THE MEDIATING ROLE OF THE PARATHYROID GLAND IN THE EFFECT OF LOW CALCIUM INTAKE ON BLOOD PRESSURE IN THE RAT

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SUMMARY

Recent reports suggest an inverse relationship between calcium intake and blood pressure. This effect could be mediated by parathormone (PTH), since a low calcium intake leads to an increase in PTH and this hormone produces an increase in intracellular calcium, raising the excitability of the muscle arteriolar cells. Wistar female rats, 56 days old, were submitted to a parathyroidectomy or to a sham operation. After that, they were placed on a normal or on a calcium-free diet during 10 weeks. Four groups of nine rats were studied: parathyroidectomized animals on a normal calcium diet, parathyroidectomized ones on a calcium-free diet, controls (sham operation) on a normal calcium diet, and controls (sham operation) on a calcium-free diet. The control calcium-free diet showed a significant increase in blood pressure values over the treatment period. The parathyroidectomized calcium-free diet group did not show any increase. The difference between these two groups regarding change in blood pressure was statistically significant. The parathyroidectomized-calcium-free group showed no weight increase during the study, while rats in the other three groups significantly increased their weight. PTH could be the mediator of the blood pressure rise observed in the calcium-deprived rats in spite of the possible confounding effect of the poor weight increase detected in the parathyroidectomized-calcium deprived animals.

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These results warrant future studies since the role of PTH in the regulation of blood pressure needs to be confirmed. This possibility, therefore, opens a new area of research in the study of the pathophysiology of hypertension.

INTRODUCTION

Recent reports indicate that calcium intake may have an effect on blood pressure. Epidemiological studies show that individuals who drink "hard water" (with high calcium content) have low blood pressure values (1, 2). An inverse relationship has been observed between calcium intake and pregnancy-induced hypertension (3).

In a recent clinical trial we observed that young healthy individuals who received a daily supplement of one g of elemental calcium had a significant reduction in diastolic blood pressure values (4). This effect was also observed in pregnant women, the blood pressure values being lower in women with the highest calcium intake (5).

Rats on a calcium-free diet showed significant increases in blood pressure values (6, 7); on the other hand, rats which spontaneously developed hypertension (SHR) showed a significant reduction in their blood pressure values when placed on a diet with a double calcium concentration (8).

Parathormone (PTH) could be the mediator of the relationship between calcium intake and blood pressure. Parathormone secretion is very sensitive to changes in calcium intake. A rise in PTH stimulates calcium influx to the cell, increasing intracellular calcium concentration in several kinds of cells such as kidney (9), bone (10), liver (11) and HeLa cells (12). An increase of intracellular ionic calcium in smooth muscle is responsible for the mechanical activation of the muscle cell (13).

In the calcium supplementation study on pregnant women, those women with higher Ca supplementation and lower blood pressure values showed lower values of serum PTH (5).

This report explores the possible role of PTH in the relationship between calcium intake and blood pressure changes in rats.

METHODS

Forty-two female Wistar rats, 56 days old, were randomly assigned to undergo either parathyroidectomy (n = 24) or a sham operation (n = 18). The parathyroidectomy (PTX) was surgically performed, removing both glands but leaving the thyroid glands as intact as possible. Similar techniques were performed on the animals included in the sham operation group, but without removing the glands. Both groups (PTX and sham operation) drank water containing 2 mg of calcium chloride per liter during three days following the operation, in order to avoid the onset of acute hypocalcemia after the glands were removed.

One week after the operation, blood samples were obtained by performing a small cut in the tails of all animals. In order to assess the efficacy of the parathyroidectomy, total calcium was measured in all samples by atomic absorption spectrophotometry. The 18 animals with

sham operation had a mean serum calcium value of 11.08 mg/dl (SD = 0.49), while the 19. rats in the parathyroidectomized group exhibited values velow 8.50 mg/dl (5 SD below the mean value of the control group). Five animals in the PTX group, with values above 8.50 mg/dl (mean 10.23 SD = 0.76), were considered to have undergone unsuccessful parathyroidectomies, and were excluded from the study. One of the PTX rats died two days after blood sampling. Therefore, the two groups (sham operation and PTX) were finally composed of 18 animals each.

On the day after the blood sample was obtained, the 18 PTX and 18 non-parathyroidectomized (non-PTX) animals were randomly assigned to a normal calcium diet group, or to a calcium-free diet group. The diet used was described in detail in a previous report (7). Basically, however, it contains 18o/o casein with 600 mg of calcium per 100 g of diet. The calcium-free diet had the same composition but the calcium was lacking. Therefore, four groups of nine animals each, were studied:

1 – Parathyroidectomized – Normal calcium diet group (PTX-Ca)

2 – Parathyroidectomized – Calcium-free diet group (PTX-noCa)

3 – Non-parathyroidectomized – Normal calcium diet group (PT-Ca) and

4 – Non-parathyroidectomized – Calcium-free diet group (PT-noCa)

The animals were kept in individual cages during the complete study period; room temperature was kept between 25 and 27°C and the natural periods of light and darkness were not altered.

Body weight and blood pressure measurements were begun on the same day as the differential diets. All the blood pressure baseline measures were obtained before the blood samples were drawn. Weekly measurements of body weight and systolic blood pressure were performed for a 10-week period. Systolic blood pressure was measured with a tail cuff and a pressure transducer linked to a physiograph via an amplifier. At the end of those 10 weeks the rats were anesthetized with ether; blood samples were taken from the abdominal aorta, the animal was sacrificed and the right femur excised.

Total serum calcium and magnesium levels were measured by atomic absorption spectrophotometry. Bones were reduced to ashes in an oven at 600°C. and total bone calcium was measured by atomic absorption spectrophotometry. All measurements were carried out in duplicate and the mean value was derived.

In order to reduce the effect of the systolic blood pressure (SBP) variability observed (Figure 1), data were also analyzed after smoothed. For that, a regression equation of SBP on time was calculated for each individual by the least squares method. The predicted SBP values of each animal were used to calculated mean group differences in SBP between the initial and final measure. A covariance analysis was performed on the mean difference of SBP, including the initial SBP as a covariate to control for regression to the mean effect. The group means of change in smoothed SBP values are presented in Table 1.

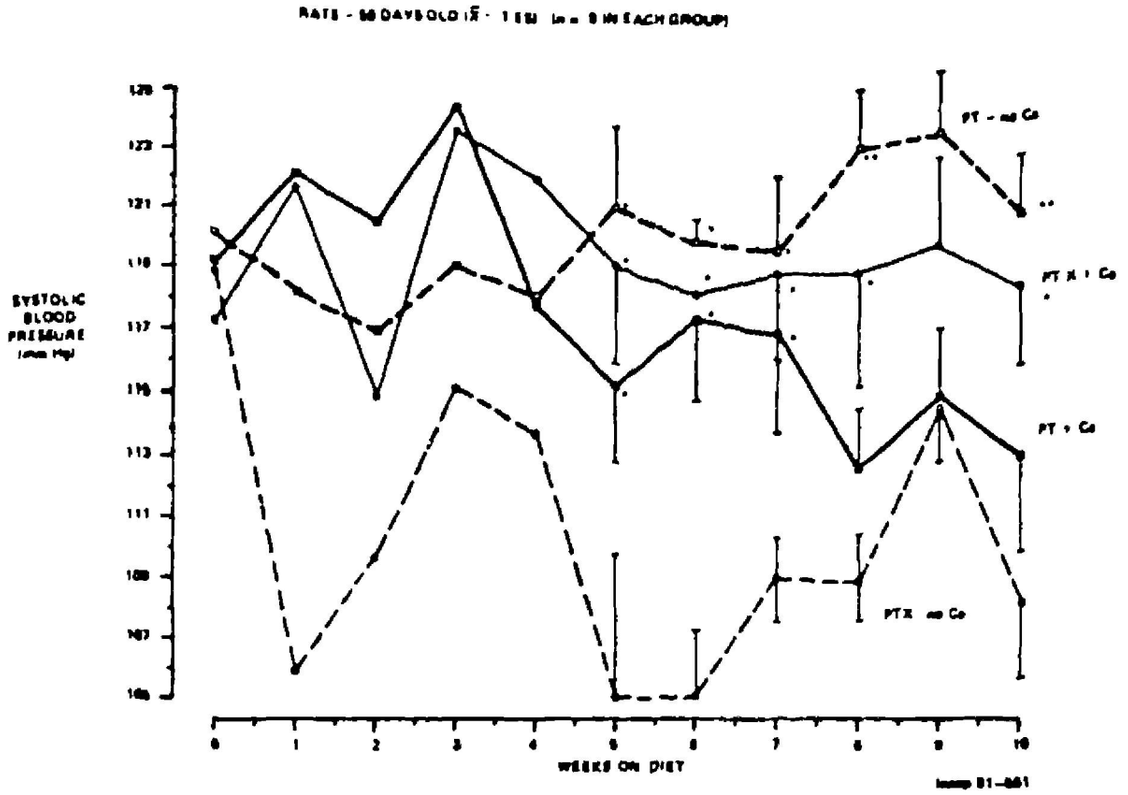

FIGURE 1

Values of systolic blood pressure during the study. The control calcium-free diet group (PT-no Ca) showed a significant increase of blood pressure values after eight weeks of diet, in relation to the control normal calcium group (PT-Ca). The parathyroidectomized calcium-free diet group (PTX-no Ca) exhibited significantly lower values of blood pressure throughout the study.

RESULTS

Mean systolic blood pressure values for the four groups throughout the study period are presented in Figure 1. Before the initiation of the diet (week 0), there were no significant differences among them. By the first week after initiation of treatment the PTX-no Ca group showed a decrease in blood pressure values, when compared to the other three groups. The blood pressure values in the PTX-no Ca group remained the lowest during the 10 weeks of study. By contrast, an increase in SBP values was observed in the control group (PT-no Ca) after eight weeks.

The overall differences among treatment groups for change in SBP were found highly significant ($F = 7.4$ $p < 0.005$). An analysis of all pairwise differences between treatment group means was performed. The t-statistics for between group differences are presented in Table 2, together with (*) indicating which differences exceeded the critical value of 1.97 computed using Duncan's K-ratio procedure. As can be observed, after adjusting for the initial values, the group with the sham operation and calcium-free diet (PT-no Ca) had a mean increase in blood pressure of 3.44 mmHg during the study. This is significantly higher than the

TABLE 1

MEAN ADJUSTED SYSTOLIC BLOOD PRESSURE DIFFERENCE (mmHg) BETWEEN INITIAL AND FINAL VALUES FOR THE FOUR STUDIED GROUPS

Group	No of rats	Mean (mmHg)	SE
PT-No Ca	9	3.44	1.95
PTX-No Ca	9	−9.67	2.05
PT-Ca	9	−5.50	2.01
PTX-Ca	9	1.37	1.95

TABLE 2

T-STATISTICS FOR TESTING PAIR-WISE DIFFERENCES IN ADJUSTED GROUP MEANS

	PT-no Ca	PT-Ca	PTX-no Ca
PT-Ca	3.20*		
PTX-No Ca	4.64*	1.45	
PTX-Ca	0.75	−2.45*	−3.90*

* Above the t-statistics critical value: 1.97.
(Calculated using Duncan's K-ratio procedure).

mean differences for the PT-Ca group (−5.50 mmHg), and than those for the PTX-no Ca group (−9.67 mmHg). There were no differences of statistical significance between the PT-Ca and PTX-no Ca groups.

At the beginning of the study the four groups showed similar body weight values, but by the first week after treatment, the PTX-no Ca group had lower weight values and weight gain, a pattern that was maintained during the rest of the study (Table 3).

The biochemical information is shown in Table 4. Mean values are compared using a one-way analysis of variance. The mean baseline calcium values of the PTX groups were, as expected, well below those of the control groups ($F = 133.28$, $p < 0.0001$).

By the end of the study the PTX-no Ca group showed a decrease in calcium serum values, while the PTX-Ca group presented little increase in relation to the initial values. Both had significantly lower values than the groups submitted to the sham operation ($F = 16.51$, $p < 0.0001$). Significantly higher magnesium values than the other three groups were detected in the control-no calcium group (PT-no Ca) ($F = 4.88$, $p < 0.01$), while it showed significantly lower calcium values in the femur than the other three groups at the end of the study period ($F = 9.26$, $p < 0.0001$).

TABLE 3

VALUES OF BODY WEIGHT DURING THE STUDY (MEAN AND STANDARD DEVIATION)
(N= 9 IN EACH GROUP)

Group	Weeks on diet										
	0	1	2	3	4	5	6	7	8	9	10
Non-parathyroidectomized non calcium (PT-no Ca)	171 (8.7)	182 (9.3)	190 (8.3)	201 (5.6)	210 (6.1)	219 (7.5)	221 (6.2)	226 (5.6)	230 (6.8)	232 (6.0)	236 (5.8)
Parathyroidectomized-non calcium (PTX-no Ca)	164 (6.8)	143 (7.4)	154 (7.5)	162 (8.7)	163 (8.0)	163 (6.5)	159 (5.0)	159 (4.5)	160 (7.0)	158 (4.6)	159 (5.9)
Non-parathyroidectomized-normal calcium (PT-Ca)	170 (8.1)	181 (8.1)	188 (4.9)	200 (5.3)	209 (6.7)	216 (7.4)	220 (7.2)	227 (7.2)	232 (7.8)	234 (9.0)	238 (9.0)
Parathyroidectomized-normal calcium (PTX-Ca)	164 (10.4)	171 (16.9)	180 (14.2)	197 (12.7)	202 (20.1)	212 (16.6)	214 (18.2)	220 (18.0)	225 (19.3)	230 (16.0)	231 (16.7)

TABLE 4

BIOCHEMICAL VALUES IN SERUM AND BONE
(MEAN AND STANDARD DEVIATION)

Group		Serum		Bone	
		Total calcium (mg/dl) (Initial value)	Total calcium (mg/dl) (Final value)	Magnesium (mg/dl) (Final value)	Calcium in femur (g/100 g net weight)
Parathyroidectomized calcium-free diet (PTX-no Ca)	n = 9	7.80 (0.44)	5.40 (2.11)	1.60 (0.41)	14.82 (1.30)
Parathyroidectomized normal diet (PTX-Ca)	n = 9	7.69 (0.54)	8.31 (1.57)	1.85 (0.32)	15.44 (0.96)
Control calcium free-diet (PT-no Ca)	n = 9	11.09 (0.55)	10.25 (1.05)	2.27 (0.58)	12.47 (1.39)
Control normal diet		11.07 (0.45)	10.47 (0.60)	1.65 (0.15)	14.78 (1.36)
	F	133.28	16.51	4.88	9.26
	P	< 0.0001	< 0.0001	< 0.01	< 0.001

DISCUSSION

In the present study additional evidence for the findings presented in an earlier report is given (7), i.e., that a calcium-free diet produces an increase in blood pressure in normal rats about five or six weeks after treatment is started. Furthermore, we have also shown that, when on a calcium-free diet, parathyroidectomized rats do not have the rise in blood pressure referred to. On the contrary, following the normal control group (PT-Ca), a decrease in blood pressure occurs.

The results are in agreement with those reported by Berthelot and Gairard (14), as their findings also revealed a protective effect of parathyroidectomy on deoxycorticosterone (DOCA) –induced hypertension in rats. This evidence suggest that the rise in blood pressure induced by a calcium-free diet or by the administration of DOCA in rats is mediated by PTH. This mechanism may explain why patients with primary hyperparathyroidism, in absence of renal damage, present a higher incidence of hypertension, with a 20o/o remittance after parathyroidectomy (15), and why pregnancy –when associated with hyperparathyroid crisis– produces a clinical picture of hypertension. Elevated uric acid values and altered mental status can also point to the diagnosis of severe preeclampsia (16).

PTH facilitates calcium ion movement from extracellular to intracellular cytolic compartments, stimulating, in the case of smooth muscles, their tone. Thus, with very limited intracellular stores, the balance of Ca entry and exit accross the cellular membrane will control not only Ca^{+} but smooth muscle tone and, as a consequence, blood pressure. A calcium-free diet will produce a compensatory rise in PTH and, therefore, a PTH-mediated increase in the intracellular concentration of Ca^{2+} as well as an increase in smooth muscle tone. In the absence of PTH, this effect cannot be produced; in this case, a low calcium intake would produce a decrease in intracellular Ca concentration and, consequently, a reduced arteriolar smooth muscle tone.

The control animals (PT-Ca) showed a decrease in blood pressure values not different from that of the PTX-no Ca group. A possible explanation for this finding is that the calcium diet resulted in lower PTH values in the PT-Ca group; this assertion may be inferred from the fact that such group had lower Mg serum values at the end of the study, similar to those of the parathyroidectomized rats.

An important confounding variable in the study herein discussed can be the low weight gain observed in the PTX-no Ca group, since weight gain and blood pressure have been reported to be associated. A similar effect on weight was notified by Larsson *et al.* (17) in parathyroidectomized rats fed a low calcium diet. In his series this phenomenom was due to a reduction in food intake rather than to an endocrinological secondary change (e.g., the adrenal cortex). In our study, while the differences in weight between the PTX-no Ca group and the other groups increased steadily throughout the course of the experiment, the differences in blood pressure occurred within the first weeks, and then stabilized. Correlations were calculated between changes in weight and blood pressure during the study in each treatment group. No significant associations were found between these two variables in any group. Therefore, this evidence suggests

that the differences in blood pressure between the PT-no Ca and PTX-no Ca groups cannot be wholly attributed to the differentiated changes in weight.

Biochemical values reflected the effect of the parathyroidectomy on Ca and Mg values. In the PTX-Ca group, a slight increase in the mean and SD of total serum Ca values was observed at the end of the study, when compared to initial values. Some animals in this group showed an increase in serum calcium values, a fact that has been reported by other authors, and explained by the development of aberrant parathyroid glands (17). The PT-no Ca group exhibited significantly higher values of serum Mg and lower values of bone calcium, a phenomenon which could be attributed to a higher activity of the parathyroid gland so as to compensate the low calcium intake.

The present report gives support to the possible role the parathyroid gland plays in the regulation of blood pressure, namely a PTH-mediated increase in intracellular calcium concentration in the smooth vascular muscle. These preliminary results warrant additional studies. The confirmation of a role of PTH in the regulation of blood pressure would open a new area of research on the pathophysiology of hypertension, and thus, on its prevention.

RESUMEN

EL ROL MEDIADOR DE LA GLANDULA PARATIROIDES EN EL EFECTO DE LA BAJA INGESTA DE CALCIO SOBRE LA TENSION ARTERIAL EN LA RATA

Estudios recientes sugieren una relación inversa entre la ingesta de calcio y la tensión arterial. Dicho efecto podría ser mediado por la paratohormona (PTH), ya que una baja ingesta de calcio produce un alza de la PTH, y ésta lleva a un aumento del calcio intracelular. incrementando la excitabilidad de la célula muscular arteriolar. Ratas hembras Wistar, de 56 días de edad, fueron sometidas a una paratiroidectomía o a una operación simulada. Luego de ello, a los animales se les suministró una dieta normal o una dieta libre de calcio durante un período de 10 semanas. Se estudiaron cuatro grupos compuestos de nueve ratas cada uno: ratas paratiroidectomizadas con dieta normal; ratas paratiroidectomizadas con dieta libre de calcio; ratas control (operación simulada) con dieta normal, y un grupo, también control (operación simulada), pero con dieta sin calcio. Las ratas del grupo testigo cuya dieta era libre en calcio, acusaron una elevación significativa de la tensión arterial. Las ratas del grupo paratiroidectomizado, con dieta sin calcio, no mostraron alza de la tensión arterial. Las diferencias en cambios de tensión arterial entre estos dos grupos fueron estadísticamente significativas. Los animales del grupo paratiroidectomizado que recibieron la dieta sin calcio, no mostraron aumento de peso, mientras que las ratas de los tres grupos restantes subieron de peso en forma significativa. De este estudio se desprende que el alza de la presión arterial observado en las ratas privadas de calcio podría estar mediado por la PTH, a pesar del efecto interferente del poco aumento de peso que se determinó en el grupo paratiroidectomizado, con dieta sin calcio. Estos hallazgos ameritan estudios que confirmen el papel de la PTH en la regulación de la tensión arterial, ya que esta posibilidad abre una nueva área en el estudio de la fisiopatología de la hipertensión.

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