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# ELEVATION OF BLOOD PRESSURE IN YOUNG RATS FED A LOW CALCIUM DIET

## EFFECTS OF NIFEDIPINE AND CAPTOPRIL

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**Abstract**—Hypertension was developed in 5-week-old male rats fed a low calcium diet, which evokes hypocalcemia and nutritional hyperparathyroidism, for 2 weeks. Blood pressure returned to normal after changing to a normal calcium diet. These changes of blood pressure were preceded by changes of calcium levels in plasma. In parathyroidectomized rats receiving a normal calcium diet, blood pressure did not rise, though the plasma calcium level decreased to a similar extent as in rats fed the low calcium diet. These findings seem to indicate that hyperparathyroidism, not hypocalcemia, is involved in the elevation of blood pressure in rats fed a low calcium diet. The elevated blood pressure was reduced by a calcium antagonist, nifedipine, but not by an inhibitor of angiotensin-converting enzyme, captopril. This may indicate that hypertension due to nutritional hyperparathyroidism responds to the calcium antagonist nifedipine and calcium supplementation, and is not dependent on renin.

There is increasing evidence for abnormal metabolism of calcium in hypertension [1, 2]. In spontaneously hypertensive rats (SHR), calcium supplementation lowered blood pressure in the younger rats and reversed the "fixed" hypertension of the adult rats [3–5], suggesting that the level of dietary calcium significantly influences the development and maintenance of increased arterial pressure. In Wistar–Kyoto rats, which are the normotensive genetic control for SHR, blood pressure was also influenced by the dietary calcium level [6]. Similarly, blood pressure in young or pregnant rats was increased by dietary calcium deficiency [7, 8]. These findings suggest that, in both experimental hypertensive rats and normal ones, calcium balance may be important in blood pressure regulation.

In experimental hypertension, abnormal findings in calcium metabolism include reduced serum ionized calcium concentrations [9], elevated levels of parathyroid hormone (PTH) [3], and enhanced urinary calcium excretion [3]. Recently, it was demonstrated that chronic PTH deficiency impeded blood pressure increase in SHR [10, 11] and that the vascular and PTH abnormalities were evident before blood pressure was significantly elevated [12]. These studies suggest that parathyroid function, which is regulated by serum calcium concentration, plays an important role in genesis of hypertension. Nevertheless, the cause–effect relationship between hyperparathyroidism and hypertension is still debatable, and has been discussed in a recent paper by Pang *et al.* [13].

Recently, using a low calcium diet to evoke hypocalcemia and nutritional hyperparathyroidism [14], we confirmed Itokawa *et al.*'s observation of increased blood pressure in calcium deficient normal Wistar rats [7]. Our present study describes some properties of the hypertension in rats fed a low calcium diet and examines the effects of two antihypertensive drugs with different vasodilating mechanisms, nifedipine and captopril, on the hypertension.

Table 1. Composition of diet

| Ingredients | % |
|---|---|
| Cerelose (Glucose $H_2O$) | 64.5 |
| Casein | 18.0 |
| Roughage celluflour | 3.0 |
| Cottonseed oil | 10.5 |
| Cystine | 0.2 |
| Choline chloride | 0.2 |
| Mineral mixture* | 4.0 |
| Vitamin mixture† | 0.1 |

* In the normal diet (milligrams per 100 g diet): $CaCO_3$ 750; $K_2HPO_4$ 707; $KH_2PO_4$ 553; KCl 1144; NaCl 418; $MgSO_4$ 318; $FeSO_4$ 35; $ZnSO_4$ 4.9; NaF 2.3; $CuSO_4$ 1.0; $MnSO_4$ 0.7; $(NH_4)_6Mo_7O_{24}$ 0.09; $CoCl_2$ 0.06; in the low calcium diet the same except for the absence of $CaCO_3$.

† Vitamin mixture (milligrams per 100 g diet): inositol 20.0; Ca-pantothenate 2.8; nicotinamide 2.0; thiamine 0.5; riboflavin 0.5; pyridoxine 0.5; folic acid 0.02; biotin 0.01; cyanocobalamin 0.002; the fat-soluble vitamins (E, K, A and D) already dissolved in the cottonseed oil.

### MATERIALS AND METHODS

*Animals.* Male Wistar rats, suckled by their mothers receiving standard laboratory diet (Labo MR Stock: Nihon Nosan Co., Ltd., Yokohama, Japan), were purchased at 3 weeks of age from Shizuoka Laboratory Animal Center (Hamamatsu, Japan). The rats were housed five per cage, at 23°, with a 12 hr light–dark cycle, and fed *ad lib.* a casein-based synthetic diet (Table 1). Three groups were

studied: intact rats receiving either normal diet (NCaD; Ca 0.3%, P 0.42%, adequate for growing rats) or low Ca diet (LCaD; Ca 0.01%, P 0.42%, known to evoke nutritional hyperparathyroidism [15], and parathyroidectomized rats receiving only the NCaD. The parathyroidectomy (PTX) was carried out under ether anesthesia.

*Blood pressure measurements.* Each rat was warmed for 10 min at 40° to dilate the tail arteries, and was restrained in a rat holder. Systolic blood pressure and heart rate were measured in the conscious rat with a tail-cuff. Five readings were averaged for each rat at each measurement.

*Administration of drugs.* Nifedipine (Sigma Chemical Co., St. Louis, MO) or captopril (Captoril®, Sankyo Co. Ltd., Tokyo, Japan) were suspended in 0.2% carboxymethyl cellulose (CMC) just before administration, and given p.o. with a gastric tube.

*Analytical procedures.* Plasma total calcium concentrations were measured by atomic absorption spectrophotometry after samples were diluted in lanthanum–HCl to eliminate interference by plasma phosphate. Plasma ionized calcium values were determined by a calcium-specific electrode. Plasma phosphate was measured by the method of Fiske and Subbarow [16]. Alkaline and acid phosphatase activities in plasma were assayed by determining the release of phenol from phenylphosphate at 37° for 10 min, using high-performance liquid chromatography with electrochemical detection [17]. Plasma PTH concentrations were determined by Intact-N-terminal Specific (INS) PTH RIA kit (Nichols Institute Diagnostics, San Juan Capistrano, CA). This assay requires 4 days incubation in the presence of chicken anti-PTH serum, 2 days with the antibody and $^{125}$I-bovine PTH(1–84) tracer, and 4 hr in the presence of second antibody precipitant. Human PTH(1–34) were used as standards.

## RESULTS

The changes in body weight, systolic blood pressure and plasma total calcium concentration in rats fed NCaD or LCaD are shown in Fig. 1. Good growth was observed in rats fed NCaD. Body weights of rats fed LCaD reached a plateau at 3 weeks, but began increasing again after changing to NCaD. In rats fed LCaD for 2 weeks, systolic blood pressure was substantially higher than that in rats fed NCaD (Fig. 1B). This difference remained significant during the following 5 weeks. The rats fed LCaD for 4 weeks returned to normal blood pressure after changing to NCaD, indicating that the hypertensive effect of LCaD is reversible. These blood pressure changes were preceded by changes in plasma calcium levels, as shown in Fig. 1C.

Table 2 shows the rats' blood pressure and plasma biochemistry. In rats fed LCaD, total and ionized plasma calcium were significantly lower than in rats fed NCaD. On the other hand, plasma alkaline phosphatase activity and PTH concentration were higher in rats fed LCaD than in rats fed NCaD, indicating that those fed LCaD had hyperparathyroidism. In parathyroidectomized rats fed NCaD, total and ionized calcium in plasma significantly decreased and were almost the same as in rats fed LCaD. However, unlike the rats fed LCaD, there was no blood pressure increase. Plasma phosphate was elevated by PTX but not influenced by LCaD.

The effects of nifedipine and captopril on systolic blood pressure and heart rate in rats fed either normal or low calcium diet for 5 weeks are shown in Fig. 2. For both types of diet, nifedipine (5 and 10 mg/kg p.o.) decreased the blood pressure and increased the heart rate. These effects were observed 1 hr after administration. Nifedipine proved more potent in reducing blood pressure in LCaD rats than in NCaD rats (Fig. 2A). Captopril (5 and 10 mg/kg p.o.) had no effect on blood pressure or heart rate in rats fed either NCaD or LCaD for 5 weeks, as shown in Fig. 2B.

## DISCUSSION

The present study observed that a decrease of dietary calcium significantly elevated blood pressure in young Wistar rats, and that the elevated blood pressure was reduced by an increase of dietary calcium. This supports the experimental observations in normotensive rats and SHR [1, 6, 7, 18] that calcium balance may be important in blood pressure regulation. However, the antihypertensive effect of calcium supplementation in rats fed LCaD was different from that in SHR. The antihypertensive effect observed in SHR fed an extremely high calcium diet (2.5–4%) [3–5] was mediated by phosphate deficiency independently of PTH [5]. The elevated blood pressure in rats fed LCaD was reduced by NCaD (0.3%) and phosphate was not involved in these changes (Table 2).

Dose alteration in serum calcium play a major role in the initial development and maintenance of high blood pressure in rats fed LCaD? PTX reduces the hypertensive effect of mineralocorticoid treatment [19] and impedes blood pressure increase in SHR [10, 11]. Therefore, PTH seems to be one of the factors which regulate blood pressure in experimental hypertension. In our experiment, the hypertension in rats fed LCaD was associated with hypocalcemia and hyperparathyroidism, but changes in plasma calcium concentration in the absence of PTH did not modify blood pressure in rats. As shown in Table 2, in parathyroidectomized rats, decreased plasma calcium did not elevate the blood pressure. This suggests that it is not the decrease of plasma calcium but rather the increase of parathyroid function which generates hypertension in rats fed LCaD.

What is the role of PTH in this kind of hypertension? One possible etiology for the hypertension is an alteration in the renin–angiotensin system, because of *in vitro* stimulation of renin release by calcium and PTH [20] and *in vivo* elevation of plasma renin activity by injection of synthetic bovine PTH [1–34] in saline-loaded dogs [21]. To assess the renin dependency of the hypertension, we examined the effect of captopril, an inhibitor of angiotensin-converting enzyme. As shown in Fig. 2B, the hypertension was not influenced by captopril (5 and 10 mg/kg, p.o.). Captopril at 10 mg/kg p.o. exhibited maximum antihypertensive activity in the two-kidney Goldblatt hypertensive rat [22]. Our results suggest

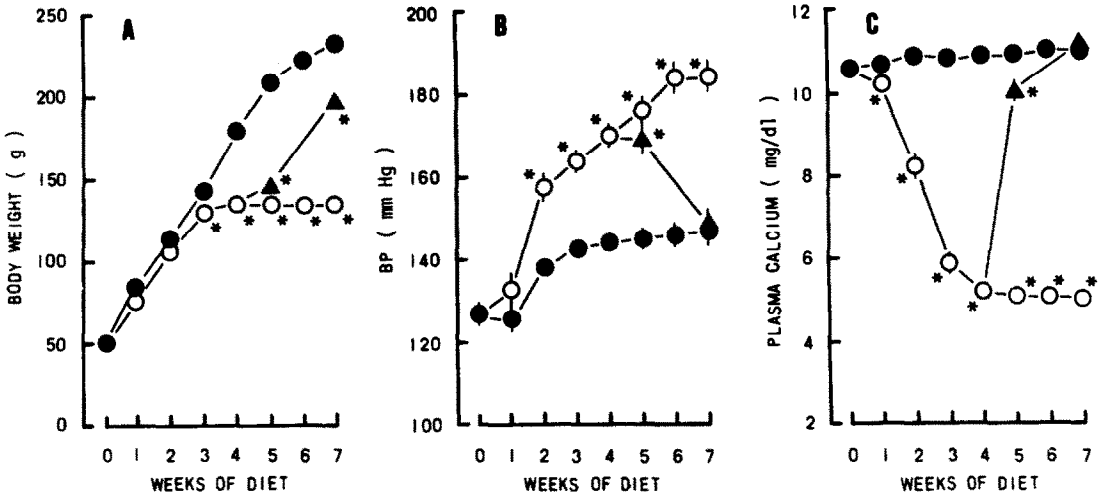


Fig. 1. Changes in body weight (A), blood pressure (B) and plasma calcium (C) in rats. Rats were fed a normal calcium diet (●) or a low calcium diet (○) for 7 weeks. Some of the low calcium diet rats were changed to a normal calcium diet after 4 weeks (▲). To investigate the calcium levels in plasma, blood samples were obtained from the tail vein with a heparinized capillary tube. Each point represents the mean value, and the bar indicates the SE (N = 6–12). The age of the rats used was 3 weeks. (*$P < 0.01$, statistical difference from the normal calcium diet group at corresponding time, by Student's *t*-test or Cochran–Cox test.)

Table 2. Influence of chronically affected parathyroid function on blood pressure, total plasma calcium, ionized plasma calcium, plasma phosphate, plasma alkaline and acid phosphatase, and plasma parathyroid hormone

| | Intact rats | | PTX rats§ |
|---|---|---|---|
| | NCaD group† | LCaD group‡ | NCaD group |
| Blood pressure (mmHg) | 149.9 ± 2.4 | 181.5 ± 3.7* | 151.0 ± 4.0 |
| Heart rate (beats/min) | 383.0 ± 9.0 | 389.5 ± 4.7 | 374.8 ± 9.2 |
| Total calcium (mg/dl) | 10.3 ± 0.1 | 4.7 ± 0.1* | 5.6 ± 0.3* |
| Ionized calcium (mg/dl) | 5.3 ± 0.1 | 2.3 ± 0.1* | 2.6 ± 0.2* |
| Phosphate (mg/dl) | 6.2 ± 0.1 | 6.4 ± 0.2 | 13.3 ± 0.5* |
| Alkaline phosphatase (nmol/min/ml) | 188.2 ± 8.0 | 559.2 ± 21.5* | 206.0 ± 6.6 |
| Acid phosphatase (nmol/min/ml) | 14.9 ± 0.7 | 14.6 ± 1.5 | 13.5 ± 1.3 |
| Parathyroid hormone (pg/ml) | 32.7 ± 4.6 | 550.5 ± 53.0* | n.d. |

† Fed NCaD (Ca 0.3%; P 0.42%) for 7 weeks.
‡ Fed LCaD (Ca 0.01%; P 0.42%) for 7 weeks.
§ Fed NCaD for 4 weeks, then parathyroidectomized, then fed NCaD for 3 weeks.
Each value represents the mean ± SE (N = 5–12). n.d.: not determined. Age of rats used was 10 weeks. *$P < 0.01$, statistical difference from the normal calcium diet group, by Student's *t*-test or Cochran–Cox test.

that hypertension associated with hyperparathyroidism is not renin dependent, which is consistent with clinical observations that elevated blood pressure is not reduced by saralasin in patients with hyperparathyroidism [23]. Resnick [24] emphasized the biochemical and clinical heterogeneity of human hypertension, and claimed that calcium supplementation in human hypertension may decrease or increase the blood pressure in low- or high-renin essential hypertension, respectively. Hypertension in rats fed LCaD, which is reversed by calcium supplementation but not by captopril, may therefore have a similar profile to low renin essential hypertension.

Treatment with calcium antagonist as well as calcium supplementation were beneficial in hypertension with nutritional hyperparathyroidism. These results are consistent with recent clinical observations that essential hypertensive patients with low renin, low ionized calcium and elevated PTH respond to the calcium antagonist nifedipine, and have significantly lower blood pressure in response to oral calcium supplementation [25]. Nifedipine reduces vascular tone by inhibiting the influx of extracellular calcium via calcium channels into smooth muscle cells [26–28]. The hypotensive effect of nifedipine was more pronounced in LCaD rats than in NCaD rats (Fig. 2A), which supports the idea

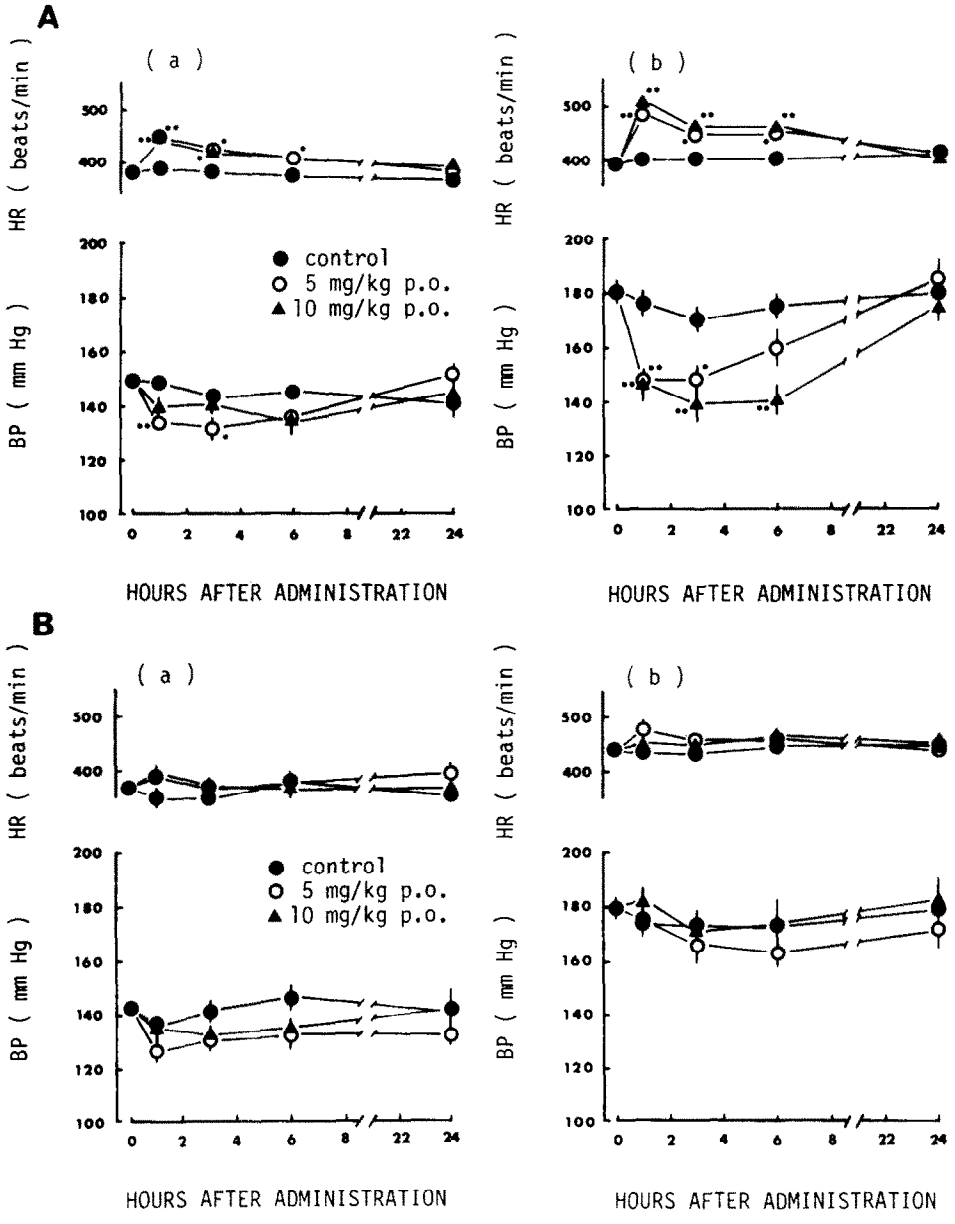


Fig. 2. Effects of nifedipine (A) and captopril (B) on blood pressure (BP, lower panel) and heart rate (HR, upper panel) in rats fed a normal calcium diet (a) or low calcium diet (b) for 5 weeks. The drug was given orally at time 0. Control rats were given the vehicle p.o. with a gastric tube. Each point represents the mean value. Bar indicates SE (N = 6). The age of the rats used was 8 weeks. (*$P < 0.05$, **$P < 0.01$, statistical difference of the observed changes at each time point, determined by Dunnett's method for comparing multiple treatments with a control, using analysis of variance.)

that the basic change occurring in vascular smooth muscle with the development of hypertension is an increase in cell membrane permeability to calcium. Since PTH enhances calcium entry into and increases calcium content of many tissues [29–31], chronic hyperparathyroidism in rats may increase calcium-influx and thereby increase vascular tone, leading to elevated blood pressure. However, in acute experiments, PTH possessed vasodilating action [32–35]. This may indicate either the difference between acute and chronic effects of PTH on the vasculature, or the involvement of another factor under chronic hyperparathyroidism, such as catecholamine and/or the release of a hypertensive substance, as yet not identified, from the hypertrophic parathyroid.

In conclusion, the hypertension in young growing rats fed LCaD may be due to nutritional hyperparathyroidism and may be a good experimental model for investigating the relationship between altered calcium regulation and elevation or maintenance of blood pressure, and for pharmacological evaluation of antihypertensive drugs. This type of

hypertension is reduced by a calcium antagonist and calcium supplementation but not by treatment with an inhibitor of angiotensin-converting enzyme.

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