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RELATION BETWEEN CALCIUM AND CARDIOVASCULAR REACTIVITY IN MINERALOCORTICOID-INDUCED HYPERTENSION IN THE RAT

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1 Previous work has shown that parathyroidectomy (PTX) decreases cardiovascular reactivity to noradrenaline in Sprague-Dawley rats with mineralocorticoid-induced (D) hypertension.

2 In order to explain this diminution of cardiovascular reactivity, we studied *in vivo* the influence of serum calcium levels on the cardiac and vascular response to noradrenaline (500 ng/kg). We used rats with or without parathyroids but fed a standard or a high-calcium (+Ca) diet that re-established a serum calcium level of about 100 mg/l. Work was performed in vagotomized, anaesthetized rats after ganglionic blockade with pentolinium and atropine sulphate.

3 Cardiac output was unchanged in all the experimental groups after 11 weeks of mineralocorticoid treatment. The enhancing effect of noradrenaline was not modified.

4 Since a serum calcium level of about 100 mg/l was sufficient to re-establish vascular reactivity to exogenous noradrenaline in the PTX-D rats, parathyroid hormone was not necessary.

5 The total and lanthanum-resistant calcium fractions of the walls of the aorta were reduced in the PTX-D rats. When serum calcium levels were re-established at about 100 mg/l, there was no difference between PTX-D and D rats.

6 It is postulated that the decreased storage of calcium in vascular smooth muscle cells of PTX-D rats depresses the vascular effect of noradrenaline.

Introduction

The calcium ion plays an important role in the contraction of vascular smooth muscle (Weiss, 1977), in which tone determines the peripheral vascular resistance and thus, with cardiac output, influences blood pressure. On the other hand, the calcium ion seems to be involved in some types of hypertension (Jones & Hart, 1975; Rorive, 1975). Many cases of hyperparathyroidism in man have been shown to be linked with arterial hypertension (Ory, David & Redmond, 1970; Rosenthal and Roy, 1972; Weil, Lagrue, Fournier & Kazandjian, 1972). In addition, Berthelot, Schleiffer & Gairard (1979) observed that during hypertension induced in the rat by treatment with deoxycorticosterone (DOCA)-plus-saline, parathyroidectomy performed previously delayed and lessened the development of hypertension. In previous work (Berthelot & Gairard, 1978), we have shown that the cardiovascular reactivity to noradrenaline decreased after parathyroidectomy and thyroparathyroidectomy in rats treated with DOCA-plus-saline.

The purpose of this work was to elucidate the mechanism of action of the parathyroid on cardiovascular reactivity. The effect of parathyroidectomy could be produced by the decrease in serum calcium and/or suppression of the parathyroid hormone (PTH), which enhances calcium transport into various cells (Borle, 1970). First, to differentiate the effect of parathyroidectomy on cardiac reactivity from that on vascular reactivity, we measured the cardiac output in these rats. We also studied the variation in cardiovascular reactivity and the calcium content of the aorta in DOCA-plus-NaCl-treated rats with or without parathyroids and in rats with normal serum calcium levels but deprived of PTH.

Methods

Male Sprague-Dawley rats weighed 120 g at the beginning of the experiment. They were fed a normal diet (a standard diet, UAR AO_4, with balanced vitamin and mineral contents, containing 0.60% calcium) or a high-calcium diet (the standard diet with the calcium content increased to 1.30% and with 12% lactose). Lactose enhances the intestinal absorption of calcium (Fournier & Dupuis, 1975). This diet allowed

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a serum calcium level of about 100 mg/l to be maintained in parathyroidectomized rats. DOCA pellets (containing a total of 100 mg in 4 pellets) or placebo pellets (control group) were implanted subcutaneously by the technique of Peterfalvi & Jequier (1960). Starting from the day of operation, the DOCA-treated groups drank 0·9% NaCl solution (saline) *ad libitum* while the control groups drank distilled water.

The rats were randomly divided into several experimental groups: normal DOCA-treated (D); normal control (C) rats; parathyroidectomized DOCA-treated (PTX-D) rats and parathyroidectomized DOCA-treated rats given the high-calcium diet (PTX-D + Ca).

Surgery was performed under anaesthesia with 30 mg/kg intraperitoneal pentobarbitone 1 week before the start of the mineralocorticoid treatment. Parathyroidectomy was done by cauterization under a binocular lens (× 5) to assure complete removal of the glands.

The normal DOCA-treated (D) and control (C) rats were anaesthetized, their skin was cut, their parathyroid glands were exposed, and the incisions were then closed. The success of parathyroidectomy was verified by ascertaining the serum calcium level.

Serum calcium

Serum calcium was determined by atomic absorption photometry (Girard & Rousselet, 1967).

Cardiovascular reactivity (CVR)

Blood pressure was measured in unanaesthetized warmed rats, by the tail-cuff method, 24 h before the study of CVR.

For the CVR study, animals were also anaesthetized with an intraperitoneal injection of pentobarbitone (30 mg/kg). In order to minimize their spontaneous blood pressure regulation, we pretreated them with pentolinium (May and Baker, 25 mg/kg, s.c.) and atropine sulphate (0.25 mg/kg, s.c.) according to the method described by Dupont & Sassard (1974). Two small polyethylene cannulae were implanted, one in the carotid artery for direct measurement of the mean blood pressure and one in the jugular vein for drug injections. At the same time, vagotomy was performed on both sides. The mean blood pressure was measured directly with a Statham 23 D B pressure probe. Cardiovascular reactivity to noradrenaline was expressed as changes in blood pressure (BP) evoked by the injection of noradrenaline (500 ng/kg).

Cardiac output

Cardiac output was measured by a thermodilution method (Houdas, Ketelers, Houdas-Heyraud & Lerche, 1969). Anaesthetized rats were injected rapidly with cold serum (0.2 ml at 20 to 23°C) in the right auricle by means of a jugular vein catheter, and the changes in temperature were measured with a copper-and-constantan thermocouple, implanted in the left carotid at the junction of the carotid and the aorta. The cardiac output was measured after gangliоplegy with or without injection of 500 ng/kg of noradrenaline. The mean of three measurements was taken as the value of cardiac output.

Aortic calcium content

The rat was killed with a guillotine. The thoracic aorta was immediately cut in half longitudinally. One part was submerged in a 0.5 mM lanthanum chloride solution ($LaCl_3$) for 5 min for later measurement of the lanthanum-resistant calcium content (Godfraind, 1976). The other part of the aorta was used for measurement of the total calcium. After being weighed (wet tissue) the aortae were placed overnight in an oven at 100°C and reweighed (dry tissue). The strips were kept at 400°C for 18 h and the calcium was determined by atomic absorption photometry.

The CVR, cardiac output, and aortic calcium content were measured 11 weeks after the start of treatment (during the sustained phase of hypertension).

The data are arithmetical means of n individual values ± s.e. mean. Statistical comparisons were made by analysis of variance.

Results

Weight

After 11 weeks of treatment with DOCA-plus-saline, there was no statistically significant difference between the results for the D and the C rats. The results for PTX-D rats and PTX-D + calcium rats were not significantly different from those for D rats (Table 1).

Blood pressure

The D rats, treated with DOCA-plus-saline for 11 weeks, were hypertensive. Their blood pressure was higher than that of the control rats ($P < 0.001$). The blood pressure of the PTX-D rats was lower than that of the D rats ($P < 0.001$). The blood pressure of PTX-D + Ca rats was also significantly lower than that of the D rats ($P < 0.001$) but was not significantly different from that of the PTX-D rats (Table 1).

Serum calcium

The high-calcium diet re-established the serum cal-

cium level at about 100 mg/l in the PTX-D + Ca rats, a level that was not significantly different from that in the D and the C rats. As expected, the PTX-D rats had lower levels of serum calcium than did the D and the PTX D + Ca rats ($P < 0.001$) (Table 1).

Cardiac output

The cardiac output increased significantly in the four groups after the injection of noradrenaline, but there was no significant difference between these groups before or after injection (Table 2).

Cardiovascular reactivity to noradrenaline

The CVR was higher in the PTX-D + Ca than in the PTX-D rats ($P < 0.05$) (Figure 1). As previously shown by some of us, the values for D rats were significantly higher than those for the PTX-D rats ($P < 0.05$). It clearly appeared that there was no difference between PTX-D + Ca and D rats.

Aortic calcium content

The total calcium and lanthanum-resistant calcium fraction in the aortic walls of the PTX-D + Ca rats were significantly higher than in those of the PTX-D rats ($P < 0.001$ and $P < 0.01$) (Table 3). Similarly, the total calcium and the lanthanum-resistant calcium fraction in the aorta of the D rats were significantly higher than in the PTX-D rats ($P < 0.001$) (Table 3). Moreover, the total calcium in the aortae of the D rats was significantly higher than in those of C rats ($P < 0.01$) (Table 3).

Discussion

Our present data clearly show that the lower CVR to noradrenaline after parathyroidectomy was due not to a difference in cardiac reactivity, but to a lowered vascular reactivity. Indeed, the variation of cardiac output after the injection of noradrenaline was not

Table 1 Body weight, blood pressure, and serum calcium in rats after 11 weeks of treatment with DOCA-plus-saline

Group	*Number of rats*	*Body weight* (g)	*Arterial blood pressure* (mmHg)	*Serum calcium* (mg/l)
C	4	443 ± 25.2	105 ± 6.4	100 ± 1.1
D	11	425 ± 8.5	163 ± 5.7***	102 ± 0.7
PTX-D	8	405 ± 14.4	128 ± 5.2*≠≠	57 ± 2.4*≠≠
PTX-D + Ca	10	396 ± 9.2	129 ± 2.4	97 ± 2.0†††

C: Control rats; D: DOCA-plus-NaCl-treated rats; PTX-D: parathyroidectomized DOCA-plus-NaCl rats; PTX-D + Ca: parathyroidectomized DOCA-plus-saline rats fed a high-calcium diet: Means ± s.e. mean
Two symbols, $P < 0.01$. Three symbols, $P < 0.001$.
* Comparison of D and C; ≠ comparison of D and PTX-D; † comparison of PTX-D and PTX-D + Ca.

Table 2 Cardiac output (ml min^{-1} kg^{-1}) in anaesthetized and ganglioplegy-treated rats before and after injection of 500 ng/kg of noradrenaline, 11 weeks after the start of DOCA-plus-saline treatment

Group	*Number of rats*	*Cardiac output after ganglioplegy*	*Cardiac output after injection of 500 ng noradrenaline*
C	4	247 ± 26.8	340 ± 15.0**
D	11	257 ± 20.9	331 ± 17.7*
PTX-D	8	263 ± 12.6	344 ± 16.0**
PTX-D + Ca	10	267 ± 21.0	356 ± 23.7*

C: Control rats; D: DOCA-plus-NaCl-treated rats; PTX-D: parathyroidectomized DOCA-plus-NaCl rats; PTX-D + Ca: parathyroidectomized DOCA-plus-NaCl rats fed a high-calcium diet. Means ± s.e. mean.
Comparison for the same group before and after injection of noradrenaline.
* $P < 0.05$; ** $P < 0.01$.

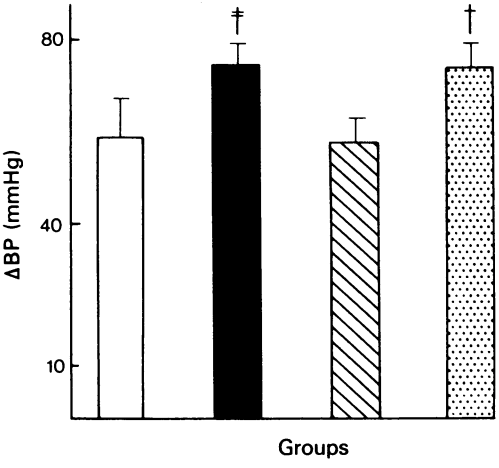

Figure 1 Cardiovascular reactivity to noradrenaline, 11 weeks after the start of mineralocorticoid treatment, expressed as change in blood pressure (BP, mmHg) after injection of noradrenaline (500 ng/kg). Control rats: open column (4); DOCA-plus-NaCl treated rats (D): solid column (14); parathyroidectomized DOCA-plus-NaCl rats (PTX-D): hatched column (11); parathyroidectomized DOCA-plus-NaCl rats fed a high calcium diet (PTX-D + Ca) stippled column (13). ‡Comparison of PTX-D and D; †Comparison of PTX-D and PTX-D + Ca.

different in D, PTX-D, and PTX-D + Ca rats. Moreover, parathyroidectomy did not alter the cardiac sensitivity to noradrenaline in the rats treated with DOCA + saline for 11 weeks. Apparently the level of serum calcium did not change the effect of noradrenaline on cardiac output. However, Reuter (1974) showed that extracellular calcium influx occurs in the cardiac action of this catecholamine. Probably very large changes in extracellular calcium concentration are necessary to change the cardiac reactivity to noradrenaline.

Our study shows the importance of the serum calcium level in the vascular reactivity to noradrenaline. The parathyroidectomized rats whose serum calcium was artificially increased by a high-calcium diet but without parathyroid hormone (PTX-D +Ca) had the same vascular reactivity as group D. On the other hand, PTX-D rats with low serum calcium levels had a decrease of vascular reactivity that confirms our previous work (Berthelot & Gairard, 1978). So it seems that the vascular reactivity to noradrenaline was influenced by the serum calcium level in the PTX-D rats.

The changes in serum calcium level could act directly by the extracellular calcium concentration or indirectly by modifying the intracellular calcium content of vascular muscle. Godfraind & Kaba (1972) showed that extracellular and intracellular calcium play a role in the *in vitro* contraction of vascular smooth muscle by the catecholamines. Noradrenaline induces a phasic contraction of the vessel followed by a tonic contraction. The first is due to the liberation of bound calcium into the cell, and the second, to an increase of extracellular calcium influx. Somlyo & Somlyo (1970) found that intracellular calcium concentration was one of the principal factors in the contractile responses in smooth muscle and that tonic shift that increased or decreased cell calcium contributed to increased or decreased reactivity.

As Jones & Hart (1975) found, the D hypertensive rats showed a total aortic calcium greater than their controls. Nevertheless the lanthanum resistant calcium fraction did not change during the same period.

Table 3 Total calcium and lanthanum-resistant calcium fraction in rat aortae after 11 weeks of treatment with DOCA-plus-saline

Group	*Total calcium (mmol/kg dry wt.)*	*Lanthanum-resistant calcium fraction (mmol/kg dry wt.)*
C	9.1 ± 0.65 (12)	5.0 ± 0.55 (4)
D	12.4 ± 0.62‡** (11)	6.0 ± 0.38 (10)
PTX-D	9.1 ± 0.50*≠≠ (11)	3.6 ± 0.31*≠ (10)
PTX-D + Ca	12.3 ± 0.52††† (12)	5.3 ± 0.40†† (9)

C: Control rats; D: DOCA-plus-NaCl-treated rats; PTX-D: parathyroidectomized DOCA-plus-NaCl rats; PTX-D + Ca: parathyroidectomized DOCA-plus-NaCl rats fed a high-calcium diet. Means ± s.e. mean.
Two symbols, $P < 0.01$. Three symbols, $P < 0.001$.
* Comparison of D and C; ≠ comparison of D and PTX-D; † comparison of PTX-D and PTX-D + Ca.

The lanthanum method is known to replace calcium ions at membrane binding sites and to inhibit greatly its transport into and out of the cell (Freeman & Daniel, 1973; Godfraind, 1976). Since activator calcium ions may be of different cellular sources (e.g. extracellular, membrane bound or intracellular), it appears that mineralocorticoid treatment changed calcium stores, probably superficial calcium, loosely bound to mucopolysaccharides which are greatly enhanced during hypertensive development (Rorive, 1975).

Indeed the PTX-D rats had a lower total calcium content and a lower lanthanum-resistant calcium fraction than did D rats. In contrast, the PTX-D + Ca rats had concentrations of total calcium and of lanthanum-resistant calcium that were not statistically different from those of D rats. High-Ca diet reestablished normal calcium serum and aortic levels. We therefore propose that the decrease in storage of (probably) intracellular calcium in vascular smooth muscle cells of PTX-D rats lessened the vascular response to noradrenaline stimulation in the mineralocorticoid-treated rats.

It appears that parathyroid hormone was not directly necessary for the complete action of exogennous noradrenaline on the cardiovascular reactivity in DOCA-plus-saline-treated rats. Nevertheless, Schleiffer, Berthelot & Gairard (1979) showed that parathyroid extract changes ^{45}Ca fluxes in isolated aortae of rats in a biphasic manner. We can also exclude any effect of calcitonin in rats fed the high-calcium diet, because we have obtained the same result with thyroparathyroidectomized rats (unpublished observations).

Nevertheless, a normal serum calcium level is not sufficient for the establishment of arterial hypertension, parathyroid hormone seems necessary: the PTX-D + Ca rats with a normal level of calcium had a much lower blood pressure than did the D rats. We have thus confirmed previous results from this laboratory (Berthelot & Gairard, 1980). Reid, Zivin & Kopin (1975) and De Champlain, Farley, Cousineau & Van Ameringen (1976) found increased concentrations of serum catecholamines in DOCA-and-saline-induced hypertension. The presence of parathyroid hormone could be necessary for the release of noradrenaline from sympathetic nerves. Study of catecholamine turnover in PTX-D rats would be of great interest.

Our conclusions on cardiovascular reactivity may be summarized as follows: the smaller change in blood pressure after the injection of noradrenaline in parathyroidectomized DOCA-plus-saline-treated rats than in D rats was a result of a decrease in vascular reactivity in the former. The changes in the concentration of lanthanum-resistant and total calcium in the aorta could explain the differences in the vascular response to noradrenaline.

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