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INHIBITORY EFFECT OF SOMATOSTATIN ON THE BASAL AND TSH-STIMULATED ^{3}H-THYMIDINE INCORPORATION INTO RAT THYROID LOBES INCUBATED IN VITRO

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The effects of somatostatin on the spontaneous and TSH-stimulated incorporation of tritiated thymidine into the rat thyroid lobes incubated in vitro were investigated. The rate of ^{3}H-thymidine incorporation was used as an index of thyroid follicular cells (TFC) proliferation. It was shown that: 1) somatostatin, at a concentration of 10^{-7}M, decreased ^{3}H-thymidine incorporation into DNA of TFC, 2) the highest somatostatin concentration, as tested in this study (10^{-6}M), produced a similar decreasing effect; the decrease, in this case, did not attain significance vs. controls, 3) somatostatin, when employed together with TSH, suppressed the stimulatory effect of the latter hormone on ^{3}H-thymidine incorporation into DNA of thyroid lobes.

Various hormones and growth factors are involved in the growth regulation of the thyroid gland. Demonstration of the presence of somatostatin in thyroid C cells (12, 13) has attracted many investigators to study the effect of this neurohormone on the hypothalamus-pituitary-thyroid axis. Somatostatin is known to be a potent inhibitor of various secretory processes. It was shown that somatostatin inhibited the basal and TRH-stimulated release of TSH (11). Loos et al. (4, 5) reported that this neurohormone decreased thyroid hormone secretion both in vivo and in vitro.

Several data indicated that somatostatin could also play the role of an antiproliferative hormone. Pawlikowski et al. (8) reported that somatostatin inhibited the mitogenic action of TRH on the anterior pituitary gland in the organ culture. However, there have been conflicting data reporting that somatostatin augments thymidine incorporation into DNA of unidentified hypophyseal cells in monolayer culture (9).

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Somatostatin exerted the antiproliferative influence not only on the normal, but also on the neoplasmatic tissues, i.e., HeLa and gerbil fibroma cells (6) and on the meningioma cells (3).

As a continuation of our previous study (14), the goal of the present paper has been to examine the effect of various concentrations of somatostatin, as well as interactions among somatostatin and TSH in their action on ^{3}H-thymidine incorporation into DNA of rat thyroid lobes in the organ culture.

MATERIALS AND METHODS

Thirty three adult male rats of Wistar strain, weighing 200 ± 10 g each, were used as donors of thyroids. The animals were killed by decapitation. The thyroid lobes collected from all the animals under sterile conditions were placed in a culture vessel on the surface of a stainless grid in such a way that the fluid under the grid moistened the thyroid explant resting on it. The explants were incubated for 4 hours in RPMI-1640 medium (Gibco) containing 15% fetal calf serum, with addition of 2 μCi/ml of ^{3}H-thymidine (specific activity 22 Ci/mM, Amersham, England) plus 10 mM Hepes buffer. The thyroid lobes in the individual groups were incubated in the presence of the following compounds:
Group I - control, the number of thyroid explants (n=6),
Group II - TSH (Ambinon, Organon), 20 mU/ml (n=7),
Group III - somatostatin (Serono), 10^{-6}M (n=7),
Group IV - somatostatin, 10^{-7}M (n=7),
Group V - TSH, 20 mU/ml + somatostatin, 10^{-7}M.
At the termination of incubation the explants were washed out with cold RPMI-1640 medium. DNA was extracted as described by Schmidt-Thannhauser (10), and determined by the diphenylamine method, according to Burton (1) in modification by Giles and Myers (2). The results were expressed as the mean counts per minute (cpm) per 1 μg of DNA. Data were statistically analyzed using a one-way analysis of variance (ANOVA). The significance of differences observed among the individual groups was subsequently determined by Newman-Keuls' test.

RESULTS

The results are shown in Figure 1.
Thyrotropin significantly increased ^{3}H-thymidine incorporation into DNA of thyroid lobes (138.2 ± 19.7; $\bar{x}$ ± SEM), when compared to controls (86.9 ± 2.7). In turn, somatostatin (10^{-7}M) significantly decreased tritiated thymidine uptake (35.4±2.0). The highest somatostatin concentration tested in this study (10^{-6}M) produced a similar decreasing effect (77.5±10.7); the decrease, however, did not attain significance in that case. Somatostatin (10^{-7}M), when employed together with TSH, suppressed

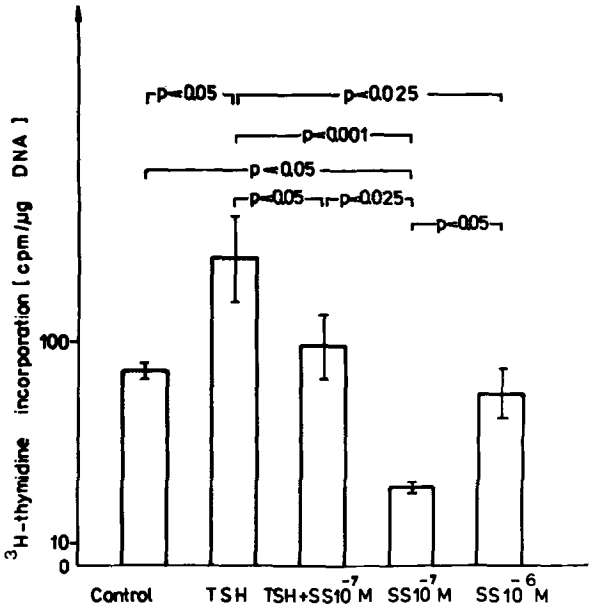

Fig. 1. Influence of somatostatin and TSH on ^{3}H-thymidine incorporation into DNA of the rat thyroid lobes. SS - somatostatin; TSH - thyrotropin. Bars represent means ± SEM; p - level of significance.

the stimulatory effect of the latter hormone on ^{3}H-thymidine incorporation into DNA of thyroid lobes (98.9±14.0).

DISCUSSION

It is generally accepted that the incorporation of tritiated thymidine into DNA reflects the rate of actual DNA synthesis and is vastly used as a sensitive method for measuring the proliferation of cells. The data presented above indicate that somatostatin, at a concentration of 10^{-7}M, decreases significantly ^{3}H-thymidine incorporation into DNA of TFC and suppresses TSH--stimulated tritiated thymidine uptake. It is noteworthy that somatostatin, at a concentration of 10^{-6}M, also decreased ^{3}H-thymidine uptake by rat thyroid lobes, but the effect in question did not attain statistical significance. The present results are concordant with the data of our previous study (14); we have found that somatostatin suppressed the basal and TSH--stimulated mitotic activity of follicular cells in the organ--cultured rat thyroid. We have also examined the effect of direct intratnyroidal *in vivo* microinjections of somatostatin and/or TSH on tritiated thymidine incorporation into thyroid lobes

while incubated in vitro (Żerek-Mełeń et al., unpublished data); we have demonstrated that somatostatin, when injected together with TSH, partially suppressed the stimulatory effect of the latter hormone on tritiated thymidine uptake by thyroid lobes.

All the a.m. findings are compatible with the antiproliferative effects of somatostatin in other tissues and organs (3, 7).

The present data speak in favour of our earlier hypothesis that somatostatin could affect the thyroid growth by paracrine interaction between C-cells and TFC.

Further studies are needed to establish firmly the role of somatostatin in the regulation of thyroid growth.

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