Studies on thyroid activity in deoxycorticosterone-salt and Goldblatt two-kidney, one-clip hypertensive rats

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Abstract. In this paper we studied the role of thyroid gland function in two experimental hypertension models with different pathophysiological mechanisms: deoxycorticosterone-salt (DOCA-salt, volume dependent) and Goldblatt 2-kidney, 1-clip (2K1C, renin dependent). DOCA-salt hypertensive rats showed lower T₃ and T₄ serum levels by the third week of induced hypertension. Goldblatt 2K1C hypertensive rats, however, exhibited normal values for both hormones. Treatment with thyroxine accelerated the evolution of hypertension and did not affect the PRA of DOCA-salt rats. Radiothyroidectomy inhibited DOCA-salt and Goldblatt 2K1C hypertension, and prevented the suppression of PRA in DOCA-salt rats, without altering PRA or serum aldosterone in Goldblatt 2K1C rats. These results suggest that: a) a thyroid depressing factor is not activated in Goldblatt 2K1C rats; b) thyroidectomy interferes with the suppressor effect of mineralocorticoid on renin secretion; and c) normal thyroid activity is required for the hypertensive effect of the renin-angiotensin-aldosterone system in Goldblatt 2K1C rats.

Moreover, differences depending on the strain of normotensive rat used as controls (Werner et al. 1975) and age-dependent variations (Rioux & Berkowitz 1977) have been observed. Sensitive and resistant Dahl rats show suppressed T₃ and T₄ serum levels when compared with rats fed a normal saline diet (McParland & Rapp 1982). In renal hypertension, induced by kidney encapsulation with latex envelopes, T₃ serum levels are reduced and the T₃ to T₄ ratio is found to be normal (Threatte et al. 1982). T₃ and T₄ serum values in deoxycorticosterone-salt (DOCA-salt) and Goldblatt 2-kidney, 1-clip (2K1C) hypertensive rats, however, have not been measured before this study.

Moreover, surgical or chemical manipulation of the thyroid gland has important effects on hypertension in rats. Surgical thyroidectomy performed simultaneously at the start of DOCA and salt treatment inhibits hypertension, reduces mortality, and prevents renal and cardiac lesions (Salgado 1954). Similar results have been observed in encapsulated hypertensive rats (Fregly 1958) and in spontaneously hypertensive rats (Rioux & Berkowitz 1977; Blumenthal et al. 1982; Rodgers & McNeil 1986).

The aim of this paper is to evaluate thyroid gland activity and the effect of thyroid function alterations in DOCA-salt and Goldblatt 2K1C
hypertensive rats: two models with well-established and different physiopathological mechanisms.

Materials and Methods

Experiment I

Thyroid hormone levels were studied in DOCA-salt and Goldblatt 2K1C hypertensive rats. The following groups were made: a) an uninephrectomized-salt group: the left kidney was removed and rats received a 1% NaCl drinking water solution (N = 7); b) a DOCA-salt group: uninephrectomized rats given a 1% saline drinking water solution and weekly 12.5 mg of deoxycorticosterone enantate injections (Cortiron Depot, Schering AG) (N = 6); c) a sham-operated group: ventral incision, left kidney handling and muscle and skin suture conducted under ether anesthesia (N = 6); d) Goldblatt 2K1C hypertensive group: rats were prepared by placing a U-shaped silver clip (internal diameter of 0.2 mm) on the left renal artery (N = 8).

After 3 weeks of treatment, all rats from the different groups were catheterized by inserting a cannula into the left carotid artery. Following a 48-h recovery period, arterial pressure was estimated in conscious rats by direct recording (Bell and Howe type 4 transducer connected to a two-channel Device MX 2 recorder) and blood samples were subsequently taken to determine T3 and T4 levels.

Experiment II

The effect of hyper- and hypothyroid function on the development of DOCA-salt hypertension was studied in three groups of rats: a) a DOCA-salt control group: uninephrectomized rats given a 1% NaCl drinking water solution and weekly 12.5 mg of deoxycorticosterone enantate injections (N = 6); b) a DOCA-salt group treated with thyroxine: rats were treated with a daily dosage of 64.3 nmol/rat of thyroxine (Merck) from the beginning of the hypertension induction (N = 6 for all 3 weeks); c) a thyroidectomized DOCA-salt group: chemical thyroidectomy was performed by injecting 800 µCi of 131I (Nuclear Iberica, Spain) before DOCA and salt treatment. The week before thyroidec¬omy, rats were fed Remington’s diet (non-iodine) to increase thyroid gland captation (N = 7 first, N = 6 second and N = 6 third week).

Systolic blood pressure of unanesthetized rats was measured using the tail-cuff technique with a pneumatic sphygmomanometer unit (Technical Instruments, Inc) connected to a two-channel recorder (Devices MX 2). After the initial acclimatization period, the rats were put into plastic holders and placed on a warm pad for each recording session. Blood pressure was measured seven times, and after eliminating the highest and lowest levels, the mean of five recordings served as the final pressure.

At the end of 3 weeks of hypertension induction, the left carotid artery was cannulated. After a 48-h recovery period, hemodynamic parameters – systolic blood pressure, pulse pressure and heart rate – were determine. Subsequently, blood samples were taken to estimate humoral parameters: plasma renin activity, T3, T4, Na+, K+ and serum osmolality. Both, hemodynamic and humoral parameters, were also determined in the three experimental groups at the first and second week of evolution.

Experiment III

To study the effect of thyroidectomy on Goldblatt 2K1C hypertension, two groups were made: a) a Goldblatt 2K1C control group (N = 10) and, b) a group of Goldblatt 2K1C thyroidectomized rats (N = 8) given Remington’s diet the week before 131I administration and clip implantation. Three weeks later both groups of rats were catheterized to measure the same parameters studied in Experiment II. Moreover, serum was drawn to measure aldosterone levels.

Analytical procedures

Total T3 measurement was performed by a solid-phase radioimmunoassay (Count-A-Count, Diagnostic Products Corporation, Los Angeles, USA). The intra-assay variation coefficient was 8.9% with a sensitivity of 0.03 nmol/l. Total T4 was measured by the PEG-accelerated double-antibody radioimmunoassay method (Diagnostic Products Corporation) with an intra-assay coefficient of variation of 4.7% and a sensitivity of 3.8 nmol/l. Plasma renin activity (PRA) was determined in duplicate by angiotensin I radioimmunoassay according to the method described by Haber et al. (1969), after plasma incubation for 1 h at 37°C, pH 6.0, using a commercially available kit (Cea-Irri-Sorin, Gaf-sur-lvette, France). The intra-assay coefficient of variation was 6.9% and a sensitivity of 3.3 pmol/l per h. Serum aldosterone was measured using a commercially available kit (Abbot, Diagnostic Products, Wiesbaden, FRG). In this study, the intra-assay coefficient of variation was 7.6% with a sensitivity of 0.083 µmol/l.

Serum electrolytes and osmolality were measured by flame photometry (Corning Instruments 435, using lithium as an internal standard) and by freezing point depression (Automatic Osmometter, Osmomette A, Precision Systems Inc), respectively.

Data analysis

Experiment I: independent means were compared using the classic Student’s t-test with equal variances, and the Welch approximation otherwise. Experiment II: one-way analysis of variance was made to compare different groups. When the variable distribution was
Fig. 1. Mean arterial pressure (MAP), T₃ and T₄ serum in sham-operated (S), uninephrectomized-salt (N), Goldblatt 2KIC (G) and DOCA-salt (D) rats after 3 weeks of treatment or operation. ★★P < 0.01, ★★P < 0.001 hypertensive groups vs control groups.

Fig. 2. Evolution of systolic blood pressure measured by tail-cuff method in the DOCA-salt control group (C-DS), thyroxine-treated group (64.3 nmol/rat/day), DOCA-salt (H-DS), and radiochemically thyroidectomized DOCA-salt (T-DS) group.
not normal, the Kruskal-Wallis test was used. The pairwise comparisons were made using the Bonferroni Method. Experiment III: the same method as used in the first experiment was followed.

Results

Experiment I
In the third week of hypertension induction, mean arterial pressure was significantly higher in DOCA-salt and Goldblatt 2K1C rats than that registered in the control uninephrectomized-salt and sham-operated groups. DOCA-salt rats showed a marked reduction in T₃ and T₄ plasma levels in comparison with plasma levels observed in uninephrectomized-salt and sham-operated rats. Goldblatt 2K1C hypertensive rats showed T₃ and T₄ values similar to those observed in control groups (Fig. 1).

Experiment II
The course of systolic blood pressure (recorded by plethysmography) in DOCA-salt, thyroxine-treated and thyroidectomized DOCA-salt rats revealed that the daily injection of 64.3 nmol/rat of thyroxine accelerates DOCA-salt hypertension and thyroidectomy inhibits it (Fig. 2). These data were later confirmed by the results obtained in direct recordings. Weekly mean systolic blood pressure values obtained by direct carotid recording of the animals throughout the experiment are illustrated in Fig. 3. Thyroxine-treated rats showed a higher blood pressure increase at the end of the first (P < 0.001) and third (P < 0.05) week than did control DOCA-salt rats. No elevation in blood pressure was detected in thyroidectomized rats (P < 0.001) in the first, second and third week when compared with control rats.

Pulse pressure was found to be increased in thyroxine-treated rats (P < 0.01 first week, P < 0.05 second and third week) and markedly reduced in thyroidectomized rats (P < 0.001 first and second week, P < 0.01 third week) when

**Fig. 3.** Weekly evolution of systolic blood pressure (SBP), pulse pressure (PP), heart rate (HR), and plasma renin activity (PRA) in DOCA-salt control group (open bars), thyroxine-treated DOCA-salt group (lined bars) and radiothyroidectomized DOCA-salt group (shaded bars). ▲ P < 0.05, ★P < 0.01, ★★P < 0.001 vs DOCA-salt control group.
compared with that observed in DOCA-salt control group. Thyroidectomized rats showed a significant decrease of heart rate (Fig. 3) at the end of the second ($P < 0.05$) and third ($P < 0.01$) week, and thyroxine-treated rats presented an increase at the end of the third week ($P < 0.05$). Fig. 3 also shows the effect of thyroxine administration on PRA in DOCA-salt rats. The results indicate that there are no differences in PRA when comparing thyroxine-treated and control DOCA-salt rats during the 3-week period. In both groups, PRA was practically suppressed in the second and third week. The PRA of thyroidectomized rats, however, was not suppressed in the second ($P < 0.05$) and third ($P < 0.05$) week.

Table 1 shows the evolution of $\text{Na}^+$, $\text{K}^+$ serum, osmolality, and $\text{T}_3$ and $\text{T}_4$ levels in the three groups of rats. No significant differences in these parameters were found in the three groups at the end of the first and second week, although a significant increase ($P < 0.01$) in serum $\text{Na}^+$ in thyroxine-treated rats at the end of the third week was recorded, $\text{T}_3$ and $\text{T}_4$ levels were elevated in thyroxine-treated rats and reduced in thyroidectomized rats during the 3 weeks studied when both are compared with DOCA-salt control rats.

**Experiment III**

The results of this experiment are presented in Fig. 4 and Table 1. Thyroidectomy in Goldblatt 2K1C rats produces important reductions in: systolic blood pressure (199 ± 6.1 mmHg in con-

| Table 1. Serum $\text{Na}^+$, $\text{K}^+$, serum osmolality (Osm), serum $\text{T}_3$ and $\text{T}_4$ levels in DOCA-salt and Goldblatt two-kidney, one-clip hypertensive (2K1C) rats. |
|---------------------------------|---------------------------------|---------------------------------|
| **Groups** | **DOCA-salt hypertension** | **Goldblatt 2K1C hypertension** |
| | **1st week** | **2nd week** | **3rd week** | **Groups** | **3rd week** |
| **Na$^+$ (mmol/l)** | | | | | |
| C-DS | 148.0 ± 1.4 | 149.6 ± 5.0 | 144.7 ± 1.7 | C-G2K1C | 146.0 ± 2.7 |
| H-DS | 145.3 ± 2.3 | 150.0 ± 4.0 | 156.0 ± 5.2** | T-G2K1C | 141.7 ± 2.9* |
| T-DS | 146.2 ± 2.5 | 148.0 ± 4.5 | 143.0 ± 1.5 | | |
| **K$^+$ (mmol/l)** | | | | | |
| C-DS | 3.5 ± 0.7 | 3.4 ± 0.5 | 3.1 ± 0.4 | C-G2K1C | 5.7 ± 0.5 |
| H-DS | 4.7 ± 1.2 | 4.2 ± 0.6 | 3.9 ± 1.1 | T-G2K1C | 4.4 ± 0.8* |
| T-DS | 3.8 ± 0.4 | 3.7 ± 0.7 | 3.2 ± 0.3 | | |
| **OsM (mosmol/kg)** | | | | | |
| C-DS | 280.2 ± 2.1 | 281.6 ± 9.3 | 284.2 ± 3.9 | C-G2K1C | 286.5 ± 4.9 |
| H-DS | 292.6 ± 8.1 | 293.0 ± 9.8 | 293.6 ± 15.2 | T-G2K1C | 281.6 ± 2.0* |
| T-DS | 285.0 ± 13.0 | 286.0 ± 7.0 | 285.8 ± 6.8 | | |
| **T$_3$ (nmol/l)** | | | | | |
| C-DS | 0.936 ± 0.16 | 0.839 ± 0.14 | 0.784 ± 0.09 | C-G2K1C | 1.71 ± 0.36 |
| H-DS | 0.284 ± 0.5** | 0.254 ± 0.4* | 0.250 ± 0.4* | T-G2K1C | 0.56 ± 0.2** |
| T-DS | 0.434 ± 0.08* | 0.425 ± 0.07* | 0.350 ± 0.1* | | |
| **T$_4$ (nmol/l)** | | | | | |
| C-DS | 47.6 ± 2.8 | 52.7 ± 14.0 | 52.7 ± 6.4 | C-G2K1C | 72.07 ± 18.01 |
| H-DS | 117.1 ± 24.4** | 139.0 ± 21.8** | 128.7 ± 16.7** | T-G2K1C | 18.01 ± 5.14** |
| T-DS | 20.2 ± 7.7* | 15.4 ± 5.1* | 12.9 ± 2.6** | | |

Data expressed as mean ± sd. * $P < 0.01$; ** $P < 0.001$ vs their respective control groups. C-DS: DOCA-salt controls (hypertensive). H-DS: thyroxine-treated (64.3 nmol/rat/day) DOCA-salt. T-DS: radiochemically thyroidectomized DOCA-salt. C-G2K1C: Goldblatt two-kidney, one-clip controls (hypertensive). T-G2K1C: radiochemically thyroidectomized Goldblatt two-kidney, one-clip.
trol Goldblatt 2K1C vs 120 ± 4.8 mmHg in thyroidectomized Goldblatt 2K1C rats, $P < 0.001$; pulse pressure (56.1 ± 8.8 mmHg in control rats vs 24.7 ± 6.3 mmHg in thyroidectomized Goldblatt 2K1C rats, $P < 0.001$); and heart rate (440.6 ± 49.7 beats/min in control rats vs 252.5 ± 39.4 beats/min in thyroidectomized rats, $P < 0.001$), whereas PRA and aldosterone serum were not significantly altered. Na$^+$ and K$^+$ serum concentration and serum osmolality were found to be reduced in thyroidectomized Goldblatt 2K1C rats in comparison with control Goldblatt 2K1C rats. T$_3$ and T$_4$ serum levels were obviously found to be reduced in thyroidectomized rats.

**Discussion**

In several models of experimental hypertension, e.g. genetic spontaneous hypertensive rats (Kojima et al. 1975), mineralocorticoid (Fregly et al. 1969) and encapsulated hypertensive rats (Threatte et al. 1982), an increase in thyroid gland weight has been reported. A sigmoid relationship was observed between the ratio of thyroid weight to body weight, and systolic blood pressure. The thyroid weight ratio increased significantly when systolic blood pressure rose to levels of 160–169 mmHg. Fregly et al. (1969) suggest that an unidentified primary factor increases blood pressure.

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**Fig. 4.**

Systolic blood pressure (SBP), pulse pressure (PP), heart rate (HR), plasma renin activity (PRA), and serum aldosterone (Ald) 3 weeks after operation, in Goldblatt 2K1C control group (open bars) and radiochemically (131I) thyroidectomized Goldblatt 2K1C group (shaded bars). ★$P < 0.001$. 

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to the threshold range without thyroid mediation, but when exceeding the threshold range, thyroid weight increases and may mediate further elevation of blood pressure.

Several parameters have been studied to determine if thyroid weight increases were secondary to hyper- or hypofunction of the gland, with T3 and T4 serum levels as those most frequently measured. In spontaneous hypertensive rats, age-dependent variations (Riox & Berkowitz 1977) and contradictory results in adult rats have been reported (Werner et al. 1975; Kojima et al. 1975; Wright et al. 1978). A decrease in T3 and T4 serum levels in Dahl-rats (McParland & Rapp 1982) and a reduction in T3 serum concentration in encapsulated hypertensive rats (Thratte et al. 1982) were observed. Our results (Fig. 1) show that DOCA-salt hypertensive rats had a marked reduction in comparison with their suitable controls, uninephrectomized-salt normotensive rats. We found no significant differences, however, in vasculorenal Goldblatt 2K1C hypertensive rats with respect to sham-operated rats (Fig. 1). The data obtained in DOCA-salt hypertension indicated that the increase of thyroid weight, previously reported in mineralocorticoid hypertension, is not due to gland hyperfunction but could be the result of a hypoactive goitre. Hence, the data do not support the fact that an increase in thyroid function plays a role in the elevated blood pressure of DOCA-salt hypertension.

Reduced thyroid activity reported in hypertensive is believed to be mediated by the action of a substance referred to as a ‘thyroid-depressing factor’ (TDF) by Thratte et al. (1982). This substance has been reported to be found in the liver (Levey 1967), spleen (Fregly & Thratte 1982), kidney (Lutherer et al. 1971) and plasma (Thratte et al. 1982). TDF is produced and released into the blood at a greater rate in hypertensive rats than in normotensive rats (Fregly & Thratte 1982). This factor reduces the uptake and binding of 131I by the thyroid gland and blocks the 131I uptake stimulated by TSH (Fregly & Thratte 1982). The normal T3 and T4 serum levels observed in Goldblatt 2K1C hypertensive rats suggest that TDF could not be activated in renin-dependent hypertension.

Radiochemical thyroidectomy prevents the development of hypertension produced by DOCA and salt treatment in uninephrectomized rats (Fig. 2 and 3), as has been reported before by Salgado (1954), using surgical and propylthiouracil procedures, and more recently by Rials et al. (1982). Furthermore, the present paper shows that thyroxine accelerates the course of DOCA-salt hypertension (Fig. 2 and 3). In vasculorenal Goldblatt 2K1C hypertension, radiothyroidectomy also blocks the elevation in blood pressure (Fig. 4). Similar results have been observed in other models of hypertension in rats: e.g. in genetic spontaneous hypertensive rats (Riox & Berkowitz 1977; Blumenthal et al. 1982; Rodgers & McNeill 1986), and induced by renal encapsulation (Fregly 1958). However, radiothyroidectomy was unable to reduce blood pressure in hypertensive dogs (Page & McCubbin 1952). Hence, our results, together with those obtained by the other authors, demonstrate that thyroidectomy prevent the development of hypertension in rats, independently of underlying pathophysiological mechanisms.

Thyroid function alterations could alter the pathophysiology of arterial hypertension through different mechanisms: e.g. affecting cardiac output (Graettinger et al. 1958), mineralocorticoid sensitivity (Taylor & Fregly 1964; Holmes & Dicala 1970) or blood volume (Graettinger et al. 1958, 1959), and sensitivity to vasoactive agents (Konen et al. 1967; Riox & Berkowitz 1977). Nevertheless, the mechanism by which thyroidectomy prevents different models of hypertension remains to be determined.

In DOCA-salt and Goldblatt 2K1C thyroidectomized rats, an important reduction in pulse pressure and heart rate was observed, whereas DOCA-salt rats treated with thyroxine show an enhancement in both parameters. These alterations are in agreement with the cardiovascular manifestations observed in hypothyroidism (Graettinger et al. 1958) and hyperthyroidism (Graettinger et al. 1959).

PRA is increased in hyperthyroidism (Hauger-Klevene et al. 1977) and in thyroxine-treated rats (Jimenez et al. 1982), and reduced in hypothyroidism and propylthiouracil-treated rats. Hauger-Klevene et al. (1977) demonstrated that kidney slices from hyper- and hypothyroid rats released more and less renin, respectively, than those obtained from euthyroid rats. On the other hand, it has been well established that DOCA and salt treatment inhibit renin secretion by Na+-mediated mechanisms (Geelhoed & Vander 1967). Our results (Fig. 3) show that the administration
of thyroxine does not significantly affect the suppressor effect on PRA produced by DOCA and salt treatment; thyroidectomy, however, prevent this suppressor effect on renin secretion.

As has been reported previously, both hypothyroidism and DOCA-salt treatment, used separately, reduced PRA. When both treatments are administered simultaneously, however, PRA is not suppressed to the same degree as was observed in DOCA-salt control rats. This paradoxical phenomenon could be explained by the data reported by Taylor & Fregly (1964) and Holmes & Discala (1970), who found that the threshold for mineralocorticoids is elevated approximately 9-fold in hypothyroid rats. Hence, in thyroidectomized rats, DOCA-treatment could produce less Na+ reabsorption and less renin inhibition.

Thyroidectomy did not significantly alter PRA or serum aldosterone in Goldblatt 2K1C hypertensive rats. Hence, an inadequate response of the renin-angiotensin system to renal ischemia cannot be implicated in the preventive effect of thyroidectomy in this model of hypertension.

In conclusion, this study demonstrates that: a) DOCA-salt rats have low levels of thyroid hormones, an alteration that is not present in Goldblatt 2K1C rats; b) treatment with thyroxine accelerates DOCA-salt hypertension without altering PRA; c) thyroidectomy prevents DOCA-salt hypertension and the suppressive effect of DOCA and salt treatment on PRA; and d) thyroidectomy, similarly, prevents vasculorenal Goldblatt 2K1C hypertension, with no changes in the activity of the renin-angiotensin-aldosterone system.

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Received July 9th, 1987.
Accepted December 17th, 1987.

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