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Effects of Hyper- and Hypothyroidism on the Basal Levels of Angiotensin I and Kinetic Parameters of Renin-Angiotensin System in Male Rats

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The basal levels of angiotensin I and kinetic parameters of renin-angiotensin system were studied under control, hyper- and hypothyroidism conditions. The serum levels of triiodothyronine (T_3) and thyroxine (T_4) and plasma angiotensin I have been measured by radioimmunoassay. Hyperthyroidism was induced by 5.5 μg/200 g of T_3 or 100 μg/200 g of T_4 administration for 12 days, and hypothyroidism by propylthiouracil (PTU) administration of 1 mg/200 g for 12 days.

Basal levels of angiotensin I and plasma renin activity (PRA) increased after T_3 injection, were not altered by T_4 and decreased after PTU administration. T_3 and T_4 induced an increase in plasma renin concentration (PRC), while PTU induced a decrease in PRC. Plasma renin substrate (PRS) decreased in hyperthyroid rats and was unchanged by experimentally induced hypothyroidism. A good correlation between T_3 serum levels and PRA was found, but there was no such correlation between T_4 serum levels and PRA.

The vascular responsiveness to a variety of pressor and depressor drugs depend of thyroid activity, finding a first relation between the thyroid function and the renin-angiotensin system (14).

The renin-release from juxtaglomerular cells is controlled by changes in sodium concentration in the macula densa, by changes in intravascular pressure in the kidney afferent arteriole and changes in adrenergic activity (4). STOUT *et al.* (20) have shown that changes in adrenergic activity are always found in cases of hyper- and hypothyroidism. These results raised the possibility that changes in renin-release may occur under those pathological conditions. In this way, HAUGER-KLEVENE *et al.* (7, 8) have found significant changes in PRA, such as an increase in hyperthyroidism and a decrease in hypothyroidism.

The present paper was undertaken in

order to study kinetic parameters of the reaction angiotensinogen-angiotensin I such as PRA, PRC and PRS and its relation to basal levels of angiotensin I in hyper- and hypothyroid rats.

Materials and Methods

Adult male rats of the Wistar strain, weighing aproximately 200 g were used. All the animals were fed the same standard diet and tap water was available *ad libitum*.

Twelve days prior to blood sampling a daily dose of propylthiouracil (PTU) was given by s.c. injection to one group of animals in order to induce hypothyroidism. The dose consisted in 1 mg of PTU (Carlo Erba) diluted with isotonic saline. To induce hyperthyroidism other group of animals was treated with 100 μg of thyroxine (T_4) and another group was treated with 5.5 μg of l-triiodothyronine (T_3). T_4 (BDH) and T_3 (Sigma) were diluted in 0.9 % NaCl solution made slightly alkaline with 0.1 N NaOH and injected s.c. daily during twelve days prior to blood sampling.

All groups of rats, seventy days old ,were anaesthetized with sodium pentobarbitone (Nembutal, Abbot) and blood samples were collected from abdominal aorta in ice-cold syringes. Plasma and serum were stored at —20° C until assayed for renin-angiotensin parameters and thyroid hormones.

T_3 and T_4 serum levels were measured by radioimmunoassay methods (The Radiochemical Centre, Amersham) in order to follow the changes of both hormones.

Two plasma aliquots were separated and 10 μl of dimercaptopropanol 0.8 M (Merck) and 20 μl 8-hydroxychinoleine 0.34 M (Merck) were added to each aliquot. Basal concentration of angiotensin I was estimated in one aliquot of these samples at 0° C and pH 6.5. In addition, another aliquot was incubated at 37° C and pH 6.5, and angiotensin I concentration was measured at 2, 4, 6 and 8 hours of incubation. Angiotensin I concentration was always analyzed by radioimmunoassay (6) (Cea-Ire-Sorin). PRA was evaluated as half the difference between the angiotensin I concentration obtained 2 hours after incubation and that existing in basal conditions and expressed in units of nmol/ml/h angiotensin I. PRS or angiotensinogen concentration was determined following the method described in detail by CAMPILLO *et al.* (3). In summary, a linear relation between the reciprocal of angiotensin I concentration at the different times of incubation versus the reciprocal of the time of incubation was obtained. The interception of this line is the reciprocal of the angiotensinogen concentration. PRC was measured as the specific velocity constant of the reaction (18). Therefore, it was evaluated from the following equation:

$$PRC = 1/t \ln [PRS/(PRS-P_t)]$$

where P_t is the angiotensin I concentration obtained in the incubation at 37° C for time t.

Results

The data in table I show changes in serum concentration of T_4 and T_3 in hyper- and hypothyroid rats. Thyroxine administration induces a significant increase in T_4 serum levels, but does not change significantly the T_3 concentration. Conversely, l-triiodothyronine injection induced a significant increase in T_3 and decrease in T_4 serum concentration. In PTU treated rats both T_4 and T_3 serum concentration decrease.

These results are considered a good estimate of changes induced experimentally in the thyroid function.

Table I. *Thyroxine (T_4) and Triiodothyronine (T_3) serum levels (nmol/l) in Hyper- and Hypothyroid rats.*
Mean values ± S.E.M. are given. N is the number of experiments.

	N	T_3	T_4
Control	8	5.6 ± 0.9	22.2 ± 1.6
Hypothyroids	8	0.25 ± 0.03 ***	6.4 ± 0.4 ***
Hyperthyroids			
Thyroxine	8	5.5 ± 0.6	161.9 ± 9.1 ***
l-Triiodothyronine	7	14.8 ± 1.4 ***	6.8 ± 0.4 ***

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Paired t-test was used.

Table II. *Angiotensin I basal and kinetic parameters of renin-angiotensin system in Hyper- and Hypothyroidism.*
Mean values ± S.E.M. × 10^{-3} are given. N is the number of experiments.

	N	Angiotensin I (nmol/ml)	PRA (nmol/ml/h)	PRC (h^{-1})	PRS (nmol/ml)
Control	8	5.0±0.6	43.5±4.0	27.3± 4.2	1,470±193
Hypothyroidism	8	3.4±0.4 ***	22.6±2.8 ***	16.5± 3.2 *	1,578±376
Hyperthyroidism					
Thyroxine	8	5.5±0.5	44.1±3.2	64.0± 6.5 ***	604± 67 ***
l-Triiodothyronine	7	8.1±0.6 **	60.3±9.0 *	80.9±16.0 **	640± 69 ***

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Paired t-test was used.

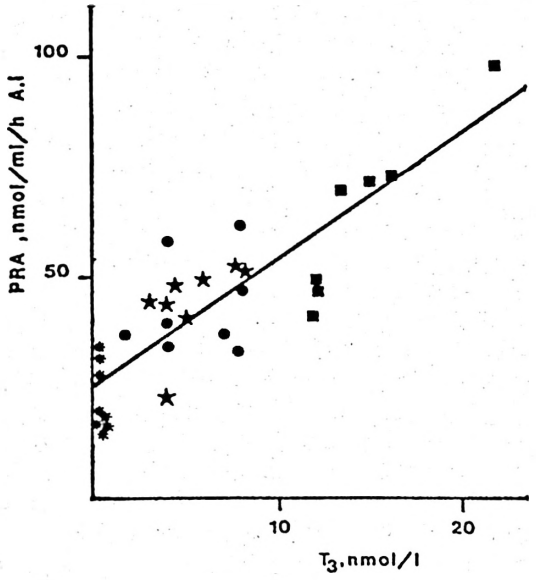

Fig. 1. *PRA as a function of T_3 serum levels after PTU (✻), T_3 (■) and T_4 (★) administration and in control rats (●).*
The regression line is $PRA = m_1\ T_3 + m_2\ T_4 + n$. The linear regression, (r) = 0.73.

Table II shows the values for the basal levels of angiotensin I and kinetic parameters of renin-angiotensin system. Basal levels of angiotensin I decreased under action of PTU, increased after T_3 administration ,and it was not affected by T_4 administration. PRA decreased in rats which were given PTU. However, T_3 increased PRA and T_4 did not induce any change in PRA. PTU induced a decrease in PRC, while both T_3 and T_4 administration increased PRC values. PRS levels were not changed by PTU, whereas a decrease after the injection of either T_3 or T_4 was found. The influence of thyroid hormones T_3 and T_4 on PRA was studied by means of linear regression analysis. No significant correlation was found when the influence of T_4 on PRA was tested. However a significant correlation was obtained between PRA and T_3 serum levels with regression line having a slope of 2.3 and intercept of 28.5 (fig. 1).

Discussion

In the study of experimental alterations of the thyroid function and the renin-angiotensin system different methods have been followed regarding the hormonal concentration doses and time of application of treatment, in order to induce hyperthyroidism (7, 8). On the other hand, the variations in the levels of thyroid hormones have not been analyzed, thus impeding to establish an interrelation between the thyroid hormones and the nature of these changes in the renin system. In this paper the administering of a daily dose of propylthiouracil induces a state of hypothyroidism reducing strongly the serum levels of T_4 and T_3, according to observations made by PASCUAL *et al.* (15). Likewise the levels of T_4 and T_3 determined after administering thyroxine and triiodothyronine prove to be a good reason for the hyperthyroidism provoked in this way.

The PRA is frequently used as the only kinetic parameter to evaluate the alterations of the renin-angiotensin system and, specifically, that of the changes in the renal renin release. However, some previous studies have proved that there are situations as those after administering dexametasone (17), treatment with estrogens (1), hypophysectomy (9), presence of activating or inhibiting factors of angiotensinogen-angiotensin I reaction and others related to the experimental conditions, such as pH and temperature (12), in which in the absence of changes in the renal renin release, alterations are produced in the PRA. In accordance with these observations, the results of the present study, obtained in hyperthyroid rats by means of administering them thyroxine, suggest that such a kinetic parameter should not be considered as a good estimate by itself, and that the determinations of other components of the system such as PRC and PRS contribute with a greater information on the study of the activity of the renin-angiotensin system. On the other hand, the basal levels of angiotensin I could be used in the same measure as the PRA to follow the changes in the system. Under the studied conditions, the PRA increase observed in thyrotoxic rats and conversely the PRA decrease in hypothyroid rats is accompanied by an increase or decrease in the basal levels of angiotensin I, respectively.

The changes in PRA and basal angiotensin I may be interpreted as a consequence of alterations in PRC during the hyper- or hypothyroidism, either by a direct action of the thyroid hormones regulating the synthesis of renal renin (8) or, indirectly, regulating its release through the changes of the adrenergic activity and the adenil cyclase activity. In this respect, the studies carried out prove the importance of the levels in plasma of catecholamines (5) and AMP_c (16) in the regulation of the renin release and the existence of a relation between the adrenergic activity (13, 19) and adenil cyclase (10, 11) and the changes of the thyroid function.

However, the importance that the levels of angiotensinogen may have in the change of PRA and basal levels of angiotensin I in hyperthyroid rats, should also be taken into consideration. Recently BOUHNIK *et al.* (2) have confirmed that the production of renin substrate takes place quickly and depends upon the action of the thyroid hormones. In accordance with these results, the increase in PRA and basal levels of angiotensin I in rats treated with triiodothyronine and the existence of a good correlation between the peripheric levels of T_3 and PRA in euthyroid, hyper and hypothyroid animals, not depending upon the levels of T_4, prove that there is a more intense action of T_3 than that of T_4 in the changes of the renin-angiotensin system and suggest that the increase in PRC in hyperthyroid rats, after administering thyroxine is produced through

the peripheric conversion of T_4 into T_3 during the drugs just before the animals are killed.

Resumen

Se estudian los niveles basales de angiotensina I y parámetros cinéticos del sistema renina-angiotensina en ratas controles, hiper- e hipotiroideas. Los niveles séricos de tiroxina (T_4) y triiodotironina (T_3), y angiotensina I en plasma se determinan por radioinmunoensayo. El hipertiroidismo es inducido por la administración de 5,5 μg/200 g de T_3 ó 100 μg/200 g de T_4 durante 12 días y el hipotiroidismo por la administración de 1 mg/200 g de propiltiouracilo (PTU) durante el mismo período.

Los niveles basales de angiotensina I y actividad renina plasmática (PRA) aumentan después de la administración de T_3, no alterándose por la administración de T_4 y disminuyen después de la administración de PTU. El tratamiento con T_3 y T_4 aumenta la concentración plasmática de renina (PRC). Por el contrario, el PTU provoca una disminución en PRC. En ratas hipertiroideas se observa un descenso en los niveles plasmáticos de substrato de renina, no existiendo cambios de este parámetro durante el hipotiroidismo. Existe una buena correlación entre los niveles séricos de T_3 y PRA, pero no entre los de T_4 y PRA.

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