The changes of the thyroid function and serum testosterone levels after long-term L-NAME treatment in male rats

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ABSTRACT. Nitric oxide is a highly reactive gas that is produced by many tissues and exerts a series of physiological and pathophysiological effects. We studied the changes of the serum testosterone, thyroxine and thyrotropin levels, thyroid and anterior pituitary weights and thyroid cGMP concentrations in male Wistar strain rats treated with estradiol benzoate (EB) (1 mg/kg, im twice a week) and nonselective NO-synthase inhibitor L-NAME (N-omega-nitro-L-arginine methyl ester) alone and with combination of these substances. We have found that L-NAME in a dose 100 mg/kg/day but not in a dose 50 mg/kg/day increased the serum thyroxine and testosterone levels and in the case of testosterone in a higher dose partially blocked its drop when administered simultaneously with EB. The serum thyrotropin levels significantly fell after L-NAME and EB treatment. The cGMP thyroid levels changed only slightly in groups treated EB and L-NAME alone and were significantly decreased in group treated with combination of these substances. The nitric oxide thus seems to be an important modulator of thyroid and testicular function. The cGMP activation cascade is not probably involved in the nitric oxide induced changes of thyroid function.

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INTRODUCTION

Nitric oxide (NO) is a reactive gas with a series of important physiological and pathophysiological functions in the human body. NO as endothelium derived relaxing factor was found to play a crucial role in the endothelium derived vasodilatation of blood vessels (1). On the contrary the overproduction of NO seems to be an important pathophysiological factor in the etiology of hypotension during the septic shock and liver failure (2, 3) and the inhibition of NO production by NO - synthase inhibitors brought a therapeutic benefit in these cases.

The expression of inducible NO-synthase isoform and both types of constitutive NO-synthase isoforms was detected in the thyroid gland and the L-NAME treatment decreased the vascular expansion during goiter formation *in vitro* by 36 % (4). NO donor treatment inhibited iodine organification and human thyrocytes growth *in vitro* (5, 6) while cGMP analogue administration had no effect, which suggested that cGMP did not mediate these effects. The inducible NO-synthase isoform was identified also in monocytes of patients with Graves' disease (7).

We have found that a thiazine dye, methylene blue, which is proposed to be a NO-synthase inhibitor (8), increased the serum thyroxine levels, decreased the serum thyrotropin (TSH) concentration and partially blocked the drop of serum thyroxine and the increase of serum TSH when administered simultaneously with the antithyroid agent carbimazole (9, 10). Methylene blue also partially inhibited the hypertrophic reaction of the anterior pituitary after estrogen treatment (11-13) and decreased serum luteinizing hormone concentrations (unpublished observations). The aim of our present study was to elucidate whether the described effects of methylene blue are mediated by modulation of NO production. That was the reason for the use of nonselective inhibitor of NO-production L-NAME.

MATERIALS AND METHODS

The experiments were performed on male rats (Wistar strain, Konárovice, Prague) with initial body weight

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180 - 200 g. The rats were kept at 22±2 C in a 12 hours light - 12 hours darkness regimen and fed on a standard laboratory diet (Larsen diet, VELAZ, Prague). The rats were divided into four groups with 10 animals in each group. The first group was made by controls, the second group was administered estradiol benzoate (EB) (Agofollin depot., Slovakofarma) in a microcrystalline suspension in a dose 1 mg/kg im twice a week, the third group received L-NAME (N-omega-nitro-L-arginine methyl ester) (Sigma) in the drinking water in a dose 50 mg/kg/day in the first experiment and in a dose 100 mg/kg/day in the second experiment, the fourth group was treated by combination of L-NAME and EB.

The animals were weighed at the beginning and at the end of the experiment. After three weeks treatment the animals were killed by decapitation. The blood was collected and the anterior pituitary (AP) and the thyroid gland were cut out, weighed and quickly frozen in liquid N_2.

The serum testosterone and thyroxine concentrations were measured by radioimmunoassay by means of commercially available RIA kits (Immunotech, :Prague). The AP and serum TSH concentrations were determined by radioimmunoassay using rat TSH NIDDK-rTSH-1-9 for radioiodination, NIDDK-rTSH-RP-3 as reference preparate and rTSH NIDDK-anti rat TSH-RIA-6 antiserum (see acknowledgment). The thyroid cGMP levels were estimated by radioimmunoassay kits (Adico, Prague). The means±95% confidence intervals were computed and the significance of differences between the means was evaluated by an analysis of variance and Dunnett's test.

RESULTS

The body, anterior pituitary (AP) and thyroid weights, thyroxine, TSH, testosterone serum concentrations, thyroid cGMP and AP TSH concentrations from the first experiment are given in Table 1. The EB treatment significantly decreased the body weight, serum thyroxine, testosterone and TSH concentrations and increased anterior pituitary weight. The treatment with L-NAME in a dose 50 mg/kg/day alone decreased the thyroid gland weights (in mg/kg), did not influence the serum thyroxine and testosterone concentrations and decreased serum TSH levels. The simultaneous administration of L-NAME did not influence changes in anterior pituitary weights and changes in hormonal levels induced by EB. The cGMP levels slightly (non significantly) fell after L-NAME and EB administration and decreased strongly in the group treated with combination of EB and L-NAME.

In the second experiment L-NAME was administered in a dose 100 mg/kg/day. The results are shown in Table 2. The EB treatment had the same effect on body, anterior pituitary weight and serum hormonal concentrations as described in experiment No. 1. The administration of L-NAME alone significantly increased the serum thyroxine and testosterone levels and decreased serum TSH concentrations. The simultaneous administration of L-NAME with EB did not influence the drop of serum thyroxine concentrations after EB but partially inhibited the EB induced decrease of serum testosterone level. The thyroid cGMP levels remained unchanged after EB treatment and L-NAME administration but dropped significantly in the

Table 1 - *The influence of estradiol benzoate (EB) and L-NAME (50 mg/kg/day) treatment on the body, anterior pituitary (AP) and thyroid weight, AP TSH levels, serum thyroxine, TSH and testosterone concentration and thyroid cGMP concentrations. Data expressed as means±95% confidence intervals.*

	Controls	EB	L-NAME	EB+L-NAME
Body weight (mg)				
initial	172.5±3.86	176.5±5.35	172.5±7.41	178.5±6.75
final	262.3±13.12	165.7±7.84*	262.6±13.32+	177.1±10.47*
AP weight (mg/kg)	32.1±1.99	158.4±34.14*	41.5±10.68+	135.8±15.02*
Thyroid weight (mg/kg)	160±18.4	179.3±27.2	119.8±17.6*+	125.8±25.2*+
Thyroxine serum (nmol/l)	68.8±7.25	40.8±4.8*	65.5±10.06+	45.5±5.78*
TSH serum (ng/ml)	0.867±0.34	0.35±0.14*	0.25±0.13*	0.524±0.16*
TSH AP (ng/AP)	7.22±1.23	2.44±0.64*	6,59±1.04+	2.24±0.37*
Testosterone serum (ng/ml)	1.99±2.5	0.348±0.15*	4.51±3.38+	0.351±0.18*
cGMP - thyroid (pmol/thyroid)	18.75±7.2	13.14±2.22	13.21±2.59	9.06±2.46*+

*statistically significant difference from control group.
+statistically significant difference from EB group (Dunnett's test).

Table 2 - *The influence of estradiol benzoate (EB) and L-NAME (100 mg/kg/day) treatment on the body, anterior pituitary (AP) and thyroid weight, AP TSH levels, serum thyroxine, TSH and testosterone concentration and thyroid cGMP concentrations. Data expressed as means±95% confidence intervals.*

	Controls (1)	EB (2)	L-NAME (3)	EB+L-NAME (4)
Body weight (g) initial	193.5±4.15	191.5±5.85	193.5±3.79	193.5±4.15
final	292±14,87	187.9±8.68*	295.1± 12.26+	176.5±8.19*
AP weight (mg/kg)	29.1±1.89	150.8±18.03*	33.1±2.15+	166.5±27.8*
Thyroid weight (mg/kg)	112.9±14.9	143±24.5*	95.5±12.4+	122±0.158
Thyroxine serum (nmol/l)	63.8±5.67	48.1±4.31*	82.7±7.89*+	47.3±3.4*
TSH- serum (ng/ml)	1.73±0.87	1.604±0.74	0.67±0.29*+	1.019±1.35
TSH - AP (ng/AP)	5.44±1.02	0.45±0.17*	5.45±0.90+	0.63±0.29*
Testosterone serum (ng/ml)	1.52±0.58	0.29±0.12*	2.61±0.75*+	0.629±0.25*+
cGMP (thyroid) (pmol/thyroid)	8.28±1.24	7.76±1.98	9.37±2.34	3.2±1.6*+

* statistically significant difference from control group.
\+ statistically significant difference from EB group (Dunnett's test).

group treated simultaneously with both substances. The anterior pituitary TSH levels in both experiments fell after EB treatment and were not influenced by L-NAME administration.

DISCUSSION

In the present study it was demonstrated that the NO-synthase inhibitor L-NAME administration increased *in vivo* the serum thyroxine and testosterone concentrations in a dose dependent manner and decreased serum TSH levels in both doses.

NO is a highly reactive gas that is produced in a series of tissues and organs. It was found that NO exerts a neurotransmitter function in central nervous system and myenteric plexus of stomach and intestine (14, 15). NO is a physiological mediator of penile erection (16). The excessive production of NO by immunocompetent cells seems to play a pathophysiological role in the development of type I diabetes mellitus (17) and septic shock (2).

Most studies that investigated the influence of NO on the thyroid function have been performed *in vitro* so far. It was found that sodium nitroprusside increases the cGMP concentration in the human thyroid cells *in vitro* without affecting thyroid hormone production (19). On the contrary Kasai et al. (6) described that NO donors administration inhibited *in vitro* the iodine organification in human thyroid cells while cGMP analogues had no effect. Motohashi et al. (5) found the inhibition of growth of human thyrocytes in culture by NO donor administration.

Our previous studies showed (9, 10, 13) that the administration of the thiazine dye, methylene blue, which is proposed to be a NO-synthase inhibitor (8), increased blood thyroxine levels and decreased the serum TSH levels. The results with another type of NO synthase inhibitor, L-NAME, are in agreement with our hypothesis that the effect of methylene blue is probably mediated through the changes of local NO production. We therefore suggest that NO is an important local mediator that stimulates the hormonal secretion in the thyroid gland by a direct mechanism independent of guanylate cyclase pathway.

There are probably two distinct effects of NO-synthase inhibitors on the hypothalamo hypophyseal-thyroid axis. The first one is the decrease of TSH secretion on the hypophyseal level, although in the second experiment it can be at least in part the result of a feedback inhibition by increased thyroxine levels. The second one is the increase of total serum thyroxine concentration. It is difficult to explain the mechanism of described changes. In the case of TSH we have found no changes in its hypophyseal contents after L-NAME administration. It is therefore possible that L-NAME influences rather hypophyseal secretion of TSH than its synthesis.

We suggest that the effects of L-NAME on the thyroid level are mediated by inhibition of local production of NO, which was found to partially block the iodine organification and growth of thyrocytes (5, 6). The decreased iodine organification as a cause of lower serum thyroxine levels is however only one of the possible mechanisms of L-NAME-

induced changes. There is also a possibility of L-NAME influence on thyroid iodine uptake, the thyroglobulin endocytosis and thyroid hormones release or peripherial deiodination. NO thus seems to have an important regulatory function in normal thyroid gland, even if the mechanism of its action is not established so far.

In both experiments we have found that L-NAME administration decreased the thyroid weight. The possible explanation of our results is that inhibition of NO local production partially blocks the TSH mediated vascularization of the thyroid tissue and therefore decreases the thyroid weight. This observation is in agreement with that of Colin et al. (4) which have found that the NO-synthase inhibitors treatment decreased the thyroid tissue vascularization by 36% *in vitro.* We suggested a similar possible mechanism in the case of the hypertrophic reaction of the anterior pituitary after EB treatment, but the pituitary weight elevations after EB administration were not influenced by simultaneous L-NAME treatment in the present study.

The increase in the serum testosterone concentration after L-NAME supports the results of Adams et al. (18) who have found that NO-donor administration decreased the serum luteinizing hormone and testosterone levels. The partial inhibition of the estrogen-induced drop in serum testosterone levels by simultaneous L-NAME administration opens the possibility that the effect of estrogen on testicular hormonal production is at least in part mediated by local changes in NO production.

Our results that show the modulation of thyroid function by NO-synthase inhibitors open the possibility of pharmacological intervention by means of NO-synthase inhibitors in the series of pathophysiological states of the thyroid gland. The further elucidation of the mechanism of the described changes in thyroid, hypophyseal and testicular function is in the scope of our future investigations.

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