CYCLIC 3',5'-ADENOSINE MONOPHOSPHATE-MEDIATED FOLLICULAR REORGANIZATION OF ISOLATED THYROID CELLS IN CULTURE

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1. Introduction

Rearrangement of isolated thyroid cells cultured as monolayer in a structural pattern resembling cross sections of the intact thyroid gland under the influence of TSH* was first demonstrated by Kerkof et al. [1]. Fayet et al. [2] recently described the conditions in which TSH induced the rearrangement in follicle-like structures of porcine thyroid cells isolated by a continuous-flow trypsinization procedure whereas cells cultured in the absence of the hormone developed as a monolayer.

Considerable evidence supports the idea that many actions of TSH on thyroid cell metabolism are mediated by the adenylcyclase–cyclic AMP system. The interaction of the hormone with specific sites of the plasma membrane [3] stimulates adenylcyclase activity and results in an increase of the concentration of cAMP in the thyroid follicular cell [4–5].

In this communication we demonstrate that the TSH-induced reorganization of isolated thyroid cells in follicle-like structures is specifically mediated by cAMP.

2. Materials and methods

Isolated thyroid cells were obtained by a continuous-flow trypsinization procedure [2]. The cells were cultured at 35° in 30 ml Falcon plastic flasks in Eagle's basal medium enriched in calf serum (15%), penicillin G (200 U./ml) and streptomycin (50 μg/ml). Each bottle contained a final volume of 5 ml and was seeded with 2.4×10^6 cells/ml. Before addition of cells, the pH was 6.8–7.0, in order to obtain a pH of 7.2–7.4 after temperature equilibrium. In these conditions, the cells covered the surface of the bottle (25 cm^2) as a monolayer in 2 to 4 days, or reorganized in follicle-like structures if TSH was added at the beginning of culturing. The formation of this typical pattern of cell rearrangement was used to follow semiquantitatively the response to TSH or to the other compounds tested.

TSH (NIH-TSH-S2 ovine, 2.2 U./mg) was obtained from the National Institutes of Health (Bethesda, MD, USA). cAMP and DBcAMP were purchased from Calbiochem (Lucerne, Switzerland), cCMP, cGMP and cUMP from Boehringer (Mannheim, Germany), 5'-AMP and adenosine from Sigma (St. Louis, MO, USA) and theophylline from Touzart et Matignon (Paris, France). All media were sterilized by filtration on Millipore membranes.

3. Results

The rearrangement of porcine thyroid cells cultured in the presence of TSH is shown in fig. 1B and compared to cells grown in the absence of the hormone which develop as a monolayer (fig. 1A). In view of the typical morphological difference between isolated and reorganized cells, the type of culture can be assessed

* *Abbreviations*:
cAMP: cyclic 3',5'-adenosine monophosphate;
DBcAMP: N^6-2'-*O*-dibutyryl cAMP;
cCMP, cGMP, cUMP: cyclic 3',5'-cytidine (guanosine or uridine) monophosphate;
TSH: thytoid-stimulating hormone.

* Unpublished results.

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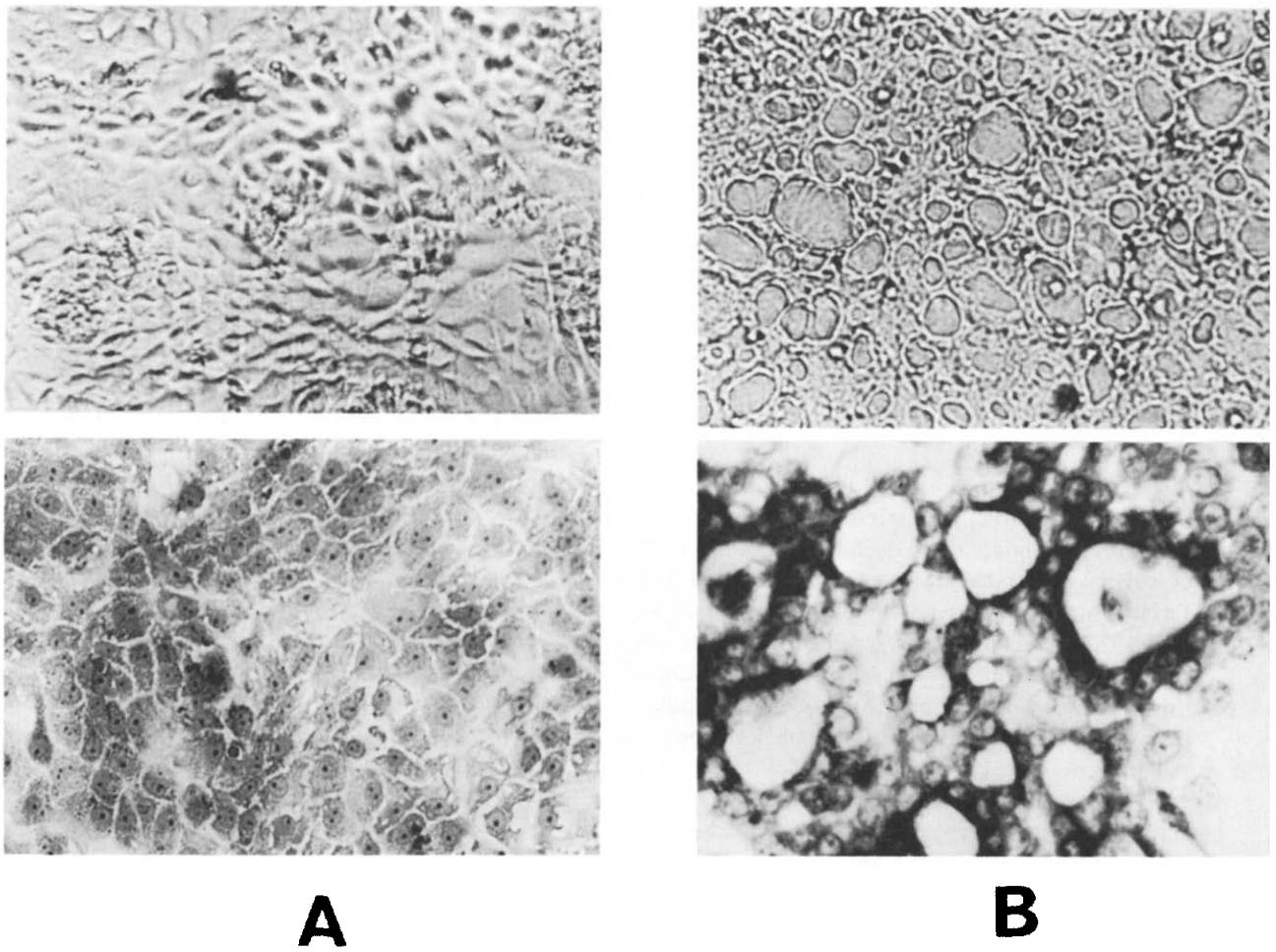

Fig. 1. Photomicrographs of porcine thyroid cells grown in the absence (A) or in the presence (B) of TSH (88 mU./ml) for four days. Top views, direct observation through the plastic culture flasks (X 125); bottom views, after staining with Giemsa (X 250).

unambiguously by light microscopic examination (X125). The tridimensional organization of the follicle-like structures of the reassociated cells has been established by electron microscopy after *in situ* fixation of 4 day-old cultures in the presence of TSH*. Rearranged cells show the typical ultrastructural aspects of follicular cells in the intact thyroid gland (numerous microvillae at the apical pole, terminal bars, material within the lumen of an electron density similar to that of colloid).

Table 1 compares the effect of increasing doses of TSH and DBcAMP on the histiotypic rearrangement of thyroid cells. From a dose of 2 mU./ml, TSH causes 100% of cell reassociation. At a concentration of 1.7×10^{-4} M, DBcAMP induces cell reassociation in all the flasks examined; 50% of positive responses are obtained for concentrations in the range 1.7 to 3.4×10^{-5} M. Cyclic AMP (table 2) is less active than its butyryl derivative and induces about 50% of positive responses at a concentration about 20-times higher. In addition, the effect of cAMP is delayed since, for suboptimal concentrations, the typical pattern of rearranged cells is only visible after four days of culturing.

Theophylline which enhances cellular cAMP level by inhibiting the phosphodiesterase specific for mono-

Table 1
Effect of TSH and DBcAMP on the association of isolated thyroid cells in follicle-like structures.

Addition	Final concentration mU./ml (TSH) or M (DBcAMP)	Positive response (%) at D_2	Positive response (%) at D_4
none	–	0 (70)*	0 (65)
TSH	100 to 40	100 (6)	100 (6)
	25 or 20	100 (8)	43 (7)
	10 or 5	100 (2)	100 (2)
	2	100 (1)	nd
	0.2 or 0.02	0 (2)	nd
DB cAMP	3.5 or 1.7×10^{-4}	100 (6)	100 (6)
	8.6 or 6.8×10^{-5}	83 (6)	83 (6)
	4.3 or 3.4×10^{-5}	50 (6)	40 (5)
	1.7×10^{-5}	50 (6)	25 (4)
	0.9×10^{-5}	0 (2)	0 (2)
	1.7 or 0.9×10^{-6}	0 (2)	0 (2)

Each bottle of cultured cells was routinely examined daily for at least 4 days. Positive responses were assessed from the presence of histiotypic cell rearrangement 2 days (D2) and 4 days (D4) after the onset of the culture. nd, not determined.

* Number of experiments in parentheses.

nucleotide 3′,5′–bonds [6], induces 100% of cell rearrangement for a minimum concentration of 5×10^{-5} M (table 2). Adenosine, 5′-AMP, cCMP, cGMP and cUMP at concentrations up to 6×10^{-3} M are inactive (table 2). At concentrations up to 1×10^{-2} M, NaF is also inactive.

4. Discussion

It is clear from these experiments that the action of TSH on the rearrangement of isolated thyroid cells in follicle-like structures is specifically mediated by cAMP, for the other cyclic mononucleotides and 5′-AMP fail to mimic TSH action. The positive effect on reassociation of the phosphodiesterase inhibitor, theophylline, strongly supports this conclusion. The higher activity of DBcAMP and its more precocious effect are probably related to its better ability to penetrate the cells and/or to its lower suspectibility to hydrolysis by the phosphodiesterase [7].

The observations related in this paper suggest that the enhancement of cAMP concentration in isolated thyroid cells triggered by TSH should be a prerequisite

Table 2
Effect of several compounds on the association of isolated thyroid cells in follicle-like structures.

Addition	Final concentration (M)	Positive response (%) at D2	Positive response (%) at D4
cAMP	6×10^{-4}	60 (5)*	60 (5)
	3×10^{-4}	0 (5)	60 (5)
	1.5×10^{-4}	0 (5)	20 (5)
	7 to 0.3×10^{-5}	0 (11)	0 (11)
Theophylline	1.1×10^{-4}	100 (3)	100 (3)
	5×10^{-5}	100 (3)	66 (3)
	2×10^{-5}	66 (3)	33 (3)
	1×10^{-5}	33 (3)	0 (3)
	5×10^{-6}	0 (6)	0 (6)
Adenosine	7.5×10^{-3} to 3.8×10^{-6}	0 (13)	0 (13)
5′-AMP	6×10^{-3} to 3×10^{-6}	0 (11)	0 (11)
cCMP	same	0 (21)	0 (21)
cGMP	same	0 (19)	0 (19)
cUMP	same	0 (19)	0 (19)
NaF	1×10^{-2} to 2.5×10^{-5}	0 (24)	0 (24)

* Number of experiments in parentheses.

to cell rearrangement. Some observations showing that thyroid cell reassociation can be obtained in the absence of TSH if high concentrations of cells are used [8, 9], might be explained by this mechanism if we consider that high cell concentrations would decrease the extracellular diffusion of cAMP from those cells which, after trypsinization of the thyroid gland, retained bound TSH.

Thyroid cell reassociation is not induced by fluoride, a potent stimulator of adenylcyclase activity in both thyroid slices and homogenates. It was reported that F^- ions stimulated glucose oxidation in thyroid slices [10–12] by a mechanism other than cAMP accumulation, for cAMP level in F^--stimulated thyroid slices was not increased [12]. Dissociation between F^-- and TSH-stimulated adenylcyclase activity was recently described [13]. If F^- does not actually induce accumulation in cultured isolated thyroid cells, as it does in thyroid slices, the idea that increased intracellular cAMP concentration is a prerequisite to histiotypic cell rearrangement will be strengthened.

The role of cAMP in thyroid cell rearrangement may be compared to its role as cell attractant (acrasin) for the cellular slime mold *Dictyostelium discoideum*. Cyclic AMP causes an aggregation of a large number of isolated myxamoebae which initiates the morphogenetic phase of its development, the differentiation into stalk and spore cells [14, 15]. A similar role of cAMP in histogenesis and differentiation of mammalian tissues, at least those in which cAMP mimics the effect of an external stimulator, is not unlikely.

The mechanism of action of cAMP on reassociation and differentiation (unpublished results) of the thyroid cell in culture is under investigation.

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