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EFFECT OF RAS-BLOCKERS AND NO-CYCLE METABOLITES ON THE RENAL FUNCTIONS OF RATS EXPOSED TO THYROXINE INJECTIONS

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Abstract

Objective. Evaluating the role of RAS and NO-dependent pathogenic mechanisms of formation of renal dysfunction in disorders of the thyroid status. ***Methods.*** First 2 groups of experimental white male outbredrats was administered thyroxine (T4) in a dose of 50 g / 100 g per body weight injected intraperitoneally on 1% starch gel base once or during 7 days. In addition, rats were administered non-selective NO-synthase inhibitor Nω-NLA. Those rats were exposed to single administration of the combined T4 (intraperitoneally, 50mg/100g body weight in 24 hours before water loading) and Nω-NLA (intraperitoneally 1 mg / 100 g body weight 30 min before water loading). As a comparison we studied a group of animals received only an equivalent dose only or T4 Nω-NLA only 1 mg / 100 g body weight in 30 min before water loading. After 7-days. of T4 administration the same rats received water solution of losartan (10 mg / l) or water solution of captopril (20 mg / l) for 24 hours after the last administration of T4. Another group of rats was treated with T4 for 7 days and afterwards was administered L-arginine in a dose 2 mg / 100 g bw per day or water solution (20 mg / l) of sodium nitrite. Rats in the control group for 7 days. was administered intragastrically gel containing no T4. Kidney function was studied in 24h after administration of T4 in terms of water 5% loading on kidneys. ***Results.*** It was found out that administaration of RAS-blockers increases creatinine clearance value after either a single or prolonged administration of T4 in rats. However, decreased renal excretion of endogenous nitrates and protein as well as prevented retention of endogenous nitrite was registered in rats only after administration of losartan after 7 days of T4 administration. Prolonged administration of T4 to the rats was accompanied by weakened renal effects of NO and activated the arginine dependent pathway of NO synthesis into the nitrite reduction, evidenced by the increase of endogenous nitrite level in blood plasma of rats treated with T4 continuously along with no pronounced corrective effect of exogenous arginine in hyperthyroid animals and elevated creatinine clearance under the influence of exogenous sodium nitrite in the hyperthyroid animals. **Conclusion.** Discovered effects of RAS blockers allow us to recommend this pharmacological agents as an effective way to slow down the progression of renal dysfunction inthyroid pathlogy.

Keywords: rat, hyperthyroidism, renal function, RAS- inhibitors, nitrites, nitrates.

Background. Thyroid pathology is a widespread human disease and the leading risk factor for renal diseases as well (Dolomatov S. et al., 2011; Zoccali C. et al., 2012; Shin DH et al., 2013). However, thyroid hormones can directly affect the state of osmotic and volemic homeostasis (Klein I., Danzi S., 2007; Moreno JM et al., 2008), and are also involved in the humoral control of renal function (Vargas F. et al., 2006; 2012). Iodothyronines are involved into regulating the activity of the renin-angiotensin-aldosterone system (RAAS) (Vargas F. et al., 2006; Iglesias P., Díez JJ, 2009) by modulating the state of renal transport of osmotically active substances and liquids (Wangensteen R. et al ., 2006; Kimmel M. et al., 2012; Williams TL et al., 2013).

Iodothyronines can also act as activators of NO-synthase (Fernández V. et al., 2009; Barreiro Arcos ML et al., 2011). It was reported about the organ specificity of the stimulating effect of thyroid hormone on endothelial NO-synthase (Hiroi Y. et al., 2006; Oztay F. et al., 2007) and inducible NO-synthase (Barreiro Arcos ML et al., 2011; Fernández V. et al., 2009) and the neuronal NO-synthase (Wangensteen R. et al., 2006). Many authors consider the pathophysiological significance of thyroid hormone stimulation of

mitochondrial NO-synthase (Venditti P., Di Meo S., 2006; Venditti P., et al., 2007; Puddu P. et al., 2007). Apparently, main effect of thyroid hormones on the nitrogen oxide cycle is to control the arginine-dependent production of NO and the oxidation products of metabolism regulation such as the NO derivates-endogenous nitrites and nitrates (Rodriguez-Gomez I. et al., 2003; 2005; Dolomatov S. et al., 2004).

Thus renotropic effects of thyroid hormones may be implemented as a direct effect on kidney parenchyma metabolism, and by modulating the activity of RAS combined with the rate of formation of NO and arginine-dependent nitrite (nitrate) -reduktase circuits nitrogen oxide cycle. Consequently, a homeostatic balance is maintained between normal activity iodothyronine renal system and PAS and NO concentrtion creating the optimum conditions for the suffucient tubular substance transport and glomerular filtration rate (Toda N. et al. 2007; Kobori H. et al. 2007). At the same time, there are still exist unresolved questions about the pathophysiological significance of RAS and NO-dependent mechanisms in the pathogenesis of renal dysfunction in disorders of the thyroid status of the organism.

In this study we tried to establish the role of RAS and NO-dependent pathogenic mechanisms of formation of renal dysfunction in disorders of the thyroid status.

Materials and methods

For the research we selected outbred albino male rats weighing 140-180 g. Sodium salt of *thyroxine (T4),* produced by "Berlin Chemie" (Germany) was injected to them intraperitoneally at the dose of 50 g per 100 g body weight on 1% starch gel base *once* (n = 10) or for **5** (n = 15) and **7** (n = 10) days.

We also researched particular renal reactions of animals on a single administration of a non-selective inhibitor of NO-synthase *Nω-NLA*. Rats of another group were exposed to single administration of the combined T4 (intraperitoneally, 50 mg / 100 g body weight 24 hours before water loading) and Nω-NLA (intraperitoneally 1 mg / 100 g body weight 30 min before water loading) (n = 12). As a comparison, control group of animals received an equivalent dose of only T4 (n = 14) or only Nω-NLA 1 mg / 100 g body weight in 30 min before water loading (n = 18).

Analysis of the role of the *RAS-dependent* mechanisms of renal functions was carried out on groups of animals treated with T4 for 7 days. with 50 g/100 g bw and then within 24 hours after completion of hormone intake administered *captopril* water solution (20 mg/l) (n = 10), or water solution of *losartan* (10 mg/l) (n = 10).

In addition, the rats of one group treated with T4 for 7 days were also daily intragastrically administered an water solution *of L-arginine hydrochloride* (n = 10) manufactured by CPP Lugansk (Ukraine) in a dose 2 mg / 100 g per body mass or water solution (20 mg / l) of *sodium nitrite* (n = 15) manufactured by Acros organics (US).

Rats in the *control group* of animals (n = 30) for 7 days was administered intragastrically gel containing no T4. Kidney function was studied 24h after administration of T4 along with 5% water loading [Pahmurny BA 1969; Berkhin EB, Y. Ivanov, 1972]. Urine was collected for 2 hours.

The experimental protocol was approved by the General Directorate of Veterinary Services (Permit number 403/17-04-09) according to Legislation on scientific andexperimental procedures (Presidential Decree 160/1991, in compliance with the Directive 86/60μ9/EEC).

After sacrifice the blood is stabilized with heparin and centrifuged for 15 min at 3000 rev / min. In the obtained samples of urine and plasma osmolality was determined by the amount of freezing point depression method on osmometer 3D3 (USA). Creatinine concentration was determined by the photometric method in the reaction with picric acid on spectrophotometer SF-46 (Russia). The concentration of nitrite and nitrate were measured by the photometric method using Griess reagent on the SF-46 in accordance with the previously described method in modification [Yemchenko NL et al., 1994]. The protein concentration of urine was recorded by photometric method in the reaction with sulfosalicylic acid on the SF-46. Indicators of renal function animals calculated in accordance with previously published methods [Pahmurny BA, 1969; Berkhin EB, Y. Ivanov, 1972].

The results were expressed as mean ± SEM.The data were statistically analyzed using one way analysis of variance (ANOVA) followed by Tukey test. The levels of significance were accepted with $p < 0.05$. Comparisons of p values between different groups were performed.

RESULTS

Table 1 represents the parameters of renal function in rats receiving only T4 and once for 7 days. It was found that the appointment of the hormone is accompanied by a moderate decrease in the value of urine output, creatinine clearance and creatinine concentration index. It was revealed that due to increased release of T4 kidneys nitrate and protein reaches a maximum to 7 days of the experiment. We emphasize that in the group of rats treated with T4 for 7 days it was detected a significant increase in the concentration of nitrite in blood plasma on the background rate of minor changes nitrite excretion by the kidneys.

Table 1. **Kidney Reaction on a single and continuous intragastric administration of thyroxine.**

Index	**Control group** *n=30*	**24h after single administration of T4** *n=10*	**Administration of T4 for 7 days** *n=15*
Diuresis, ml / h / 100 g	2,1±0,1	1,8±0,2	1,7±0,1 $p_1<0,05$
Creatinine clearance, µl / min	561±7	449±17	390±17 $p_1<0,01$ $p_2<0,05$
Urine Nitrites µmol / l	1,4±0,1	1,8±0,1	1,9±0,2 $p_1<0,01$
Urine Nitrates µmol / l	14,5±0,2	23,9±1,9	41,2±2,5 $p_1<0,01$ $p_2<0,01$
Urine protein, mg / l	16±1	62±3	93±11 $p_1<0,01$ $p_2<0,01$
The osmolality of the urine, mOsm / kg H_2O	107±1	144±9	121±5 $p_1<0,05$ $p_2<0,05$
Excretion of nitrites, µmol / hr / 100 g	0,0032±0,0001	0,0038±0,0003	0,0037±0,0002
Excretion of nitrates, µmol / hr / 100 g	0,027±0,001	0,041±0,010	0,079±0,006 $p_1<0,01$ $p_2<0,01$
Protein excretion, mg / hr / 100 g	0,036±0,001	0,097±0,008	0,149±0,018 $p_1<0,01$ $p_2<0,01$
Excretion of OAS, mOsmol / hr / 100 g	0,221±0,001	0,259±0,007	0,214±0,005 $p_1<0,01$
The osmolality of blood plasma, mOsm / kg H_2O	301±1	295±2	299±2
Serum creatinine, µmol / l	67±1	91±2	98±7 $p_1<0,01$
Blood plasma nitrites µmol / l	4,9±0,1	4,2±0,2	9,5±0,9 $p_1<0,01$ $p_2<0,01$
Blood plasma nitrates, µmol/l	7,2±0,1	8,9±0,3	7,8±0,8
Creatinine concentration index	17,9±0,1	14,7±0,2	12,3±0,4 $p_1<0,01$ $p_2<0,01$

n-number of observations;
p_1-significant difference in comparison with the intact animals;
p_2- significant difference in comparison with the single administration of thyroxine.

In our investigation we revealed that watersolution of captopril in rats who previously received a single dose of T4 of 50g/100g per body mass (**Table 2**), prevents a decrease in creatinine clearance, increases the concentration of creatinine index and reduces the nitrate concentration in the blood plasma accompanied by elevated nitrate urine secretion. However, a combination of captopril and T4 assignment on one side reduces renal loss of protein and on another side stimulates the excretion of osmotically active substances (OAS).

Table 2. **Kidney reaction on a single administration of thyroxine T4 in terms of ACE-blockade by captopril**

Index	**Control group** *n=30*	**Administration of T4 only** *n=10*	**Administration of T4+captopril** *n=10*
Diuresis, ml/h/100 g	2,1±0,1	1,8±0,2	2,2±0,1
Creatinine clearance, µl / min	561±7	349±17	790±39 p_1<0,01 p_2<0,01
Urine Nitrites µmol / l	1,4±0,1	1,8±0,1	3,1±0,4 p_1<0,01
Urine Nitrates µmol / l	14,5±0,2	23,9±1,9	29,8±2,6 p_1<0,01 p_2<0,05
Urine protein, mg / l	16±1	93±3	43±3 p_1<0,01 p_2<0,05
The osmolality of the urine, mOsm / kg H_2O	107±1	114±9	149±11 p_1<0,01
Excretion of nitrites, µmol / hr / 100 g	0,0032±0,0001	0,0038±0,0003	0,0068±0,0002 p_1<0,01 p_2<0,01
Excretion of nitrates, µmol / hr / 100 g	0,027±0,001	0,041±0,010	0,068±0006 p_1<0,01 p_2<0,01
Protein excretion, mg / hr / 100 g	0,036±0,001	0,187±0,008	0,085±0,009 p_1<0,01 p_2<0,01
Excretion of OAS, mOsmol / hr / 100 g	0,221±0,001	0,229±0,007	0,298±0,005 p_1<0,01 p_2<0,01
The osmolality of blood plasma, mOsm / kg H_2O	301±1	298±2	286±2 p_1<0,01 p_2<0,01
Serum creatinine, µmol / l	67±1	91±2	65±3 p_2<0,01
Blood plasma nitrites µmol / l	3,9±0,1	7,2±0,2	4,0±0,3 p_2<0,01
Blood plasma nitrates, µmol/l	7,2±0,1	8,9±0,3	3,0±0,4 p_1<0,01 p_2<0,01
Creatinine concentration index	17,9±0,1	14,7±0,2	20,1±0,4 p_1<0,01 p_2<0,01

n-number of observations;
p_1-significant difference in comparison with the intact animals;
p_2- significant difference in comparison with the administration of thyroxine.

Meanwhile, after the consumption of the AT1-receptor blocker water solution an **angiotensin-II-losartan** group of rats additionally treated for 5 days by T4 **(Table 3)** demonstrated a significant elevation of creatinine clearance, decreased level of proteinuria combined with increased renal clearance of nitrites and decreased nitrite anions level in the blood plasma compared with group of rats only treated with T4. However, the administration of losartan in the group of rats treated with T4 accompanied by increased renal OAS excretion compared with group of rats treated with T4 only. At the same time, in this group of animals nitrate renal excretion rates were significantly lower than in rats treated with T4 alone.

Table 3. **Losartan Effect on renal transport of substances in white rats treated with T4**

Index	**Control group** *n=30*	**Hyperthyroidism** *n=15*	**Hyperthyroidism +Losartan** *n=10*
Diuresis, ml / h / 100 g bw	2,1±0,1	1,8±0,1	1,6±0,2 $p_1<0,05$
Creatinine clearance, µl / min	561±7	365±15	904±37 $p_1<0,01$ $p_2<0,01$
Urine Nitrites, µmol / l	1,4±0,1	1,5±0,3	3,9±0,4 $p_1<0,01$ $p_2<0,01$
Urine Nitrates µmol / l	14,5±0,2	39,7±0,7	19,2±3,9 $p_1<0,01$ $p_2<0,01$
Urine protein, mg / l	16±1	101±4	61±7 $p_1<0,01$ $p_2<0,05$
The osmolality of the urine, mOsm / kg H_2O	107±1	119±7	149±11 $p_1<0,01$ $p_2<0,01$
Excretion of nitrites, µmol / hr / 100 g	0,0032±0,0001	0,0035±0,0002	0,0063±0,0005
Excretion of nitrates, µmol / hr / 100 g	0,027±0,001	0,074±0,002	0,032±0,004
Protein excretion, mg / hr / 100 g	0,036±0,001	0,154±0,010	0,097±0,008
Excretion of OAS, mOsmol / hr / 100 g	0,221±0,001	0,217±0,006	0,238±0,007
The osmolality of blood plasma, mOsm / kg H_2O	301±1	298±1	300±2
Serum creatinine, µmol / l	67±1	101±6	53±3 $p_1<0,05$ $p_2<0,01$
Blood plasma nitrites µmol / l	4,9±0,1	8,9±0,7	3,3±0,2 $p_1<0,01$ $p_2<0,01$
Blood plasma nitrates, mcmol/l	7,2±0,1	7,1±0,6	8,9±0,9

n-number of observations;
p_1-significant difference in comparison with the intact animals;
p_2- significant difference in comparison with hyperthyroidism.

The influence of sodium nitrite upon excretory renal function in **rats with experimental hyperthyroidism (Table 4)** show a distinct increase in the volume of urine output in comparison with hyperthyroid rats and with euthyreoid animals treated with sodium nitrite. We want to stress out that combined administration of T4 and sodium nitrite significantly increased creatinine clearance, diminished levels of nitrites in plasma and reducticed of protein concentration in urine in comparison with the group of animals treated only with T4. In addition, the combined concentration of T4 and nitrite anions also positively affects renal protein loss, OAS and nitrites excretion and reduces the concentration index of creatinine.

Table 4. **Renal functional indices in white rats received combination of thyroxine and sodium nitrite**

Index	**Hyperthyroidism** *n=15*	**Hyperthyroidism + sodium nitrite sol.** *n=15*	**Hyperthyroidism + sodium nitrite sol.** *n=15*
Diuresis, ml / h / 100 g bw	1,7±0,2	4,9±0,6 p_1<0,01 p_2<0,01	1,9±0,2
Creatinine clearance, µl / min	390±17	597±29 p_1<0,01 p2<0,01	747±33
Urine Nitrites, µmol / l	1,9±0,2	1,6±0,2 p_2<0,01	3,5±0,3
Urine Nitrates, µmol / l	41,2±2,5	22,7±1,3 p_1<0,01 p_2<0,01	65,3±4,2
Urine protein, mg / l	93±11	41±6 p_1<0,01 p_2<0,01	121±13
The osmolality of the urine, mOsm / kg H_2O	121±5	142±12 p_1<0,01 p_2<0,01	110±7
Excretion of nitrites, µmol / hr / 100 g	0,0037±0,0002	0,0078±0,0009	0,0065±0,0007
Excretion of nitrates, µmol / hr / 100 g	0,079±0,006	0,119±0,008 p_1<0,01	0,124±0,010
Protein excretion, mg / hr / 100 g	0,149±0,018	0,207±0,019 p_1<0,01	0,229±0,011
Excretion of OAS, mOsmol / hr / 100 g	0,214±0,005	0,610±0,017 p_1<0,01 p_2<0,01	0,208±0,011
The osmolality of blood plasma, mOsm / kg H_2O	299±2	297±1	298±1
Serum creatinine, µmol / l	98±7	74±3 p_1<0,01 p_2<0,01	59±3
Blood plasma nitrites, µmol / l	9,5±0,9	5,9±0,5 p_1<0,01 p_2<0,01	3,7±0,3
Blood plasma nitrates, µmol/l	7,8±0,6	6,8±0,6 p_2<0,01	15,5±1,3
Creatinine concentration index	12,3±0,4	7,8±0,2 p_1<0,01 p_2<0,01	23,6±0,9

n-number of observations;
p_1-significant difference in comparison with hyperthyroidism;
p_2- significant difference in comparison with euthyroidism combined with sodium nitrite;

Results of the reaction of the kidneys of animals with the combined administration of T4 and non-selective inhibitor of NO-synthase Nω-NLA **(Table 5)** demonstrated that a single injection of pre-T4 contributes to the weakening effect renotropic inhibitor. In particular, during combined administration of T4 and Nω-NLA we observed a moderate decrease in urine output and creatinine clearance cantreversary to euthyroid rats in that group decline in GFR and urine output were very significant. We draw attention to the fact that in the group with the combined administration of T4 and Nω-NLA it was observed the lowest levels of proteinuria. Moreover, in this group there is no drastic decrease of renal clearance parameters of chemically stable metabolite of nitric oxide - endogenous nitrates and nitrites. While the introduction of Nω-NLA in euthyroid rats leads to a drastic reduction of these indicators.

Table 5. **Effect of the intraperitoneal injection of NO-synthase blocker at rat kidneys within 24 hours after a single administration of T4 combined with 5% water loading.**

Index	**Euthyroidism + NO blocker** *n=18*	**T4 only** *n=14*	**T4+ NO blocker** *n=12*
Diuresis, ml / h / 100 g	0,6±0,1	1,9±0,1	1,3±0,1 p_1<0,01 p_2<0,01
Creatinine clearance, µl / min	102±9	439±29	328±17 p_1<0,01 p_2<0,01
Urine Nitrites µmol / l	1,7±0,1	1,7±0,1	2,0±0,1
Excretion of nitrites, µmol / hr / 100 g	0,0010±0,0002	0,0034±0,0005	0,0026±0,0004 p_1<0,01
Urine Nitrites µmol / l	2,9±0,2	21,5± 1,8	31,7±1,6 p_1<0,01 p_2<0,01
Excretion of nitrates, µmol / hr / 100 g	0,0017±0,0003	0,0409±0,0011	0,0417±0,0012 p_1<0,01
Urine protein, mg / l	207±5	51±4	45±4 p_1<0,01
Protein excretion, mg / h / 100 g	0,125±0,009	0,098±0,007	0,056±0,004 p_1<0,01 p_2<0,01
The osmolality of the urine, mOsm / kg H_2O	235±3	138±16	159±6 p_1<0,01
Excretion of OAS, mOsmol / hr / 100 g	0,139±0,011	0,265±0,009	0,201±0,008 p_1<0,01 p_2<0,05
Standardized excretion of nitrites, µmol / ml of the filtrate	$(1,58\pm0,06)\times10^{-4}$	$(1,14\pm0,13)\times10^{-4}$	$(0,88\pm0,06)\times10^{-4}$ p_1<0,01
Standardized excretion of nitrates, µmol / ml of the filtrate	$(0,2\pm0,1)\times10^{-3}$	$(1,3\pm0,2)\times10^{-3}$	$(1,8\pm0,3)\times10^{-3}$ p_1<0,01
Standardized protein excretion, mg / ml of the filtrate	$(20,2\pm0,9)\times10^{-3}$	$(3,4\pm0,3)\times10^{-3}$	$(2,6\pm0,4)\times10^{-3}$ p_1<0,01
Standardized excretion OAS, mOsm / ml of the filtrate	$(22,7\pm1,1)\times10^{-3}$	$(10,6\pm0,4)\times10^{-3}$	$(7,5\pm0,3)\times10^{-3}$ p_1<0,01 p_2<0,05
The osmolality of blood plasma, mOsm / kg H2O	288±2	298±2	291±2
Blood plasma nitrites, µmol / l	1,6±0,1	2,2±0,1	4,0±0,1 p_1<0,01 p_2<0,01
Blood plasma nitrites, µmol / l	7,1±0,6	8,1±0,4	2,7±0,3 p_1<0,01 p_2<0,01

n-number of observations;
p_1-significant difference in comparison with the euthyroid animals;
p_2- significant difference in comparison with animals injected on T4.

Table 6 contains systematized renal parameters of animals treated for 7 days only with T4 or T4 in combination with arginine. It was found that the combined intake of T4 and arginine has no significant effect on the amount of urine output and creatinine clearance, compared with rats treated with thyroxine alone. Meanwhile, the co-administration of arginine and T4 increases the rate of renal excretion of nitrites, nitrates and OAS and slightly reduces the amount of renal protein loss. Arginine supplement administered to hyperthyroid rats prevents the retention of nitrite anions in blood plasma, however, this is accompanied by a vivid accumulation of nitrates in the extracellular fluid of the body.

Table 6. **Arginine effect on renal functions in white rats during experimental hyperthyroidism**

Index	**Hyperthyroidism group** *n=15*	**Hyperthyroidism + arginine** *n=10*	**Euthyroidism + arginine** *n=10*
Diuresis, ml / h / 100 g bw	1,7±0,1	1,9±0,1	2,2±0,1
Creatinine clearance, μl / min	390±17	440±37 $p_2<0,01$	627±33
Urine Nitrites, μ mol / l	1,9±0,2	4,7±0,5 $p_1<0,01$ $p_2<0,01$	3,1±0,2
Urine Nitrates, μ mol / l	41,2±2,5	51,4±3,7 $p_1<0,01$	51,9±7,4
Urine protein, mg / l	93±11	64±5 $p_1<0,01$ $p_2<0,01$	34±2
The osmolality of the urine, mOsm / kg H_2O	121±5	149±13 $p_1<0,05$ $p_2<0,01$	112±8
μmol / hr / 100 g .	0,0037±0,0002	0,0119±0,0015 $p_1<0,01$ $p_2<0,01$	0,0065±0,0006
Excretion of nitrates, μ mol / hr / 100 g	0,079±0,006	0,097±0,008 $p_1<0,05$	0,106±0,013
Protein excretion, mg / h / 100 g	0,149±0,018	0,118±0,014 $p_1<0,05$ $p_2<0,01$	0,072±0,004
Excretion of OAS, mOsmol / hr / 100 g.	0,214±0,005	0,278±0,010 $p_1<0,01$ $p_2<0,01$	0,231±0,009
The osmolality of blood plasma, mOsm / kg H_2O	299±2	294±1	299±1
Serum creatinine, μmol / l	98±7	125±13 $p_1<0,05$ $p_2<0,01$	65±1
Blood plasma nitrites, μmol / l	9,5±0,9	5,9±0,1 $p_1<0,01$	5,4±0,3
Blood plasma nitrates, μmol / l	7,8±0,8	26,9±2,2 $p_1<0,01$ $p_2<0,01$	16,3±1,6

n-number of observations;

p_1-significant difference in comparison with the hyperthyroid animals;

p_2- significant difference in comparison with hyperthyroid animals receiving arginine.

DISCUSSION

The obtained results lead to the conclusion that the acute kidney response to the exogenous T4 administration is characterized by the rapid growth of renal excretion of nitrites and nitrates, a steady decrease in GFR, increased allocation of kidney proteins and OAS leading to the elevated nitrate concentrations in blood plasma. After 24 hours of a single T4 administration rat kidney excretion rate of nitrates but not nitrites exceeded the control group levels along with fixed low values of GFR together with observed increased standartized renal excretion level of OAS and proteins. In the context of long-term administration of T4 in rats we revealed the statistically significant decrease in GFR, the increase of proteinuria and renal OAS excretion reduction followed by nitrite anion retention in the extracellular fluid of the body. These results indicate a steady decline of glomerular filtration rate and increased proteinuria which in turn demonstrate the pilot kidney-related symptoms of hyperthyroidism. Analyzing the possible pathogenetic mechanisms of pathological disorders in the renal parenchyma, we identified two possible causes: the restructuring of the NO-cycle and the induction of intrarenal RAS in response to the exogenous T4 administration.

The results enlighten the fact that nitrite anions may be involved in the regulation of renal activity, providing adaptive changes in the functional state of the body in response to the exogenous thyroxine. In particular, in the group with hyperthyroid rats revealed increased indices of nitrite and nitrate in plasma, reduced sensitivity of the kidneys to NO-synthase blocker, no pronounced nephroprotective effect of arginine and higher renal parenchyma sensitivity to sodium nitrite than in the group of euthyroid rats. On the one hand, the evidence suggests the strengthening of the regulatory role of nitrite (nitrate) -reductase loop cycle of nitric oxide (Reutov VP et al., 1994; 1998; Crawford JH et al., 2006; Hill BG et al., 2010) in the adaptive response of the kidney to the introduction of exogenous thyroxine. On the other hand, increased production and retention in the extracellular fluid metabolites of NO - anions nitrite and nitrate can be regarded as one of the pathogenic mechanisms leading to the disruption of homeostatic functions of the kidney. According to the literature, the excess of NO-synthase activity can be observed in some pathological processes leading to the formation of various chemical compounds possesing high cytotoxic properties, which include active forms of nitrogen as well as products of proteins nitrosylation and free amino acids (Turko IV, Murad F., 2002; Pattillo Ch.B. et al., 2011). Cyclic process of nitrosylation / denitrosylation of proteins is normally involved in the regulation of metabolic processes in the cell, and its intensity is controlled by mitochondria (Koeck Th. Et al., 2004). However, under pathological conditions, the balance of the formation rate and metabolic clearance of nitroproteins can become violated (Koeck Th. et al., 2004; Pacher P. et al., 2007). Therefore, increased intrarenal nitric oxide production in patients can be considered as an indicator of unfavorable prognosis of renal failure of different ethiology (Meenakshi SR, Agarwal R., 2013). It is reported that the restriction of NO-synthase activity favorably affects the functional state of the kidneys in renal dysfunction of toxic origin (El-Moselhy MA, El-Sheikh AA, 2014), and infectious diseases of the kidneys (Quoilin C. et al., 2014), as well as during reperfusional renal injury (Lempiäinen J. et al., 2013). The main contribution to the increased synthesis of cytotoxic compounds of nitrogen activating the inducible NO-synthase (Turko IV, Murad F., 2002; Pacher P. et al., 2007). Perhaps the retentional effects of endogenous nitrite and nitrate on various organs and tissues may be qualitatively different. Though the small levels of nitrite anions posses cytoprotective properties on hepatocytes or cardiomyocytes but may cause severe structural alterations of proximal nephrocytes (Basireddy M. et al., 2006). These facts lead to the conclusion that the observed in the early stages of the experimental hyperthyroidic retention of nitrites and nitrates can be regarded as an important pathogenetic mechanism, which subsequently induces structural renal impairment. Indeed, pathologic analysis of the dynamics of structural damage to the kidneys of rats with chronic hyperthyroidism showed that the most pronounced feature of the structural changes of renal parenchyma is necrosis of tubular epithelium (Dolomatov SI et al., 2011).

Analysis of the effects of renal RAS blockers showed that, firstly, captopril and losartan have a positive impact on the kidney in violation of thyroid status, increasing the value of GFR, decreasing proteinuria, reducing renal excretion rates of nitrates, preventing retention of nitrites in the extracellular fluid. Secondly, the effects of the introduction of renal RAS blockers hyperthyroid rats indicated that the early stages of the current experimental hyperthyroidism restructuring of the kidneys is reversible. Consequently, the performance of the kidney changes in experimental hyperthyroidism in the early stages is not related to the reduction in existing renal parenchyma (Dolomatov SI et al., 2011) even due to the imbalance of regulatory control mechanisms of renal function.

Intrarenal RAS activation in hyperthyroidism is associated with impaired regulation of vascular tone and homeostasis related to volemic parameters (Klein I., Ojamaa K., 2001; Fommei E., Iervasi G., 2002). Meanwhile, our experiment shows the direct stimulating effect of thyroid hormones on the secretion of such key components of the RAS as renin (Vargas F. et al., 2012). Growth of NO production in response to stimulation of the RAS is designed to mitigate the potential vasoconstrictor Ang II preventing high blood pressure (Rodriguez-Gomez I. et al., 2005). Necessity for greater control over the activities of the kidney by internal humoral systems, in particular, the RAS may arise as a result of damage to the renal parenchyma. As a reaction to structural damage to the kidney, the activation of the RAS is associated with adaptive changes in the parameters of GFR, renal blood flow and tubular reabsorption (Goodfriend Th. L. et al. 1996; Volpe M. et al. 2002), the metabolic processes in the preserved renal parenchyma (Adler S . et al. 2001; Welch WJ et al. 2005), starting reparative mechanisms (Yang J. et al. 2002; Chen J. et al. 2006).

Own results suggest that the key kidney response to exogenous T4 is a RAS stimulation of nitric oxide cycle which occurs on early stages of disorders of the organism thyroid status when a distinct structural damage of renal tissue is no detected yet. Perhaps this restructuring activity RAS cycle and NO occurs due to the stimulating effect of T4 on various parts of the RAS (Segarra AB et al., 2006; Carneiro-Ramos MS et al., 2006) and the cycle of NO (Ozcan O. et al., 2005; Venditti P. et al., 2007). At the same time, long-term activation of the RAS can generate conditions for structural and functional disorders of the

kidney in further turms of hyperthyroidism (Kobori H. et al., 1998; Dolomatov SI et al., 2011). Perhaps, in this situation, RAS activation deterrence can happen only due to to the use of pharmacological blockers of the RAS. The validity of this conclusion is supported by the literature on single beneficial effect of ACE inhibitors on the functional state of the cardiovascular system and kidneys of rats with experimental hyperthyroidism (Dolomatov SI et al. 2005; Xiao P. al., 2011; Dolomatov SI et al., 2011; Kim BH et al., 2012). At present time the role of RAS in the pathogenesis of renal dysfunction has been poorly studied and the feasibility of using blockers of the RAS to contain the progression of renal disease in hyperthyroidism still remains the subject of debate (Vargas F. et al., 2012).

The results of our own observations illustrate that in this case RAS blockers posses strong nephroprotective properties. Furthermore, according to pathomorphological studies RAS blockers reduce the severity of parenchymal renal injury in rats with chronic experimental hyperthyroidism (Dolomatov SI et al., 2011). In our studies we assumed that sustainable inappropriate activation of intrarenal RAS is an independent pathogenetic mechanism of the formation and progression of renal failure due to the effects of Ang-II, designed to limit the cell cycle and to reduce nephrocyte reparative potential tissue, as well as to increase production of reactive oxygen and nitrogen combined with stimulation of pro-inflammatory action and tissue fibrosis, retention of sodium and fluid, induction of endothelial dysfunction, violationg systemic and intrarenal hemodynamic parameters, etc. (Rüster Ch., Wolf G., 2006; Zhuo JL, Li XC, 2007; Kobori H. et al., 2007; Kelsen S. et al., 2008; Wilcox CS, Pearlman A., 2008; Hosojima M. et al., 2009). Observered improvement of renal protein loss regulation is additional stimulating factor for RAS kidney activity (Wolf G. et al., 2004). The difficulty is in the fact that the stimulation of the RAS may be accompanied by an increase in renin secretion by cells not only the by the juxtaglomerular cells but also increased production of intracellular components of the RAS by the tubular epithelium when significant amounts produced in the kidneys Ang II- exert their effects by an autocrine mechanism spreading onto intraorganic and in the systemic circulation (Kobori H. et al., 2007; Li XC, Zhuo JL, 2008). Pharmacological correction of this form of activation of intrarenal RAS is not sufficient due to low efficiency of existing RAS inhibitors towards the components of the system, localized in the cells of the renal parenchyma (Mogi M. et al., 2007), and its laboratory analysis requires the development of fundamentally new diagnostic test systems (Kobori H. et al., 2009). Perhaps stimulation level RAS intracellular level tubular epithelium performs an important role in the pathophysiological mechanisms of formation and progression of renal dysfunctions during hyperthyroidism illustrating why RAS blockers do not lead to a complete normalization of renal excretion endogenous proteins and nitrates, and partly explains some differences nephrotropic effects of losartan and captopril. Recalling that chronic co-administration in rats T4 and RAS blockers with different efficiency minimizes, but does not completely cancel the structural damage of the nephron (Dolomatov SI et al., 2011).

Thus, the mechanisms of direct (Ichihara A. et al., 1998; Kobori H. et al., 2001; Vargas F. et al., 2006; 2012) or indirect (Klein I., Ojamaa K., 2001; Fommei E ., Iervasi G., 2002; Flanagan ET et al., 2008), stimulation of thyroid hormone production and angII- NO, allows us to consider these compounds as mediators TG responsible for a wide spectrum of the observed changes in the kidneys at the early stages experimental hyperthyroidism. In our opinion, in this coordinate system occupy a special place endogenous nitrites and nitrates. Renal Transport Mechanisms of nitrites and nitrates, as well as their role in regulating humoral control systems intrarenal insufficient elucidated. Own the results of studies demonstrate that endogenous nitrites and nitrates should also be considered as humoral mediators TG initiating restructuring activities in the early stages of kidney hyperthyroidism involved in the pathophysiological mechanisms responsible for decreased GFR, changes in renal transport OAB, liquid and strengthening of proteinuria.

In addition to the previously discussed aspects, we draw attention the fact that the introduction of exogenous T4 rats is accompanied by clear signs of activation of phylogenetically ancient mechanisms of physiological regulation. In particular, the previously listed arguments support the restructuring of the homeostatic functions of the kidney in rats after T4 (Dolomatov S. et al., 2013) in the direction of phylogenetically older renal mechanisms controlling water-salt metabolism in accordance with the general principles of evolution homeostatic role of the kidneys in vertebrates animals (Natochin Yu, 1988). In addition, a significant strengthening of the role of nitrite (nitrate) -reduktase loop in a cycle of nitric oxide, in our opinion, is also possible to consider as a return to phylogenetically more ancient mechanism which perform a primary role in the homeostasis of the lower vertebrates. The life cycle of these animals is closely linked to water - an important source of nitrite (nitrate) substrates which activate reduction mechanisms of nitric oxide synthesis (Dolomatov SI et al., 2013; 2014). In mammals external source of exposure to the substrates of nitrite (nitrate) -reductase segment of nitrogen oxide cycle is closely integrated into the enterohepatic recirculation loop of NO metabolites (Jansson E. et al., 2008; van Faassen EE et al., 2009).

Normally, human has very high absolute parametres of mass transfer of metabolites NO (mainly in the form of nitrates) in the enterohepatic recirculation loop (Dolomatov SI et al., 2012). However, the changes in these parametres in pathology, including the thyroid gland diseased, have hardly been studied.

CONCLUSIONS

1.It was found out that thyroxine administration in rats leads to a steady decline in glomerular filtration rate values, regulates renal excretion of proteins and osmotically active substances, increases the value of renal excretion of endogenous nitrates.

2. It was revealed that prolonged administration of thyroxine in rats is accompanied by a weakening of the renal effects of arginine-dependent pathway of NO synthesis amid increasing nephrotropic effects of nitrites, mainly at the level of tubular nephron.

3. The administration of the renin-angiotensin system blockers has a beneficial effect on the activity of the kidneys of rats treated with thyroxine, increasing the value of the glomerular filtration rate, weakening the pace of renal protein loss, normalizing the indicators of renal transport of osmotically active substances, reducing the renal excretion of nitrates and preventing retention of nitrites in plasma.

4.Discovered effects of RAS blockers allow us to recommend this group of pharmacological agents as an effective way to curb the progression of renal dysfunction in hyperthyroidism and background hormone replacement therapy with hypothyreosis.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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