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Effects of Two Different Experimental Situations of Hypothyroidism on Serum Aldosterone Concentration and Plasma Renin in Rats

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When hypothyroidism is induced surgically in early steps of development in the rat, an increase in serum aldosterone concentration (AC), in absence of changes in plasma renin activity (PRA), is observed. In contrast, in propylthiouracil (PTU) induced hypothyroidism, in adult animals, both AC and PRA decrease.

Potassium iodide (KI) or triiodo-L-thyronine (T_3) administration to thyroidectomized rats restores AC to normal levels, increasing PRA during the latter treatment.

A close relationship between AC and plasma renin concentration (PRC) is observed in these experimental situations.

The decrease in urinary aldosterone concentration (AC_u), and the relation found between AC/AC_u ratio and T_3 concentration, suggest that metabolic clearance of aldosterone might be related to peripheric T_3 levels in thyroidectomized animals, treated with KI or T_3.

These observations support the hypothesis, previously reported, which suggests different mechanisms involved in the control of aldosterone and renin release during the two different types of hypothyroidism.

Key words: Aldosterone, Renin, Hypothyroidism, Potassium iodide, Triiodo-L-thyronine.

The effect of thyroid hormones on various components which constitute the renin-angiotensin system (RAS) has been studied in the last few years. The results obtained in these studies have shown that thyroid hormones play an important role in this system, controlling the production of renin substrate within the liver (2, 18) and regulating the liberation of renal renin (10).

In situations of pharmacological hypothyroidism, induced by administra-

tion of propylthiouracil, a reduction in the secretion of aldosterone has been described (6), which correlates closely with the alterations observed in plasma renin activity (PRA) in the same experimental situation (12).

The aim of the present work has been to study the changes in serum and urine levels of aldosterone in animals in which hypothyroidism has been produced by thyroidectomy, at an early age, since the variations found in the RAS in these animals differ from those described in animals in which hypothyroidism was induced in later stages of development (14).

Materials and Methods

Animals and treatments. A group of 19 male albino-Wistar rats were surgically thyroidectomized at the fourth week of life. In order to ensure complete thyroid removal, 250 μCi of ^{131}I was administered to each animal in the week after thyroidectomy. The animals were fed with a deficient iodine diet (less than 25 μg/kg) and 0.1 % Ca-lactate in drinking water until sacrifice, at the 70th day of life. Signs of tetany were looked for. Seven animals received no treatment (T), while other two groups, of 6 animals each one, received daily, the last week of life, 3 mg/100 g b.w. of KI (Merck) i.p. (T+KI) and 3.3 μg/100 g b.w. of triiodo-L-thyronine (Sigma) s.c. (T+T_3), respectively. In another group, of 8 animals, pharmacological hypothyroidism was induced by daily administration 0.5 mg/100 g b.w. of propylthiouracil (Carlo Erba) i.p., for the twelve last days of life (PTU). Seven animals were used as reference group and were fed with a standard diet and tap water *ad libitum* (E).

Collection of samples. Before being killed the animals were put into individual metabolic cages and the 24 hour-urine was collected for each experimental group.

Blood samples were taken, under sodium pentobarbitone anaesthesia (Nembutal, Abbot Lab.), from artery aorta and divided in two aliquots. Both aliquots were then immediately centrifuged at 2.500 *g* for 15 min at 4° C. The plasma obtained, in presence of 50 μl of 0.16 M EDTA, and serum samples were stored at −20° C for subsequent use.

Renin-aldosterone assay. Angiotensin I concentration (AI) was determined by radioimmunoassay as described elsewhere (9): 10 μl of 0.8 M dimercaptopropranol and 20 μl of 0.34 M 8-hydroxiquinoleine sulphate were used as inhibitors of converting enzyme and angiotensinases.

The basal AI and the amounts generated, during plasma incubation at 37° C and pH 6.5, at 2 hourly intervals up to 8 h, were measured. PRA was determined from basal AI and that generated during 2 h of incubation, and the specific velocity constant of reaction was used to deduce PRC (20).

Aldosterone concentration in serum extracts (AC) and hydrolyzed urine (AC_u) samples were analyzed by radioimmunoassay (19). For serum aldosterone measurement, 100 μl of serum were extracted with 2 ml methylene chloride. The tubes were placed in a vortex and mixed at high speed for 5 min, centrifuged at 2.000 *g* for 15 min and the aqueous layer removed by suction. The solvent fraction was dried in a water bath at 37° C under a nitrogen stream. For urinary aldosterone measurement, 2 ml of 0.2 N hydrochloric acid were added to 1 ml of sample, mixed and incubated at 30 ± 2° C for 16 to 20 h.

Thyroid hormones determination. Thyroxine (T_4) and T_3 serum levels were

measured by radioimmunoassay to follow the changes of both hormones in these experimental groups. The results have been published elsewhere (14).

Sodium and potassium concentrations. Plasma and urinary sodium and potassium were measured by flame photometry.

Statistical methods. They were performed by Student's t-test and regression analysis.

Results

Figure 1 shows the changes in PRA, AC and AC_u following the induction of surgical and pharmacological hypothyroidism. In pharmacological hypothyroidism the PRA falls markedly, while in thyroidectomized animals the decrease observed is negligible. The administration of potassium iodide to thyroidectomized rats did not produce any changes in the kinetic parameter PRA. However, after triiodo-L-thyronine administration there was a significant increase.

In PTU-treated animals a decline in AC was observed, in marked contrast to the thyroidectomized animals, where AC increased sharply, and in thyroidectomized animals, treated with potassium iodide or triiodo-L-thyronine, where it returned to normal levels.

AC_u only showed a significant decrease in thyroidectomized rats. It recovered its normal levels following potassium iodide or triiodo-L-thyronine administration.

No significant changes in serum or urine electrolyte levels in pharmacologi-

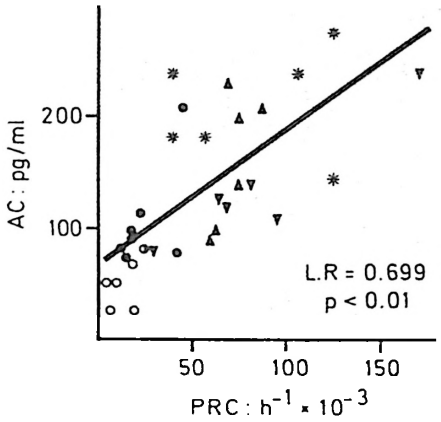

Fig. 2. *Relationship between serun aldosterone concentration (AC) and plasma renin concentration (PRC) in euthyroid (●), pharmacologically hypothyroid (o) and thyroidectomized (*) animals, and in these treated with KI (▲) or triiodo-L-thyronine (▼).*

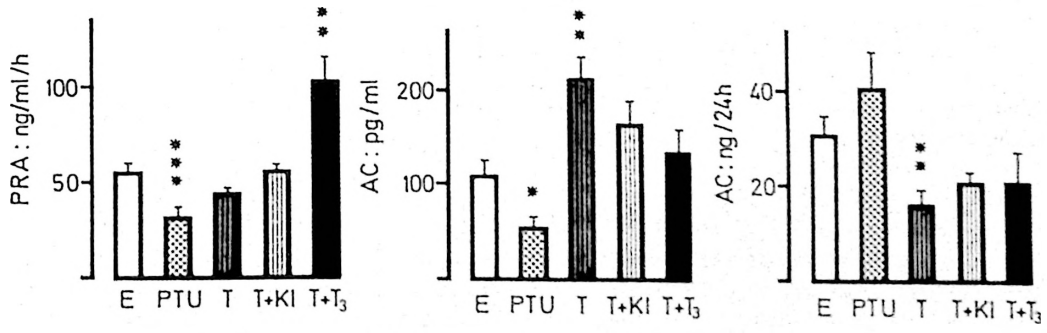

Fig. 1. *Plasma renin activity (PRA), serum aldosterone concentration (AC) and urinary aldosterone concentration (AC_u) in euthyroid (E), pharmacologically hypothyroid (PTU) and thyroidectomized (T) animals, and in these treated with KI (T + KI) or triiodo-L-thyronine (T + T_3). (*$p < 0.05$, **$p < 0.01$; ***$p < 0.001$).*

Table I. *Serum and urine levels of sodium and potassium in euthyroid (E), pharmacologically hypothyroid (PTU) and thyroidectomized (T) animals.*
Mean values ± SEM are given. N is the number of experiments. Unpaired t-test was used (* $p < 0.05$; ** $p < 0.01$).

	N	Serum		Urine	
		Na^+ (mEq/l)	K^+	Na^+ (mEq/24 h/kg)	K^+
E	7	139.8 ± 0.3	4.6 ± 0.1	2.04 ± 0.16	3.77 ± 0.26
T	6	138.9 ± 0.9	5.2 ± 0.1 **	3.10 ± 0.29 *	1.82 ± 0.17**
T + KI	6	137.8 ± 0.5	4.9 ± 0.1	3.04 ± 0.39 *	3.13 ± 0.08
$T + T_3$	6	137.6 ± 1.4	4.4 ± 0.1	2.65 ± 0.23	2.78 ± 0.34
PTU	7	140.0 ± 0.4	4.6 ± 0.1	2.56 ± 0.11	3.38 ± 0.16

cally hypothyroid animals were observed (table I), whereas there were significant alterations in thyroidectomized animals; the serum potassium concentration increased, while the potassium urine decreased. Triiodo-L-thyronine and potassium iodide administration to thyroidectomized animals restored serum levels to normal, but only after triiodo-L-thyronine administration did the urinary potassium return to normal. The thyroidectomized animals showed a slight natriuretic effect, which disappeared following triiodo-L-thyronine treatment.

A relationship between PRA and AC was observed (figure 2), with a 49 % determination coefficient.

Table II shows simple determination coefficients (R_1) for PRC-AC and PRC-AC/AC_u, and multiple determination coefficients (R_2) in which T_3 influence is considered in both cases.

Table II. *Statistical values of determination coefficient according to thyroid changes.*
R_1: simple determination coefficient. R_2: multiple determination coefficient. Other details are described in the text.

		Serum (AC)	Urine (AC/AC_u)
T	R_1	—	0.16
	R_2	—	0.17
T + KI	R_1	0.31	0.40
	R_2	0.54	0.67
T + T3	R_1	0.88	0.08
	R_2	0.96	0.12

Discussion

Numerous studies have revealed the existence of a relationship between the thyroid and adrenal glands. These reports, mainly referred to glucocorticoids secretion, have shown that hyper- and hypothyroidism produce an increase and a decrease of the adrenal gland activity, respectively (4, 8). However, there are few, and sometimes contradictory, data reported about the action of thyroid hormones upon mineralocorticoids metabolism (1, 16).

On the other hand, it is well known that thyroid hormones, especially the triiodothyronine, exert an important role on the renin-angiotensin system activity, regulating the hepatic synthesis of angiotensinogen (14), and renal renin release (13). As a consequence of these actions an increase in PRA during hyperthyoridism, and a decrease of it during hypothyroidism, have been described in patients and animals (11).

Although in sodium deficiency the primacy of the RAS is discussed (17), this system has been accepted one of the principal stimuli in regulating the synthesis and release of aldosterone.

Because a close relation between PRA and T_3 exists in these conditions (12), parallel changes in PRA and PRC could be expected in both pharmacological and surgical hypothyroidism. However, the results obtained in this work do no support this hypothesis. While in pharmacologically induced hypothyroidism there is a decrease in PRA and AC, in thyroidectomized animals an increase in AC, in absence of changes in PRA, is found.

The absence of correlation between PRA and AC observed in this case would suggest that RAS is not involved in the control of synthesis and release of aldosterone, but in rats PRA has been reported to be an inadequate index to evaluate RAS (15).

The existence of a correlation between PRC and AC, in all the experimental situations studied, support the fact that RAS is really involved in the regulation of aldosterone synthesis and release, explaining a 49 % of the changes observed in AC, although it is not the only factor in regulating AC metabolism in thyroidectomized animals.

The AC decrease in pharmacological hypothyroidism may result either from a direct action of PTU on the adrenal cortex or an indirect action mediated through RAS. The second hypothesis could be supported by the decrease in PRA, as consequence of the reduced adenyl-ciclase and adrenergic activities (11, 13), and a concomitantly decreased aldosterone secretion associated with a reduced total pressor activity of kidney extracts from hypothyroid rats (6).

No change in serum or urine electrolyte levels that might affect directly aldosterone secretion rate, was observed in these animals.

In thyroidectomized animals the AC increase observed may be either a consequence of an increase of aldosterone secretion or from a decrease in its hepatic catabolism and/or renal clearance. The first possibility could be kept in mind since an increase in serum potassium levels and a slight natriuretic response were observed. In addition there was a good correlation between PRC and AC, which further supports these results.

It is well known that the increase of serum potassium promotes aldosterone secretion and diminishes renal renin release (5). In these animals the observations suggest that the natriuretic response, due to renal immaturity and lower tubular sensitivity to aldosterone (21), is a predominant stimulus upon renal renin release.

It is likely that thyroidectomy modifies aldosterone metabolism in liver and/or its renal clearance. Although urinary aldosterone, measured by radioimmunoassay, represents only a 0.5 % of its urinary metabolites (3, 7), the AC/AC_u ratio, used in this work as an index of hepatic metabolism and/or renal clearance, changes in thyroidectomized animals after KI or T_3 administration, showing a dependence from circulating thyroid hormones. For lower levels of T_3, produced by extrathyroidal synthesis after KI administration, a relation of this hormone with AC/AC_u ratio is observed. However, after T_3 treatment, this relation disappears.

While KI administration to thyroidectomized animals restores AC only, T_3 treatment restores AC and urinary sodium excretion to normal levels, increasing PRA. This increase in PRA is an effect of T_3 mediated by increasing angiotensinogen synthesis from liver, as has been reported previously (2).

The results show that the control of aldosterone synthesis and/or release, in hypothyroid animals, is different, depending on whether hypothyroidism has been induced in early steps of development or in adult animals.

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Resumen

Tras la inducción del hipotiroidismo, en los primeros estados de vida en la rata, por tiroidectomía quirúrgica, se observa un incremento en la concentración de aldosterona en suero (AC), en ausencia de cambio en la actividad renina plasmática (PRA). Por el contrario, durante el hipotiroidismo inducido, en animales adultos, por administración de propiltiouracilo (PTU), la AC y PRA están descendidas.

La administración de ioduro potásico (KI) o triiodo-L-tironina (T_3) a los animales tiroidectomizados restablece la AC, aumentando la PRA en el caso del último tratamiento.

Una buena correlación fue observada entre la AC y los cambios en la concentración de renina en plasma (PRC) inducidos en estas situaciones experimentales.

El descenso en la concentración de aldosterona en orina (AC_u) en animales tiroidectomizados, así como la relación entre la razón AC/AC_u y T_3, sugieren que el aclaramiento metabólico de aldosterona está relacionado con los niveles periféricos de T_3 en animales tiroidectomizados, y tratados con KI o T_3.

Estos resultados soportan previas observaciones en las que se pone de manifiesto que en el control de secreción de aldosterona y renina, en ambos casos de hipotiroidismo, están involucrados mecanismos diferentes.

References

1. Boler, R. K. and Moore, N. A.: *Horm. Res.*, **16**, 209-218, 1982.
2. Bounhik, J., Galen, F. X., Clauser, E., Menard, J. and Corvol, P.: *Endocrinology*, **108**, 647-650, 1981.
3. Christy, N. P.: In «The Thyroid» (Werner, S. C. and Ingbar, S. H., eds.). Harper and Row, Hagerstown, 1978, p. 780.
4. Corvol, P., Oblin, M. E., Degoulet, P., Fressinaud, P. and Menard, J.: *Endocrinology*, **100**, 1008-1013, 1977.
5. Flamenbaun, W., Kleinman, J. G., MacNeil, J. S., Hamburger, R. J. and Kotchen, T. A.: *Amer. J. Physiol.*, **229**, 370-375, 1975.
6. Fregly, M. J., Cade, R. J., Waters, I. W., Straw, J. A. and Taylor, R. E.: *Endocrinology*, **77**, 777-784, 1965.
7. Guelpa-Decorzant, C., Riondel, A. M., Chabert, P. R. and Valloton, M. B.: *J. Steriod Biochem.*, **10**, 365-369, 1979.
8. Guyton, A. C.: In «Medical Physiology». Saunders, Philadelphia, 1976, p. 1014.
9. Haber, E., Koerner, T., Page, L. B., Kliman, B. and Purnode, A.: *J. Clin. Endocr.*, **29**, 1349-1355, 1969.
10. Hauger-Klevene, J. H. and Levin, G. M.: *Medicina* (Buenos Aires), **36**, 219-222, 1976.
11. Hauger-Klevene, J. H., De Vito, E. and Fasciolo, J. C.: *Acta Physiol. Latinoam.*, **27**, 37-41, 1977.
12. Jiménez, E., Montiel, M., Narváez, J. A. and Morell, M.: *Rev. esp. Fisiol.*, **38**, 149-154, 1982.
13. Jiménez, E., Montiel, M., Narváez, J. A. and Morell, M.: *Rev. esp. Fisiol.*, **38**, 35-40, 1982.
14. Jiménez, E., Montiel, M., Narváez, J. A. and Morell, M.: *Acta Endocrinol.*, (Copenh.), **105**, 505-510, 1984.
15. Jiménez, E., Montiel, M., Narváez, J. A., Miranda, M. T., Parras, L. and Morell, M.: *Horm. Metabol. Res.*, **16**, 315-318, 1984.
16. Luestcher, J. A., Cohn, A. P., Camargo, C. A., Dowdy, A. J. and Callaghan, A. M.: *J. Clin. Endocrinol. Metab.*, **23**, 873-880, 1963.
17. McCaa, R. E., Langford, H. G., Montalvo, J. M., Andy, O. J., Read, V. H. and McCaa, C. S.: *Hypertension*, Suppl. I., **3**, 74-80, 1981.
18. Murakami, E., Hiwada, K. and Kobuku, T.: *Jpn. Cir. J.*, **45**, 1078-1082, 1981.
19. Ogihara, T., Iimura, K., Nishi, K., Aranawa, Y., Tagari, A., Kurata, K., Miyai, K. and Kumahara, K.: *J. Clin. Endocrinol. Metabol.*, **45**, 726-731, 1977.
20. Ryan, J. W., McKenzie, J. A. and Lee, M. R.: *Biochem. J.*, **108**, 679-685, 1968.
21. Taylor, R. E. and Fregly, M. J.: *Endocrinology*, **75**, 33-41, 1964.