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GOLGI SECRETION AND FOLLICULAR GENESIS IN RAT FOETAL THYROID

Claude Penel, Lionel Remy[x], Suzanne Rua[x], Evelyne Mazzella[x]

Lab. Endocrinologie Cellulaire and [x] Lab. Histologie, Médecine-Nord, Marseille, France

ABSTRACT

16 day-old Wistar rat foetal thyroid glands were incubated with various thyrotropin concentration and investigated by electron microscopy to study Golgi secretion and follicular lumina genesis. The secretory vesicles size distribution and the evolution with time of the volume - and surface densities of vesicles and follicular lumina were obtained by stereological analysis. In our experimental conditions, it was shown that Golgi secretion was heterogeneous and biphasic and that the follicular lumina genesis mainly originated from the transfer and the coalescence of the biggest vesicles.

INTRODUCTION

Following a previous study on the morphogenetic effect of thyrotropin on rat foetal thyroid gland in vitro (Remy et al., 1980), a stereological analysis was performed in the aim to quantify the influence of Golgi secretion on thyroid follicular lumina genesis.

MATERIAL and METHODS

14 day-old rat foetal thyroid gland from the same mother were explanted and placed on millipore filters in Corning plastic dishes filled with 1 ml Eagle's medium (Eagle, 1955) supplemented with glutamin (2 mU), penicillin G (200 U/ml)

and streptomycin (50 µg/ml). Some glands were cultured with TSH (20 mU/ml or 80 mU/ml). After various incubation times, the glands were fixed with glutaraldehyde 1 % in phosphate buffer (0.1 M, pH 7.35). Ultrathin sections (90 nm) from different levels of the gland were investigated in a Siemens Elmiskop 101 electron microscope.

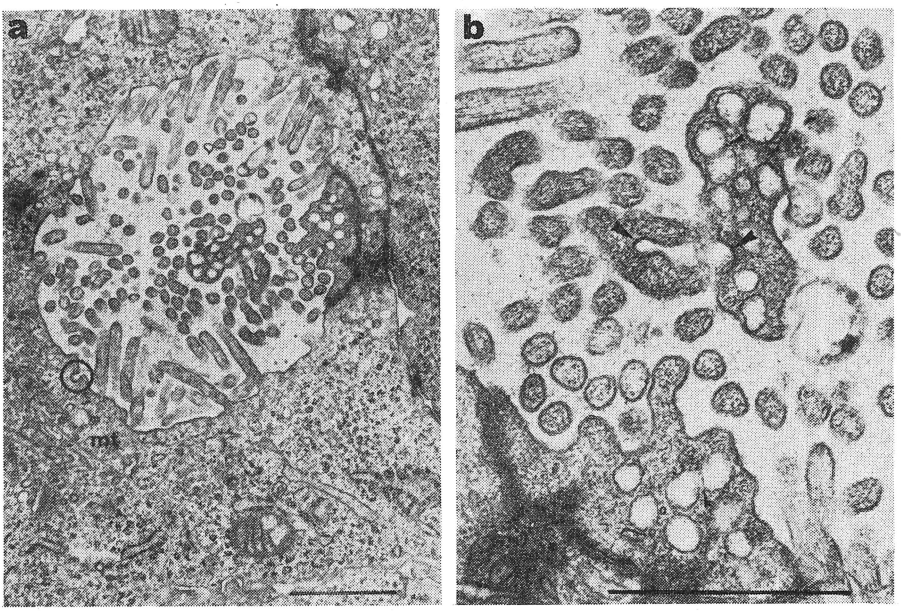
For each incubation time, 40 micrographs (final magnification 20 000) were obtained by an UR sampling (Cruz-Orive and Weibel, 1980). The size distribution (diameter) of the Golgi vesicles and of the nuclei were determined by a modified Wicksell transform (Penel et al., 1981). The first moments of the vesicles size distribution ($\overline{D}$, $\overline{D^2}$, $\overline{D^3}$) were calculated by numerical integration. The numerical (N), surface (Sv) and volume (Vv) densities of vesicles were determined by the relationships

$$N = \frac{No}{pd^2} \times \frac{1}{\overline{D} + t - 2h}$$ (see Weibel, 1979, p. 341), $Sv = \pi N\overline{D^2}$ and $Vv = \frac{\pi}{6} N\overline{D^3}$.

Volume (V_F) and surface (S_F) densities of follicular lumina were obtained by point- and intersection-counting (Mathieu et al., 1981). The mean cell volume was calculated by the ratio of the mean nuclear volume versus the volume density of the nucleus in the cell.

RESULTS

1) An ultrastructural analysis demonstrated that (i) follicular lumina were the result of the coalescence of Golgi vesicles (Fig. 1) and (ii) follicular lumina formation was enhanced by TSH. 2) A stereological study showed that whatever the TSH concentration and the incubation time, the size distribution of Golgi vesicles (Fig. 2) was composed of a spectrum of two normal and one lognormal laws whose parameters were respectively : Amplitude A = 0.07, 0.37, 0.56 , Mean M = 49.9, 109.4, 140.3 nm , Standard Deviation SD = 6.6, 11.1, 37.1 nm . The diameter range lay between 30 nm and 270 nm with two maxima at 50 and 110 nm. The mean vesicles diameter was 120 nm.



Figs I a, b - Formation of follicular cavity

a - follicular cavity mt : microtubules
o : exocytotic pit X 15 000
b - magnification of previous figure
vesicle fusion and vesicle exocytosis
X 33 000

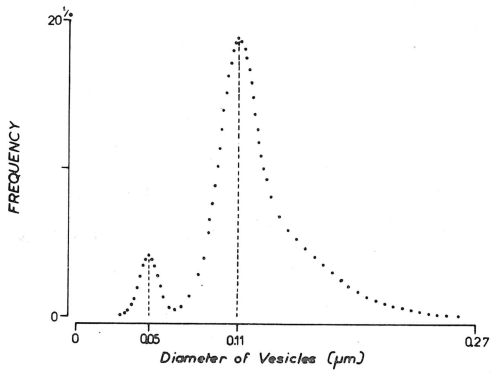
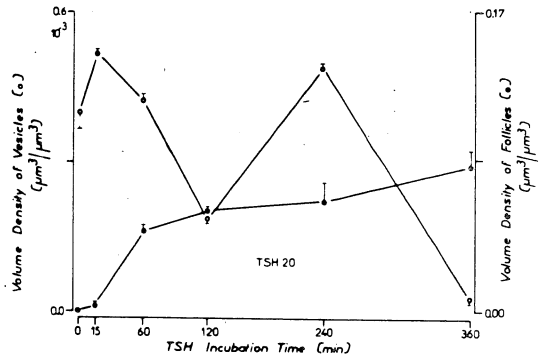
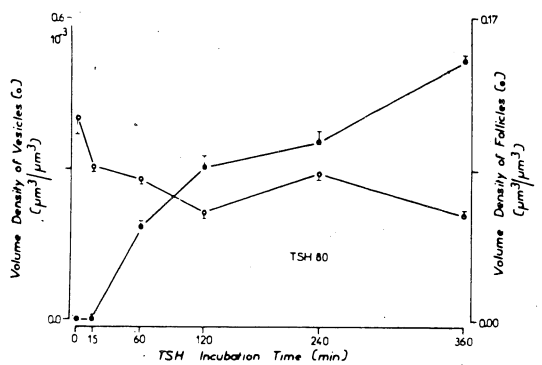


Fig. 2 - Vesicle size distribution

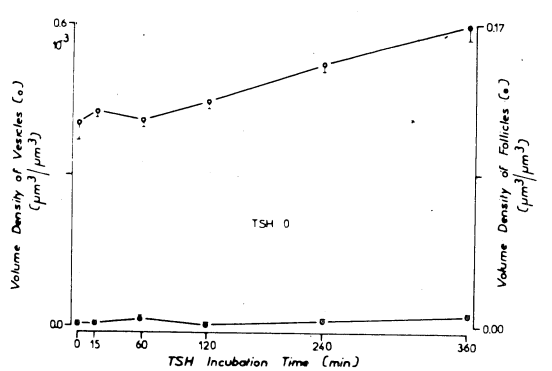
a - 20 mU TSH/ml



b - 80 mU TSH/ml



c - control (no TSH)



Figs 3 a, b and c - Evolution of the volume density of vesicles and follicular lumina

Whatever the TSH concentration, the evolution with time of the volume densities of vesicles (Vv) and follicular lumina (V_F) were similar Fig. 3 a, b (the same evolution was observed for the surface densities) : from 0 to 120 mn V_F increased whilst Vv decreased. From 120 to 240 mn V_F increased more slowly than Vv and from 240 to 360 mn the inverse occurred. Without TSH and whatever the incubation time, V_F was very low and remained constant whilst Vv continuously increased between 120 and 360 mn (Fig. 3 c).

Finally, it must be emphasized that whatever the TSH concentration and the incubation time, the mean cell volume was constant ($474 \pm 13\ \mu m^3$).

DISCUSSION

As suggested by the analysis of the vesicles size distributions, two populations (normal) of vesicles could be secreted: some small vesicles (M = 49.9 nm) and some larger one (M = 109.4 nm).

The third vesicles population (lognormal) would result from the vesicles coalescence phenomenon which was observed on micrographs. It must be emphasized that the smallest vesicles were not able to fuse each other since the smallest vesicles population was symetrical and not mixed with the other one. These two kinds of vesicles could represent two different pathways of secretion in foetal thyroids.

The evolution with time of the volume density of vesicles (Vv) and of follicular lumina (V_F) shows : (i) without TSH, a significant Vv increase after a delay of 120 mn (ii) with TSH 20 mU, an early Golgi secretion (< 15 mn). This latter phenomenon was not observed with TSH 80 mU/ml, nevertheless, in this case TSH receptors could be temporary saturated. Thus, in our experimental conditions, Golgi secretion appears to be biphasic with an early secretion (TSH dependent) and a lat er one not induced (but increased) by TSH. After each secretory period, the Vv decrease could result from an "impoverishment" of the Golgi membrane pool. Finally, it must be emphasized that with TSH only, a continuous Vv increase can be observed suggesting a constant exocytotic flux of secretory vesicles in

the follicular lumina.

For conclusion and according to our experimental conditions : (i) Golgi secretion was biphasic with an early TSH dependent secretion (<15 mn) and a latter one, not induced (but increased) by TSH. (ii) Golgi secretion was heterogeneous with two normal populations of vesicles which could represent two different pathways of secretion. (iii) The continuous TSH dependent increase of follicular lumina mainly originated from the transfer and the coalescence of the largest vesicles.

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