# SPECIES-SPECIFIC INTERACTION OF GROWTH HORMONES WITH ERYTHROCYTE MEMBRANES

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## 1. Introduction

Very recently various experimental observations have added weight to the suggestion that hormones act at the membrane level [1, 2].

Insulin covalently linked to Sepharose beads causes metabolic alterations on isolated fat cells than can only be explained by assuming an interaction of the insoluble hormone with the cell membranes [1]. Direct evidence of this type of phenomenon has also been obtained: human growth hormone (HGH), in physiological concentrations, induced a change in the elipticity of human erythrocyte membranes and the ultraviolet spectrum of the hormone was modified. No changes could be detected when bovine growth hormone (BGH) was used in place of the human protein, thus suggesting a species-specific interaction [2].

We have attempted detection of the presumable binding of growth hormone to the erythrocyte membrane by following the rate of exchange-out of a tritium label previously introduced in the hormone. The assumption was made that any interaction would slow down the process and, accordingly, should be detected as excess radioactivity in the hormone. The results obtained support this hypothesis and confirm the existence of a species-specific interaction between growth hormones and erythrocyte membranes.

## 2. Materials and methods

HGH was prepared by the method of Roos et al. [3] and BGH by the method of Dellacha and Sonenberg [4]. Erythrocyte membranes were obtained by the method of Dodge et al. [5], from fresh human, rat and guinea-pig blood. Dialyses were carried out in the countercurrent dialysis apparatus of Craig and Stewart [6]. Radioactivity measurements were performed in a Packard Tri-Carb liquid scintillation spectrometer. Solutions for counting were prepared by mixing 50 $\mu$l of sample with 10 ml of a scintillation mixture containing 0.1 g of 1,4-bis-2(4-methyl-5-phenyloxazolyl)-benzene, 4 g of 2,5-diphenyloxazole and 50 ml of Bio-Solv (formula BBS-3, Beckman Instruments, Inc.) per liter of toluene. Membranes were counted under a phase microscope.

The experiments were conducted as follows: 0.4 ml of hormone solution (2 mg per ml in Krebs-Ringer phosphate buffer, pH 7.4) plus 5 $\mu$l of tritiated water (100 mCi/ml) was incubated 4 hr at 20°, in duplicate; then 0.1 ml of membrane suspension ($10^9$ cells/ml, in Krebs-Ringer phosphate buffer, pH 7.4) was added to one of the tubes and 0.1 ml of buffer to the other. After 15 min at 20° both solutions were dialyzed. The retentates were allowed to stand 2 hr at 20° and then dialyzed a second time. The tritium activity in the retentates was measured. A third tube with membranes but no hormone was carried through the same procedure to establish the amount of tritium retained by the membranes.

The duration of the exchange-in and -out periods, as well as the amount of tritiated water used, were adopted taking into account the known behaviour of HGH and BGH under these conditions and the practical capability of our dialysis equipment, as previously established by us [7].

The radioactivity retained by the hormone incubated with membranes was compared with the radioactivity

Table 1
Excess tritium retained by human and bovine growth hormones incubated with triated water in the presence of human, rat or guinea-pig erythrocyte membranes.

| No. | Incubation of: | Exp. no. | Erythrocyte membranes from: Human 1 | 2 | 3 | 4 | 5 | 6 | 7 | Rat 8 | 9 | Guinea pig 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | HGH Plus Membranes | | 60±1.6 | 51±1.7 | 45±1.3 | | | | | 48±2.8 | | 31±1.7 | |
| 2 | Membranes | | 6±1.3 | 0 | 0 | | | | | 6±2.4 | | 3±1.6 | |
| 3 | (HGH Plus Membranes)-Membranes | | 54±2.0 | 51±1.7 | 45±1.3 | | | | | 42±3.7<br>33±2.7 | | 28±2.3<br>29±1.7 | |
| 4 | HGH | | 40±1.5 | 40±1.3 | 29±1.2 | | | | | | | | |
| 5 | BGH Plus Membranes | | | | | 41±1.3 | 47±1.2 | 39±1.2 | 51±2.9 | | 59±2.1 | | 60±1.4 |
| 6 | Membranes | | | | | 5±1.1 | 0 | 0 | 1±2.4 | | 5±1.7 | | 5±1.2 |
| 7 | (BGH Plus Membranes)-Membranes | | | | | 36±1.7 | 47±1.2 | 39±1.2 | 50±4.0 | | 54±2.7 | | 55±1.8 |
| 8 | BGH | | | | | 40±1.3 | 46±1.2 | 44±1.2 | 49±2.9 | | 42±2.0 | | 59±1.4 |
| | Significance of 3>4 or 7>8 | | H.S. | H.S. | H.S. | N.S. | N.S. | N.S. | N.S. | S. | H.S. | N.S. | N.S. |
| | $p$ | | <0.002 | <0.002 | <0.002 | ⩾0.1 | >0.1 | ⩾0.1 | >0.1 | <0.02 | <0.002 | ⩾0.1 | ⩾0.1 |

In-exchange: 4 hr at 20°; out-exchange: 2 hr at 20°. Values expressed in cpm/ml ± standard error. The statistics for one-side limit with confidence probability [8] were applied. N.S. = not significant; S = significant; H.S. = Highly significant.

of the hormone incubated alone. Excess of the former was interpreted as evidence of interaction. This experimental design requires that the amount of dilution occurring during the dialyses be of the same magnitude in all tubes; only under these circumstances do the results truly indicate interaction. Constancy of dilution was assessed by counting the number of membranes per ml in the final retentates of each experiment. The average difference was 1.4±0.4% (S.E.) (9 paired experiments). This result was interpreted as a satisfactory proof that the errors introduced by non-constant dilution were negligible.

## 3. Results and discussion

Table 1 shows the results obtained in comparative experiments performed with HGH and BGH incubated with human, rat and guinea-pig erythrocyte membranes.

The excess of tritium label, indicating interaction, occurred in such a fashion as to make highly improbable that it arose due to non-specific adsorption of the proteins on the membranes. In fact, there is a perfect correlation with the known species specificity of action of the hormones. Only HGH is active in human beings, and showed excess of tritium in presence of human erythrocyte membranes. The rat is responsive to human and bovine hormones and its erythrocyte membranes showed a significant interaction with both proteins, whilst the guinea-pig erythrocyte membrane did not interact with either, in accordance with the known refractoriness of this species to all growth hormones so far tested [9].

The present experiments confirm and extend the observations made by Sonenberg [2] by optical methods, and both studies point to the existence of structural requirements for the interaction of growth hormones and erythrocyte membranes. It is not known if this interaction suffices to initiate the variety of metabolic alterations that characterize growth hormone action, but it can be mentioned that, by analogy with what happens with insulin [1]. HGH and BGH chemically bonded to cellulose or Sepharose have substantial activity *in vivo* [10].

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