

## Effects of octreotide on propylthiouracil-induced goiter in rats: a quantitative evaluation

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**Summary.** To evaluate the possible antigoirogenic effect of somatostatin, the influence of long-acting somatostatin analog - octreotide - on experimental goiter developed in rats treated with propylthiouracil was examined. Goiter formation was assessed by measurement of the main histological compartments of the thyroid as well as by morphometric analysis of the vascularization and blood supply of the gland. Although treatment with octreotide did not prevent the goiter formation, it clearly reduced blood supply and vascularization of the thyroid and counteracted propylthiouracil-induced increase in the relative volume of follicular epithelium. To conclude, the somatostatin analog - octreotide - is effective in reduction of goiter vascularisation. This finding provides a rationale for the clinical trials of the treatment of hypervascular goiter by somatostatin analogs.

**Key words:** Somatostatin analogs, Octreotide, PTU, Thyroid gland, Vascularization, Morphometry

### Introduction

Somatostatin is one of the important physiological regulators of thyrotropin (TSH) secretion. In addition to the effect exerted at the pituitary level, somatostatin also acts at the level of the thyroid gland. It has been found that somatostatin is able to inhibit TSH-induced secretion of thyroid hormones (Loos et al., 1977; Ahren et al., 1978). In our laboratory we have found that somatostatin directly suppresses the basal and TSH-stimulated mitotic activity of the organ-cultured rat thyroid lobes (Żerek-Meleń et al., 1987). The *in vitro* antiproliferative effects of somatostatin have also been shown in respect to human thyrocytes (degli Umberti et al., 1991) as well as to the rat thyroid follicular cell line FRTL5 (Tsuzaki and Moses, 1990). Somatostatin analog - octreotide - has been found to inhibit the proliferation

of human thyroid cancer cell lines (Hoelting et al., 1996). The presence of somatostatin receptors has been shown in human thyroid cancers *in vitro* (Ain and Taylor, 1994) as well as *in vivo* (Postema et al., 1996). The binding of radiolabeled octreotide has also been found in normal and goitrous thyroid gland in human subjects (Becker et al., 1995). These data suggest that somatostatin may counteract the goiter formation, since the latter depend on increased proliferation of thyrocytes and is often connected with enhanced TSH secretion. It is also worth recalling that goitrogenesis is accompanied by enhanced neo-vascularization, and somatostatin exhibits an anti-angiogenetic activity (Barrie et al., 1993). To prove the possibility that somatostatin may counteract the goitrogenesis, we have evaluated the effect of long-acting somatostatin analog, octreotide (SMS), on experimental goiter developed in rats treated with propylthiouracil (PTU). PTU-induced goiter is a commonly used model of the experimental goiter in rodents (Denef et al., 1989)

### Materials and methods

Twenty-four male Wistar rats were used, weighing 170-240 g each at the beginning of the experiment. The animals were divided into 3 equal groups and fed with standard laboratory diet. Propylthiouracil (6-n-propyl-2-thiouracil [Sigma]) was administered as a 0.1% solution in drinking water in 2 groups. Since the 3rd day of PTU administration the rats were injected subcutaneously either with 10 µg of octreotide (Sandoz) or with 0.9% NaCl solution every 12 hours. After 8 days of the experiment the animals were weighed and sacrificed by prolonged ether anaesthesia and the thyroids were carefully collected and weighed. After proper histological procedures the paraffin sections (8 µm thick) were subjected to morphometric analysis, which included assessment of the vascularization and blood supply of the thyroids as well as the measurement of the relative volumes of thyroid compartments.

The analysis of the vascularization and blood supply of the thyroid tissue was carried out with use of the digital image analyzer (IBAS for Windows, KONTRON,

Germany) coupled with a black-white camera mounted on a light microscope. The microscopic slides of glands stained according to the method of Seyle with Poely's modifications (Poely et al., 1964) were analyzed. Five to ten fields (1.31 sq. mm each) were randomly selected and analyzed for each animal. The image of each analyzed field was recorded into the computer memory twice: using red and subsequently green filter. That procedure facilitated distinguishing red erythrocytes from surrounding (green) tissue.

The main histological compartments of the thyroid gland (epithelium, colloid and stroma) were also quantitatively evaluated and their relative volumes were assessed. The slides stained with hematoxylin and eosin were analyzed with the use of a computer system for microscopic image analysis and the computer program "Compartments" - designed for quantitative analysis of histological compartments (Klencki et al., 1997). The point-counting method was applied, which has been shown to allow for quantitative assessment of the relative volumes of the histological compartments of the thyroid gland (without significant error) when a sufficient (usually 500 or more) number of points is analyzed (Palkovits, 1963). The square grid of points was used (the distance between neighboring points was 30  $\mu\text{m}$ ; 63 points were classified in each analyzed field; the magnification was approx. 0.5  $\mu\text{m}/\text{pixel}$ ) and at least 600 points were classified for each thyroid.

We assessed the following parameters of vascularization: a) blood supply: sum of areas of all profiles of red cells, expressed as percentage of the total field area; b) total vessel area: sum of areas of all vascular profiles in a field, expressed as percentage of the total field area; c) total vessel perimeter: the sum of all vessel perimeters in a field (given in microns in one square mm); d) vessel number: mean number of vessel profiles in a field (calculated as number of vessels crossing one cubic millimeter of the tissue); e) degree of vessel filling: the ratio of blood supply to total vessel area; f) mean vessel

diameter: mean diameter of the circles inscribed into each vessel profile (given in microns); and g) mean vessel area: mean area of a vessel profile (given in square microns).

The values of mean vessel diameter and mean vessel area concern only vessel profiles totally included in the measuring frame and not crossing the border of an analyzed field. Mean vessel number was recalculated to one cubic millimeter of tissue volume.

Moreover, the following morphometric parameters of the thyroid gland were estimated: a) relative volumes of the colloid, the epithelium and the stroma of the thyroid gland; b) relative weight of the thyroid gland; and c) relative weights of the thyroid compartments, calculated as the products of the relative thyroid weight and relative volumes of compartments.

The statistical significance of the obtained results was assessed with the use of a non-parametric Mann-Whitney U test, as the variances of analyzed parameters were significantly different or their distribution was not normal.

## Results

### Morphometric evaluation of the thyroid tissue compartments

As expected, the treatment with PTU resulted in a sharp increase of the thyroid gland weight (18.75 $\pm$ 10.6 mg vs. 12.7 $\pm$ 3.6 mg in controls). PTU also affected the examined morphometric parameters of thyroid tissue compartments (follicular epithelium, colloid and stroma) (see Figs. 1, 2). Relative weight and relative volume of follicular epithelium was significantly increased in PTU-treated rats. The relative weight, but not the relative volume of stroma, was also increased. In contrast, the relative volume (but not the relative weight) of colloid was decreased.

The treatment with SMS failed to affect the PTU-

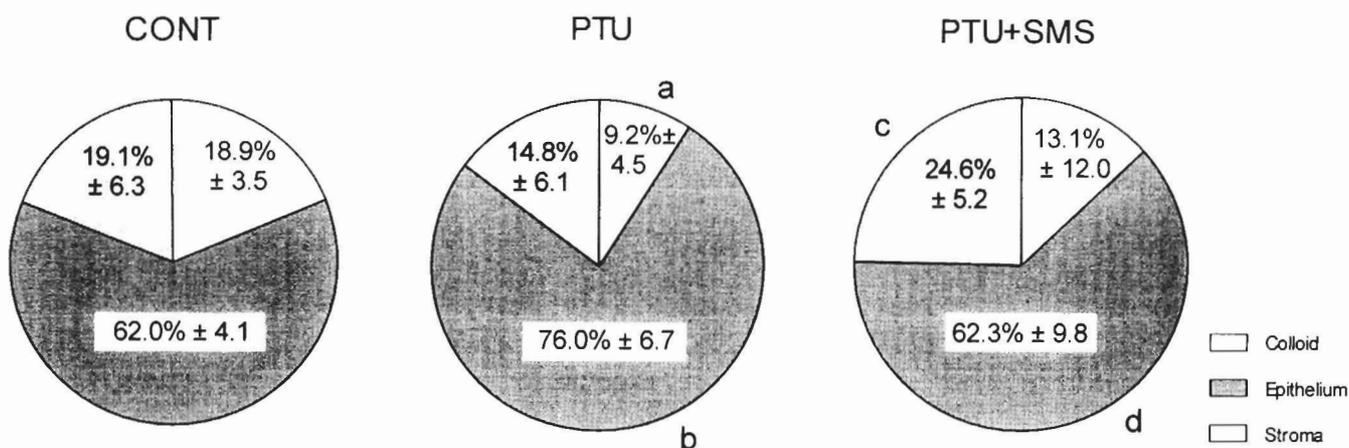


Fig. 1. Mean relative volumes of thyroid compartments  $\pm$  SD. Letters denote statistical significances: a,  $p < 0.001$  vs controls; b,  $p < 0.005$  vs controls; c,  $p < 0.05$  vs PTU; d,  $p < 0.01$  vs PTU.

induced weight increment of the thyroid gland. However, it influenced some morphometric parameters of the gland. Namely, it prevented the PTU-induced increase in the relative volume of follicular epithelium and enhanced the relative weight and the relative volume of the stroma (Figs. 1, 2).

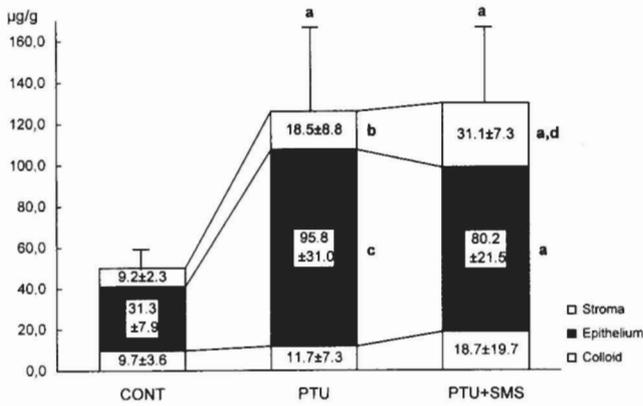
*Parameters of vascularization*

The treatment with PTU resulted in a sharp alteration of almost all examined parameters of vascularization. The density of vascularization, the total vessel area, and the mean area occupied by red blood cells (blood supply) were strongly increased (Figs. 3-5). In turn, the mean vessel diameter and the mean area of the vessel profile remained unchanged (data not shown). The simultaneous treatment with SMS partially

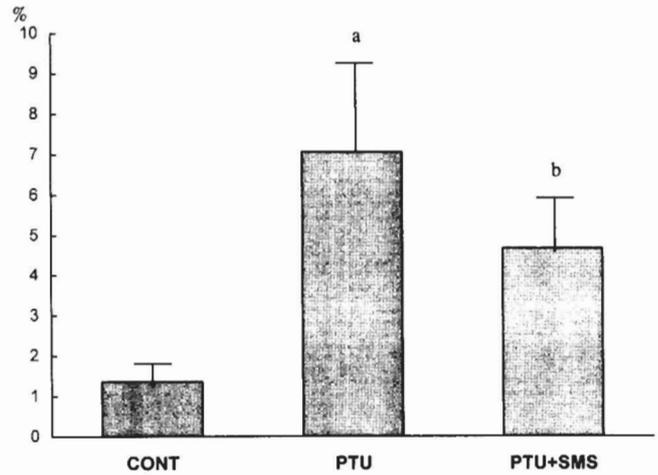
prevented all the above mentioned alterations.

**Discussion**

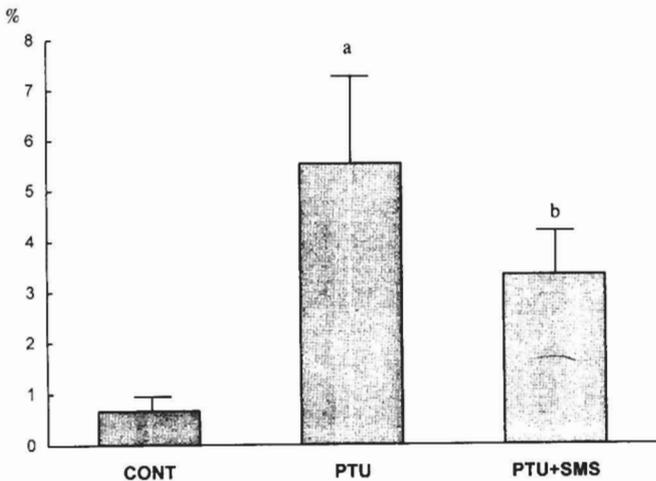
As expected, the treatment of animals with PTU resulted in dramatic changes in thyroid weight and morphology. These changes also included vascularization. Although simultaneous treatment with SMS did not prevent the goiter formation (at least in the dosage used in this study), it counteracted PTU-induced increase in the relative volume of follicular epithelium. Unexpectedly, SMS evoked a further increase the PTU-induced increment in the relative weight and relative volume of stroma. This alteration may be explained as a result of the diminishment of the gland parenchyma. The effects of SMS treatment on the vascularization seem to



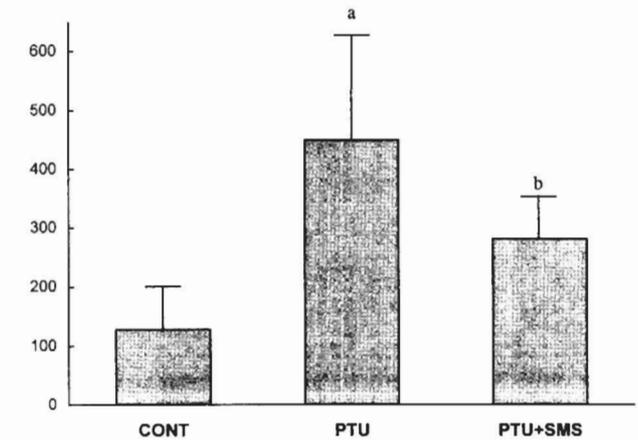
**Fig. 2.** Mean relative thyroid weight (total bars) ± SD and mean relative compartment weights (partial bars) ± SD, in µg/g of body weight. Letters denote statistical significances: a, p<0.005 vs controls; b, p<0.01 vs controls; c, p<0.001 vs controls; d, p<0.05 vs PTU.



**Fig. 3.** Mean total vessel area ± SD. Letters denote statistical significances: a, p<0.001 vs controls; b, p<0.01 vs controls and p<0.05 vs PTU.



**Fig. 4.** Mean blood supply of the thyroid ± SD. Letters denote statistical significances: a, p<0.001 vs controls; b, p<0.01 vs controls and p<0.05 vs PTU.



**Fig. 5.** Mean vessel number ± SD. Letters denote statistical significances: a, p<0.001 vs controls; b, p<0.01 vs controls and p<0.05 vs PTU.

be more pronounced than those concerning other aspects of thyroid morphology. The effects of SMS on the morphometric parameters of vascularization roughly corroborate with those observed earlier in the anterior pituitary gland (Pawlikowski et al., 1997). To sum up, the findings presented in this study clearly indicate that simultaneous SMS treatment reduces blood supply and vascularization of the PTU-induced goiter. The effect of SMS may be exerted indirectly, on the hypophyseal level, or directly, on the level of the thyroid gland. Although the TSH levels were not estimated in this study, the suppression of TSH secretion by somatostatin and octreotide is a well-known phenomenon (Yang et al., 1996). SMS also might act at the peripheral level, by inhibiting the intrathyroidal secretion of angiogenic factors and/or by suppressing directly the proliferation of endothelial cells (Grant et al., 1993). Obviously, all the above-mentioned mechanisms may be involved in the SMS action on intrathyroidal vascularization. Irrespective of the mechanism in question, the effectiveness of SMS in diminishing the vascularization of the experimental goiter provides a rationale for the clinical trials of the treatment of the hypervascular goiter in humans.

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