Epidermal growth factor controls the proliferation and the expression of differentiation in canine thyroid cells in primary culture

Pierre P. Roger and Jacques E. Dumont

Institute of Interdisciplinary Research (IRIBHN), School of Medicine, Free University of Brussels (ULB), Campus Erasme, Route de Lennik 808, B-1070 Brussels and *Biology Department, Euratom, Brussels, Belgium*

Received 14 June 1982

Thyrotropin *Thyroid* *Epidermal growth factor* *Iodide transport* *Cell multiplication* *Differentiation*

1. INTRODUCTION

Epidermal growth factor (EGF) is a potent mitogen for many different cells of various origins, in vitro and in vivo [1–3]. In this regard, it has received an hormonal status [4]. However, the effects of EGF on other cell functions, e.g., differentiation, have been little studied.

Dog thyroid follicular cells in primary culture in monolayer constitute a model which allows the investigation of the poorly known [5] regulation of proliferation and differentiation in this tissue. These cells cultured in a high serum-supplemented medium respond to TSH by increasing their cyclic AMP content [6,7]. TSH increases cell proliferation [7,8] and when multiplication rate decreases, the expression of differentiated functions (e.g., iodide uptake and organification) (submitted). Both TSH effects are reproduced by agents which enhance the intracellular cyclic AMP levels [7, submitted].

Here, we investigate, in dog thyroid cells cultured in a low serum-hormone-supplemented medium, the effects of EGF on proliferation and on iodide trapping and organification.

2. MATERIALS AND METHODS

2.1. *Materials*

Collagenase (150 U/mg) was purchased from Worthington Chemical Co. (Freehold NJ). Basal medium Eagle (BME), Dulbecco's modification of minimum essential medium (DMEM), Ham's F12 medium, MCDB 104 medium [9], glutamine, penicillin–streptomycin and amphotericin B (fungizone) were obtained from Flow Labs. (Irvine); foetal bovine serum was from Eurobio (Paris).

Bovine insulin, transferrin, hydrocortisone, murine EGF, pituitary FGF were purchased from Collab. Res. (Waltham MA). Glycyl–histidyl–lysyl acetate and somatostatin were Sigma products (St Louis MO). Bovine TSH (1 U/mg) was obtained from Armour Pharmaceutical Co. (Kankakee MI). All the other products and reactives were the best commercially available.

2.2. *Culture*

The dog thyroid cells were cultured as in [7]. Briefly, the thyroid tissue was digested by collagenase so that the resulting suspension consisted mainly of fragmented and intact follicles. These follicles were seeded in 35 mm Petri dishes, and, in 1 day, adhered to the substratum while a monolayer developed. The seeding was realized so that, in general, $\sim 2 \cdot 10^5$ cells attached to the dish after 1 day and 1 medium change. The cells were cultured in the following mixture which constitutes the control medium:DMEM + F12 + MCDB104 (2:1:1) with 2 mM glutamine, supplemented [10] by 10 μg insulin/ml, 1.25 μg transferrin/ml, 20 ng hydrocortisone/ml, 10 ng glycyl–histidyl–lysyl acetate/ml, 10 ng somatostatin/ml, 40 μg ascorbic acid/ml and 1% foetal bovine serum. Antibiotics, penicillin 100 U/ml, streptomycin 100 μg/ml and amphotericin B 2.5 μg/ml were also added. The Petri dishes were maintained in a water-saturated incubator at 37°C in an atmosphere of 5% CO_2 in air. The medium was changed after 1 day and subsequently every other day. After 1 day, TSH or other agents were added to some dishes and their presence maintained throughout the culture period.

Published by Elsevier Biomedical Press
00145793/82/0000–0000/$2.75

2.3. *Measurement of cell multiplication*

Cell multiplication curves were obtained from cell DNA measurements. Our cell DNA assay described in [7], uses the increase of fluorescence of ethidium bromide when complexed with nucleic acids.

2.4. *Active transport of iodide*

Trapping of iodide was evaluated by the uptake of radioiodide at equilibrium. The cells in the Petri dishes were incubated for 2 h with $Na^{131}I$ (2 μCi/ml) in 10^{-6} M KI in BME at 37°C. Mercapto –methyl–imidazole (1 mM) was added to block iodide organification. The cells were then rapidly rinsed with BME 3 times, scraped and counted in a γ-counter. The radioactivity was normalized to the cellular DNA measured from the same dishes. Trapping was also measured as the ratio of radioiodide in cells incubated with mercapto–methyl –imidazole and in cells incubated with mercapto –methyl–imidazole and $NaClO_4$ 1 mM. This ratio provides a good estimate of the commonly used C/M ratio (where C is the radioactivity in the cells and M the radioactivity of a corresponding volume of medium) [11].

2.5. *Iodide organification*

The incorporation of radioiodide into proteins (PBI) was measured by the radioactivity of the trichloroacetic acid (5%) precipitate of cells incubated as for iodide uptake estimations, but for 5 h and without mercapto–methyl–imidazole. The results were also normalized to the cellular DNA.

Results in the graphs were expressed as means ± range of measurements from duplicate Petri dishes.

3. RESULTS

3.1. *Effects of TSH*

Cultured in a low serum hormone supplemented medium, canine thyroid cells responded to TSH (1 mU/ml). The hormone enhanced the proliferation; the final cell density of TSH-treated cells was twice that of the control cells (fig.1). On the other hand, when the proliferation rate decreased, TSH stimulated two differentiated functions: the active transport of iodide (fig.2), and its organification (fig.3).

3.2. *Effects of EGF*

Added 1 day after seeding and maintained throughout the culture period, EGF (25 ng/ml) stimulated thyroid cell proliferation. The effects of TSH and EGF were similar but not fully additive (fig.1). In the same experiment (fig.2) EGF (25 ng/ml) inhibited by 82% at day 9 and by 66% at day 15 the iodide transport of TSH-treated cells. The basal level was also lowered by EGF. The binding of iodide to proteins which was increased by TSH when the cells reached the stationary phase, was also inhibited at day 15 by EGF (fig.3). Similar effects of EGF on proliferation were obtained in the 9 experiments done. Effects on the iodide trapping were always reproduced in 5 experiments, and inhibition of the iodide organification was observed 3 times.

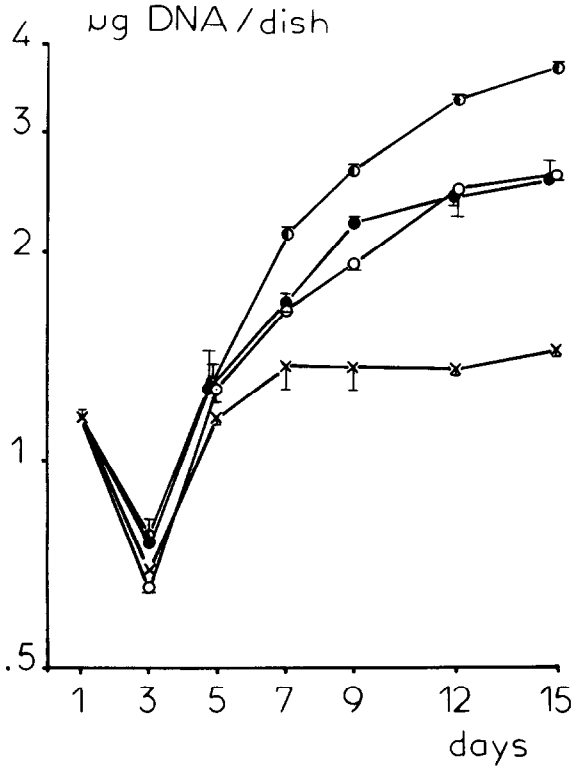

Fig.1. Stimulation of proliferation of canine thyroid cells by TSH and EGF present throughout the culture period for 1 day: (X) control medium; (•) TSH 1 mU/ml; (○) EGF 25 ng/ml; (◐) EGF 25 ng/ml + TSH 1 mU/ml.

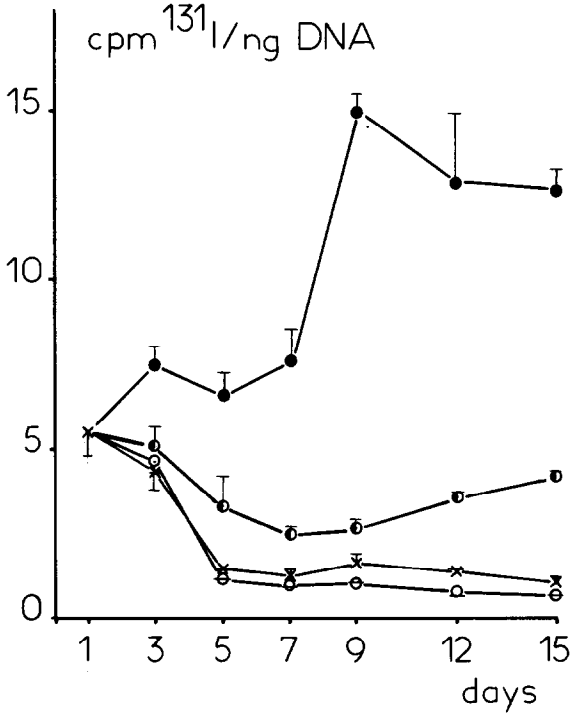

Fig.2. Active transport of iodide measured in parallel with the multiplication shown in fig.1. cpm ^{131}I/ng DNA should be multiplied by ~4 to obtain an absolute evaluation of the *C/M* ratio: (X) control medium; (•) TSH 1 mU/ml; (○) EGF 25 ng/ml; (◐) TSH 1 mU/ml + EGF 25 ng/ml.

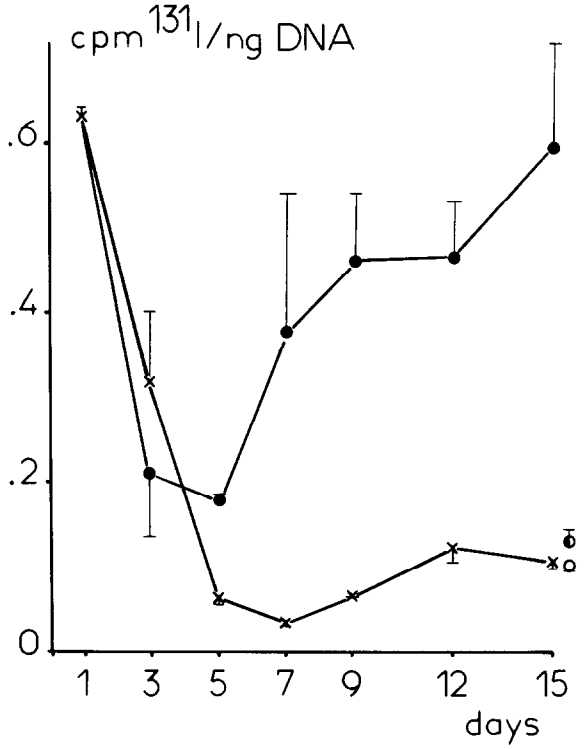

Fig.3. Iodide organification (PBI). Same culture as in fig.1,2: (X) control; (•) TSH 1 mU/ml; (○) EGF 25 ng/ml; (◐) TSH 1 mU/ml + EGF 25 ng/ml.

4. DISCUSSION

The cells used in this work are almost exclusively the thyroid follicular cells [7]. The present study first demonstrates the mitogen effect of EGF on such canine cells. The EGF effect on the proliferation of other hormone-responsive systems has been shown [12–14], but conflicting data exist in the case of the thyroid gland. According to [15], bovine thyroid cells are unresponsive to EGF; however no effect of EGF on the proliferation of the rat thyroid cell line in [10] was observed. But, in both cases, the experimental conditions of the assay were not reported. In contrast, while this work was in progress, a potent mitogenic effect of EGF on sheep thyroid cells in culture was described [16]. The results in [16] and ours in two different experimental systems, are thus similar and mutually confirmatory.

Our results also show an unexpected effect of EGF on the differentiation characteristics of our thyroid cells. EGF strongly inhibits the induction by TSH of the active transport of iodide and of the binding of iodide to proteins. Both functions are considered as rate-limiting steps of the thyroid hormone synthesis. The de-differentiating effect of EGF does not appear directly related to a stimulation of proliferation. The iodide trapping and organification inhibitory effects still occur when proliferation has considerably decreased (at day 15, in this experiment). However, 10% fetal bovine serum or pituitary fibroblast growth factor (50 ng/ml) which, as EGF, stimulate the proliferation of our thyroid cells, do not decrease their iodide uptake capacity when multiplication slows down (not shown). A de-differentiating action does not seem to be a general property of EGF. In fact, an inhibi-

tory effect on hormone synthesis has only been observed in a very few cases, namely on the steroidogenesis by gonadal cells [17] and on the synthesis of growth hormone-stimulated by T4 in the GH3 cells [18]. In contrast to these observations, a differentiation-inducing action of EGF was shown in mammary cells [19], and an organ maturing role is attributed to EGF in the fetal lung and in the secondary palate [20].

There are indications for an interrelationship between EGF and the thyroid gland. T4 stimulates the synthesis of EGF in the mouse submaxillary gland [21]. EGF inhibits T4 stimulation of growth hormone synthesis in glial cells [18]. In the thyroid itself, presence of EGF has been demonstrated in human tissue at 5 ng/g wet wt [22], and an effect of EGF is observed in sheep [16] and dog thyroid cells in culture. Although these observations were obtained from different species, our results, in this context, suggest the possibility that EGF plays an important specific role in the regulation of thyroid growth and differentiation.

ACKNOWLEDGEMENTS

We are grateful to Mrs D. Leemans for typing the manuscript. This work was done within the framework of Euratom contract 230-76-1310B, and partially thanks to grant 3.4536.81 of the Fonds de la Recherche Médicale. P.P.R. is a research fellow of the Institut pour l'Encouragement de la Recherche Scientifique dans l'Industrie et l'Agriculture (IRSIA).

REFERENCES

[1] Carpenter, G. and Cohen, S. (1979) Annu. Rev. Biochem. 48, 193–216.
[2] Rudland, P.S. and Jimenez de Asua, L. (1979) Biochim. Biophys. Acta 560, 91–133.
[3] Gospadorowicz, D. (1979) Mol. Cell. Biochem. 25, 79–110.
[4] Hollenberg, M.D. (1979) Vitamin. Hormone. 37, 69–110.
[5] Dumont, J.E. and Lamy, F. (1980) in: Comprehensive Endocrinology (De Visscher, M. ed) The Thyroid Gland, pp. 153–167, Raven Press, New York.
[6] Rapoport, B. (1976) Endocrinology 86, 1189–1197.
[7] Roger, P.P., Hotimsky, A., Moreau, C. and Dumont, J.E. (1982) Mol. Cell. Endocrinol. 26, 165–176.
[8] Rodesch, F. and Dumont, J.E. (1967) Exp. Cell. Res. 47, 386–396.
[9] Winand, R.J. and Kohn, L.D. (1975) J. Biol. Chem. 250, 6534–6540.
[10] Mc Keehan, W.L., Mc Keehan, K.A., Hammond, S.L. and Ham. R.G. (1977) In Vitro 13, 399–415.
[11] Ambesi-Impiombato, F.S., Parks, L.A.M. and Coon, H.G. (1980) Proc. Natl. Acad. Sci. USA 77, 3455–3459.
[12] Gospadorowicz, D. and Bialeki, H. (1979) Endocrinology 104, 757–764.
[13] Taylor-Papadimitriou, J., Purkis, P. and Fentiman, I.S. (1980) J. Cell Physiol. 102, 317–321.
[14] Yang, J., Guzman, R., Richards, J., Imagawa, W., Mc Cormick, K. and Nandi, S. (1980) Endocrinology 107, 35-41.
[15] Gospadorowicz, D., Greenburg, G., Bialecki, H. and Zetter, B.R. (1978) In Vitro 14, 85–118.
[16] Westermark, K. and Westermark, B. (1982) Exp. Cell Res. 138, 47–55.
[17] Hsueh, A.J.W., Welsh, T.H. and Jones, P.B.C. (1981) Endocrinology 108, 2002–2004.
[18] Johnson, L.K., Baxter, J.D., Vlodawsky, I. and Gospadorowicz, D. (1980) Proc. Natl. Acad. Sci. USA 77, 394–398.
[19] Tonelli, Q.J. and Sorof, S. (1981) Differentiation 20, 253–259.
[20] Gospadorowicz, D. (1981) Annu. Rev. Physiol. 43, 251–263.
[21] Walker, P., Weichsel, M.E. jr, Hoath, S.B., Poland, R.E. and Fisher, D.A. (1981) Endocrinology 109, 582–587.
[22] Hirata, Y. and Orth, D.N. (1979) J. Clin. Endocr. Metab. 48, 667–672.