

## POSSIBLE EFFECTS OF MELATONIN ON THE KIDNEY OF HYPERTHYROID RATS

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*The aim of this study was to investigate structural changes that occur in the kidney of rats with hyperthyroidism and the effect of melatonin on these changes.*

*The rats were divided into 3 groups; group I was designated as the control, group II was injected daily with 3,3,5-triiodo-L-thyronine (T<sub>3</sub>) and group III was injected daily with T<sub>3</sub>+melatonin. After 40 days, tissue specimens of kidney were removed and examined by light and electron microscopy.*

*In the proximal tubule of the T<sub>3</sub> injected group, basement membrane thickening, microvillus irregularity and evidence of basal folding were observed. In the glomerule, basal lamina thickening, irregularity of pedicels and somewhere an increase of mesangium were recorded. Moreover, in the melatonin injected group, the findings were similar to the T<sub>3</sub> injected group.*

*In conclusion, it was observed that hyperthyroidism caused structural changes in the kidney and melatonin had no important effects on these changes.*

*Key words: hyperthyroidism, kidney, melatonin, microscopy*

### INTRODUCTION

Hyperthyroidism is causes a hypermetabolic state induced by increased thyroid hormone secretion (Kumar *et al.* 1992). Healthy thyroid gland secretes 90% thyroxine (T<sub>4</sub>) and 10% tri-iodothyronine (T<sub>3</sub>). However, an important portion of thyroxine is transformed to tri-iodothyronine in the blood and peripheral tissues. T<sub>3</sub> is four times more active than T<sub>4</sub>. Thyroid hormones have two main effects in the body; 1) they increase the overall metabolic rate, 2) they stimulate growth in children (Guyton 1986). Thyroid hormones accelerate the basal metabolic rate, specifically oxidative metabolism, by inducing mitochondrial enzyme activity in target tissues. Moreover, it was reported that extreme thyroid hormone concentrations induced tissue damage (Asayama *et al.* 1987, Venditti *et al.* 1997). When the much hormone is secreted, the basal metabolic rate rises and protein synthesis increases. Within one week following thyroid hormone administration about 100 or more intracellular enzymes increase. When thyroid hormone is injected experimentally, the size and number of mitochondria also increase. Total

membrane area of the mitochondria are widened in accordance with the rise in metabolic rate. In this way, ATP production, as the energy source for cell functions, is accelerated. When thyroid hormone is injected in very high doses, mitochondria swell irregularly and much heat but very little ATP are produced because oxidative phosphorylation is uncoupled from electron transport. One of the enzymes which increases in response to thyroid hormone is Na-K ATPase (Guyton 1986).

Melatonin (N-acetyl-5-methoxytryptophol) is an indole amine synthesized during the night in the pineal gland (Pierrefiche *et al.* 1993). Melatonin is metabolized principally in the liver and secondarily in the kidney (Erlach and Apuzzo, 1985; Reiter 1981).

Melatonin has endocrinological and non-endocrinological effects. Endocrinological actions of melatonin affect the reproductive system and organs such as the thyroid and adrenal gland. Melatonin has an inhibitory effect on the hypothalamus-hypophysis-gonad systems (Reiter 1991; Cagnacci 1996). Pinealectomy increases T<sub>4</sub> secretion. On the contrary, melatonin decreases T<sub>4</sub> secretion and suppresses plasma T<sub>4</sub> (Erlach and Apuzzo 1985). Melatonin also possesses unique properties as a free radical scavenger, which strongly suggests a protective, especially, neuroprotective role (Pierrefiche *et al.* 1993; Hardeland *et al.* 1993).

The aims of this study were to investigate structural changes which may occur in the kidney of rats with hyperthyroidism and the effect of melatonin on the changes.

#### MATERIALS AND METHODS

Adult male Wistar rats (weighing 250-300 g, n=30) were kept under conditions of controlled temperature ( $21 \pm 1^\circ\text{C}$ ) and photoperiod (07.00 to 19.00 h). Feed (standard pellet diet) and tap water were supplied *ad libitum*.

The animals were divided into 3 groups. Group I (n=10) was designated as the control (sham). They received 2 ml saline daily for 20 days intraperitoneally (ip) and then ip saline+subcutaneously (sc) 10% ethanol (0.1 ml) for daily 20 days. To induce hyperthyroidism, the rats in group II were given ip injections of 3,3,5 triiodo-L-thyronine (T<sub>3</sub>) (Sigma) (10 µg/kg, 24 h) in 2 ml saline for 20 days and then T<sub>3</sub>+0.1 ml ethanol was injected sc for 20 days. The rats in group III were administered ip injections of 3,3,5 triiodo - L thyronine (T<sub>3</sub>) (Sigma) (10 µg/kg, 24 h) in 2 ml saline for 20 days and then T<sub>3</sub>+melatonin (6 mg/kg in 0.1 ml 10% ethanol sc; Sigma) was injected sc for 20 days.

At the end of the experiment, all animals were weighed and then blood samples were collected under general anesthesia for biochemical analysis. The kidneys were removed. For electronmicroscopy small pieces were fixed in 2.5 glutaraldehyde in 0.2 M phosphate buffer (pH 7.4) at 4°C. They were postfixed in phosphate-buffered 1% osmium tetroxide. After dehydration in ethyl alcohol, the specimens were embedded in Araldite Cy 212. Thin sections were cut on an ultramicrotome, stained with uranyl acetate and lead citrate, and examined under a Carlzeiss-900 electron microscope. For light microscopy, other pieces of kidney were fixed 10% neutral formalin solution, dehydrated in alcohol and embedded in paraffin. 5 µm-thick sections were stained with Crossman's trichrome stain.

## RESULTS

At the end of study, the mean body weight of the T<sub>3</sub> injected group had decreased compared to the control. There was a slight increase in body weight of the melatonin injected group (Table I).

The mean concentration of T<sub>3</sub> in the serum of rats receiving T<sub>3</sub> was higher than in the control animals and those receiving T<sub>3</sub>+melatonin (Table II).

Table 1. Mean ( $\pm$ SD) body weight of rats in groups I, II and III after treatment.

Groups	n	Mean $\pm$ SD
Group I	10	315,10 $\pm$ 9,49
Group II	10	296,00 $\pm$ 7,75**
Group III	10	300,00 $\pm$ 5,77*

\* p=0.001 compared to group I.

\*\* p<0.001 compared to group I using one-way ANOVA.

n= number of rats in each group.

Table 2. Mean ( $\pm$ SD) level of T<sub>3</sub> in groups I, II and III after treatment.

Groups	n	Mean $\pm$ SD
Group I	10	0,46 $\pm$ 0,03
Group II	10	2,13 $\pm$ 0,60**
Group III	10	0,98 $\pm$ 0,22*

\* p<0.05 compared to group I.

\*\* p<0.001 compared to the others group using one-way ANOVA.

n= number of rats in each group.

At the light microscopic examination, Crossman's trichrome stain of the T<sub>3</sub> injected group showed an increase of kidney connective tissue compared to the control (Fig. 1A,1B). An increase of connective tissue was also present in the melatonin injected group.

Electron microscopic examination revealed regular microvilli, thin basement membrane and mitochondria in the proximal tubules of the control group (Fig. 2). However, in the proximal tubules of the T<sub>3</sub> injected group, the basement membrane had thickened and microvilli irregularity and evidence of basal folding were observed (Fig. 3). In the glomerular structure of the control group, podocyte and pedicels showed regularity and the mesangium was normal (Figure 4). In the glomerular structure of the T<sub>3</sub> injected group, basement membrane thickening, irregularity of pedicels and increase of mesangium were

observed (Figure 5). Moreover, in the melatonin injected group, the pedicels were lost and the podocyte lay directly on the basement membrane due to nephropathy. The other findings were similar to the T<sub>3</sub> injected group (Figure 6).

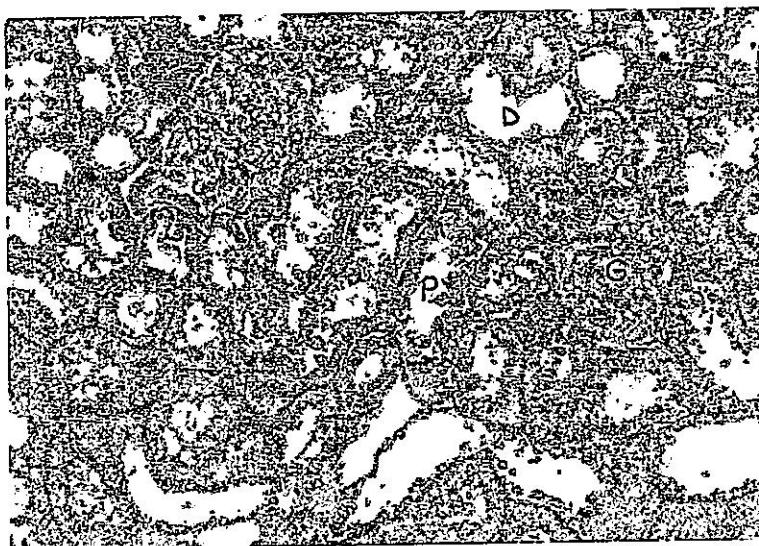


Figure 1A: Light micrograph of the kidney in a control rat. P: Proximal tubule D: Distal tubule G: Glomerule Crossman's trichrome stain X 10.

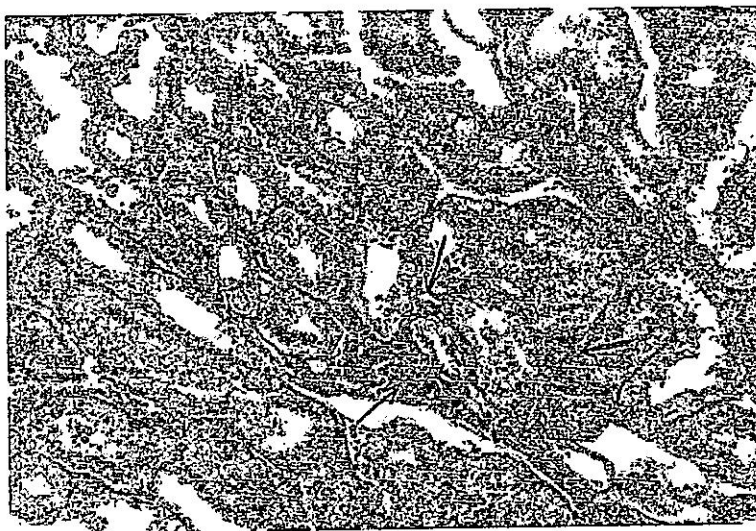


Figure 1B: Light micrograph of the kidney in a T<sub>3</sub> injected rat. Hyperthyroidism caused an increase of connective tissue (arrows). Crossman's trichrome stain X 10.

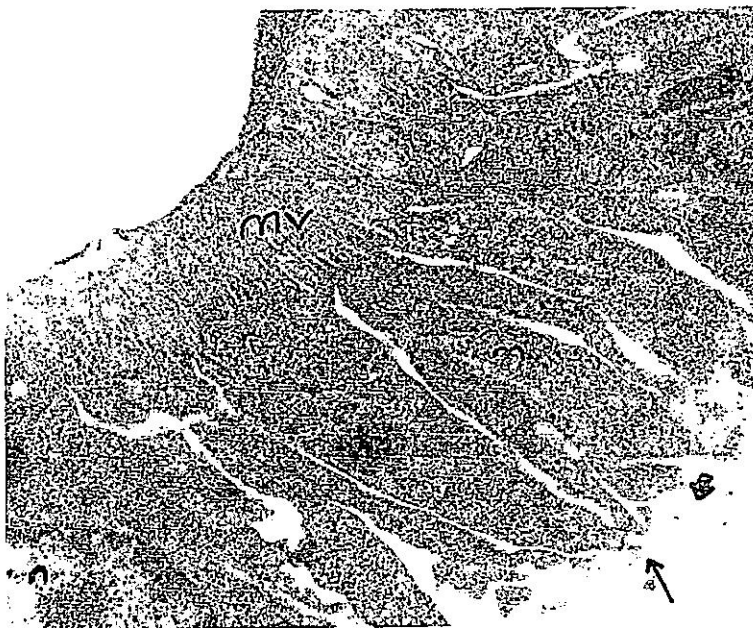


Figure 2. Electron micrograph of the proximal tubule in a control rat.  
 mv: microvillus m: mitochondria, n: nucleus, → : basement membrane, (↪): basal folding.  
 Lead citrate and uranyl acetate. original magnification X 7000.

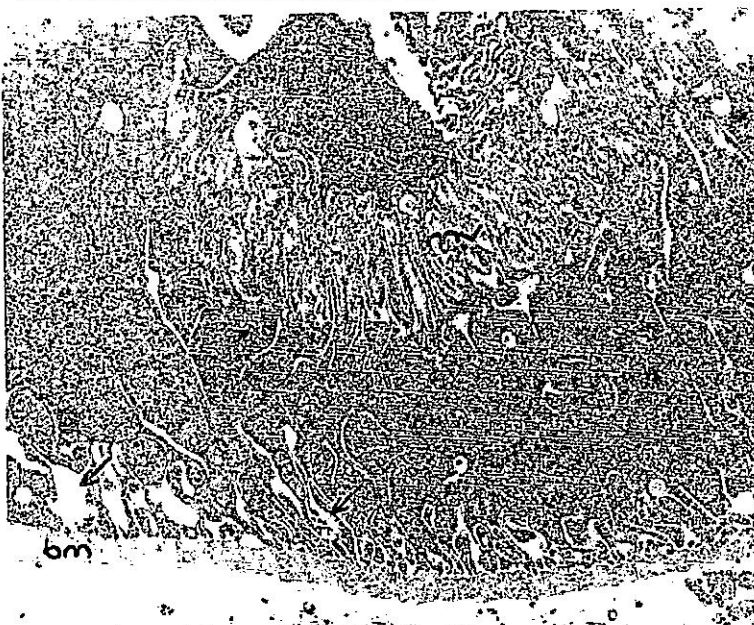


Figure 3. Electron micrograph of the proximal tubule in a  $T_3$  injected rat. Hyperthyroidism caused irregularity in microvilli (mv), basement membrane thickening (bm) and evident basal folding (arrows), m: mitochondria. Lead citrate and uranyl acetate, original magnification X 4400.

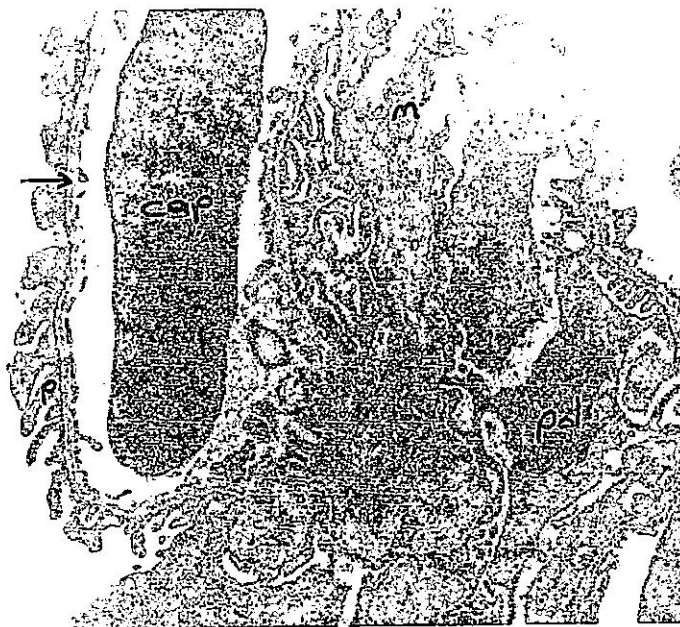


Figure 4. Electron micrograph of the glomerule in a control rat. → : basement membrane, p: pedicels, pd: podocyte, m: mesangium, cap: capillary, e: endothelium. Lead citrate and uranyl acetate, original magnification X 7000.



Figure 5. Electron micrograph of a glomerule in a T<sub>3</sub> injected rat. Hyperthyroidism caused thickening in the glomerular basement membrane (→), increase in the mesangium (m), and irregularity in pedicels (→). Lead citrate and uranyl acetate, original magnification X 4400.





Figure 6. Electron micrograph of a glomerule in a  $T_3$ +melatonin injected rat. The findings were similar to those in the  $T_3$  injected groups. → : glomerule basement membrane thickening, cap: capillary, pd: directly laid podocyte on basement membrane. Lead citrate and uranyl acetate, original magnification X 7000.

#### DISCUSSION

There are few histological studies about thyroid hormone related structural changes in the kidney. Studies have mostly been at the physiological level. It was reported that thyroxine affected tubular function (Oakley *et al.* 2000).

Becker and coworkers (2000) observed that hyperthyroidism increased glomerular filtration rate (GFR) proportion, while methimazole used for therapy decreased this proportion in cats hyperthyroid. Cats injected with thyroxine showed an increase in thyroxine, GFR and effective renal blood flow (ERBF) and a significant decrease in serum creatinine and blood urea nitrogen (BUN) values. Administration of high doses of exogenous thyroxine to cats resulted in significant stimulation of renal function (Adams *et al.* 1997).

In another study, hyperthyroid rats gained less weight and had lower blood glucose despite an increase in food intake.  $T_4$  treatment induced a significant increase in glucose synthesis by renal tubule fragments and this suggested that renal gluconeogenesis contributed to enhance glucose production in hyperthyroidism (Piementa and Silva 1999).

In our study, it was observed that the body weight decreased compared to controls in both the  $T_3$  injected group and the  $T_3$ +melatonin injected group.

Other studies showed that hyperthyroidism resulted in structural changes in skeletal muscle, myocardium and liver (Zaiton *et al.* 1993; Kazakov *et al.* 1986; Parmacek *et al.* 1986; Callas and Cannon 1974).

It has been shown that melatonin, has an inhibitory endocrine effect on thyroid gland functions. Pinealectomy caused an increase of T<sub>4</sub> release and hypertrophy in the thyroid gland. However; melatonin decreased T<sub>4</sub> release and suppressed plasma T<sub>4</sub>, and also increased TSH (Erich and Apuzzo 1985).

An inhibitory effect of melatonin on the hypophysial-thyroid axis has often been reported. Thus, Viriend and Wasserman (1986) observed that injection of melatonin to hypothyroid rats decreased TSH. It was suggested that melatonin was one of the neural systems that regulated TRH release (Viriend and Wasserman 1986). In the present study, the increase in serum T<sub>3</sub> was decreased by melatonin.

Capasso *et al.* (1999) showed that thyroid hormones affected proximal tubular sodium transport and this effect was only partially mediated by the action of thyroid hormones on Na-K-ATPase activity. Nevertheless, hypothyroid patients had a decrease in glomerular filtration rate and renal plasma flow which was completely reversed by thyroxine administration, whereas hyperthyroid subjects exhibited a significant increase in both parameters (Capasso *et al.* 1999).

It was found that renal hypertrophy was induced by hyperthyroidism, but the mechanism is not fully understood. Kobori *et al.* (1998) showed that the kidney-to-body weight ratio increased in hyperthyroidism. Radioimmunoassays and reverse transcriptase-polymerase chain reactions revealed increased renal renin and angiotensin II levels and enhanced renal renin mRNA expression in the hyperthyroid groups. The authors suggested that thyroid hormone activated the intrarenal renin-angiotensin system via enhancement of renal renin mRNA expression, which then led to renal hypertrophy (Kobori *et al.* 1998).

Different materials related to protective systems have been used in experimental hyperthyroidism. It was reported that vitamin E decreased T<sub>3</sub> and T<sub>4</sub> level in hyperthyroidism (Adali *et al.* 1999, Seven *et al.* 1996). In another work, the effect of melatonin on antioxidant enzyme activity and renal tubular necrosis induced by gentamicin was studied. In the groups injected with gentamicin there was widespread tubular necrosis but in the other groups there was a marked reduction in the extent of tubular damage. These results suggested that melatonin prevented the tubular necrosis induced by gentamicin in rats, presumably because it was a potent antioxidant and restored antioxidant enzyme activity in the rat kidney (Ozbek *et al.* 2000).

In conclusion, it was observed that hyperthyroidism caused structural changes in the kidney but that melatonin had no important effect on these changes.

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## MOGUĆE DELOVANJE MELATONINA NA BUBREGI HIPERTIREOIDNIH PACOVA

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### SADRŽAJ

Cilj ovih ispitivanja je bio da se utvrde strukturne promene koje nastaju u bubrezima hipertiroidnih pacova i ispita efekat melatonina na njih. Kod hipertireodnih životinja uočene su promene u proksimalnim tubulima koje su se manifestovale zadebljanjem bazalne membrane i nepravilnim oblikom mikrovila. U glomerulima je bazalna membrana takođe bila zadebljana uz nepravilan oblik pedicela i povećanje mezangiuma. Kod hipertireoidnih pacova tretiranih melatoninom uočene su slične promene tako da nije dokazano da ovaj hormon ima značajan efekat na strukturne promene u bubregu hipertiroidnih pacova.