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Evidence for the Alteration of Plasma Calcium by Synthetic Glucocorticiod Dexamethasone

N. Venkat Appa Rao*, Rakhee Dubey and Balarko Chakraborty
Department of Zoology, St. Xavier's College, Ranchi -834 001,India.
Email: venkap@rediffmail.com

Abstract : The present investigation deals with the effect of dexamethasone, a synthetic glucocorticoid on plasma calcium concentration in guinea pigs. The administration of dexamethasone @ 2.5 mg/kg body weight for seven consecutive days results to an elevation of plasma calcium concentration from a mean value of 4.51±0.106 mol eq/L to a mean value of 5.85±0.102 mol eq/L, an increase of about 30% which is statistically significant. The probable mode of action for dexamethasone is suggested.

Keywords : Dexamethasone, Guineapigs, Plasma calcium , Hypercalcemia.

Introduction

Calcium is one of the most important elements in the body of mammals including humans, involved in numerous physiological functions. It is the main structural component of bone and is also readily available to serve a variety of extracellular and intracellular activities e.g., its involvement in blood coagulation, nerve impulse conduction (Widdicombe, 1985), muscle contraction (Andrew, 1975), cell signaling (Clapham, 1995; Berridge *et al*., 2000; Carafoli *et al*., 2001), lymphocyte activation (Stefan, 2007), inhibition of renin secretion from kidney (Kurtz and Wagner, 1999) and mitochondrial functioning (Brini, 2003).

The homeostasis of calcium is classically maintained by parathormone [PTH], calcitonin, 1, 25 $(OH)_2$ D_3 and more recently discovered PTH related peptide [PTHrP] (Hadley and Levine, 2009). However, there exist a large number of non-classical modulators which also directly or indirectly influence the blood calcium concentration. One such modulator is glucocorticoid.

Effects of glucocorticoids in different mammals vary with species hence the results are often contradictory. Studies in rats have shown that glucocorticoids increase bone mineralization (Yasumura, 1976; Yasumura *et al*., 1976). The effects glucocorticoids on bone density in rats also varied with the dose of glucocorticoids administered (Jee, *et al*., 1970). On the other hand, rabbits lose bone rapidly with glucocorticoid therapy (Storey, 1961; Thompson and Urist, 1973). Limited reports are available on guinea pigs regarding glucocorticoid treatment and calcium regulation (Follis, 1951; Sobel, *et al*., 1960), although, guinea pigs and man both have cortisol is a predominant glucocorticoid. It seems that they would be a good animal model to use in such kind of studies. The present authors, therefore, took an attempt to investigate the effect of a synthetic glucocorticoid (dexamethasone, 9-fluoro-11β, 17, 21-trihydroxy-16a-methylpregna-1, 4-diene-3, 20- dione) on plasma calcium level in guinea pigs.

Materials and methods

For the present investigation, eighteen guinea pigs of both the sexes (Mammalia, Eutheria, Rodentia) weighing about 250-300 grams were procured from the local market of Ranchi and brought to the laboratory. They were acclimated to laboratory conditions for a period of fifteen days and fed with leaves of cauliflower and spinach *ad libitum*.

The animals then were divided into three groups. From the animals of Group 1 the blood was collected on day one and this group was treated as normal / control. Group 2 animals were injected with 2% normal saline alone for seven consecutive days, while group 3 individuals were treated with dexamethasone (Dexona, Cadila laboratories) intra-peritoneally @ 2.5 mg/kg body weight daily (Rao *et al.*, 1994) at 10 am for seven

* **Corresponding author :**

consecutive days. On the seventh day, one hour after the last injection, the blood was collected from individual each group. Group 2 treatment with saline was to assess the variation if any due to stress in blood parameters during the experimental phase.

All the samples were analyzed to measure the plasma calcium concentration together to minimize the inter assay variation if any, following the method of Tinder (1969).

The protocol is as follows :

Altogether 29 test tubes were taken and labeled as T_1-T_5 as blank, T_6-T_{11} as standard, T_{12} -T_{17} as group-1(1-6), T_{18}-T_{23} as group-2(7-12) and T_{24}-T_{29} as Group 3 (13-18).

InT_1-T_5, 0.2 ml of distilled water is taken and considered as blank.

In T_6-T_{11}, 0.2 ml of calcium solution is added in increased concentration; 1 mol eq/L, 2 mol eq/L, 4 mol eq/L, 6 mol eq/L, 8 mol eq/L and 10 mol eq/L respectively, these will help us to prepare a standard curve when required.

In T_{12} -T_{17}, 0.2 ml of samples of Group-1, in T_{18}-T_{23}, 0.2 ml of samples of Group-2 and in T_{24}-T_{29}, 0.2 ml of samples of Group-3 were added.

Now in each test tube 5 ml of calcium reagent 1 is added and incubated for half an hour at 37°C, cooled and centrifuged at 2000 rpm, the supernatant so formed were discarded.

To each test tube 1 ml of EDTA solution was added, the mixture is heated in water bath for one hour, cooled and in each test tube 3 ml of colour reagent was added. The colour so developed was measured by Systronic spectrocolorimeter at 450nm.

For measurement with the blank, zero was fixed and Optical Density (OD) values of different standard were measured followed by the samples. Considering the OD values the concentration was estimated by using the following formula:

$$\text{Concentration of Sample} = \frac{\text{OD value of sample}}{\text{OD value of standard}} \times 5 = \ldots\ldots\ldots \text{ mol eq/L}$$

The data obtained were statistically evaluated to paired sample t-test (Zar, 2006).

Results

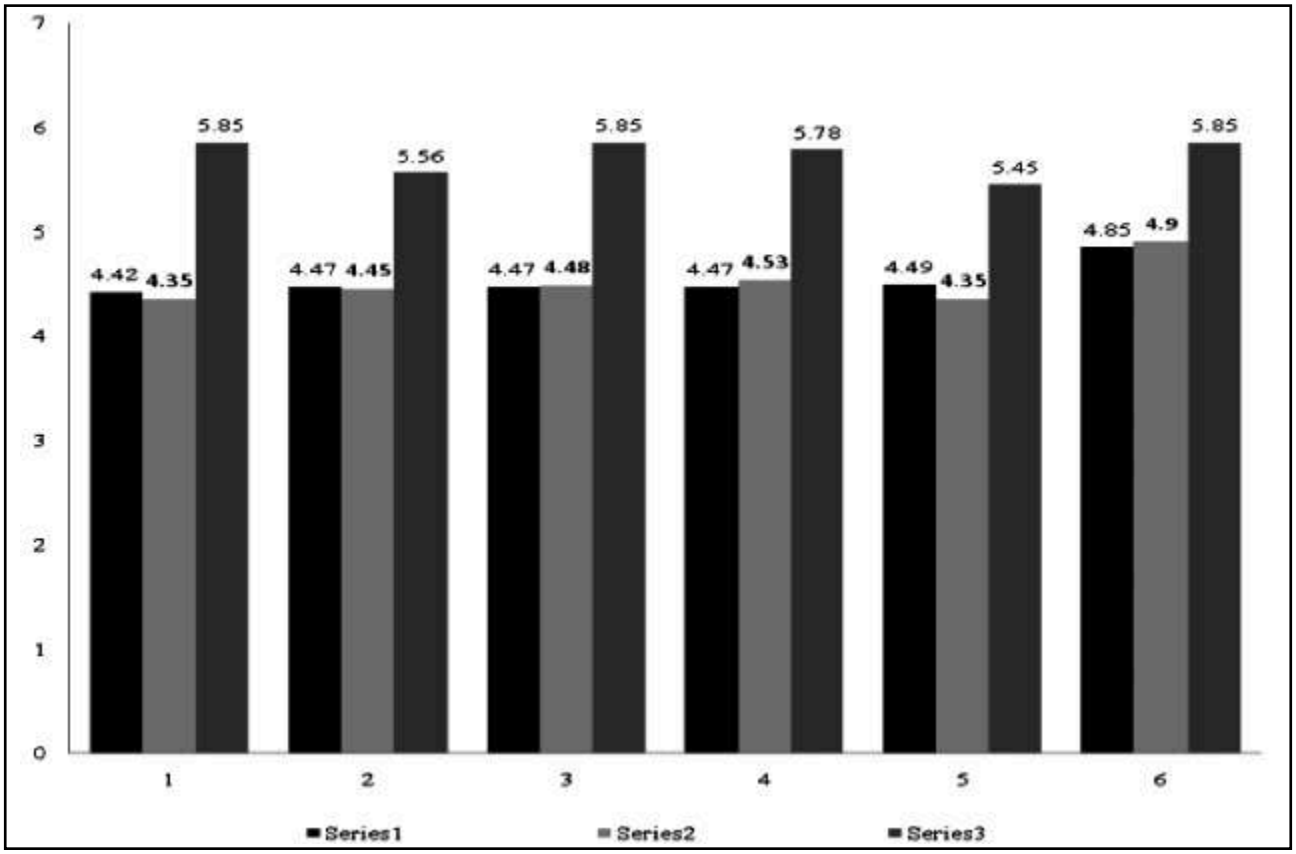
In normal/ control group of individuals, the plasma calcium concentration was found to be an average value of 4.52±0.104 mol eq/L on the first day while on last day it was 4.51±0.106 mol eq/L. However, the administration of dexamethasone for seven consecutive days@2.5 mg/ kg body weight caused a sharp increase in the level of plasma calcium from an average value of 4.51±0.106 mol eq/L to an average value of 5.85±0.102 mol eq/L an increase of about 30% (Table 1 & 2 and Fig. 1). The statistical analysis also indicates the elevation of plasma calcium due to the administration of dexamethasone @ 2.5 mg/kg body weight was a significant at less than 1% level (Table 2).

Table: 1. showing the mean calcium concentration in different treatment groups

Experimental Groups	Group-1. Normal on 1st day	Group-2. Normal (Saline treated) on last day	Group-3. Dexamethasone treated
Mean value of plasma calcium concentration	4.52±0.104 mol eq/L	4.51±0.106 mol eq/L	5.85±0.102 mol eq/L

Table: 2.showing the paired sample t-test values between different treatment groups.

	Gr.1: Gr.2	Gr.1: Gr.3	Gr. 2: Gr.3
t-value	-0.58882 (insignificant)	-14.3878**	-14.8257**



Series 1 Individuals of Gr. 1. on first day

Series 2 Individuals of Gr.2. saline treated

Series 3 Individuals of Gr.3. dexamethasone treated

Figure: 1. showing the plasma calcium concentration in different experimental groups expressed in mol. eq/L (one to one comparison)

Discussion

It is well established that glucocorticoids are anabolic steroids for hepatic cells and catabolic steroids for skeletal muscles as well as for adipose tissue (Hadley and Levine, 2009). Glucocorticods are widely known for their gluconeogenic, anti-inflammatory and anti-allergic activity (Norris, 2006). In recent past, multiple functional roles for synthetic glucocorticod, dexamethasone (9-fluoro-11β,17,21-trihydroxy-16a-methylpregna-1,4-diene-3,20-dione) is ascertained as it is often used in the treatment of asthma (Walsh, 2005), rheumatoid arthitis (Boers , 2004) and tissue/organ transplantation (DuPont *et al.*, 1984) .It is also used in post dental surgery, in countering the side effects of anti tumor treatment, in carcinomatous metastatic spinal cord compression (Sørensen *et al.*,1994), in counteracting development of edema in brain tumors (Kaal and Vecht, 2004) and in the treatment of congenital adrenal hyperplasia (Rivkees and Crawford, 2000). Such wide spread use of glucocorticoids also influences the calcium balance in the body. Glade and Krook (1982) and Patschan *et. al.* (2001) have observed that long term administration of glucocorticods causes osteoporosis and osteonecrosis. On the other hand, Klein *et. al*. (1977) and Cosman *et al*. (1994) have reported the decreased calcium absorption form dietary source and increased urinary elimination of calcium after the administration of dexamethasone. Weinstein *et al.* (1998) and O'Brien *et al.* (2004) have found that glucocorticoids are involved in inhibition of osteoblastogenesis and in the promotion of apoptosis of osteoblasts and osteocytes. On the contrary, Hirsch *et al.* (1998) have observed calcitonin like anti-hypercalcemic property of glucocorticoid but in parathyrodectomised rats.

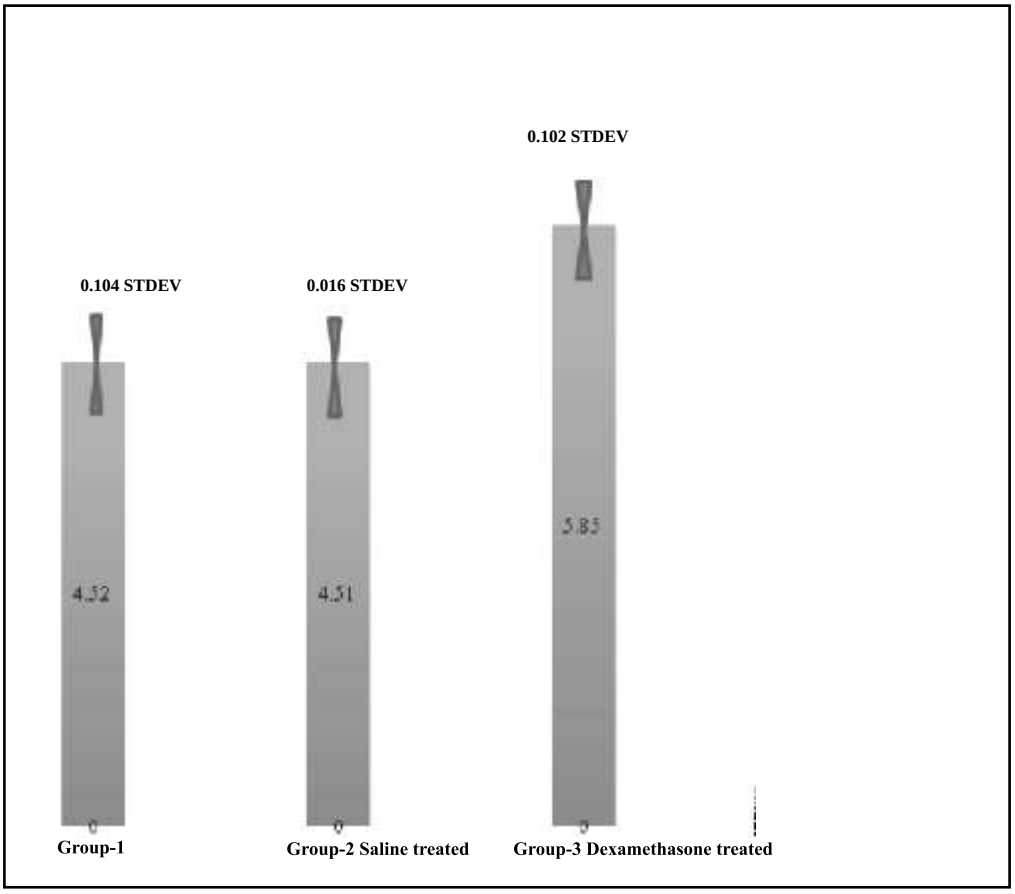


Figure: 2. indicating mean values of calcium in different experimental groups expressed in mol. eq/L

In the present investigation a significant increase in plasma calcium concentration has been observed after the administration of dexamethasone @ 2.5 mg/kg body weight for seven consecutive days. The probable action seems to be through demineralization of bone as dexamethasone is a potent apoptotic agent for osteoblasts and osteocytes. It can also be assumed that the action requires the presence of circulating parathyroid hormone hence in parathyrodectomised rats dexamethasone unable to cause hypercalcemic effect (Hirsch *et al.*, 1998) because parathormone and glucocorticoids act synergistically (Zhang *et al*., 1993).

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