Parathyroid hormone- and deoxycorticosterone acetate-induced hypertension in the rat

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Summary

1. Hypertension induced by treatment with deoxycorticosterone acetate and sodium chloride was studied in male Sprague–Dawley rats and related to parathyroid hormone secretion.

2. Lack of parathyroid hormone (due to parathyroidectomy) or decreased parathormone secretion (due to a high-calcium diet) partially inhibited the development of arterial hypertension.

3. In contrast, in thyroparathyroidectomized rats supplemented with thyroxine, the administration of parathyroid hormone rapidly elevated arterial blood pressure.

4. Maintaining a physiological concentration of serum calcium in the absence of parathyroid hormone (by feeding a high-calcium diet to parathyroidectomized rats) was not sufficient to establish mineralocorticoid hypertension.

5. These results show that parathyroid hormone is necessary for the complete development of mineralocorticoid hypertension.

Key words: arterial hypertension, calcium, deoxycorticosterone acetate, parathyroid hormone.

Abbreviations: DOCA, deoxycorticosterone acetate; $T_4$, DL-thyroxine.

Introduction

Arterial calcium has been found to be raised during experimental arterial hypertension, including that of renal origin (Tobian & Chesley, 1966; Rorive, 1975) and mineralocorticoid origin (Jones & Hart, 1975). Calcium ions play an important part in the mechanism of contractile proteins of vascular smooth muscle and condition peripheral vascular resistance, thereby, with cardiac output, influencing blood pressure. Hyperparathyroidism has also been found in more hypertensive people than in a population with normal blood pressure (Rosenthal & Roy, 1972). The hypertension of a female patient with primary hyperparathyroidism disappeared after a partial parathyroidectomy (Blum, Kirsten & Worth, 1977). Christensson, Hellstrom & Wengle (1977) showed that 80% of the subjects with normal kidney function, raised systolic arterial blood pressure and serum calcium concentration were hyperparathyroid. Primary hyperparathyroidism in man thus seems to be associated with an increase in blood pressure.

In preliminary experiments in rats with mineralocorticoid-induced hypertension, we have found the following disorders of calcium metabolism: increased urinary calcium (Gairard, Stoclet & Miss, 1973), decreased calcium turnover (Stoclet, Miss-Pages, Berthelot & Gairard, 1975) and increased parathyroid activity (Berthelot, Desplan, Pernot & Gairard, 1978). Thus, in the present work, our aim was to study the relation between parathyroid hormone and the development of hypertension artificially induced by the administration of the mineralocorticoid deoxycorticosterone acetate (DOCA). For this reason we have observed the evolution of mineralocorticoid hypertension in rats with or without parathyroid hormone and in rats with normal serum calcium.
concentrations but deprived of parathyroid hormone.

Methods

Animals

Male Sprague–Dawley rats weighing 120 g at the beginning of the experiment were fed on a standard diet (UAR AO, Chow, O3 Commentry France) equilibrated in vitamins and mineral components, containing 0.60% Ca and 0.24% Na. DOCA pellets (Roussel-UCLAF) (25 mg of DOCA/pellet) or placebo pellets (control group) were implanted subcutaneously (Peterfalvi & Jequier, 1960). Starting from the day of operation, DOCA-treated groups drank 0.9% NaCl solution ad libitum and the control group drank distilled water.

The rats were randomly divided into the following experimental groups: group 1, normal DOCA-treated and normal control rats; group 2, parathyroidectomized, DOCA-treated and parathyroidectomized control rats; group 3, thyro-parathyroidectomized, DOCA-treated rats with or without a supplement of DL-thyroxine (T₄) (Roche); group 4, thyro-parathyroidectomized, DOCA-treated rats supplemented with DL-thyroxine and receiving parathyroid hormone; group 5, thyro-parathyroidectomized control rats with or without a supplement of T₄. In addition, part of each group was fed on a high-calcium diet.

Surgery

Surgery was performed under anaesthesia with pentobarbital (30 mg/kg, administered intraperitoneally) 1 week before the start of mineralocorticoid treatment. Parathyroidectomy by cauterization with a hot wire and surgical thyro-parathyroidectomy were performed under a binocular magnifier (x5) to assure complete removal of the glands. The efficacy of the operations was verified by measuring the serum calcium concentration 1 week after parathyroidectomy or thyro-parathyroidectomy, and also by following the body weight after thyro-parathyroidectomy. Rats underwent sham operations, during which they were anaesthetized, their parathyroid glands were exposed, and their incisions were closed.

High-calcium diet

The amount of calcium in the standard diet was increased by 30% by adding CaCl₂, and that of lactose by 27%. The CaCl₂ and lactose did not change the amount of food and drink ingested. Lactose was used because it favours the intestinal absorption of calcium (Fournier & Dupuis, 1975). This diet maintained serum calcium at about 2.5 mmol/l in parathyroidectomized and in thyro-parathyroidectomized rats.

Administration of hormones

DL-Thyroxine was given subcutaneously on alternate days (5 µg/animal for the first 3 weeks, and 8 µg/animal thereafter).

Parathyroid hormone (Parathyroid hormone Lilly, lot no. ODX92) was injected subcutaneously into thyro-parathyroidectomized, DOCA-treated rats twice a day (30 United States Pharmacopea units/day) for 11 days to maintain a serum calcium concentration of about 2.5 mmol/l.

Analytical procedures

Blood samples were drawn from the orbital sinus, collected in heparin-treated refrigerated tubes and centrifuged immediately.

C-terminal serum immunoreactive parathyroid hormone was measured by radioimmunoassay with bovine parathyroid hormone as antigen in guinea pig, and pure bovine parathyroid hormone radioactively labelled with ¹²⁵I. C-terminal immunoreactive parathyroid hormone was considered an index of the production of the hormone (Milhaud, Julienne, Chalmettes & Moukhtar, 1976).

Blood pressure measurements

Systolic arterial pressure was measured on unanaesthetized warmed rats by the tail-cuff method at intervals of 1 or more weeks for 10 weeks after the start of DOCA treatment; mineralocorticoid-induced hypertension is generally thought to be established by the end of that period.

Statistical analysis

The data presented are arithmetic means of n individual values ± SEM. Statistical comparisons were made by using analysis of variance (Schwartz, 1963).

Results

Effect of parathyroidectomy

Parathyroidectomy reduced the hypertensive effect of mineralocorticoid treatment at the end of
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FIG. 1. Blood pressure: (a) in normal control rats (○), n = 10, and in normal DOCA-treated rats (●), n = 12; (b) in parathyroidectomized control rats (▾), n = 6, and in parathyroidectomized, DOCA-treated rats (▼), n = 8. Vertical lines show SEM. *P < 0.05; **P < 0.01; ***P < 0.001.

FIG. 2. Blood pressure: (a) in normal control rats (○), n = 12, and in normal DOCA-treated rats (●), n = 11; (b) in normal control rats that were fed on a high-calcium diet (○), n = 7, and in normal DOCA-treated rats that were fed on a high-calcium diet (●), n = 10. Vertical lines show SEM. *P < 0.05; **P < 0.01; ***P < 0.001.

week 2. The blood pressure of parathyroidectomized rats treated with DOCA was significantly lower (P < 0.001) than that of normal DOCA-treated rats, but higher than that of control parathyroidectomized rats. In untreated rats, parathyroidectomy had no significant effect on blood pressure (Fig. 1a, 1b).

Effect of a high-calcium diet in normal rats

In the normal rats, a high-calcium diet reduced the hypertensive effect of mineralocorticoid treatment. The blood pressure of the DOCA + calcium-treated rats was significantly lower during the entire 10 weeks than that of the DOCA-treated rats (P < 0.001). In untreated rats, the high-calcium diet induced a biphasic effect: an increase in blood pressure in week 2 (P < 0.001) and a decrease in week 4 (P < 0.001) (Fig. 2a, 2b). As expected, the high-calcium diet lowered immunoreactive parathyroid hormone. During week 2 of treatment the results were: DOCA-treated rats, 1.18 ± 0.10 ng/ml (n = 8); DOCA + calcium-treated rats, 0.73 ± 0.14 ng/ml (n = 8), P < 0.05.
Effect of high-calcium diet in parathyroidectomized and thyroparathyroidectomized rats

In parathyroidectomized rats (Fig. 3a, 3b), the high-calcium diet did not affect blood pressure during the first 10 weeks of mineralocorticoid treatment, but decreased it by the end of week 10 (P < 0.001). In the untreated rats, the high-calcium diet increased blood pressure in week 2 (P < 0.05), and then provoked no further variation. This diet increased the serum calcium concentration to about 2.5 mmol/l. The serum calcium of high-calcium-treated parathyroidectomized rats was of significantly higher concentration than that of normal calcium-treated parathyroidectomized rats (P < 0.01).

In the thyroparathyroidectomized rats (Fig. 4a, 4b) the high-calcium diet did not affect blood pressure in either treated or untreated rats, whether or not they were given a T₄ supplement (Fig. 4c, 4d), but it increased the serum calcium by about 2-5 mmol/l. Again the same difference in serum calcium was observed as in the preceding group.

In summary, blood pressure was not related to serum calcium concentration in these experimental groups.

Effect of administration of exogenous parathyroid hormone

During the administration of parathyroid hormone the thyroparathyroidectomized, DOCA-treated rats had a serum calcium concentration about 2-5 mmol/l and this was no different from the normal DOCA-treated group.

After parathyroid hormone had been given for 3 days, the blood pressure rose rapidly (thyro-parathyroidectomized, DOCA-treated rats supplemented with thyroxine vs thyroparathyroidectomized, DOCA-treated rats supplemented with thyroxine and receiving parathyroid hormone: P < 0.001) and stayed high throughout the remaining 8 days of administration.

Three days after the onset of the parathyroid hormone treatment there was no longer any significant difference between the blood pressure of the DOCA rats and that of the thyroparathyroidectomized, DOCA-treated rats supplemented with thyroxine and receiving parathyroid hormone. At any given time during this experiment the DOCA-treated rats always showed significantly higher blood pressure than did the thyroparathyroidectomized DOCA-treated rats supplemented with thyroxine (Fig. 5).

Discussion

The relation between parathyroid hormone and mineralocorticoid-induced hypertension was demonstrated by the partial inhibition of arterial hypertension when parathyroid hormone was absent (parathyroidectomized rats) or when less of the hormone was secreted (high-calcium diet). Indeed, parathyroidectomy slowed the development of hypertension but did not completely prevent the hypertensive effect of DOCA treatment, since the parathyroidectomized--DOCA-treated rat had higher blood pressure than did the parathyroidectomized control rats. Furthermore,
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we have shown in another study (Berthelot, Schleiffer & Gairard, 1979) that the parathyroid only plays a role during the onset of hypertension. The decrease of parathyroid hormone, which was induced by the high-calcium diet as shown by serum immunoreactive parathyroid hormone concentrations, also partially inhibited the development of hypertension during DOCA treatment. However, in this experiment we cannot completely exclude a possible effect of calcitonin. The high-calcium diet could increase the release of calcitonin, and it has been shown that a large dose of salmon calcitonin (5 MRC units/g), during 5 weeks, inhibited the development of hypertension in response to the administration of DOCA in the rat (Aldred, Luna, Zeedyk & Bastian, 1976).

Parathyroid hormone thus seems to be one of the factors that conditions the level of mineralocorticoid-induced hypertension. Certainly it favours the hypertensive effects of DOCA treatment. The administration of this hormone to DOCA-treated rats lacking both parathyroid and thyroid glands and supplemented with T₄ (and therefore without any calcitonin) was sufficient to increase the blood pressure and bring it back to a pressure close to that in normal DOCA-treated rats. Moreover, we have shown that in thyroidectomized and autografted parathyroid–DOCA-treated rats, arterial hypertension could develop (Berthelot et al., 1979). In addition, the alteration in serum calcium

**Fig. 4.** Blood pressure: (a) in thyroparathyroidectomized, DOCA-treated rats fed on a normal diet (□), n = 7, or on a high-calcium diet (○), n = 7; (b) in thyroparathyroidectomized, control rats fed on a normal diet (□), n = 4, or on a high-calcium diet (○), n = 4; (c) in thyroparathyroidectomized, DOCA-treated rats supplemented with thyroxine fed on a normal diet (□), n = 5, or on a high-calcium diet (○), n = 8; (d) in thyroparathyroidectomized control rats supplemented with thyroxine fed on a normal diet (□), n = 3, or on a high-calcium diet (○), n = 5. Vertical lines show SEM.
concentrations in the absence of parathyroid hormone did not modify the development of hypertension, since in the rats deprived of parathyroids or of both thyroid and parathyroids, and supplemented or not with T₄, the return of serum calcium concentrations to about 2-5 mmol/l did not increase the blood pressure. Moreover, at week 10, the blood pressure was significantly reduced with high-calcium diet in parathyroidectomized–DOCA-treated rats, compared with normal calcium diet in parathyroidectomized–DOCA-treated rats. This observation could be explained by assuming that the diet provoked secretion of calcitonin, since the effect was not seen in similarly treated thyroparathyroidectomized rats (without calcitonin). So a quasinormal concentration of serum calcium in the absence of parathyroid hormone is not sufficient for the establishment of mineralocorticoid hypertension.

There are few observations on the relation between parathyroid hormone and experimentally induced hypertension. Lehr (1959) showed that, in adrenalectomized and nephrectomized rats, parathyroidectomy inhibited the arterial and cardiac necrosis usually provoked by treatment with DOCA. Moreover, Salgado & Green (1957) showed that surgical thyroparathyroidectomy performed before mineralocorticoid treatment inhibited the development of hypertension. Nevertheless, parathyroid hormone did not seem to be the only hormone of mineral metabolism that affected mineralocorticoid-induced hypertension. Calcitonin has been described as an anti-arrhythmic drug in man (Merzon, Glezer, Doviner, Briskin & Chertkova, 1974) and inhibited the development of mineralocorticoid hypertension in rats (Aldred et al., 1976). These findings suggest that calcium-regulating hormones may play a role in the development of hypertension.

Such observations raise the question of the role of parathyroid hormone in this kind of hypertension. It is logical to consider that parathyroid hormone may affect the movement of calcium across the vascular cell membrane, and may also affect the toxicity of arteries, which, unlike bone cells, are not generally considered to be target organs of this hormone. In vitro, parathyroid hormone increases the turnover of calcium in rat kidney cells (Borle, 1971; Borle & Uchikawa, 1978) and of ⁴⁴Ca in myocytes of rat thoracic aorta (Schleiffer, Berthelot & Gairard, 1979). Thus parathyroid hormone might increase intracellular calcium and its availability to myocytes. This would induce greater tonicity in the blood vessels or a more intense contractile response of arteries to catecholamines, which increase at the onset of mineralocorticoid-induced hypertension (De Champlain, Farley, Cousineau & Van Amerigen, 1976).

In conclusion, parathyroid hormone is necessary for the complete development of mineralocorticoid hypertension. This result raises the question of the mechanism of action of parathyroid hormone in the hypertensive process.

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References


