Vol. 82, No. 3, 1978 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
June 14, 1978 Pages 811-819

INHIBITION OF THYROTROPIN BINDING TO HUMAN THYROID PLASMA MEMBRANES BY THYROID HORMONES AND "REVERSE TRIIODOTHYRONINE" [1,2].

Wojciech Kiełczyński and Janusz Nauman

Department of Biochemistry, Medical Center of Postgraduate Education, 01-813 WARSAW, MARYMONCKA 99, POLAND

Received April 13,1978

SUMMARY: Triiodothyronine, reverse triiodothyronine and thyroxine were found to inhibit ^{125}I labelled thyrotropin binding to human thyroid plasma membranes in vitro. Both the thyrotropin binding and the effect of the above iodoaminoacids on this binding were pH, temperature and time dependent. 50% inhibition of thyrotropin binding was observed at 2×10^{-7}M concentration of reverse triiodothyronine or thyroxine and at 1.1×10^{-6}M concentration of triiodothyronine. The kinetic studies of thyrotropin binding revealed that the maximal capacity of receptor sites for the pituitary hormone is unaffected by the presence of thyroid hormones. On the other hand the association and dissociation constants for thyrotropin binding changed when iodoaminoacids were present in the incubation medium /Ka $8.13 \times 10^{7}M^{-1}$ vs $1.6 \times 10^{8}M^{-1}$ and Kd 1.14×10^{-8}M vs 4.55×10^{-9}M respectively, depending on the pH/. The double reciprocal plots showed competitive mechanism of inhibition. The present study suggest that triiodothyronine, reverse triiodothyronine and thyroxine are able to modify the thyrotropin binding to membrane receptors.

INTRODUCTION: It is well known that triiodothyronine /T_3/ and thyroxine /T_4/ affect the thyroid function by regulating the pituitary thyrotropin /TSH/ secretion. In recent years the existence of some direct effect of thyroid hormones on thyroidal responsivenes to TSH has been suggested /1/. Takasu, et al. /2/ and Shimizu, et al. /3/ have demonstrated that T_3 inhibits the TSH-induced activation of adenylate cyclase as well as TSH-induced endocytosis. Since the TSH binding to thyroid membrane receptors is generally regarded to be the first step of pituitary hormone action the aim of

1/ Presented in part at the 8th European Thyroid Association Lyon /France/ 26 - 30 Sept. 1977.
2/ This study was supported by 10.4.2.01.5.10 Grant from the Polish Academy of Sciences,.

0006-291X/78/0823-0811$01.00/0

the present study was to find out whether thyroid hormones directly influence this reaction.

Although reverse triiodothyronine /rT_3/ is regarded to be biologically inactive, the possible effect of this iodo-aminoacid on thyroid membrane TSH binding was also investigated.

MATERIALS AND METHODS: Human TSH for labelling was gifted to us by Byk-Mallincrodt Company, TSH standard A was obtained from MRC, London. Chromatographically pure standards of L-T_3, L-rT_3 and L-T_4 were prepared by Dr Henning Company /West Berlin/. All other chemicals were purchased from commercial sources and were of highest purity grade available. hTSH was labelled with ^{125}I /Amersham U.K./ by a slightly modified method of Greenwood et al. /4/ to a specific activity of 75 - 120 mCi/mg. Protein concentration was measured by the method of Lowry et al. /5/ using bovine albumin as a standard. Thyroid tissue was obtained from surgically treated patients with Graves' disease. Crude plasma membrane fraction was prepared as previously described elsewhere /6/. Membranes were divided into small aliquots and used immediately or stored frozen at -20°C prior to use.

Receptor assay. Experiments were carried out in small polystyrene tubes. The incubation medium contained 5 fmoles of ^{125}I-TSH, 100 µg of membrane proteins in 25 mM Tris-HCl 0,4% bovine serum albumin, final pH 4,75 or 6,0. The amount of plasma membranes used was kept within the linear phase of binding when evaluated as a function of membrane protein concentrations. The incubation medium was enriched with either 500 fmoles of native TSH or with 100 pmoles of T_3, rT_3 or T_4. In saturation experiments concentration of labelled TSH varied from 1 - 200 fmoles per sample. In displacement studies concentration of native TSH varied from 10 fmoles to 2 pmoles and the concentration of T_3, rT_3 and T_4 varied from 1 to 500 pmoles/sample. The final volume of the sample was 100 µl. The samples were incubated for 15 minutes at 22°C. The reaction was stopped by rapid dilution with 1 ml of icecold 25 mM Tris-HCl. The tubes were then centrifuged at 10000 x g for 10 minutes, the supernatant was discarded and the radioactivity of the resulting pellet was counted in a LKB - Wallac automatic spectro-gamma counter.

Nonspecific binding of ^{125}I-TSH was measured in on incubation mixture with 2 pmoles of native TSH/tube added and in tubes without membrane proteins. In all experiments nonspecific binding was below 5%. Since in our experimental conditions 23 to 29% of ^{125}I-TSH were bound specifically by equal amounts of membrane proteins depending on the specific activity of the label used, for statistical reasons the final results are expressed as percentage of the maximal binding. For each experiment the results shown were the mean of those obtained in quintiplicate /most of them/ or triplicate vessels. In addition all experiments were carried out at least three times and only those findings that were consistent are presented.

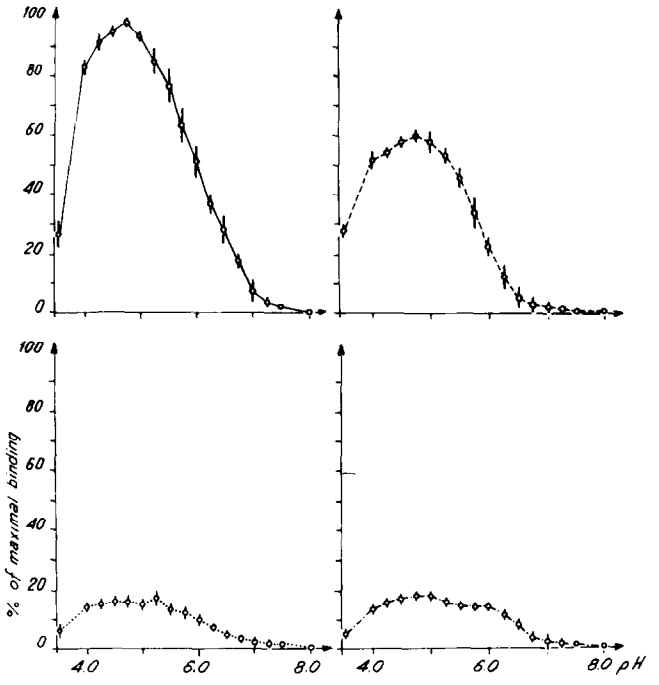

Figure 1. Effect of pH on the binding of hTSH to thyroid plasma membranes /——/ in the presence of T_3 /---/ rT_3 /..../ or T_4 /-.-.-/. Mean $\pm$S.D., n=15. Incubation medium contained 25 mM Tris-HCl and 0,4% BSA, from pH 3,5 to pH 8,0.

RESULTS: Effect of pH. Maximal binding of ^{125}I-TSH to crude thyroid membrane fraction was observed at pH 4,75, however parallel studies were also made of the effect of thyroid hormones at pH 6.0. As shown in Figure 1, 100 pmoles of T_3 added to the medium caused a decrease in the membrane binding of labelled TSH to 60%. The effect of rT_3 was equal to that of T_4. When the incubation medium was enriched with 100 pmoles of either rT_3 or T_4, the binding of ^{125}I-TSH dropped to 20%.

Effect of incubation time and temperature. The binding of labelled TSH to human thyroid plasma membranes was studied

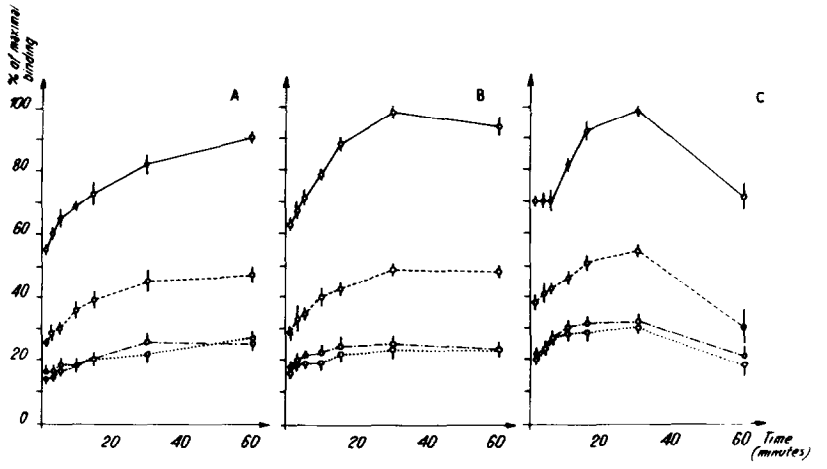

Figure 2. Effect of time and temperature on the binding of hTSH to thyroid plasma membranes /——/ and in the presence of T_3 /----/, rT_3 /..../ or T_4 /-.-.-/. Mean ± S.D., n=12.
A = Incubation temperature = 4°C
B = Incubation temperature = 22°C,
C = Incubation temperature = 37°C.

at 4°C, 22°C and 37°C, respectively. At all temperatures the binding was very rapid. At 22°C and 37°C, after 30 minutes of incubation, dissociation of TSH - membrane complex was observed /Figure 2/. At all temperatures used significant effect of T_3, rT_3 and T_4 on TSH membrane binding was noted.

Effect of different concentration of iodoaminoacids on membrane ^{125}I-TSH binding. The inhibitory effect of different concentrations of T_3, rT_3 and T_4 on membrane ^{125}I-TSH binding is shown in Figure 3.

A significant decrease in TSH binding was observed when 4 pmoles of rT_3 or T_4 were added to the medium. The inhibition of TSH binding was found to be complete when the concentration of these iodoaminoacids reached 300 pmoles per tube. The inhibitory effect of T_3 was weaker and complete inhibition

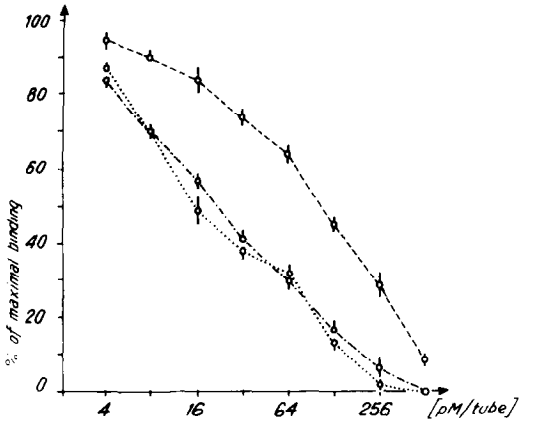

Figure 3. Influence of increasing amounts of T_3 /----/, rT_3 /..../ or T_4 /-.-.-/ on hTSH binding to thyroid plasma membranes. Mean $\pm$ S.D. n = 15.

of TSH binding was achieved when concentration of this thyroid hormone exceeded 500 pmoles per tube.

Kinetic parameters of ^{125}I-TSH - thyroid hormone interactions, in the presence of T_3, rT_3 and T_4.

Data from saturation studies performed at both pH 4.75 and 6,0 were used to draw Scatchard /7/ and Lineweaver - - Burk /8/ type plots /Figure 4 and 5/.

The analysis of both plots revealed that the values of the association constant /Ka/, dissociation constant /Kd/ and of the maximal capacity of membranes TSH binding sites /Bmax/ were in the same range when calculated either from the Scatchard or Lineweaver - Burk plots.

The values obtained at pH 4,75 and 6,0 were also in the same range /Table I/. In the presence of rT_3 or T_4 the Ka value for TSH was lower by a factor of 10, while in the presence of T_3 no significant change in Ka for TSH was observed.

DISCUSSION: The results of the present study show that

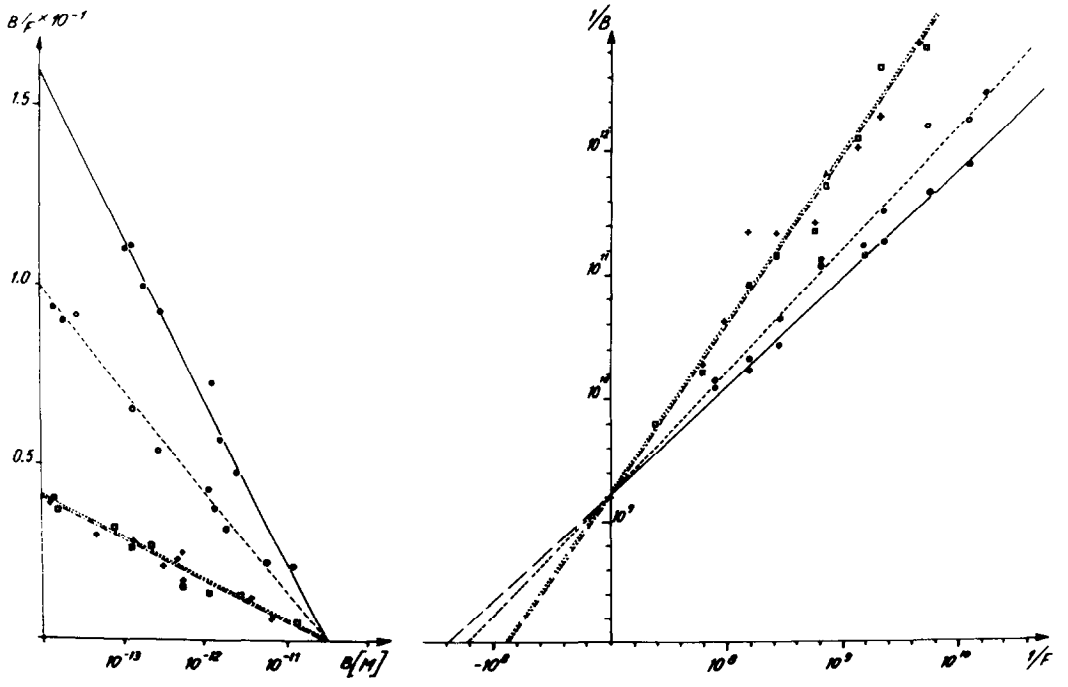

Figure 4. Scatchard /left/ and Lineweaver-Burk /right/ plots of hTSH /●——● / to thyroid plasma membranes in the presence of T_3 /○---○ /, rT_3 /+....+/ or T_4 /□-.-.-□ / at pH 4,75.

Table I. Kinetic data of hTSH binding to human thyroid plasma membranes in the absence or presence of thyroid hormones or reverse T_3.

	pH = 4.75		pH = 6.00	
	Scatchard	Lineweaver-Burk	Scatchard	Lineweaver-Burk
B max.	4.5×10^{-10} M	4.5×10^{-10} M	2.6×10^{-10} M	2.6×10^{-10} M
K_A TSH	3.60×10^{8} M^{-1}	3.61×10^{8} M^{-1}	2.40×10^{8} M^{-1}	2.39×10^{8} M^{-1}
K_A TSH/T_3	2.20×10^{8} M^{-1}	2.18×10^{8} M^{-1}	1.61×10^{8} M^{-1}	1.60×10^{8} M^{-1}
K_A TSH/rT_3	8.77×10^{7} M^{-1}	8.77×10^{7} M^{-1}	8.13×10^{7} M^{-1}	8.13×10^{7} M^{-1}
K_A TSH/T_4	8.77×10^{7} M^{-1}	8.77×10^{7} M^{-1}	8.13×10^{7} M^{-1}	8.13×10^{7} M^{-1}
K_D TSH	2.78×10^{-9} M	2.77×10^{-9} M	4.17×10^{-9} M	4.19×10^{-9} M
K_D TSH/T_3	4.55×10^{-9} M	4.58×10^{-9} M	6.23×10^{-9} M	6.25×10^{-9} M
K_D TSH/rT_3	1.14×10^{-8} M	1.14×10^{-8} M	1.23×10^{-8} M	1.23×10^{-8} M
K_D TSH/T_4	1.14×10^{-8} M	1.14×10^{-8} M	1.23×10^{-8} M	1.23×10^{-8} M

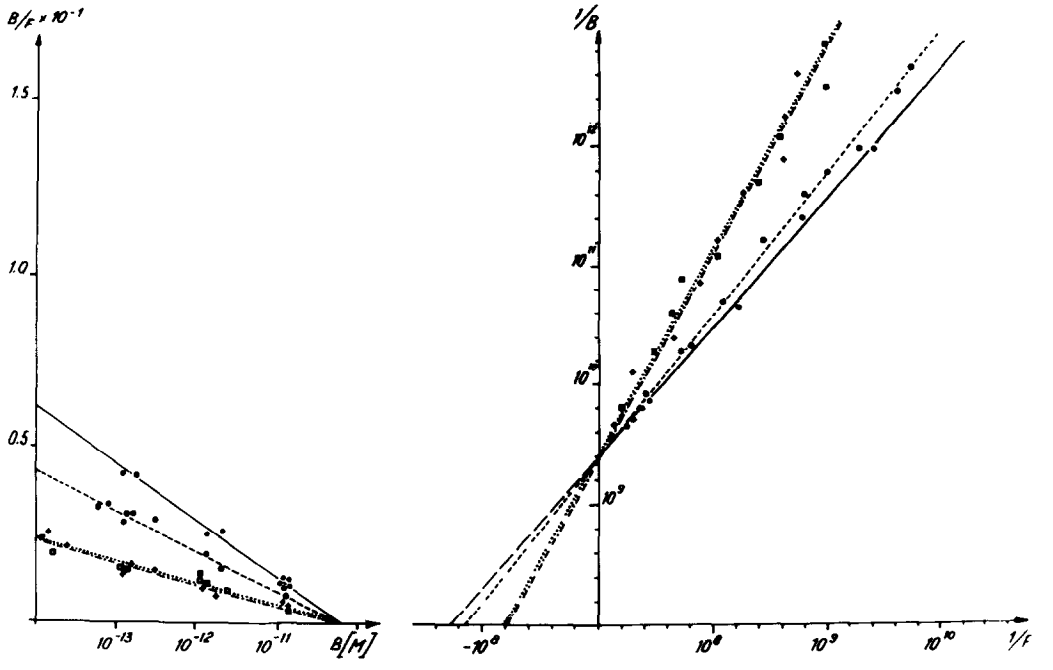

Figure 5. Scatchard /left/ and Lineweaver-Burk /right/ plots of hTSH binding /●——● / to thyroid plasma membranes in the presence of T_3 /o---o/, rT_3 /+....+/ or T_4 / □-.-.-□ / at pH 6,0.

in vitro both thyroid hormones and rT_3 competitively inhibit ^{125}I-TSH binding to human thyroid plasma membranes. TSH binding and the inhibitory effect of iodoaminoacids studied was pH, temperature and time dependent. In contrast to other autors /9,10/ who observed maximal TSH binding at pH 6,0 in our experimental conditions, maximal thyrotropine binding to membrane was found to occur at pH 4,75, a value close to that reported by Bryson et al. /11/. To exclude the possibility that a low pH might change the biological and physico-chemical properties of membrane receptors, TSH or iodoaminoacids, all experiments were carried out in parallel at pH 6,0 and the results obtained clearly demonstrate that

the differences were quantitative rather than qualitative in nature.

To exclude the possibility that the inhibitory effect of T_3, rT_3 and T_4 was not due to their binding or interaction with TSH itself two types of preliminary experiments were performed. ^{125}I-TSH was incubated with iodoaminoacids for 15 minutes at 22°C as in the receptor assay and the mixture was chromatographed on a previously calibrated Sephadex G-25 column. The TSH eluted from the column had binding properties similar to the untreated hormone.

On the contrary, preincubation of plasma membranes with T_3, rT_3 or T_4 resulted in decreased binding of TSH.

Our experiments have clearly shown that rT_3 and T_4 are more potent inhibitors of TSH binding than T_3 is. This is because T_3 in contrast to both rT_3 and T_4 have two iodine atoms in the outer ring of thyronine. The iodination of the outer ring can be significant for the iodoaminoacids - thyroid membrane receptor interrelationship. Although the physiological relevance of the in vitro experiments is limited, the results of the present study can at least suggest that not all factors influencing the action of TSH on the thyroid are thoroughly understood, even fully recognized at present.

Acknowledgments. Autors wish to express their gratitude to Dr H. Steinmans from Henning Co., West Berlin for sending us standards of T_3, rT_3 and T_4, to W.H.O. International Lab. for Biological Standards - London, for TSH standard A, and to Dr Naegele from Byk-Mallinckrodt - Dietzenbach for offering us hTSH for labelling.

REFERENCES

1. Shishiba, Y., Takaishi, M., Miyachi, Y. and Ozawa, Y. /1975/ Endocrinol. Japon. 22, 367 - 371.
2. Takasu, N., Sato, S., Tsukui, T., Yamada, T., Furihata, R. and Makiuchi, M. /1974/ J. Clin. Endocr. Metab. 39, 772-778.
3. Shimizu, T. and Shishiba, Y. /1975/ Endocrinol. Japon. 22, 55-60.

4. Greenwood, F.C., Hunter, W.M. and Glover, J.S. /1963/ Biochem. J. 89, 114-123.
5. Lowry, O.H., Rosenbrough, N.T., Farr, A.L. and Randall, R.J. /1951/ J. Biol. Chem. 193, 265-275.
6. Nauman, J. and Wasilewska, A. /1976/ in Biochemical Basis of Thyroid Stimaulation and Thyroid Hormone Action /A. von zur Mühlen and H. Schleusener, eds./ pp. 128-132. Georg Thieme Publishers, Stuttgart.
7. Scatchard, G. /1949/ Ann. N. Y. Acad. Sci. 51, 660-672.
8. Lineweaver, H. and Burk, D. /1934/ J. Am. Chem. Soc. 56, 658-666.
9. Tate, R.L., Schwartz, H.I., Holmes, J.M., Kohn, L.D. and Winand, R.J. /1975/ J. Biol. Chem. 250, 6509-6515.
10. Goldfine, I.D., Amir, S.M., Ingbar, S.H. and Tucker, G. /1976/ Biochim. Biophys. Acta 448, 45-56.
11. Bryson, J.M., Joasoo, A. and Turtle, J.R. /1976/ Acta Endocrinol. 83, 528-538.