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Research Article

Maternal treatment with dexamethasone during lactation alters serum electrolyte and adrenal gland morphology in male offspring of wistar rats

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ABSTRACT

Background: Maternal treatment with dexamethasone during development altered glucocorticoid activity in Wistar rat offspring. Increased glucocorticoid levels may affect serum electrolyte levels and the architecture of the adrenal cortex. This study was designed to investigate the effects of maternal treatment with dexamethasone during lactation on serum electrolytes and structure of the adrenal gland. **Methods:** Twenty lactating dams were divided into 4 groups of 5 animals each. Group 1 was administered 0.02 ml/100g/day normal saline through lactation days 1-21. Group 2, 3 and 4 were administered 100 µg/kg/day dexamethasone (Dex) at lactation days 1-7, 1-14, and 1-21 respectively. The male offspring were sacrificed at 12weeks of age for evaluation of serum electrolytes and architecture of the adrenal gland. **Results:** The results showed that there was no significant difference in the serum Sodium and Chloride ion concentration in all the treatment groups when compared with control. Serum Potassium ion concentration was significantly reduced in the Dex1-14($p<0.05$) and Dex1-21($p<0.01$), when compared with control. The serum calcium level was also significantly increased ($p<0.05$) in all the treatment groups administered with dexamethasone when compared with control. In addition, histology of the adrenal gland revealed that there was thickening of the capsule. The Zona glomerulosa was not so prominent. There was also a focal area of necrosis in the Zona glomerulosa. **Conclusion:** The results from this study suggest that maternal treatment with dexamethasone during lactation may reduce serum potassium ion concentration but increase serum calcium ion concentration.

INTRODUCTION

Dexamethasone is a fluorinated synthetic glucocorticoid with 25-fold glucocorticoid activity compared to cortisol (Tegethoff *et al*., 2009). Exposure of developing offspring to excess glucocorticoids from the mother may occur through specific and non-specific routes. The specific routes are those conditions peculiar to pregnancy or lactation, which may include the use of synthetic glucocorticoids for organ maturation in women with threatening preterm delivery (Singh *et al*., 2012) and in preterm infants (Wang *et al*., 2010). Synthetic glucocorticoids may also be administered to reduce female genital virilisation in congenital adrenal hyperplasia (Peiser *et al*., 2010; Clayton and Brook, 2012). However, women are also given synthetic glucocorticoids for other conditions that are not limited to pregnancy and lactation (non-specific). These conditions include asthma and other auto immune diseases (Singh *et al*., 2012).

Epidemiological reports in human and animal studies have shown that exposure to excess glucocorticoids may precipitate cardio-metabolic diseases (Drake *et al*., 2007). Studies in rats have shown that prenatal exposure to dexamethasone can have deleterious effects on the phenotypic outcome of organs such as the liver (Drake *et al*., 2007, Jeje and Raji, 2015), kidney, brain and heart (Singh *et al*., 2012). This may in the longer

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term contribute to adult onset diseases including hypertension, insulin resistance and Type-2 diabetes mellitus, among others (Drake *et al*., 2005, 2007).

It has been reported in literature that maternal exposure to excess glucocorticoids during gestation/lactation can alter activities at the hypothalamic-pituitary-adrenal axis in the offspring (Kapoor *et al*., 2006, Jeje and Raji, 2015). This alteration may lead to an increase in circulating basal corticosterone levels in the offspring (Jeje and Raji, 2015).

Glucocorticoids act physiologically at glucocorticoid receptors, but when in excess they can act non-physiologically on the mineralocorticoid receptors. The binding of glucocorticoids to mineralocorticoid receptors may affect homeostasis of the extracellular fluid and electrolyte balance (Sapolsky *et al.,* 2000; Aron *et al.,* 2007); however, little is known about the electrolyte distribution and histopathology of adrenal glands in the male offspring following maternal treatment with dexamethasone during lactation. Thus, this study was designed to evaluate the effects of maternal treatment with dexamethasone during lactation on serum electrolyte concentration and histology of the adrenal gland.

MATERIALS AND METHODS

Drug

Dexamethasone 21-Phosphate disodium salt purchased from Sigma Aldrich Chemical UK was used for this study. A dose of100 µg/Kg/day of dexamethasone was administered to the drug-treated groups (Drake et al., 2005).

Experimental animal

Twenty female Wistar rats weighing 150-180g purchased from the Central Animal House of the University of Ibadan were used for this study. The animals were housed in the Department of Physiology Animal House, University of Ibadan, Ibadan, Nigeria. After two weeks of acclimatization, animals in proestrus were exposed to male breeders overnight and the presence of sperm in their vaginal lavage on the morning after mating confirmed mating. The day on which spermatozoa were found in vaginal lavage was marked as gestation day 1(Gd1). After mating had been established, animals were randomly divided into four groups of 5 animals each and they were treated during lactation as described below (Table 1). Administration was between 09.00am and 10.00am daily. 100 µg/kg/day dexamethasone was administered to the drug-treated groups and 0.02 ml/100g/day normal saline was administered to the control. All treatments were administered subcutaneously. The litter size was standardized to 5pups/dam. All protocols involved in the animal experiments were conducted in accordance with ethical laws guiding animal care and use at the University of Ibadan.

The male offspring were allowed to grow to adulthood (12wk of age). Blood was collected from the orbital sinus before sacrifice. Rats were sacrificed by cervical dislocation. Adrenal tissues were carefully removed and fixed in 10% formalin for preparation of tissue histology. Serum was prepared by centrifuging the blood in non-anticoagulant tubes at 3000 RPM for 10 minutes.

Evaluation of serum electrolyte levels

The serum electrolytes were assessed by spectrophotometry using commercially available kits. Serum Sodium, Potassium and Chloride ion concentrations were assessed using Micropoint Diagnostic Kit (sourced from the United States) for Sodium, Potassium and Chloride ions respectively. Calcium ion concentration was assessed using Randox Diagnostic Kit (sourced from the United Kingdom) for calcium ion. These were done according to the manufacturer manuals.

Histopathology

Histology of adrenal gland was performed following hematoxylin and eosin staining techniques.

Statistical analysis

Data were expressed as mean ± Standard Error of Mean (Mean ± SEM). Statistical comparisons were performed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test to compare the means of the different treatment groups. Differences between the treatment groups with a p- value < 0.05 were considered significant. Data were analyzed with the use of Graphpad Prism Version 5.0 for Windows (GraphPad® Software, San Diego, CA, USA).

RESULTS

Effects of maternal treatment with dexamethasone during lactation on serum electrolyte distribution of male offspring at 12 weeks of age

The results showed that maternal dexamethasone treatment at lactation days 1-14 in rats significantly ($p< 0.05$) reduced serum potassium ion concentration but significantly ($p<0.05$) increased serum calcium ion concentration in the male offspring at adulthood. However, there was no significant change in serum sodium and chloride ion concentration.

Effects of maternal treatment with dexamethasone during lactation on serum electrolyte distribution of male offspring at 12 weeks of age

Histopathology of the adrenal gland in the Dex 1-7 group revealed a thin layer of Zona glomerulosa with normal zona fasciculate. A similar observation was

seen in the groups that were administered in the first 14 days and throughout lactation.

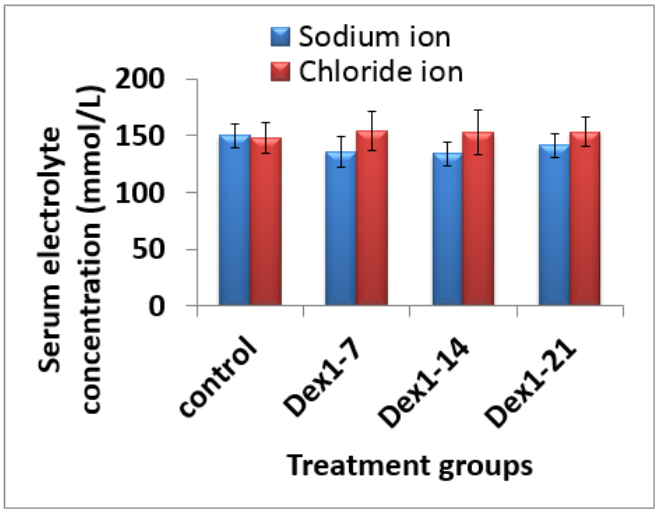

Fig. 1: Serum sodium and chloride ion concentration in male offspring at 12 weeks of postnatal life (n=6; Dex= Dexamethasone).

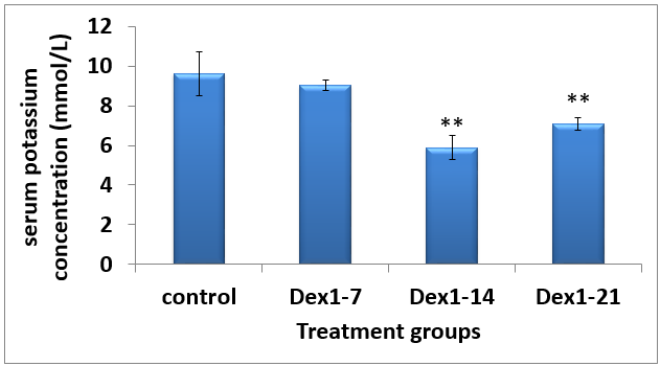

Fig. 2: Serum Potassium ion concentration in male offspring at 12 weeks of postnatal life (n=6), **p=0.001 was considered significant from the control; Dex= Dexamethasone

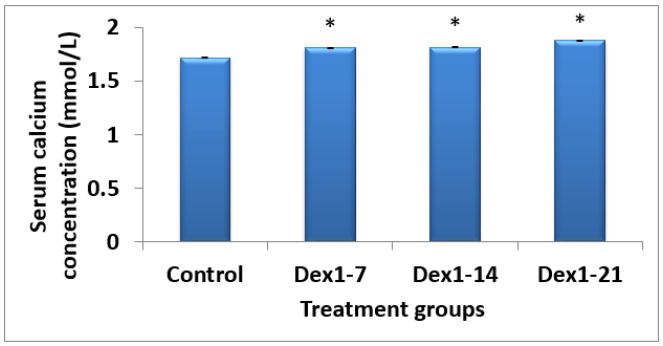

Fig. 3: Serum Calcium ion concentration in male offspring at 12 weeks of postnatal life (n=6), *p<0.05 was considered significant from the control); Dex= Dexamethasone

DISCUSSION

In this study, the serum electrolyte distribution in the male offspring following maternal treatment with dexamethasone during lactation was evaluated. The results showed a significant reduction in serum potassium ion concentration with an increase in serum calcium ion levels.

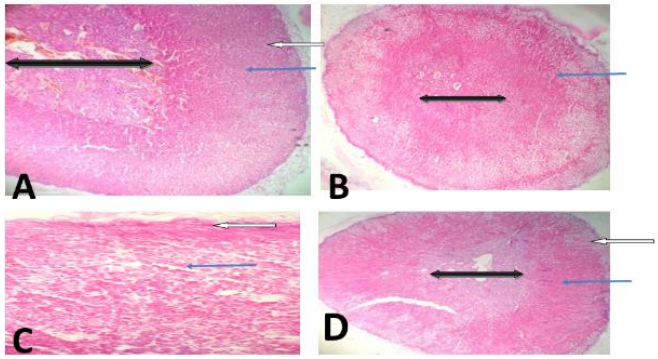

Fig. 4: Photomicrograph of the adrenal gland (A) control (B) Dex1-7 (C) Dex 1-14 and (D) Dex1-21. Black arrow: Adrenal medulla; White arrow: Zona glomerulosa; Blue arrow: Zona fasciculate. Zona glomerulosa is not so prominent in B, C and D. (H&E, X40); Dex= Dexamethasone

The observed hypokalaemia in the offspring of groups treated with dexamethasone is similar to what is obtainable in Cushing's syndrome (a clinical condition characterised by excess glucocorticoids) (Aron *et al*., 2007). Maternal dexamethasone treatment during gestation has been reported to increase both basal and stimulated glucocorticoid levels in the offspring in rats (Kapoor *et al*., 2006). Similar increase in serum corticosterone has also been observed in our laboratory due to exposure during lactation (Jeje and Raji, 2015). Therefore, it is possible that the alteration in serum electrolytes [K^+ and Ca^{2+}] is secondary to increased corticosteroid activity in the offspring.

Excess corticosterone increased the affinity of the hormones at both glucocorticoid and mineralocorticoid receptors. Binding of glucocorticoids to mineralocorticoid receptors exert mineralocorticoid effects, which is mainly electrolyte balance (Aron *et al*., 2007). The increased calcium ion concentration observed in this study may be due to osteoporosis associated with glucocorticoid excess (Aron et al., 2007). However, in patients with Cushing's syndrome, glucocorticoids may also induce hypercalciurea, which helps in maintaining the serum calcium concentration. In addition, neonatal weight has been reported to exhibit a positive association with whole body bone minerals (Gale et al., 2001), including calcium. The association is regardless of adult life style risk factors such as cigarette smoking, alcohol consumption, dietary calcium intake and physical activity (Gale et al., 2001).

According to Scaffer *et al*., (1975), the ultra-structural study of the adrenal gland in Cushing's syndrome suggests an inhibition in the activities of the zona

glomerulosa. This may be secondary to increases in the activities of the zona fasciculatae in the adrenal cortex. In agreement with this report, it was also observed in this study that the zona glomerulosa was less developed in all the groups treated with dexamethasone during lactation.

In conclusion, maternal treatment with dexamethasone in rats during lactation may alter serum electrolyte and architecture of the adrenal gland, especially zona glomerulosa in the male offspring. Establishing if the reduction in serum potassium is responsible for increased prevalence of cardio-metabolic diseases reported in the offspring of mothers exposed to dexamethasone during development requires further research.

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