SHORT COMMUNICATION

Graves’ IgG stimulation of continuously cultured rat thyroid cells: a sensitive and potentially useful clinical assay


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ABSTRACT. A continuously cultured line of normal rat thyroid (FRTL) cells can be stimulated by immunoglobulin preparations from patients with Graves’ disease as measured by increases in intracellular cAMP levels. Responsiveness is concentration-dependent but is delayed in time relative to thyrotropin. Additionally, the cells respond to Graves’ immunoglobulins which have no long-acting thyroid stimulator (LATS) activity and are negative when adenylate cyclase stimulation in human thyroid membrane preparations is assayed. No correlation exists between the stimulation activity and the ability of a Graves’ immunoglobulin preparation to inhibit thyrotropin binding; cells are responsive even in the presence of such inhibitor activity.

INTRODUCTION

Measurements of thyroid-stimulating autoantibodies in patients with Graves’ disease are of major interest in predicting disease activity, yet few assays exist which are reliable, sensitive, and readily adaptable to clinical settings. The LATS assay, for example, is fraught with all the difficulties of a bioassay; it is positive in only 40% to 50% of cases; and does not measure a significant number of stimulators detected by measurements of immunoglobulin-enhanced adenylate cyclase activity in human membrane, slice, or cell preparations (1). In this last respect, assays using thyroid slices (1) or primary cultures of human thyroid cells (2, 3) are more sensitive and have a higher positive correlation with the disease state; however, problems with all the assays still exist. Thus, human thyroid material is not readily or only episodically available in most clinical settings, and human material, when available, has a high degree of variability in response (4), a problem which may relate to the individualistic history of each tissue source.

Recently, a continuous line of functioning cultured rat thyroid cells, maintained in the presence of thyrotropin, has been described, the FRTL cell line (5). In the present report we show that withdrawal of thyrotropin from the growth medium of confluent cells does not alter cell viability but does decrease their cAMP content. We further show (i) that within 24 hours and for as long as 10 days these cells become exquisitely sensitive to thyrotropin as measured by the ability of the hormone to increase intracellular cAMP levels and (ii) that these cells become a sensitive means of assaying the stimulatory activity of human autoimmune immunoglobulins from patients with Graves’ disease. The existence of these cells as a line under continuous culture, together with the preliminary results presented herein, suggest that they may offer, for the first time, (i) a convenient, sensitive, and reliable means of detecting Graves’ disease stimulating autoantibodies as well as (ii) an assay tool readily adapted to the clinical setting of most thyroid groups.

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MATERIALS AND METHODS

Thyrotropin was either a crude preparation or a purified preparation identical to that previously used (6); the latter was homogeneous in the ultracentrifuge (molecular weight ~27,500) and contained 25±2 IU/mg. Immunoglobulin preparations from patients with Graves' disease, other patients, or normal subjects, were the same as previously described (7). The patient diagnosis in each case used the same clinical and laboratory criteria used previously (7).

The FRTL rat cells used in this report are a continuous line of functioning rat thyroid cells which have been adapted to grow in 5% calf serum as well as a mixture of thyrotropin, insulin hydrocortisone, transferrin, somatostatin and glycyl-L-histidyl-L-Lysine acetate (6H growth medium (5)). Cells were dispersed in 24-well plates (Costar or Falcon) and grown to confluency (~1 x 10⁵ cells). Medium was replaced every fourth day as appropriate. To initiate experiments the 6H medium was replaced with medium composed of all constituents above except thyrotropin (5H media). Unless otherwise noted, cells were used 24 hours after 5H were added to the cell cultures.

Assays of intracellular cAMP levels adapted a previously detailed procedure (2). The 5H media was removed and replaced with a Hanks basal salts solution containing 3-isobutyl-L-methyl-xanthine, 0.5 mM final concentration, and thyrotropin or immunoglobulin preparations as noted. Incubations were at 37°C in a 5% CO₂ atmosphere for the times noted.

Medium was removed by suction, and 300μl of cold absolute ethanol (~20°C) was added. After 30 minutes at ~20°C, the cells were scraped and the mixture transferred to 1.5 ml Brinkman microfuge tubes with one wash (0.5 ml) of cold absolute alcohol. The cell debris was pelleted by centrifugation (5 minutes), and aliquots of the supernatant were dried and reconstituted with assay buffer, 0.05 M sodium acetate, pH 7.2. The cAMP was measured using a radioimmunoassay (Becton Dickinson), while the DNA content was measured in the pellet (2). Results are expressed as picomoles/dish, since under the conditions described, DNA content varies from well to well by < 5% (6.5μg/well). Experiments are in duplicate as are the cAMP measurements in each well. The results are the average of these.

Assays of adenylate cyclase activity in human thyroid membrane preparations, LATS bioassays, and the radioreceptor assay to measure inhibition of thyrotropin binding are the same as described elsewhere (7).

RESULTS

FRTL rat thyroid cells, cultured to «confluency» in the presence of thyrotropin (the 6 hormone (6H) mixture) (5), have a high intracellular cAMP level which can be enhanced only 2-fold by additional thyrotropin at concentrations in the range of 200 to 500μU/ml (Fig. 1A); the relatively low response or desensitized state of these cells has been related to an ADP ribosylation pathway regulating adenylate cyclase activity (8).

Fig. 1 - Effect of thyrotropin and Graves' disease immunoglobulins on the cAMP levels of FRTL cells. (A) Effect of different thyrotropin concentrations on rat thyroid cells grown in the presence of thyrotropin (6H) or after its absence for 24 hours (5H). cAMP was measured 30 minutes after TSH addition. The insert depicts the effect on cAMP level of the 5H medium as a function of time; the control value is the cAMP level of 6H cells. (B) Effect of a Graves' disease immunoglobulin (4 mg/ml) on FRTL cells as a function of time compared with TSH (150 μU/ml) and a normal immunoglobulin preparation (4 mg/ml). The insert presents the effect of the Graves' disease immunoglobulin as a function of immunoglobulin concentration after 60 minutes of incubation by comparison to a normal immunoglobulin. In a(A) and (B), cAMP levels were measured in 24-well Costar plates, the values representing the cAMP level in each well of confluent cells. Each well contained the same cell number ± 5% as measured by DNA content (6.5 μg). Assay error was less than ± 5%.