LIPOLYTIC ACTIVITY OF BOVINE GROWTH HORMONE BOUND TO SEPHAROSE BEADS

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1. Introduction

Hechter and Lester [1] and Krahl [2] ten years ago formulated the general concept that the first site of action of circulating hormones is the plasma membrane of their target cells. According to this view the effects of certain hormones may involve an intricate relationship with the external membrane which would then cause distinct structural and metabolic changes in the cells.

Several experimental observations have further substantiated this hypothesis for vasopressin [3], thyrotropin and insulin [4], and ACTH [5].

The possibility that the polypeptide hormones may act on the external side of the plasma membrane has prompted various authors to explore the biological action of these hormones bound to insoluble macromolecular polymers presumably incapable of entering the cells: Schimmer et al. [6] have assayed ACTH bound to cellulose on adrenal cells and have obtained a clear stimulation of the production of ketosteroids; Cuatrecasas [7] found that insulin covalently bound to large beads of Sepharose effectively increased the utilization of glucose and suppressed the hormone stimulated lipolysis of fat cells; Turkington [8] found stimulation of RNA synthesis in mammary cells by insulin and prolactin bound to Sepharose and the steroidogenic potential of ACTH linked to agarose was found to be identical to that of free hormone on adrenal cells [9].

The results described in this paper on the lipolytic action of bovine growth hormone (BGH) bound to large Sepharose beads suggests that this hormone may also exert its effect through interaction with the plasma membrane of its target cells.

2. Material and methods

Bovine growth hormone (BGH) was prepared by the procedure of Dellacha and Sonenberg [10]. This protein was homogeneous in the ultracentrifuge and gave the usual pattern of highly pure preparations in polyacrylamide-gel electrophoresis. Its biological activity, as measured by the tibia test [11] was 1.6 USP units/mg. Sepharose 4 B (beads, diameter between 40 μ and 190 μ), was from Pharmacia, Uppsala.

The attachment of BGH to Sepharose was done as follows: Sepharose was activated by cyanogen bromide according to Porath et al. [12], 10 mg of BGH were added to 2 ml of activated Sepharose beads in 2 ml of 0.1 M sodium bicarbonate adjusted to pH 10.0 with 1 M sodium hydroxide. The slurry was stirred overnight at 4°, then thoroughly washed over a sintered glass funnel with 0.1 M sodium

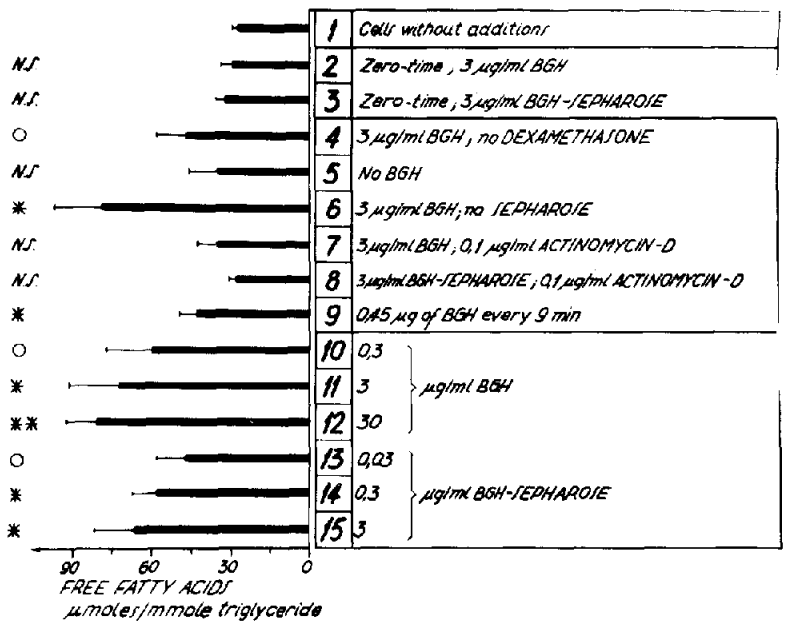

Fig. 1. Lipolytic activity of free BGH and BGH bound to Sepharose beads. All incubations were carried out in triplicate. The complete system consisted of 0.5 ml of free fat cell suspension (about 5×10^5 cells/ml), 0.06 μg of dexamethasone, variable amounts of BGH or BGH-Sepharose in a final vol of 3 ml of Krebs-Ringer buffer with 4% of bovine serum albumin and 0.1% of glucose. Gas phase: 95% O_2 5% CO_2. All Erlenmeyer flasks contained the same amount of Sepharose as the one with greatest concentration of BGH-Sepharose. Additions and omissions are indicated. Incubations were at 37°, in a Dubnoff shaker set at 5 strokes per sec. At the end of 180 min 0.20 ml of 1 M sulfuric acid was added to each flask; final pH: 2.0. Results given are averages plus the standard error of the mean. The triglyceride content was used as a measure of the number of cells present in each flask (1 g of adipose tissue contained 1 mmole of triglycerides and yielded 0.8 ml of free fat cell suspension). The statistics used to establish the significance of the differences (paired comparisons) between all groups and the incubate of cells without additions (exp. no. 1), was the Student-t-test. N.S.: $p \geq 0.10$; ○: $p < 0.10$; ✱: $p < 0.05$; ✱✱: $p < 0.01$.

bicarbonate until the filtrate was free of absorbancy at 280 nm. This in turn was followed by washings with 500 vol of 8 M urea, 0.1 M acetic acid, distilled water and phosphate-buffered saline, pH 7.40. Aliquots of the final slurry were hydrolyzed for 24 hr at 110° with 6 M hydrochloric acid and the free amino acids quantitatively estimated in a Beckman 120 analyzer. The pattern obtained corresponded closely to the amino acid composition of native BGH and it could be estimated that 1.03 mg of hormone were attached per ml of Sepharose beads.

Lypolytic activity was measured as indicated oy Fain et al. [13] on free fat cells [14] from epidimal adipose tissue from rats fasted for 24 hr. The cells ranged in size between 50 μ and 100 μ diameter; their integrity was controlled by light microscopy. The liberated non-esterified fatty acids were automatically titrated in a pH-stat, following the procedure of Dole and Meinertz [15], standardized with palmitic acid.

3. Results and discussion

Fig. 1 gives the results of the measurement of lipolytic activity of free BGH and BGH bound to Sepharose beads. Analysis of the results indicates: the fat cells used do not have intrinsic lipolytic activity and the presence of BGH or BGH-Sepharose at zero time does not affect this value (exp. 1, 2 and 3). Growth hormone alone has a marked lipolytic tendency; dexamethasone had no significant activity (exp. 4 and 5) but potentiated the response of growth hormone (exp. 4 and 11). The maximum

Table 1
Molar ratios of free amino acids arising from hydrolysis of the BGH-Sepharose used in experiments 15 and 3 (fig. 1).

Asp	0.61	Gly	1.08
Thr	0.73	Ala	1.07
Ser	0.76	Val	1.09
Glu	0.93	Ile	1.05
Pro	1.20	Leu	1.24
Tyr	0.85	Phe	0.96

Average ratio: 0.96

charge of Sepharose does not affect the lipolytic response (exp. 6 and 11); actinomycin D blocks completely the lipolytic activity stimulated by BGH or BGH-Sepharose (exp. 7, 8 and 1–3), thus discounting the possibility of the presence of a lipid mobilizing factor [16] in the preparations of the hormone; the lipolytic action of 3 μg/ml of BGH added at the beginning of the incubation is much greater than that observed when BGH was added in portions at regular intervals throughout the incubation period (exp. 9 and 11); these experiments suggest that BGH-Sepharose is not a reservoir of BGH from which the fat cells obtain a regular supply of free hormone; both free BGH and BGH-Sepharose have clear lipolytic activities (exp. 10–15). The biological potency of BGH was established by using a 6-point parallel line assay (symmetrical design) [17] between both groups. The conclusion is that the assay is statistically valid; the slope of the semi-logarithmic dose-response plot is low but the overlapping of the responses is good. Parallelism and linearity are satisfactory. The biological potency of BGH-Sepharose is 39.5%, on a weight basis, relative to free BGH.

The BGH-Sepharose used in experiments 3 and 15 (fig. 1) was recovered by centrifugation and rinsed with 0.2 M sodium hydroxide to free the beads of cell-remnants. Both samples were then hydrolyzed with 6 M hydrochloric acid for 24 hr at 110° along with a control of unincubated BGH-Sepharose. The free amino acids were estimated in an automatic analyzer. Some neutral amino acids could not be quantitated due to artifacts arising probably from hydrolytic products of the Sepharose beads. The basic amino acids were not determined. The results presented in table 1 are expressed as the ratios of those values obtained for BGH-Sepharose from exp. 15 over those of BGH-Sepharose from exp. 3. The average ratios of these samples to the control BGH-Sepharose were 1.07 and 1.03, respectively. The values quoted in table 1 strongly suggest that neither the intact hormone nor a significant fragment of it is liberated from its binding to Sepharose during its action on the cells.

These experiments support the concept that cytoplasmic metabolic and nuclear biosynthetic activities can be stimulated by a propagation of events initiated by the growth hormone acting at the level of the cell membrane. Direct evidence of this primary type of interaction has been obtained by optical methods [18–20] and by the rate of exchange of a radioactive label in the hormone [21].

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