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Effects of the hormones of hypothalamic-pituitary-thyroid axis on tissue factor and tissue factor pathway inhibitor plasma levels in rats

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Introduction Tissue factor (TF) is a critical initiator of the physiologic and pathologic coagulation that binds FVIIa to form TF/ FVIIa complex, which triggers the coagulation protease cascade (Tilley & Mackman, 2006, Steffal *et al* 2006). TF originates mainly from vascular endothelium (and all tissues, (Erez *et al* 2008, Morel *et al* 2008). Tissue factor pathway inhibitor (TFPI) is the major physiological inhibitor to TF-mediated coagulation process (Al-Mugeiren *et* al 2006, Golino *et al* 2003, Hedner *et al* 2008). TFPI binds to factor Xa and inhibits TF/ FVIIa (Bajaj *et al* 2001, Rosing *et al* 2008. De Smedt *et al* 2009). TFPI originates mainly from vascular endothelium (Bajaj *et al* 2001, Kothari *et al* 2009).

Aim The aim of the present study was to examine the effects of the hormones of the hypothalamic - pituitary - thyroid axis on plasma levels of the tissue factor and the tissue factor pathway inhibitor.

Material and methods The study was carried out on 65 male Wistar rats, weighing 200–220 g. The animals were fed by standard briquette food and received water *ad libitum*. Procedures involving animals and their care were conducted in conformity with the requirements of the European Convention for the Protection of Experimental Animals (Protection of animals used for experimental purposes, Council Directive 86/609/EEC of November 1986). The animals were divided into five identical groups – one control group (injected with saline) and four experimental groups, injected as follows: the first group was injected by thyrotropin releasing hormone (TRH), acetate salt (Sigma, minimum 97%), in a dose of 0.06 mg/kg b.w.; the second group - by thyroid stimulating hormone (TSH), bovine (in a substance with activity of 2 MU/mg, Sigma), in a dose of 1 MU/kg b.w.; the third and the fourth group, respectively by liothyroninum (trijodthyroninum – T_3) and levothyroxinum (Thyroxin – T_4) (substances produced by VEB Berlin – Chemie, Germany), in a dose of 0.08 mg/kg b.w. each.

The animals were injected s.c. once daily for three consecutive days.

The necessary blood volume was acquired by a cardiac puncture under ether narcosis. Sodium citrate (0.11 mol) was used as anticoagulant (blood/citrate ratio 9:1). The following parameters were determined: tissue factor (TF) in pg/ml, free tissue-factor pathway inhibitor (free TFPI) in ng/ml, tissue-factor pathway inhibitor activity (activity TFPI) in % and prothrombin time (PT) in sec. All parameters examined were measured by commercial enzyme-linked immunosorbent assay (ELISA) kits of Diagnostica Stago (France) and America Diagnostica inc. (USA).

All data obtained have been analyzed by variation analysis using Student-Fisher's *t*-test.

Results

1. Influence of TRH, TSH, T3, and T4 on TF level (Fig. 1).

TRH decreased TF level by 29.11 % ($p < 0.001$), TSHH – by 65.42 % ($p < 0.001$), T_3 – by 25.14 % ($p < 0.001$), and T_4 – by 56.68% ($p < 0.001$).

2. Influence of TRH, TSH, T3, and T4 on free TFPI level (Fig. 2).

TRH increased free TFPI level by 83.10% ($p < 0.001$), TSHH– by 148.68% ($p < 0.001$), T_3 – by 64.84% ($p < 0.001$), and T_4 – by 134.00% ($p < 0.001$).

3. Influence of TRH, TSH, T3, and T4 on activity TFPI level (Fig. 3).

Autonomic & Autacoid Pharmacology
2010, 30, 101–165

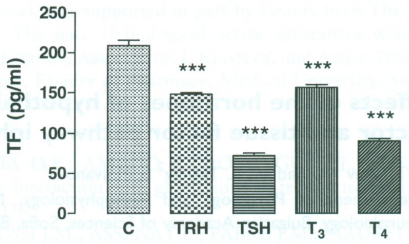

Figure 1 Effects of TRH (0.06 mg/kg b.w.), TSH (1 MU/kg b.w.), T_3 (0.08 mg/kg b.w.), and T_4 (0.08 mg/kg b.w.), applied s.c. to male Wistar rats once daily for three consecutive days on TF level (pg/ml).
TF - Tissue factor; TRH – thyrotropin-releasing hormone; TSH – thyroid-stimulating hormone; T_3 – Trijodthyroninum, T_4 – Thyroxin, C– control saline-injected rats
***$p < 0.001$.

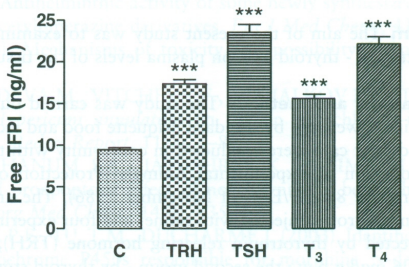

Figure 2 Effects of TRH (0.06 mg/kg b.w.), TSH (1 MU/kg b.w.), T_3 (0.08 mg/kg b.w.), and T_4 (0.08 mg/kg b.w.), applied s.c. to male Wistar rats once daily for three consecutive days on free TFPI (ng/ml).
Free TFPI – free Tissue factor pathway inhibitor; TRH – thyrotropin-releasing hormone; TSH; – thyroid-stimulating hormone; T_3 – Trijodthyroninum, T_4 – Thyroxin, C – control group rats, injected with saline ***$p < 0.001$.

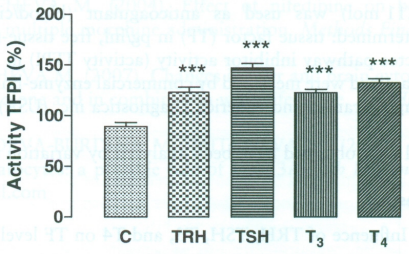

Figure 3 Effects of TRH (0.06 mg/kg b.w.), TSH (1 MU/kg b.w.), T_3 (0.08 mg/kg b.w.), and T_4 (0.08 mg/kg b.w.), applied s.c. to male Wistar rats once daily for three consecutive days on activity TFPI (ng/ml).
Activity TFPI – Tissue factor pathway inhibitor activity; TRH – thyrotropin-releasing hormone; TSH – thyroid-stimulating hormone; T_3 – Trijodthyroninum, T_4 – Thyroxin, C – control group rats, injected with saline
***$p < 0.001$.

TRH increased activity TFPI level by 37.97% ($p < 0.001$), TSH – by 64.99% ($p < 0.001$), T_3 – by 37.41% ($p < 0.001$), and T_4 – by 48.51% ($p < 0.001$).

4. Influence of TRH, TSH, T3, and T4 on PT (Fig. 4).

Autonomic & Autacoid Pharmacology
2010, 30, 101–165

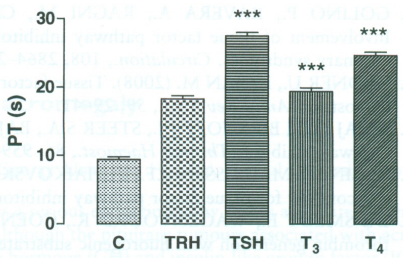

Figure 4 Effects of TRH (0.06 mg/kg b.w.), TSH (1 MU/kg b.w.), T_3 (0.08 mg/kg b.w.), and T_4 (0.08 mg/kg b.w.), applied s.c. to male Wistar rats once daily for three consecutive days on PT (sec).
PT- prothrombin time; TRH – thyrotropin-releasing hormone; TSH – thyroid-stimulating hormone; T_3 – Trijodthyroninum, T_4 – Thyroxin, C – control group rats, injected with saline ***$p < 0.001$.

TRH increased PT by 94.19% ($p < 0.001$), TSH – by 192.19% ($p < 0.001$), T_3 – by 105.95% ($p < 0.001$), and T_4 – by 162.10% ($p < 0.001$).

Discussion The analysis of the effect of the hypothalamic-pituitary-thyroid (HPT) axis hormones on plasma level of TF, presented on Fig. 1, shows a significant decrease of TF plasma level under the influence of TRH, TSH, T_3, and T_4 as compared to the control group of rats. As far as TF is known to trigger the extrinsic pathway of coagulation (7), it could be suggested that the hormones used can cause hypo coagulability. It is noticeable that TRH and T_3 similarly decreased TF, while TSH and T4 also showed similar and even more pronounced effects. Taking into consideration that T_4 has a stronger effect compared to T_3, and the fact that TSH evoked the most significant effect on TF level, it is conceivable that TSH exerts its effects both through the thyroid axis and directly, influencing plasma TF level. The results presented on Figure 2 and Figure 3 reveals a hypocoagulability, as well. TRH, TSH, T_3, and T_4 exerted pronounced unidirectional effect on free TFPI and activity TFPI – parameters, which objectively point to TFPI involvement in hemocoagulation balance. Having in mind that TFPI is the principle inhibitor of TF-initiated coagulation (5) it is reasonable to state that the significant increase of free TFPI (Figure 2) and activity TFPI (Figure 3) leads to a misbalance and hindered coagulation without bleeding. Considering the TFPI mechanism of action (6) it might be assumed that the increased levels of free TFPI and activity TFPI are probably of major importance for the observed decrease of TF (Fig. 1). The changes of prothrombin time (Fig. 4), influenced by TRH, TSH, T_3, and T_4, also point to hypocoagulability.

Conclusion In conclusion, the hormones of HPT axis TRH, TSH, T_3, and T_4, produced a pronounced hypocoagilability in rats by significantly reducing TF and increasing the plasma levels of free TFPI and activity TFPI.

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Autonomic & Autacoid Pharmacology
2010, 30, 101–165

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