109 Lukens Drive. New Castle, DE 19720

The effect of human growth hormone on cells

Introduction

The binding properties and specificity of a newly developed drug are normally studied in a cell free system [1]. However, after this phase the biological effects of the drug need to be tested on cells or tissue pieces. Such tests where different kinds of responses are to be expected require methods which can be used to detect events such as cell death, increased cell growth or stimulated cellular metabolism. Microcalorimetry is such a universal technique. Its use in cell growth experiments [2] and in the monitoring of cell death upon addition of anticancer drugs [3] has been described in detail. This particular application note deals with the effects of human growth hormone on non-growing cells known to have the growth hormone receptor in the membrane. When growth hormone binds to its receptor, a series of events, the signal transduction, leading to a gene product takes place which requires an overall stimulation of the energy metabolism. The subsequent result of the signal transduction cascade is a triggered cell growth. Cell growth following addition of growth hormone to resting cells has been measured earlier. The work presented here [4] shows for the first time how cells are metabolically activated upon addition of growth hormone. Moreover, the subsequent cell growth could be derived from the same set of experiment.

Experimental

Cells transfected with the human growth hormone receptor were cultured in suspension and used in all studies of cell activation and cell growth presented here. The cell growth was stopped prior to the experiment by taking away one component in the medium, interleukin 3. A Thermal Activity Monitor (TAM) set at 37 °C equipped with at least two 4 ml stirred titration vessels was used for microcalorimetry. The vessels were loaded with about 3 ml of 10^6 cells suspended in cell culture medium before they were transferred to the measuring position of the TAM. After thermal equilibrium had been obtained, growth hormone or phosphate buffered saline (to the reference) was added to the vessels and the heat flow was recorded as a function of time for several hours. The growth hormone was diluted in pure phosphate buffered saline prior to injection.

Thermometric AB
Spjutvägen 5A
SE-175 61 Järfälla, Sweden
Tel +46 8 564 72 200
Fax +46 8 564 72 220
mail@thermometric.se
www.thermometric.com

Dr. A. Schön*
Arne Schön Text&Data
Östanvägen 10
SE-17771 Järfälla, Sweden

*An affiliate of Lund University

Fields of application

Biology - Pharmacology, Tissue, Animal cells

Instrument configuration

2277	Thermal Activity Monitor (TAM)
5510	Prethermostat
2277-201	4 ml Ampoule Microcalorimetric Unit
2251-310	4 ml Stainless Steel Titration Ampoule
2277-301	Stainless Steel Ampoule - as reference
2277-401	Power Supply for Stirring
2277-131	Digitam Software
6120	Lund Syringe Pump

References

1. Hallén, D. Thermometric Application Note 22024 (1997)
2. Bäckman, P., Kimura, T., Schön, A., and Wadsö, I. (1992) J. Cell. Physiol. 150, 99-103.
3. Bermudez, J., Bäckman, P., and Schön A. (1992) Cell Biophys. 20, 111-123.
4. Schön, A, and Walum, E. (1997) 37th American Society for Cell Biology Annual Meeting, Washington D.C.

Results

Figure 1 shows the results from three vessels. A horizontal line represents perfect steady-state with no net growth or death of cells. A positive slope was obtained for trace amounts of added growth hormone (less than 25×10^{-12} moles per litre) and corresponds to activated metabolism and cell growth. A negative slope was only obtained for the reference, hence, the cells could not tolerate starvation conditions for periods of several hours. The activation of the metabolism reflecting the excess energy required for signal transduction is shown in Fig. 2. For an addition of 5 nmoles per litre of growth hormone, the metabolic rate is speeded up by more than 10 % during 10 min. A steady-state is then reached and after another 20 min the growth starts. Such an immediate activation upon addition of growth hormone has never been observed earlier. To elucidate which metabolic pathways that were activated, measurements of oxygen consumption and lactate production were performed as well (the results are not presented here). In accordance with earlier studies where cells of lymphoid origin were activated by other means, only an increased lactate production could be observed upon addition of growth hormone. No increase in oxygen consumption was observed. Note: the energy of binding of the growth hormone to its receptor cannot be measured in this system containing living cells (there are too few receptors per cell).

Conclusions

Microcalorimetry was used successfully as a bioassay for the detection of the utterly small metabolic activation following binding of growth hormone to its cell surface receptor. From the same set of experiments it was also possible to extract information regarding the triggered cell division following the addition. Moreover, during the course of these studies, a special technique enabling the study of cells adhered to glass plates was developed. Microcalorimetry can thus be considered to be a technique that can be used to test a vast number of therapeutically important substances on cells and tissues.

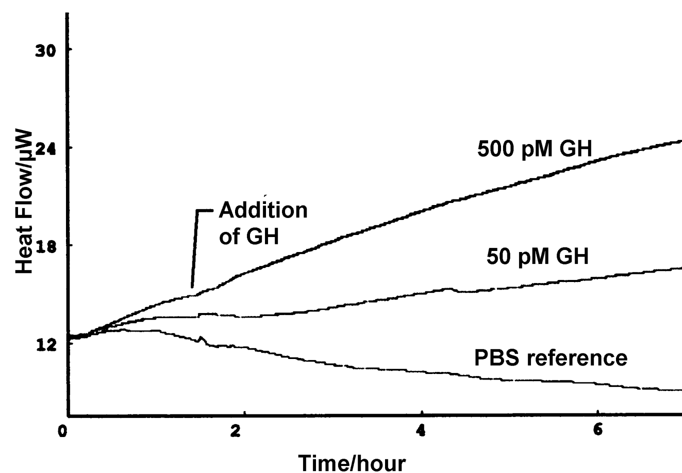

Figure 1. *The effect on growth on suspension cells, Ba/F3:6, upon addition of different concentrations of hGH (a positive slope indicates growth). As shown in the figure, the reference cells seem to decrease their metabolic rate with time (negative slope).*

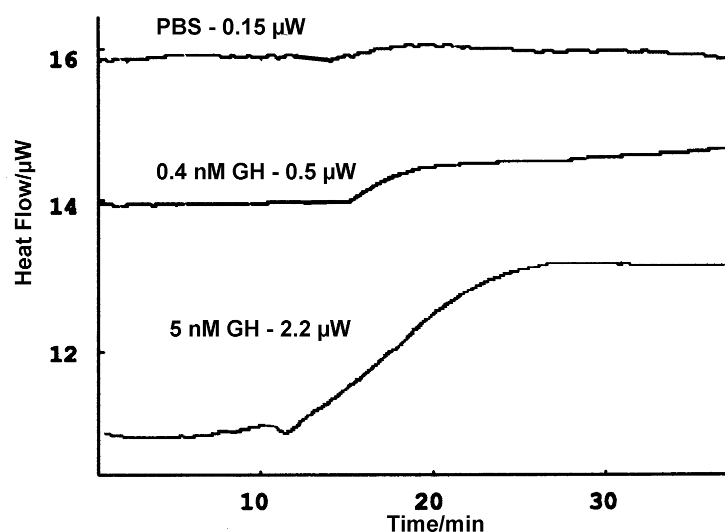

Figure 2. *The area around the time of injection hGH has been zoomed. This shows a metabolic shift dependent on the concentration of hGH which directly reflects the energy demand caused by the cellular signal transduction. The maximum is obtained for concentrations between 1 and 10 nM hGH, which gives an increase of the heat flow of 10% of the basal metabolism.*