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The Effect of Experimental Hyperthyroidism on Renal and Adrenal Weight Increase in Mice

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Summary

The mouse kidneys are enlarged after the administration of thyroxine and this influence is not mediated through androgens. The administration of thyroxine increased the weight of the adrenals and the level of plasma corticosterone. Besides the direct effect of the thyroid hormones on the kidney, our findings indicate that the excess of triiodothyronine and thyroxine stimulates the activity of adrenals indirectly and evokes hyperadrenocorticism which could be related to the action of adrenal steroids on kidney function and kidney growth. In accordance with the above mentioned hypothesis it has been shown that aminoglutethimide, a potent blocker of adrenal steroidogenesis, decreases the level of plasma corticosterone and inhibits the enlargement of the kidney in hyperthyroid mice in spite of the high serum thyroxine values.

Key words
Thyroxine - Kidney hypertrophy - Adrenal enlargement - Plasma corticosterone - Aminoglutethimide

Introduction

Renal growth is influenced by many hormonal and nutritional factors (Nomura *et al.* 1985). The thyroid hormone has been reported to stimulate renal growth as judged by an increase in organ weight, DNA synthesis and mitotic activity (Pacovský 1959, Bradley *et al.* 1974, Stephan *et al.* 1982). Kidney weight is decreased more by thyroidectomy than by castration (Kochakian *et al.* 1954). Renal compensatory hypertrophy occurs after uninephrectomy in the hypothyroid rat but it is impaired when compared to renal compensatory hypertrophy occurring in euthyroid controls. The mechanism of the renotrophic action of thyroxine is complex and several factors are probably involved. There is an evidence from animal studies that the excess of circulating thyroid hormones results in adrenal enlargement (Peterson 1958).

The aim of the present study was to demonstrate whether there is any association between kidney and adrenal weight in the presence of hyperthyroidism

due to administration of thyroxine and whether corticosterone affects the enlargement of the kidney.

Material and Methods

Studies were performed on male mice of the H strain (Velaz, Prague). Animals were fed a standard laboratory diet (Velaz, Prague) containing 23 % protein and water *ad libitum* and were kept in an indirectly illuminated room with controlled temperature (24±2 °C). The animals were divided into six groups of 8 animals each: 1. intact animals, 2. 21 days castrated animals, 3. intact animals fed L-thyroxine for 21 days, 4. 21 days castrated mice fed L-thyroxine for 21 days, 5. intact animals fed L-thyroxine and aminoglutethimide for 21 days, 6. intact animals fed aminoglutethimide for 21 days. L-thyroxine (Henning Berlin) and aminoglutethimide (alpha-ethyl-alpha-p-amino-phenyl-glutarimide), Eüpten R, CIBA, were mixed to a diet in a dose of about 5 μg/mouse/day of thyroxine and 20 mg/mouse/day of aminoglutethimide. The animals were weighed before and after the experiment and their food consumption was measured daily. No scraps were left so that amounts of the drug eaten corresponded to the dose of thyroxine and aminoglutethimide indicated above. At the end of the experiment, after 21 days administration of the drug, mice were killed in the morning and the blood was withdrawn and the adrenals, seminal vesicles and kidneys were removed, cleaned and weighed on a Roller-Smith torsion balance. Kidneys were immediately placed in ice-cold 0.4 mol/l perchloric acid and homogenized in a Potter-Elvehjem homogenizer. The total nucleic acids were determined using the method of De Deken-Grenson and De Deken (1959). DNA was determined spectrophotometrically (Burton 1956). For estimation of DNA we used a standard from Lachema Brno (purified from fish sperm). RNA was determined by subtraction of the DNA value from the total nucleic acid value. Serum level of thyroxine and triiodothyronine were measured by RIA using a kit made by URVJT Košice (Czechoslovakia). The concentration of corticosterone in the plasma was determined by the RIA method (Mihaly *et al.* 1982).

The results are presented as means ± S.D. Significant differences between groups were determined by analysis of variance, followed by Duncan's multiple range test (Duncan 1955).

Results

Intact and castrated mice with experimental hyperthyroidism had significantly higher blood thyroxine and triiodothyronine concentrations than intact or castrated animals without thyroxine. The administration of aminoglutethimide to intact mice caused a significant decrease of blood thyroxine in comparison with intact animals. However, the simultaneous administration of aminoglutethimide with thyroxine increased the serum thyroxine concentration above the levels seen in both intact animals and mice with experimental hyperthyroidism (Tab. 1).

The administration of thyroxine was followed by an increase in plasma corticosterone in intact and castrated mice. A significant decrease in plasma corticosterone was observed after administration of aminoglutethimide. Simultaneous administration of thyroxine and aminoglutethimide completely inhibited the effect of thyroxine on plasma corticosterone levels (Tab. 1).

Castration of male mice produced the expected decrease in weight and RNA content of the mouse kidney (Tab. 2). The induction of hyperthyroidism in mice during 21 days after castration caused a significant increase of the weight and RNA content of the kidney as compared with castrated animals without thyroxine. The increase in weight of the kidney in intact mice after thyroxine administration was accompanied by a proportional increase in RNA. The kidney DNA content rose by only 9 %. The administration of aminoglutethimide to intact animals significantly

Table 1

The adrenal weight and blood thyroxine, triiodothyronine and corticosterone in intact and castrated mice with and without thyroxine or aminoglutethimide

	Adrenals mg/100 g b.w.	Corticosterone nmol/l plasma	Thyroxine nmol/l serum	Triiodothyronine nmol/l serum
1. Intact	12.4±1	228.2±115	59.9±7.9	1.99±0.5
	3,4,5,6	*3,4,6*	*3,4,5,6*	*3,4,5,6*
2. Castrate	12.7±2	208.0±80	57.6±6.8	1.80±0.6
	3,4,5,6	*3,4,6*	*3,4,5,6*	*3,4,5,6*
3. Intact	17.5±1	439.1±161	167.3±43.3	5.63±3.5
+ Thyroxine	*1,2,5,6*	*1,2,6*	*1,2,5,6*	*1,2,5,6*
4. Castrate	23.6±6	430.5±109	169.3±37.2	5.65±3.3
+ Thyroxine	*1,2,5,6*	*1,2,6*	*1,2,5,6*	*1,2,5,6*
5. Intact	37.9±7	225.0±112	296.0±5.7	9.30±1.4
+ Thyroxine	*1,2,3,4,6*	*3,4,6*	*1,2,3,4,6*	*1,2,3,4,6*
+ Aminoglutethimide				
6. Intact	26.8±3	138.6±66	42.0±5.3	1.24±0.6
+ Aminoglutethimide	*1,2,3,5*	*1,2,3,4,5*	*1,2,3,4,5*	*1,2,3,4,5*

Mean values ± S.D.; the numbers of groups with significantly different means ($p<0.01$) are given in italics

Table 2

The body weight, kidney and seminal vesicle relative weights, RNA and DNA content of the kidneys in intact and castrated mice with and without thyroxine or aminoglutethimide

	Body g	Kidney mg/100 g b.w.	RNA mg	DNA mg	Seminal vesicle mg/100 g b.w.
1. Intact	40.3	1873±112	3.34±0.3	2.78±0.3	4.24±0.48
	6	*2,3,4,5,6*	*2,3,4,5,6*		*2,4,6*
2. Castrate	40.2	1161±59	1.50±0.1	2.48±0.1	0.56±0.03
		1,3,4,5	*1,3,4,5*		*1,3,5,6*
3. Intact	37.7	2323±134	3.98±0.4	3.02±0.4	4.30±0.42
+ Thyroxine		*1,2,4,5,6*	*1,2,4,5,6*		*2,4,6*
4. Castrate	39.7	1572±73	2.40±0.2	2.70±0.2	0.61±0.08
+ Thyroxine		*1,2,3*	*1,2,3,6*		*1,3,5,6*
5. Intact	39.3	1602±137	2.44±0.3	2.78±0.2	4.26±0.28
+ Thyroxine		*1,2,3,6*	*1,2,3,6*		*2,4*
+ Aminoglutethimide					
6. Intact	36.2	1466±105	1.57±0.3	2.55±0.3	3.03±0.47
+ Aminoglutethimide	*1*	*1,3,5*	*1,3,4,5*		*1,2,3,4*

Mean values ± S.D.; the numbers of groups with significantly different means ($p<0.01$) are given in italics

decreased the weight of kidneys by 23 % as compared with the controls. The RNA content of the kidney was affected in a similar way as the weight. Morphological observations made under a light microscope revealed no structural differences between intact and aminoglutethimide-treated kidneys. The simultaneous administration of aminoglutethimide and thyroxine completely inhibited the effect of thyroxine on kidney growth and RNA content (Tab. 2).

The induction of hyperthyroidism in castrated and intact mice significantly increased the weight of the adrenals. As anticipated adrenal weight rose markedly after aminoglutethimide. A further increase of the weight of adrenals was observed after the simultaneous administration of aminoglutethimide and thyroxine (Tab. 1).

Discussion

Thyroxine-induced hypermetabolism is followed by a general tissue proteocatabolic reaction. The only organs the size of which increases are the adrenals, the kidney and the heart (Schreiber *et al.* 1985). Our results in intact thyreotoxic mice confirm earlier findings which showed significant kidney growth in rats with experimental hyperthyroidism (Kochakian *et al.* 1954, Bradley *et al.* 1974, Stephan *et al.* 1982). The influence of thyroxine on kidney is not mediated through the androgens. Administration of thyroxine produced significant changes in mouse kidneys in both intact and castrated animals without androgens. The mechanism of origin of kidney hypertrophy produced by thyroxine is still unknown and several factors are probably involved. Thyroid hormones act directly on the nuclei of sensitive cells to regulate protein synthesis and growth and stimulate sodium reabsorption in the proximal tubule and in Henle's loop (Somjen *et al.* 1981). In the kidney triiodothyronine binds to nuclear receptors, modulate RNA and protein synthesis and enhances tubular reabsorption of sodium (Murayama *et al.* 1985).

The administration of thyroxine in our experiments was foliowed by an increase in the weight of the adrenals and increased levels of plasma corticosterone. Hypermetabolism due to the administration of thyroxine is a chronic stress situation which was shown to activate the brain-pituitary unit and decrease its sensitivity to corticosteroid feedback inhibition, so that high ACTH and corticosterone levels can be maintained throughout the period of stimulation (Keller-Wood and Dallman 1984). It was shown that an intact pituitary is necessary for obtaining adrenal hypertrophy following thyroid administration in animals (Peterson 1958). This observation would also seem to rule out the possibility that an excess of circulating thyroid hormone acts directly on the adrenal cortex to increase cortical secretion independently of the pituitary. An excess of adrenal steroids increases sodium reabsorption and potassium secretion both being due to active transport (Wright 1977). There is evidence that glucocorticoids (Bates and Garrison 1974) and mineralocorticoids (Moraski 1966) stimulate renal growth as judged by the increased weight of this organ. High plasma corticosterone level in our mice treated with thyroxine could be related to the action of adrenal steroids on kidney function and growth. The fact that aminoglutethimide, a potent blocker of adrenal steroidogenesis, in spite of a high blood thyroxine, decreases the plasma corticosterone and inhibits enlargement of the kidney in hyperthyroid mice, is in accordance with the above mentioned theory. There is no doubt that aminoglutethimide inhibits the renal growth reaction to administration of thyroxine,

but it is not certain whether the inhibition of adrenal steroidogenesis is the only route. Inhibition of steroidogenesis in the testes (Broulík and Stárka 1980) is another possibility for explaining of aminoglutethimide action. However, in our experiments administration of thyroxine completely restored the decrease in the weight of seminal vesicles induced by aminoglutethimide administration.

Aminoglutethimide is a goitrogen and decreases plasma thyroxine and triiodothyronine levels (Gower 1974). High plasma thyroxine and triiodothyronine levels after simultaneous administration of aminoglutethimide and thyroxine to intact animals are due to the action of aminoglutethimide which decreases the rate of thyroxine and triiodothyronine turnover.

Besides demonstrating a direct effect of thyroid hormones on the kidney our findings indicate that excess of triiodothyronine and thyroxine indirectly stimulates the activity of adrenals and evokes hyperadrenocorticism which could be related to the action of adrenal steroids on kidney function and kidney growth. Whether this is a parallel reaction of the two organs to hyperthyroidism or whether it is a causal relationship between adrenal and kidney hypertrophy deserves further investigation.

References

BATES R.W., GARRISON M.M.: Hormonal interactions among ACTH, cortisone and dexamethasone upon size of kidney, liver and adrenal. *Proc. Soc. Exp. Biol. Med.* **146:** 725–728, 1974.

BRADLEY S.E., STEPHAN F., COELKE J.B., REVILLE P.: The thyroid and the kidney. *Kidney Int.* **6:** 346–365, 1974.

BROULÍK P., STÁRKA L.: Effect of aminoglutethimide on androgenic functions in mice. *Endocrinol. Exp.* **14:** 67–71, 1980.

BURTON K.: A study of the conditions and mechanism of the diphenylamide reaction for the colorimetric estimation of DNA. *Biochem J.* **62:** 315–323, 1956.

DE DEKEN-GRENSON M., DE DEKEN R.H.: Elimination of substances interfering with nucleic acid estimation. *Biochem. Biophys. Acta* **31:** 195–206, 1959.

DUNCAN D.B.: Multiple range and multiple F tests. *Biometrics* **11:** 1–4, 1955.

GOWER D.B.: Modifiers of steroid hormone metabolism. A review of their chemistry, biochemistry and clinical applications. *J. Steroid Biochem.* **5:** 501–523, 1974.

KELLER-WOOD M.E., DALLMAN M.F.: Corticosterone inhibition of ACTH secretion. *Endocr. Rev.* **5:** 119–122, 1984.

KOCHAKIAN C.D., REED B.A., EISCHEID A.M.: Changes in tissue weights and enzymes produced by thyroidectomy and testosterone. *Am. J. Physiol.* **177:** 413–417, 1954.

MIHALY K., MAKARA G.B., STARK E.: RIA for plasma corticosterone. In: *Advances of Steroid Analysis*. S. Görög (ed.), Academiai Kiadó, Budapest, 1982, pp. 217–221.

MORASKI R.: Renal hyperplasia in the intact rat. *Proc. Soc. Exp. Biol. Med.* **126:** 838–841, 1966.

MURAYAMA N., YOSHIDA K., TORIKAI S., SAKURADA T., ASANO Y., YOSHINAGA K.: Localization of thyroxine 5'-monodeiodinase activity in the renal proximal tubules in rabbits and rats. *Horm. Metab. Res.* **17:** 197–200, 1985.

NOMURA K., DEMURA H., SHIZUME K.: Stimulation of renal deoxyribonucleic acid synthesis by a pituitary-derived renotropin and its inhibition by testosterone and thyroxine. *Endocrinology* **116:** 616–621, 1985.

PACOVSKÝ V.: *Thyroid Gland and Kidney*. SZdN (Avicenum), Prague, 1959. (in Czech)

PETERSON R.E.: The influence of the thyroid on adrenal cortical function. *J. Clin. Invest.* **37:** 736–743, 1958.

SCHREIBER V., KAPITOLA J., KÖLBEL F.: Thyroxine cardiomegaly in rats is not hypertension bound. *Physiol. Bohemoslov.* **34:** 97–101, 1985.

SOMJEN D.F., ISMAIL-BEIGI F., EDELMAN I.S.: Nuclear binding of triiodothyronine and effects on QO_2, Na-K-ATPase and alpha GPDH in liver and kidney. *Am. J. Physiol.* **240**: E146–E154, 1981.

STEPHAN F., REVILLE P., LAHARPE F., KÖLLBACK H.H.: Impairment of renal compensatory hypertrophy by hypothyroidism in the rat. *Life Sci.* **30**: 623–631, 1982.

WRIGHT F.S.: Sites and mechanisms of potassium transport along the renal tubule. *Kidney Int.* **11**: 736–743, 1977.

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