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# DIETARY CALCIUM DEPRIVATION INCREASED THE LEVELS OF PLASMA CATECHOLAMINES AND CATECHOLAMINE-SYNTHESIZING ENZYMES OF ADRENAL GLANDS IN RATS

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**Abstract**—Rats on calcium-deficient diets developed hypocalcemia, hyperparathyroidism and hypertension and showed an increase in plasma catecholamines. Adrenal gland catecholamines were decreased while tyrosine hydroxylase (TH) and dopamine β-hydroxylase (DBH) were found to be increased, as compared to controls. In contrast, no significant differences were found between controls and parathyroidectomized rats in plasma catecholamines, and catecholamines, TH and DBH of the adrenal gland. These findings seem to indicate that the genesis of hypertension in rats on a low calcium diet is secondary to hyperparathyroidism caused by a low calcium diet. Furthermore, some relation between catecholamines and parathyroid hormone seems to exist in the regulation of blood pressure in rats.

There is increasing evidence that abnormal metabolism of calcium leads to hypertension. In spontaneously hypertensive rats (SHR), calcium supplementation lowers blood pressure in the younger rats and reverses the "fixed" hypertension of the adult ones [1, 2], suggesting that the dietary levels of calcium significantly influence the development and maintenance of increased arterial pressures. In Wistar–Kyoto rats which are the normotensive controls of SHR, the blood pressure is also influenced by the dietary calcium intake [3]. Similarly, the blood pressure in pregnant rats increases when dietary calcium is limited [4]. These results suggest that calcium levels influence blood pressure regulation in both SHR and normal rats.

The changes observed in calcium metabolism as a result of dietary calcium deficiency in rats were a reduction in serum of ionized calcium concentration [5], elevated levels of parathyroid hormone (PTH) [1], and enhanced urinary calcium excretion [1]. In early studies, we found that a low calcium diet causes hypocalcemia, nutritional hyperparathyroidism and hypertension in normal Wistar rats [6]. It was also demonstrated that chronic PTH deficiency impeded blood pressure increase in SHR [7] and that the vascular and PTH abnormalities were evident before blood pressure was significantly elevated [8]. These studies suggested that parathyroid function, which is regulated by serum calcium concentration, may play an important role in the genesis of hypertension. The present study indicates that hyperparathyroidism caused by a calcium deficient diet results in increased plasma catecholamines and increased catecholamine biosynthesis in the adrenal glands.

## MATERIALS AND METHODS

Three-week-old male Wistar rats were raised at 23° room temperature, with a 12 hr light/dark cycle, and fed *ad lib.* a casein-based synthetic diet [6]. Three groups were studied: rats receiving either a normal diet (0.3% Ca and 0.42% P, adequate for growing rats) or a calcium-deficient diet (0.01% Ca and 0.42% P, known to evoke a nutritional hyperparathyroidism [9]), and parathyroidectomized (PTX) rats receiving a normal diet. PTX was carried out under ether anesthesia. Catecholamines and tyrosine hydroxylase (TH) activity were assayed using high performance liquid chromatography (HPLC) with electrochemical detection (ECD) [10, 11]. Dopamine β-hydroxylase (DBH) activity was assayed by HPLC-ECD [12]. Protein was assayed by the method of Lowry *et al.* [13].

## RESULTS

Catecholamine levels in the plasma of controls, calcium deficient and PTX rats are shown in Table 1. The results indicate that 7 weeks of dietary calcium deprivation in rats increased significantly plasma norepinephrine (NE) and epinephrine (EN) concentrations compared to those on a normal calcium diet. On the other hand, the PTX rats showed decreased plasma NE concentration, but no difference in plasma EN concentration. Catecholamine levels in the adrenal glands of all three groups are also shown in Table 1; 7 weeks of dietary calcium deprivation significantly reduced NE and EN levels of the adrenal glands compared to those on a normal calcium diet.

Activities of TH and DBH in the adrenal glands of all three groups are shown in Table 2. TH activities of rats on low calcium diet were significantly higher than PTX rats on normal calcium diets. There was an approximately 1.5-fold increase in the enzymatic activity of TH and DBH in rats on low calcium diets. The increase in TH activity in the adrenal glands of rats on low calcium diet may have been caused by

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Table 1. Catecholamine levels in plasma and adrenal glands of rats

| | Plasma (pg/mL) | | Adrenal glands (μg/g) | |
|---|---|---|---|---|
| | NE | EN | NE | EN |
| Control* | 304 ± 88 | 138 ± 46 | 170 ± 38 | 701 ± 156 |
| Ca-deficiency† | 470 ± 88‖ | 283 ± 66¶ | 147 ± 21¶ | 485 ± 105¶ |
| PTX‡ | 224 ± 57§ | 110 ± 34 | 184 ± 12 | 651 ± 58 |

* Normal diet (0.3% Ca and 0.42% P) for 7 weeks.
† Low calcium diet (0.01% Ca and 0.42% P) for 7 weeks.
‡ Normal calcium diet for 4 weeks, parathyroidectomized (PTX), then placed on a normal diet for 3 weeks.
Each value represents mean ± SD (N = 6–14) at the age of 10 weeks.
Statistical difference from the controls; §$P < 0.02$, ‖$P < 0.005$ and ¶$P < 0.001$.

Table 2. Activities of TH and DBH in adrenal glands of rats

| | TH activity | DBH activity |
|---|---|---|
| | (nmol/min/g) | |
| Control* | 174 ± 30 | 103 ± 29 |
| Ca-deficiency† | 246 ± 40§ | 176 ± 39§ |
| PTX‡ | 197 ± 46 | 113 ± 23 |

* Normal diet (0.3% Ca and 0.42% P) for 7 weeks.
† Low calcium diet (0.01% Ca and 0.42% P) for 7 weeks.
‡ Normal calcium diet for 4 weeks, parathyroidectomized (PTX), then on a normal diet for 3 weeks.
Each value represents mean ± SD (N = 6–14) at the age of 10 weeks.
Statistical difference from the controls; §$P < 0.001$.

an increase in the amount of the enzymes within the gland. In contrast, PTX rats showed lowered plasma NE and EN and unchanged NE, EN, TH and DBH in the adrenal glands (Tables 1 and 2).

## DISCUSSION

As reported previously [6], hypertension that develops in rats due to a low calcium diet accompanies dietary hyperparathyroidism as well as hypocalcemia. Elevation of blood pressure was not observed in PTX rats. In the present study, we found that plasma catecholamines and TH and DBH activities in the adrenal glands increased in rats on low calcium diets but not in PTX rats, suggesting that hyperparathyroidism but not hypocalcemia, may be related to increased catecholamines. The present results also agree with the earlier observations of Baksi and Hughes [14] that adrenal catecholamine levels decrease in rats following dietary calcium deprivation [14]. The results may underscore the importance of both central and peripheral NE in the development of essential hypertension [15]. The mechanism underlying the relationship between hyperparathyroidism and enhanced catecholamine metabolism remains to be elucidated, and the functional role of changes in plasma catecholamine remains unexplained.

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