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TRIIODOTHYRONINE AND THYROXINE STIMULATE THROMBOCYTOPOIESIS IN RATS

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It is known that iodine-containing hormones of thyroid gland are of essential importance for the growth, development and metabolic homeostasis of the organism [4,5]. This circumstance necessitates their comprehensive investigation.

Recent studies demonstrate that these hormones influence significantly upon erythropoiesis regulation, too. Thalia et al. [13] find out that triiodothyronine stimulates proliferation and differentiation of erythroid precursors. This effect is mediated by β-adrenergic receptors [8]. It is reported that hypothyroidism is accompanied by a strongly expressed anaemia [7].

At the same time, the relation of thyroid status to thrombocytopoiesis is insufficiently clarified yet. On the basis of clinical observations can be accepted that there is a tendency towards thrombocytopenia in hypothyroidism [6].

The aim of the present work is to study the influence of triiodothyronine and thyroxine on thrombocytopoiesis in rats. Usage of both hormones could enable not only to characterize more comprehensively the relation of thyroid gland to thrombocytopoiesis but also to reveal the most probable mechanism of their action.

MATERIAL AND METHODS

Our study covered 90 white male rats of Wistar breed (with body mass of 170-200 g) and 45 white male mice of Swiss breed (with body mass of 20-25 g). Rats were divided into two equal groups. Effect of Liothyroninum (Trijothyroninum-T_3) and of Levothyroxinum (Thyroxin - T_4) on thrombocytopoiesis was assessed on the first animal group divided into 3 subgroups of equal number of animals. Hormones used were in the form of a substance (production of VEB Berlin-Chemie, GDR). They were applied in a dose of 2 mg/kg b.m. intraperitoneally for 3 consecutive days. Control rats were injected with hormonal vehicle (ethanol and physiological saline). Thrombocytopoiesis level was estimated according to the following parameters: according to thrombocyte count, determined after the phasic-contrast method described by Lisichkov [1]; according to the percentage of ^{75}Selenomethionine (^{75}Se-M) incorporated into newly-formed thrombocytes - after Penington's method in the modification of Negrev and Ganchev [12] as well as according to bone-marrow megakaryocyte (MKC) changes determined after four-degree classification of Levine at al. [10].

Effect of both T_3 and T_4 on thrombocytopoietin biosynthesis was estimated in the second group of rats which were divided and injected after the aforedescribed way. Thrombocytopoietin activity (TPA) of plasma obtained was determined after Penington's method modified by Negrev and Ganchev [12]. Male mice (in 3 groups of 15 mice each) were used as recipients correspondingly to donor groups. TPA level was estimated according to the changes of thrombocyte count and percentage of ^{75}Se-M incorporated into newly-formed thrombocytes of recipient mice.

Blood amount required for analysis was obtained by decapitation of animals carried out on the 72nd hour for thrombocytopoiesis and on the 96th hour for TPA assessment.

Results obtained were processed by the methods of variation analysis.

Table 1

Changes of bone-marrow megakaryocytes in rats under the influence of T_3 and T_4

Injected substance	Megakaryocytes - Stages I[st]	% difference	II[nd]	% difference	III[rd]	% difference	IV[th]	% difference	Total MKC	% difference
Liothyroninum (Trijodthyronin-T_3) (n = 15)	9.87 + /-0.39	+ 89.80 (p < 0,001)	5.87 + /-0.24	+ 91.20 (p < 0,001)	3.67 + /-0.16	+ 112.14 (p < 0,001)	2.13 + /-0.22	+ 99.06 (p < 0,001)	21.60 + /-0.64 (p < 0,001)	+ 95.12
Levothyroxinum (Thyroxin- T_4) (n = 15)	9.40 + /-0.33	+ 80,77 (p < 0,001)	5.40 + /-0.28	+ 75.90 (p < 0,001)	3.87/-0.26	+ 123.70 (p < 0,001)	2.27 + /-0.26	+ 112.15 (p < ,001)	20.93 + /-0.70 (p < 0,001)	+ 89.07
Ethanol and physiological saline (control) (n = 15)	5.20 + /-0.29		3.07 + /-0.18		1.73 + /-0.19		1.07 + /-0.24		11.07 + /-0.61	

Data are presented as x +/- Sx to 1000 megakaryocytes. Percentage difference is calculated towards control values. Sign (+) means an increase. n is the number of animals in the coresponding group.

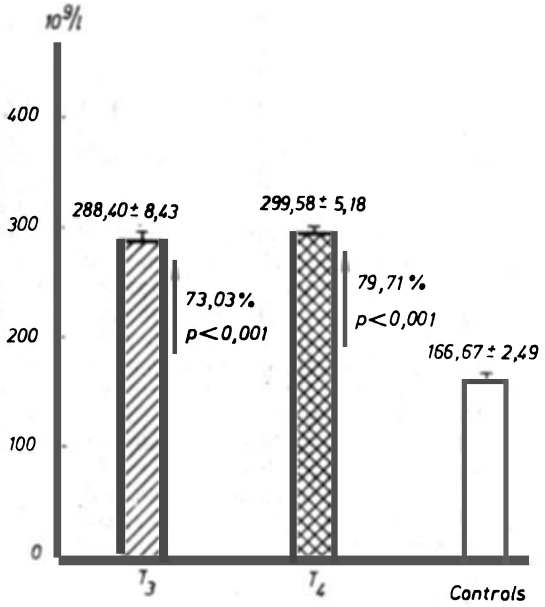

Fig. 1 Changes of thrombocyte count in male rats under the influence of T_3 and T_4. Data are presented as x +/- Sx. Percentage difference is calculated towards controls. Vertical lines on the columns indicate the standard error of the arithmetic mean.

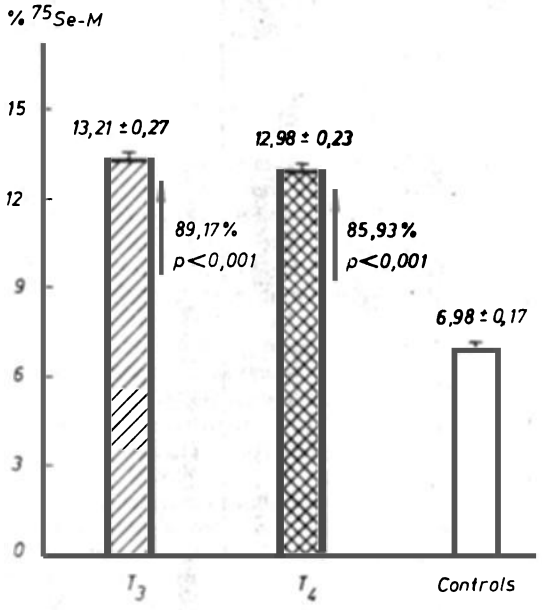

Fig. 2 Changes of the percentage of ^{75}Se-M incorporated in newly-formed male rat thrombocytes under the influence of T_3 and T_4.

RESULTS AND DISCUSSION

Influence of both T_3 and T_4 on the thrombocytopoiesis is shown on fig.1 and fig.2 as well as on table 1. It is obvious that these hormones increase significantly not only thrombocyte count (fig.1) - by 73.03 per cent ($p < 0,001$) and by 79.71 per cent ($p < 0,001$) but also the percentage of ^{75}Se-M incorporated into newly-formed thrombocytes (fig. 2) - by 89.17 per cent ($p < 0,001$)

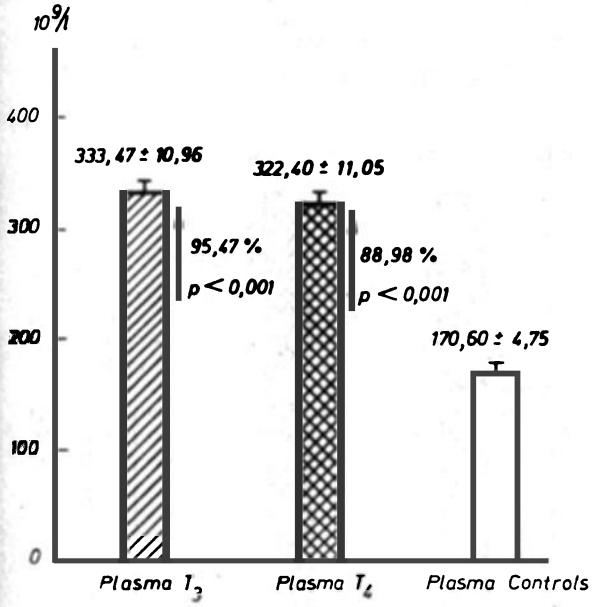

Fig. 3 Changes of thrombocyte count in recipient-mice under the influence of plasma from donors treated with plasma T_3 and plasma T_4. Data are presented as $x + /- Sx$. Percentage difference is calculated towards control values - after injection with plasma from donors treated by hormone solvents. Vertical lines of columns indicate the standard error of arithmetic mean.

85.93 per cent ($p < 0,001$), respectively. Table 1 indicates clearly that both T_3 and T_4 enhance significantly not only the total MKC number (by 95.12 per cent - $p < 0,001$ and by 89.07 per cent - $p < 0,001$, respectively) but also the number of cells in all stages (from I^{st} till IV^{th} stage).

The influence of iodine-containing hormones of the thyroid gland on plasma obtained from donors treated with T_3 and T_4 increase in recipients not only thrombocyte count (fig. 3) - by 95.47 per cent ($p < 0,001$) and 88.98 per cent ($p < 0,001$), respectively, but also the percentage of ^{75}Se-M incorporated into newly-formed thrombocytes (fig. 4) - by 89.45 per cent ($p < 0,001$) and 90.81 per cent ($p < 0,001$), respectively.

Analysis of these results reflecting thrombocytopoietic changes influenced upon by T_3 and T_4 demonstrates that all parameters examined are strongly elevated. Having in mind the significance of these parameters [3] we can accept that this is a direct evidence of the activating effect of T_3 and T_4 on thrombocytopoiesis in rats. It strikes on table 1 that strong total MKC count increase is accompanied by a significant increase of the number of all stages (from the I^{th} till the 4^{th} stage). It is known that enhancement of the number of all cells of the megakaryocyte line presents an expression of stimulated proliferation and differentiation [9] that enables the assumption that hormones used possess an activating effect on these processes. Significant increase of the number of the youngest MKC - of first stage - is a very important fact as far as it is a manifestation of enhanced mitotic activity of their immediate precursors [15]. That is why one can suppose that stimulating influence of T_3 and T_4 is realized mainly at megakaryocyte precursor level.

Investigation of plasma TPA could clarify how hormones stimulate thrombocytopoiesis - directly, at megakaryocyte precursor level, or indirectly, by means of thrombocytopoietin. Fig. 3 and fig. 4 indicate that plasma from donors treated with T_3 and T_4 enhances strongly these parameters in recipients, i.e. thrombocyte count and percentage of ^{75}Se-M incorporated into newly-formed thrombocytes being thus an indicator that they posses an increased TPA. This enables to assume that both T_3 and T_4 activate thrombocytopoietin biosynthesis as a specific humoral regulator of thrombocytopoiesis [13]. Having in mind that, on one hand, $beta_1$ - adrenergic receptors influence positively upon thrombocytopoiesis [2], and, on the other hand, thyroid hormones enhance their density in various tissues [8] we can suppose that although slightly probably these receptors are related to enhanced thrombocytopoiesis in this case.

It is noteworthy, too, that all parameters of thrombocytopoiesis and plasma TPA examined are

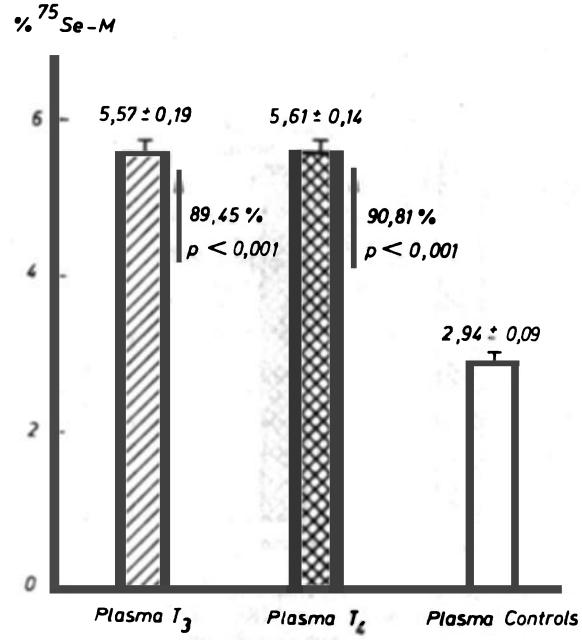

Fig. 4 Changes of the percentage of ^{75}Se-M incorporated in newly-formed thrombocytes in recipient-mice under influence of plasma T_3 and plasma T_4

enhanced to an almost equal degree under the influence of T_3 and T_4. It is possible that T_4 realizes its stimulating effect on thrombocytopoiesis after conversion into T_3 - a fact established in other tissues and processes [14].

In conclusion, we could accept that both T_3 and T_4 stimulate significantly thrombocytopoiesis in rats. It results most probably from the strongly enhanced biosynthesis of thrombocytopoietin as a specific humoral regulator of this process. There are no significant differences in the biological activity of T_3 and T_4 in thrombocytopoiesis at all.

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ТРИИОДТИРОНИН И ТИРОКСИН СТИМУЛИРУЮТ ТРОМБОЦИТОПОЭЗ У КРЫС

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РЕЗЮМЕ

Изучается влияние трийодтиронина и тироксина на основные показатели тромбоцитопоэза тромбоцитопоэтиновой активности плазмы крови у крыс самцов.

Устанавливается, что трехдневное применение этих гормонов вызывает сильное увеличение числа тромбоцитов, процента ^{75}селенометионина и числа костно-мозговых мегакариоцитов. Увеличение стоимостей этих показателей устанавливается на всех стадиях - от первого до четвертого. Тромбоцитопоэтиновая активность плазмы крови тоже показывает повышение.

Авторы полагают, что триодтиронин и триоксин в нормальных условиях стимулируют значимо тромбоцитопоэз у крыс. Очевидно, это является результатом сильно повышенного биосинтеза тромбоцитопоэтина. Существенных различий биологической активности триодтиронина и триоксина и ее влияния на тромбоцитопоэз не устанавливается.