Vol. 118, No. 2, 1984 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
January 30, 1984 Pages 385-391

INSULIN MODULATES THYROTROPIN-INDUCED FOLLICLE RECONSTRUCTION AND IODINE METABOLISM IN HOG THYROID CELLS CULTURED IN A CHEMICALLY DEFINED MEDIUM

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Received November 11, 1983

SUMMARY: Effects of insulin on cultured hog thyroid cells were examined in a chemically defined medium supplemented with thyrotropin. Thyrotropin-induced follicle reconstruction and recovery of functional activities were stimulated by the addition of insulin to the culture medium. The iodide uptake and organification at the maximum stimulation exceeded 3 times those of the control values. The minimum effective dose of insulin was 60 μU/ml. The insulin stimulation occurred after a latency period of 24 h. Since no growth promoting effect of insulin was observed during the cultivation period in the present study, insulin appears to act in concert with thyrotropin in regenerating the characteristic properties of the differentiated cells during cultivation.

It is known that isolated thyroid cells reassociate into functinal follicles when they are cultivated under certain conditions. One of the reliable culture conditions for morphogenesis in vitro is the continuous presence of TSH in the culture medium (1-5), although some culture systems not containing TSH have also been reported (6-10).

In a previous study on the effect of serum on cultured thyroid cells, we found that 0.5 to 1 % serum added to the culture medium stimulated TSH-induced morphogenesis and recovery of functional activities, whereas a higher concentration of serum inhibited them (11). This finding led us to look for physiological factors affecting the TSH action.

In the present paper, we describe how insulin enhances the TSH induced-follicle reconstruction and recovery of iodine metabolism of isolated thyroid cells cultured in a chemically defined medium.

MATERIALS AND METHODS

1) Isolation and cultivation of thyroid cells. Hog thyroid glands were obtained from a local slaughterhouse. Thyroid glands from 5- to 6- month old hogs were treated to prepare isolated follicles as reported previously (12). The procedure of cell isolation from the follicles will be described

Abbreviations used are: TSH, thyrotropin; HBS-CMF, Ca^{2+} and Mg^{2+} free Hanks' balanced salt solution; HEPES, N-2-hydroxyethyl-piperazine-N-ethane sulfonic acid; TES, N-tris(hydroxymethyl)methyl-2O-aminoethane sulfonic acid; TCA, trichloroacetic acid.

0006-291X/84 $1.50

elsewhere in detail. Briefly, the isolated follicles were incubated for 10 min. in HBS-CMF containing 0.1 % trypsin and 0.02 % EDTA at 37 C. To hydrolyze the collagen fibers liberated from the follicles during the trypsinization, a quarter volume of basal culture medium containing 0.2 % collagenase was added and incubated for another 30 min. The cells were spun down at 55 x g for 3 min. and filtered through double-layered stainless steel sieves to remove large cell clumps. One hundred thousand cells were cultivated with 150 µl of the basal culture medium at 37 C in each well in 96-well microplates. The basal culture medium was Dulbecco's modified Eagle medium (pH 7.5) supplemented with KI (0.01 µg of I^-/ml), 15 mM HEPES, 10 mM TES and antibiotics. When indicated, 2 mU/ml of highly purified bovine TSH and/or various doses of bovine pancreatic insulin were added to the culture medium.

2) Determination of iodinating activity of cultured cells. To determine iodide uptake and organification activities of the cultured cells, $Na^{131}I$ (6,000 to 10,000 cpm) was added to the culture medium at the time indicated and incubated for 1 h at 37 C. After being chilled in an ice bath, the microplates were centrifuged at 250 x g for 5 min at 4 C. After the supernate was removed, the cells were washed twice with HBS-CMF, dissolved in 0.1 ml of 0.1 N NaOH and transfered to small test tubes. They were counted in a Beckman Gamma 8000 γ-counter (Beckman, Palo Alto, CA) to determine the iodide uptake activity of the cultured cells. After the counting, 50 µl of 50 % TCA was added to precipitate proteins. The washed TCA precipitate was counted to measure iodide organification. DNA contained in the TCA precipitate was analyzed according to the method of Singh and Siminovitch (13). The cellular activities were expressed as ^{131}I/µg of DNA.

3) Hormones and Chemicals. Bovine TSH (biological activity: 20 U/mg based on the follicle reconstructing activity in culture) was purified by the method of Yora and Ui (14). $Na^{131}I$ was a product of the Commissariat a l'Énergie Atomique, France. New-born calf serum was purchased from MA Bioproducts, collagenase (CLS II) (135 U/mg) from Worthington Biochemicals (Freehold, NJ), and trypsin (Type II) from Sigma Chemical Co. (St. Louis, MS).

RESULTS

1) Insulin stimulates TSH-induced follicle reconstruction. Isolated thyroid cells were cultivated in serum-free basal medium. Whenever TSH was present, follicles were reconstructed, although the size and the number of follicles were somewhat smaller than those obtained in the basal medium enriched with 1 % serum. The addition of 30 mU/ml of insulin instead of serum resulted in the formation of follicles with markedly enlarged lumens either in rotated suspension cultures (data not shown) or stationary cultures (Fig. 1). The effect of insulin was even stronger than that of 1 % new-born calf serum. On the other hand, the addition of insulin alone caused no follicle formation.

2) Insulin stimulates TSH-induced recovery of iodine metabolism in cultured cells. Iodide uptake and organification are characteristic functions of thyroid follicles in vivo. When the cells isolated from the follicles were cultivated in the absence of TSH, the functional activities disappeared within a few days, whereas the presence of TSH induced the increase in the activities as previously reported (11). Fig. 2 shows

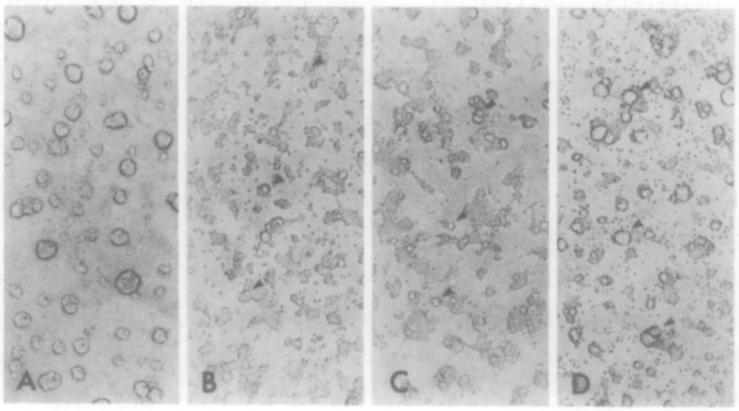

Fig. 1 Effect of insulin on TSH-induced follicle reconstruction from cultured thyroid cells. Hog thyroid cells were cultivated for 3 days in a stationary culture system as described in MATERIALS AND METHODS. Phase-contrast microscopic photographs (X 53) of the cultured cells are shown. Arrow heads indicate reconstructed follicles. A, culture with no addition; B, + TSH (2mU/ml); C, + TSH + 1 % new-born calf serum; D, + TSH + insulin (10 μg/ml)

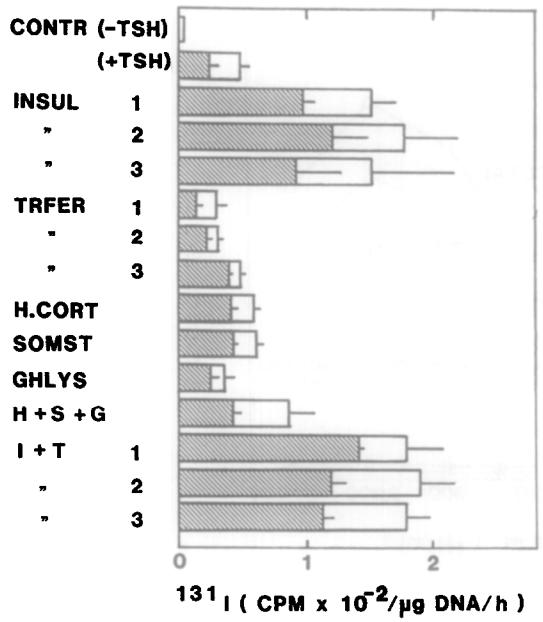

Fig. 2 Effect of insulin on TSH-induced iodine metabolism. Hog thyroid cells were cultured for 3 days with various agents and incubated with $^{131}I^-$ for 1 h as described in MATERIALS AND METHODS. Each column indicates a mean ± SE of triplicate results of ^{131}I incorporation into cells and a shadowed part that of into TCA precipitate.
CONTR, control; INSUL, insulin (1, 0.3 mU/ml; 2, 3 mU/ml; 3, 30 mU/ml); TRFER, transferrin (1, 0.05 μg/ml; 2, 0.5 μg/ml; 3, 5 μg/ml); H.CORT, hydrocortisone (5 ng/ml); SOMT, somatostatin (10 ng/ml); GHLYS, glycylhistidyllysine (10 ng/ml); H+S+G, H.CORT+SOMST+GHLYS; I+T, INSUL (1, 2, 3 same as INSUL 1, 2, 3) +TRFER (5 μg/ml)

that the simultaneous addition of 30 mU/ml of insulin to the culture medium strikingly enhances these TSH-induced activities when cells are cultivated for 3 days. The effect of insulin was specific since no appreciable effect was observed with transferrin, hydrocortisone or somatostatin. In accordance with the results of the morphological study, insulin did not affect the functional activities unless TSH coexisted.

3) Characterization of insulin effect. Fig. 3 indicates the dose dependency of the insulin effect observed 3 days after seeding. Although the lowest dose (0.48 μU/ml) of insulin tested appeared to cause a slight increase in iodide uptake and organification, no dose dependency was observed up to 12 μU/ml. The minimum dose which caused a distinct stimulation was 60 μU/ml, and the extent of stimulation increased up to 3 mU/ml in a dose-dependent fashion.

Time course studies indicated that the first 24 h of cultivation was a latency period for the insulin effect (Fig. 4). Maximum stimulation of iodide uptake and organification was observed 3 days after seeding and the activities at 3 mU/ml of insulin exceeded 3 times the control values obtained in the absence of insulin.

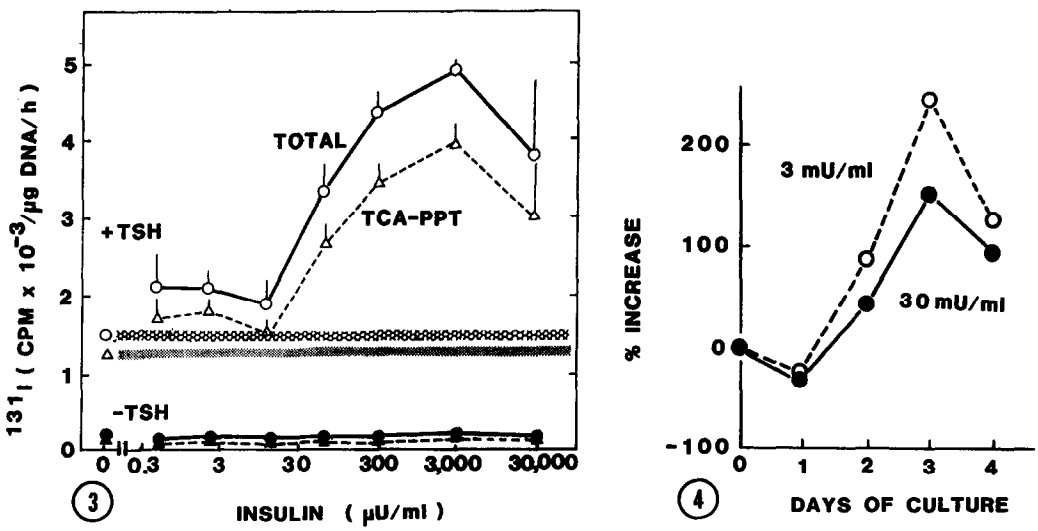

Fig. 3 Effect of insulin dose on the stimulation of TSH-induced iodine incorporation. Hog thyroid cells were cultured with the increasing doses of insulin in the presence (○, △) and absence (●, ▲) of TSH. ^{131}I incorporation into cells (——) and into TCA precipitate (- - -) were measured as described in the legend to Fig. 2. Shadowed lines (▩, ▩) indicate the control levels. The values are the mean ± SE of triplicate experiments.

Fig. 4 Time course of the insulin effect on TSH-induced iodine incorporation. Hog thyroid cells were cultured for the time indicated with 30 mU/ml (●—●), 3 mU/ml (○--○) of insulin or without insulin. Insulin effect was expressed as the percent increase of TSH-induced ^{131}I incorporation into cells following the addition of insulin. The values were calculated from the mean of tripricate experiments.

In contrast to the results mentioned above, no insulin effect at all was detected when insulin (30 mU/ml) was present in the medium together with labeled iodide during the period for the measurement of the iodine metabolism, instead of the whole period of cultivation.

As shown in Fig. 5, the presence of as much as 30 mU/ml of insulin throughout the cultivation period of 4 days caused no appreciable increase in the total amount of cellular DNA, suggesting that the addition of insulin had no distinct effect on cell growth.

DISCUSSION

The present paper is the first one to indicate that the physiological doses of insulin cooperate with TSH in regenerating the morphology and function of differentiated thyroid cells cultivated in a chemically defined medium. In our culture system, no stimulatory effect of insulin or TSH on cell proliferation was observed for at least 3 to 4 days from the beginning of cultivation.

On the other hand, insulin is known as an agent in promoting cell growth which is assumed to antagonize differentiation of cultured cells. In fact, Roger and Dumont reported that in their culture systems, the growth of thyroid cells was dependent on the presence of insulin as well as TSH, whereas the functional activities could not be recovered at least for several days (4, 16). However, after confluence was reached, they found also that TSH induced the partial recovery of iodide uptake activity. Although they did not pay attention to the effect of insulin coexisting in the medium, insulin might have acted in concert with TSH also in causing redifferentiation after confluence was reached. On the other hand, our system is supposed to have fulfilled the basic requirements for the

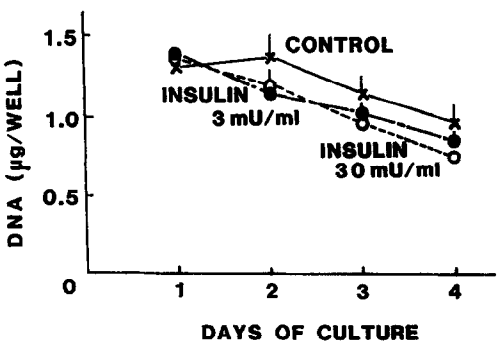

Fig. 5 Timecourse of the change in cellular DNA. Hog thyroid cells were cultivated as described in the legend to Fig. 4. Total cellular DNA of the cells cultured with 30 mU/ml (O--O), 3 mU/ml (●--●) of, and without insulin (×——×) were determined as described in MATERIALS AND METHODS. All the cultures were supplemented with 2 mU/ml of TSH. The values are the mean ± SE of triplicate experiments.

redifferentiation of cells. Presumably, this situation enabled insulin to cooperate with TSH in recovery of functions from the earlier stage of cultivation.

A physiological relationship between insulin and thyroid functions has been suggested on the basis of the fact that various abnormalities in thyroid function were found in diabetes mellitus. Bagchi et al. reported a decreased thyroidal response to TSH in the diabetic mouse and its recovery following the administration of insulin (17). Although morphological changes were not involved in that case, their results were not inconsistent with our present results. They assumed that suppressed glucose metabolism caused by impaired glucose entry in diabetic animals resulted in decreased production of hydrogen peroxide or an oxidizing agent for iodide organification, which was responsible for the decreased sensitivity to TSH. If this is the case, the effects of insulin should be observed without a long latency period. However, since there was a latency period of 24 h and no acute effect of insulin was observed, in our system in vitro, the stimulatory effect of insulin cannot be explained by the stimulation of substrate entry. The mechanism of the insulin effect remains to be elucidated.

The effect of 1 % newborn calf serum was not very different from that of insulin in its stimulation of TSH-induced follicle reconstruction and functional activities (11). However, the content of insulin in 1 % new-born calf serum used was found to be less than 1/500 of the minimum effective dose of insulin. This suggests that serum component(s) other than insulin participate(s) in the enhancement of the TSH action. This problem is under investigation.

ACKOWLEDGEMENTS: We wish to thank Prof. N. Ui for his helpful discussion and critical review of this manuscript. We also thank Miss M. Kimura for typing this manuscript. This work was supported in part by a Grant-in-Aid from the Ministry of Education, Science and Culture, Japan.

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