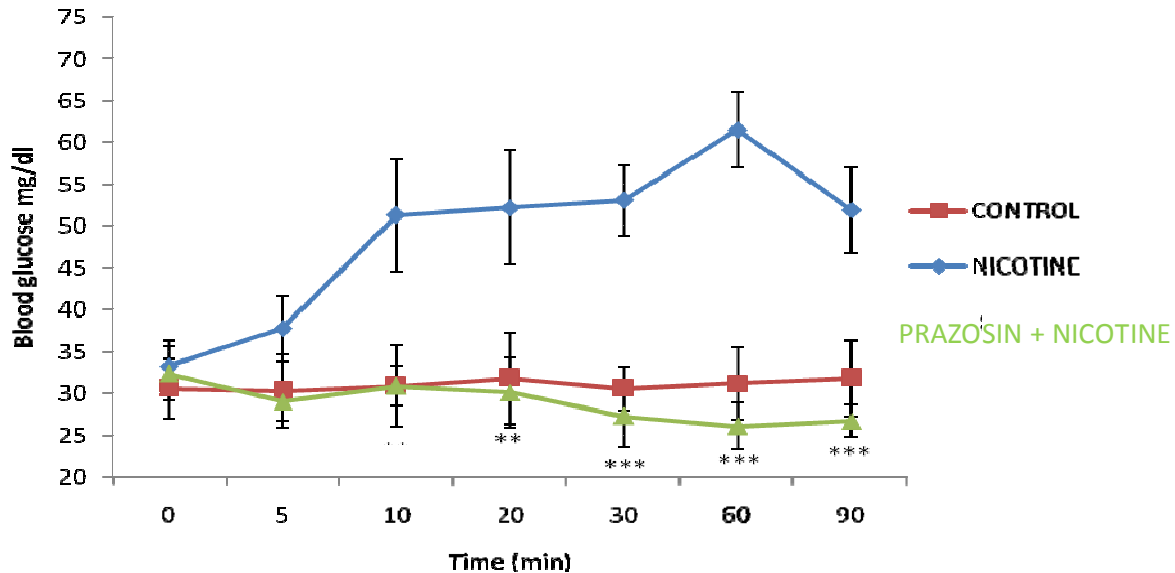Pre-treatment with prazosin prevented the increase in

Figure 3. The effects of nicotine (50 µg/kg) on blood glucose levels in untreated and prazosin treated (0.2 mg/kg) toads. Prazosin produced a significant reduction on nicotine-induced hyperglycaemia when compared with the untreated toads. Each data points represent mean ± S.E.M.
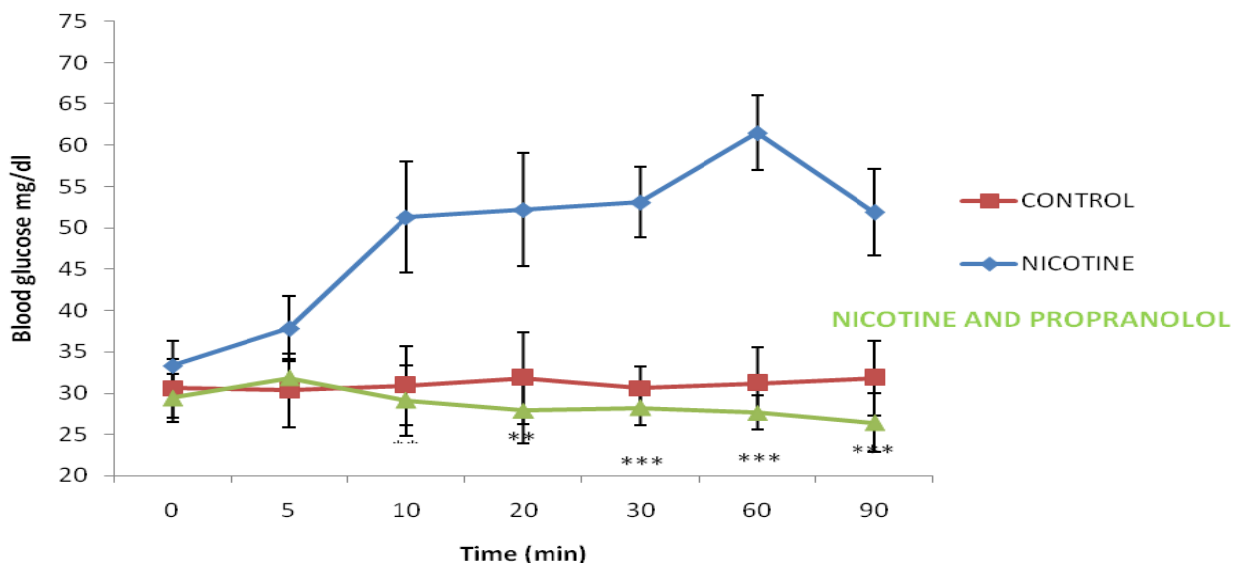

Figure 4. The effects of nicotine (50 µg/kg) on blood glucose levels in untreated versus propranolol- treated (0.5 mg/kg) toads. Propranolol (0.5 mg/kg) abolished the increase in nicotine- induced hyperglycemia.This was followed by decline in blood glucose levels in the remaining post-injection observation period. Each data point represents mean ± S.E.M. Asterisks (*) indicate values that are significantly different (*P ≤ 0.05), (**p≤0.01) and (***p≤0.001) from the nicotine (untreated group).

glucose levels induced by nicotine injection (Figures 3 to 6) while propranolol pre-treatment abolished the increases in glucose levels caused by nicotine injection. The glucose levels fell below basal level throughout post-injection period. A combination of both blockers completely abolished nicotine-induced hyperglycaemia in toad compared with the untreated toads.

DISCUSSION

The findings of the present study in which nicotine infusion caused a rise in blood glucose level of *B. regularis* is consistent with its known pharmacological effect on blood glucose (Oyebola et al., 2009). Since amphibian saline had no effect on blood glucose, the increase in blood glucose
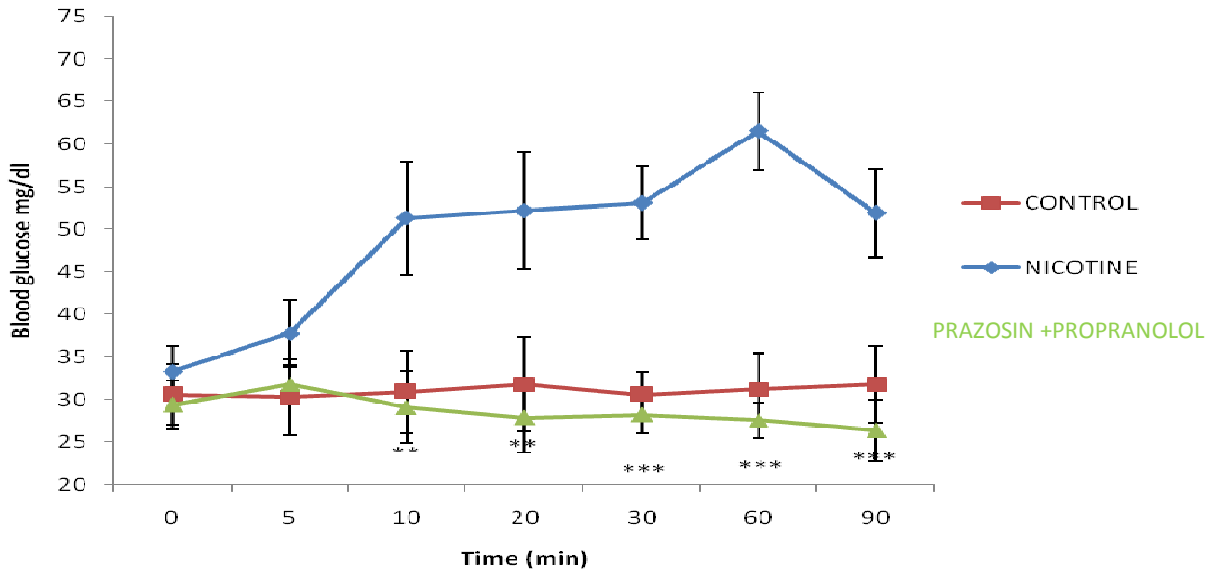
Figure 5. The effects of nicotine (50 µg/kg) on blood glucose levels in untreated and in toads treated with both prazosin (0.2 mg/kg) and propranolol (0.5 mg/kg). Combination of both blockers completely abolished nicotine hyperglycaemia in *Bufo regularis.* Each data point represents mean ± S.E.M. Asterisk (*) indicate values that are significantly different (*P ≤ 0.05), (**p≤0.01) and (***p≤0.001) from the nicotine (untreated) group. N=7 for each observation.
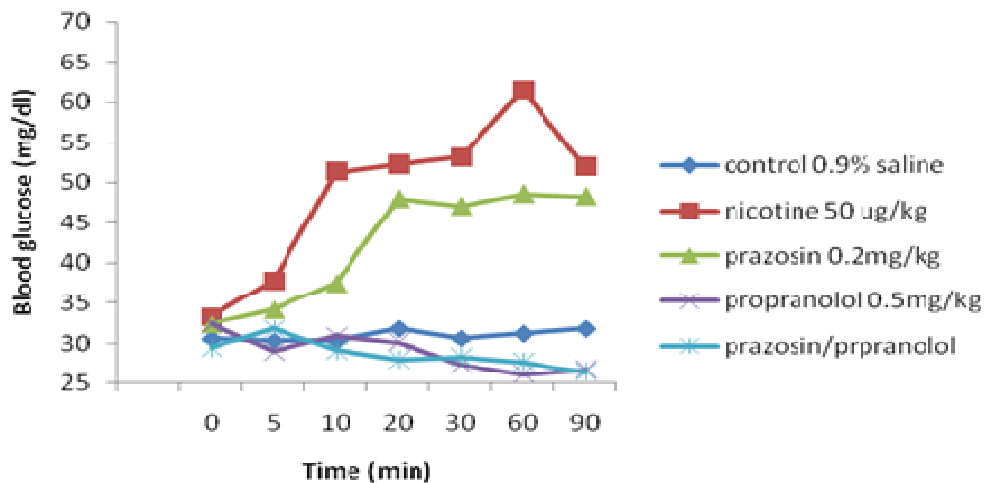

Figure 6. The effects of 0.7% amphibian saline, nicotine (50 µg/kg), prazosin treated (0.2 mg/kg), propranolol treated (0.5 mg/kg) and combination of both prazosin (0.2 mg/kg) and propranolol (0.5 mg/kg) treated toads on glucose levels. The points are mean ± S.E.M. N=7 for each observation.

Table 1. Liver and muscle glycogen content (Mg glycogen/100 g fresh liver and muscle; mean ± S.E.M) in toads infused with 0.7% saline and nicotine (50 µg/kg).

Treatment (1 h)	Liver glycogen	Muscle glycogen
Control 0.7% saline	111.62±47.98	42.31±4.99
Nicotine (50 µg/kg)	7.35±0.87***	5.02±1.04***

Asterisk(*) indicates significantly different values (***P<0.001). n=6 for each infusion.

level in nicotine-injected group could not be due to the stress of nicotine injection. Previous studies in dogs (Tsujimoto et al., 1965; Grayson and Oyebola, 1985) and cats (Milton, 1966) showed that nicotine injection induces hyperglycaemia indirectly through adrenaline release from the adrenal medulla.The hyperglycemia observed during nicotine injection results from an increase in glucose production. The results of the present study in which nicotine caused significant reduction in liver and muscle

glycogen levels suggests that both hepatic and muscle glycogen must have contributed to glucose production which results in a rise in glucose levels following administration of nicotine. This finding agrees with previous reports that nicotine rapidly increases blood glucose by mobilizing hepatic and muscle glycogen stores by adrenergic actions (Tsujimoto et al., 1965; Mlton, 1966; Benowitz, 1986; Molimard, 2013). Additionally, we report that the onset of glycaemic response to nicotine was gradual and the blood glucose levels continued to increase reaching peak levels 60 min post-injection. The sustained nicotine-induced hyperglycaemia was similar to what was previously reported in rats (Oyebola and Alada, 1993) and rabbits (Oyebola et al., 2009). This may suggest similar trends in glucose metabolism in rabbits, rats and toads following glycaemic response to nicotine.

The results of the present study in which prazosin an alpha adrenergic blocker caused profound decrease in nicotine - induced hyperglycemia in *B. regularis* suggested the role of α-adrenergic receptors in nicotine hyperglycaemia. This was consistent with the observations in dogs (Grayson and Oyebola, 1985), rabbits (Oyebola et al., 2009) but contrasts to the findings in rats (Oyebola and Alada, 1993). The studies in dogs (Grayson and Oyebola, 1985) and rabbits (Oyebola et al., 2009) showed that prazosin abolished nicotine-induced hyperglycaemia whereas prazosin merely attenuated nicotine hyperglycaemia in rats (Oyebola and Alada, 1993).

The abolition of nicotine hyperglycemia by propranolol indicated that the ability of nicotine to cause a rise in blood glucose level was mediated through the beta adrenergic receptors. This observation agrees with the studies in dogs (Grayson and Oyebola, 1985), rats (Oyebola and Alada, 1993) and in rabbits (Oyebola et al., 2009). Since beta receptors are present in the skeletal muscles (Arnold and Selbens, 1968; Hendler and Sherwin, 1984) some amount of the glucose released after nicotine administration could have been produced from lactate in the skeletal muscle.

However, the complete abolition of nicotine hyperglycaemia by combination of prazosin and propranolol appears to confirm the involvement of both alpha and beta adrenoceptors in mediating nicotine induced hyperglycaemia in the toad. This finding agrees with the observation in dogs (Grayson and Oyebola, 1985), and rabbits (Oyebola et al., 2009), but contrasts the study report in rats (Oyebola and Alada, 1993). The differences in observations may be due to species variation with respect to the receptors involved in nicotine-induced hyperglycaemia in different animals.

In conclusion, the above results suggest that both alpha and beta adrenergic receptors play roles in nicotine-induced hyperglycaemia in the common African toad *B. regularis.*

Conflict of Interests

The author(s) have not declared any conflict of interests.

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