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Effect of NO-Precursor (L-Arginine) and NO-Synthase Inhibitor (L-NAME) on the Secretion of Thyroid Hormone and Thyrotropin (TSH) in Female Rats

Wpływ prekursora NO (L-argininy) oraz inhibitory syntazy (L-NAME) na wydzielanie hormonów tarczycy i tyreotropiny u szczurzych samic

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Abstract

Background. Nitric oxide (NO) plays an important role in various biological processes. It has been suggested that NO may modulate the functions of the endocrine system. The aim of this study was to examine the role of nitric oxide in total triiodothyronine (T3), free thyroxine (fT4) and TSH secretion in adult female Wistar rats.

Material and Methods. The animals were treated with L-NAME (10 mg/kg IP) and L-arginine (100 mg/kg IP) daily for 7 consecutive days. On the day 8th plasma TSH concentration was estimated by immunoenzymatic micro-particle method, while thyroid hormones, T3 and fT4, were determined using radioimmunoassay method.

Results. The plasma TSH concentration was very significantly diminished, following L-arginine treatment. On the other hand L-arginine was without effect on T3 and fT4 plasma level. L-NAME administrated for 7 days did not produce any significant changes in the plasma concentration of T3, fT4 and TSH.

Conclusions. These findings may indicate that long-term L-arginine exposure decreases TSH secretion under basal condition in female rats. However, the results of this study need further investigation that might be able to confirm whether L-arginine action on TSH secretion is indeed mediated via NO (Adv. Clin. Exp. Med. 2004, 13, 1, 23–26).

Key words: nitric oxide, thyroid hormone, tyreotropin.

Streszczenie

Wprowadzenie. Tlenek azotu (NO) jest ważnym regulatorem różnych biologicznych procesów, przypuszcza się, iż NO może modulować funkcje układu dokrewnego.

Cel pracy. Celem pracy było zbadanie roli NO w wydzielaniu trijodojodotyroniny (T3), wolnej tyroksyny (fT4) oraz tyreotropiny (TSH) u szczurzych samic szczepu Wistar.

Materiał i metody. Zwierzętom podawano jeden raz dziennie przez 7 kolejnych dni L-NAME (10 mg/kg i.p.) oraz L-argininę (100 mg/kg i.p.). W ósmym dniu dokonywano pomiaru stężeń w osoczu TSH metodą immunoenzymatyczną oraz T3 i fT4 metodą radioimmunoanализową.

 Wyniki. L-arginina powodowała bardzo istotny spadek stężenia TSH w osoczu, nie wywierała natomiast istotnego wpływu na stężenia hormonów tarczycy (T3, fT4). L-NAME podawany przez 7 dni nie powodował istotnych zmian w stężeniach T3, fT4 oraz TSH.


Słowa kluczowe: tlenek azotu, hormony tarczycy, tyreotropina.

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Nitric oxide (NO) plays an important role in several biological systems, e.g. prevents the adhesion and aggregation of platelets [1], modulates the neurotransmitter release, synaptic plasticity, morphogenesis and gene expression [2].

The expression of NOS has been detected in various structures connected with the endocrine system. On the basis of experimental and clinical studies, it has been suggested that NO may modulate the endocrine secretion. Localization of NADPH-diaphorase reactivity, used as a marker for NOS was found in the chick and mouse thyroid gland [3], as well as in the anterior pituitary cell cultures [4]. Moreover, human thyrocytes are able to produce NO [5] and endogenous NOS inhibitor, asymmetric dimethylarginine (ADMA), which regulates NOS activity [6]. Stimulatory effect of NO donors on thyroid peroxidase (TPO) activity in the cultures of primary human thyrocytes [7] and their inhibitory effect on iodine organification by human open thyroid follicles [5] may provide evidence for the role of NO in the regulation of thyroid hormone synthesis. Thus, NO may be an important autocrine and paracrine factor in the regulation of endocrine function under physiological condition. On the other hand, the expression of iNOS was found in both human tumor cells of thyroid papillary carcinoma [8] and monocytes from patients with Graves’ disease [9].

Little is known about a possible role of NO in mechanisms controlling the hormone secretion at the hypothalamic-pituitary-thyroid gland axis level. Thus, the aim of this study was to examine the role of NO in the regulation of thyroid hormone secretion. The results were statistically analyzed using Mann-Whitney’s U-test with significance level of p < 0.01. The results are presented as mean ± SD and median.

Material and Methods

The experiments were carried out on adult female Wistar rats, weighing 230–250 g. They were kept at a temperature of about + 22°C and under a 12-hr alternating light-dark cycle. The animals received standard pelleted food and had a free access to tap water. All the experiments were performed in the morning and blood was obtained at about 9.00–10.00 am.

The rats were divided into three groups (7 to 8 animals each):

- I – Control group in which rats were treated with 0.9% NaCl at a dose 1ml/kg of body weight,
- II – Second group in which rats were treated with L-arginine at a dose 100 mg/kg of body weight,
- III – Third group received L-NAME (N^G-nitro-L-arginine methyl ester) at a dose 10 mg/kg of body weight.

Rats were treated with the drugs by intraperitoneal injection (IP). Substances were administered chronically, once a day for seven consecutive days. On the 8th day (24 h after the last injection) of the experiment all the animals were exsanguinated by heart puncture under a light ether anaesthesia. Plasma was separated by centrifugation at 4°C, 12 000 rpm for 10 min. Blood plasma was stored in −18°C for the hormone analysis. The levels of the hormones were measured with commercial type kits. Thyroid hormones, total T_{3} (T_{3}) and free T_{4} (fT_{4}) were determined using radioimmunoassay (RIA) method, while TSH level was measured by means of immunoenzymatic microparticle method (Abbot). L-NAME and L-arginine were obtained from Sigma.

Statistical Analysis

The results were statistically analyzed using Mann-Whitney’s U-test with significance level of p < 0.01. The results are presented as mean ± SD and median.

Results

The control plasma levels of hormones were as follows: TSH – 3.77 mIU/ml, fT_{4} – 0.8 ng/dl, T_{3} – 0.69 ng/ml (Tab. 1).

Administration of L-arginine 100 mg/kg IP caused significant decrease of TSH plasma concentration from 3.77 to 0.72 mIU/ml (p = 0.00031). Plasma fT_{4} and T_{3} concentrations did not show any substantial changes, following L-arginine treatment.

There are no significant alterations in the plasma level of TSH, fT_{4} and T_{3} after L-NAME 10 mg/kg IP, in comparison with the control group (Tab. 1).

Discussion

The observed very significant decrease of TSH plasma level after L-arginine (the substrate for NO synthesis), chronically administrated IP may be caused by NO inhibiting influence on the release of TSH from the pituitary. It is possible that the pituitary or hypothalamic mechanisms, controlling the TSH secretion are associated with the NO system. It is very probable that NO regulates the secretion of TSH directly at the pituitary gland.
level, but the indirect action should also be considered e.g. NO as a modulator of thyrotropin releasing hormone (TRH) secretion. The NO synthases have been detected in structures connected with the hypothalamo-pituitary hormonal axis [10, 11]. TRH in a dose-dependent fashion stimulated nitrites and nitrates (the oxidation products of NO) release from the anterior pituitary cell cultures (GH3 cells), which was abolished by NOS inhibitor, L-NAME [4]. In humans, L-NAME significantly reduced TSH increase induced by TRH, but did not change the basal secretion of TSH. These results also suggest the mediation by NO of TSH-releasing action of TRH [12].

Ciro et al. [12] reported that NO appears to be without effects on the dopaminergic control of TSH secretion, L-NAME did not change TSH response to the dopaminergic antagonist, metoclopramide.

In contrast to results obtained with L-arginine, NO donor, nitroglycerine administered sublingually had no effect on the basal TSH concentration in young males [16].

In our experiment the plasma concentrations of T3 and fT4 were not markedly changed after L-arginine chronic treatment. Moreover, NO donor, sodium nitroprusside had no effect on the basal or TSH-stimulated T3 secretion in primary human thyrocytes [17].

Blockade of NO synthase activity with the arginine derivative L-NAME (10 mg/kg/day) was without effect on the plasma TSH concentration, not confirming our observation with NO precursor, L-arginine. This discrepancy between L-NAME and L-arginine effect is difficult to explain at present. Thus, it remains to be tested whether a higher dose of L-NAME as well as other NOS inhibitors may influence TSH secretion. Haluzik and co-workers found that L-NAME in a dose 100 mg/kg/day but not in a dose 50 mg/kg/day significantly increased the serum TSH level during long-term administration in male rats [18]. As in our chronic experiment, an acute study with a single administration of L-NAME did not change the basal secretion of TSH in humans [12]. In our experimental model the decrease of TSH secretion was not reflected by the expected changes of plasma T3, fT4 levels.

These observations together with those of others may further explain the role for the L-arginine-NO-signalling pathway in the modulation of endocrine function.

References


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