Interaction of Human Growth Hormone and Human Erythrocyte Membranes as Demonstrated by Circular Dichroism

Martin Sonenberg

Division of Endocrinology, Sloan-Kettering Institute for Cancer Research, New York, New York

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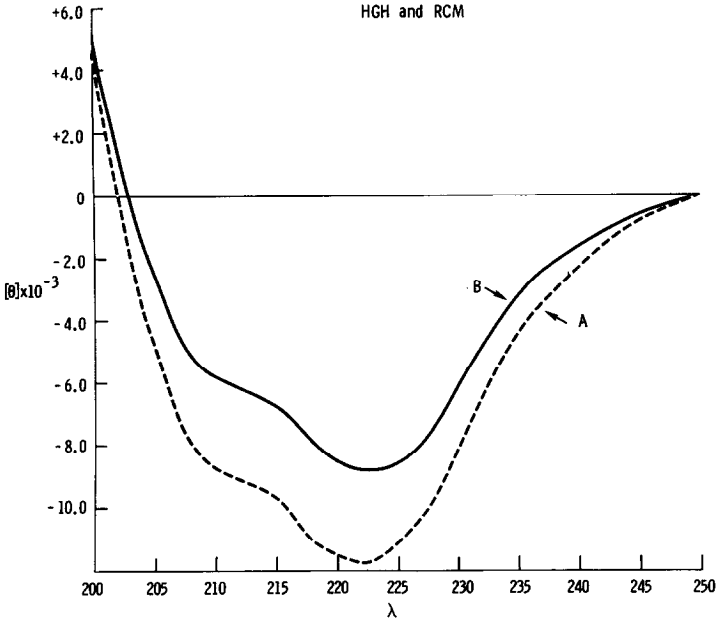
Summary. Human growth hormone in physiological concentrations induced a change in the ellipticity of human erythrocyte membranes at 222 mμ from -11,800 deg cm^2/decimole to about -8,800 deg cm^2/decimole. Bovine growth hormone, bovine serum albumin, bovine insulin or cortisol were without effect. This effect was demonstrable in the presence of phosphate buffer but not in distilled water. Erythrocyte membranes modified the near ultraviolet spectrum of human growth hormone. None of the effects observed could be accounted for by distortions due to light scattering or absorption flattening.

Although there is a significant body of information which suggests that hormones act on membranes there is little direct physico-chemical evidence in isolated systems which indicate an interaction between hormone and membrane. The observation (6) that human growth hormone (HGH) inhibits erythrocyte glucose consumption *in vitro* suggested that the readily available erythrocyte membrane might interact with human growth hormone. We have noted an alteration in the circular dichroism spectra of human erythrocyte membranes with the *in vitro* addition of human growth hormone.

Methods. Erythrocyte membranes were prepared by the method of Dodge et al. from fresh human blood. Protein concentrations of the membrane preparations were determined from the OD_{280} of 2-chloroethanol solutions prepared from measured amounts of the original membrane suspension (4). Circular dichroism (CD) measurements were made on a Cary Model 60 recording spectropolarimeter with CD attachment 6002. All measurements were made at ambient temperature in dilute (0.005 M) phosphate buffer

pH 7.4 in double cylindrical tandem cells, each compartment of 10 mm. light path. The data are expressed in terms of mean residue ellipticity (θ) in deg. cm^2/decimole. Spectrophotometric determinations were performed on a Cary 11 recording spectrophotometer with the same tandem cells employed for the CD determinations. Membranes were counted under a phase microscope.

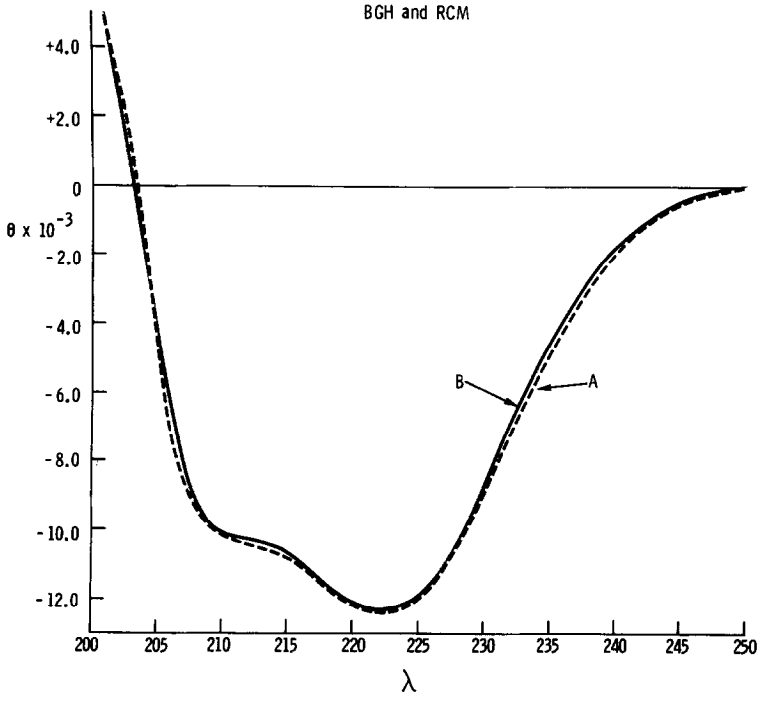


LEGEND FIGURE 1

Circular dichroism spectra between 200 and 250 mμ of human erythrocyte membranes (6×10^6/ml^3) and human growth hormone (4.8×10^{-7} M) in 0.005 M phosphate buffer, pH 7.4. A - hormone and membranes in separate compartments of tandem cell. B - hormone and membranes in same compartment of tandem cell.

Results. In Figure 1 are shown the CD spectra of human erythrocyte membranes separated from and in contact with HGH at 4.8×10^{-7} M. The $n\text{-}\pi^*$ transition appears at 222 mμ in both spectra but in the presence of HGH there is approximately a 25% decrease in the value of the negative ellipticity of the membrane optical activity. This effect of

HGH could be demonstrated with concentrations as low as 4.8×10^{-11} M, but the response was not proportional to the concentration of HGH. At similar concentrations (10^{-10} M to 10^{-7} M) bovine growth hormone (BGH) (Fig. 2), bovine serum albumin (BSA) and insulin did not alter the CD spectra of the membranes. Cortisol (2×10^{-5} M) was also without effect. When erythrocyte membranes were washed free of phosphate buffer with distilled water, no changes in the near or far U-V CD spectrum were demonstrable on addition of HGH.

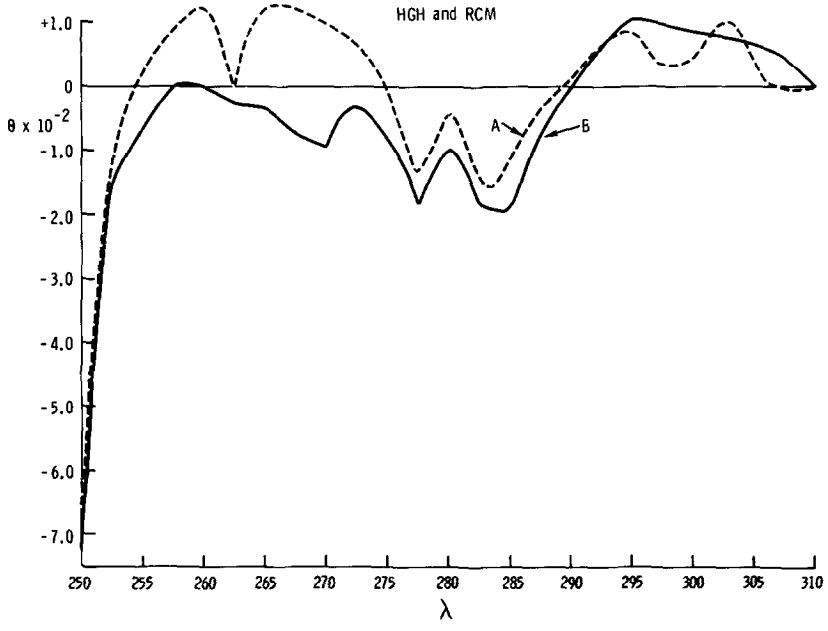


LEGEND FIGURE 2

Circular dichroism spectra between 200 and 250 mμ of human erythrocyte membranes (6×10^6/ml^3) and bovine growth hormone (4.8×10^{-7} M) in 0.005 M phosphate buffer, pH 7.4. A - hormone and membranes in separate compartments of tandem cell. B - hormone and membranes in same compartment of tandem cell.

Near ultraviolet CD spectroscopy of membranes alone revealed no definite optical activity. HGH had the previously observed (7) near

ultraviolet CD spectra. When placed in the same optical cell as erythrocyte membranes there were no changes in the peaks and troughs at 302 mμ, 295 mμ, 282 mμ and 277 mμ (Fig. 3). The positive ellipticity below 275 mμ with peaks at 266 mμ and 260 mμ became negative on interaction of the membranes and HGH (Fig. 3).



LEGEND FIGURE 3

Circular dichroism spectra between 250 and 310 mμ of human erythrocyte membranes (2.4×10^7/ml^3) and human growth hormone (4.8×10^{-5} M) in 0.005 M phosphate buffer, pH 7.4. A - hormone and membranes in separate compartments of tandem cell. B - hormone and membranes in same compartment of tandem cell.

There was no apparent change in light scattering as indicated by the similar spectra of membranes separate from and in contact with HGH. The OD_{700} readings were 0.027 and 0.026 for the membranes alone and in contact with HGH respectively. In addition, no apparent change was discernible on phase microscopy of these two samples.

Discussion. The interaction of HGH and human erythrocyte membranes has structural requirements both of the growth hormone and the membrane. The change in the far ultraviolet CD spectrum in the presence of HGH but not BGH, BSA, insulin or cortisol suggests a specific effect of HGH on human erythrocyte membranes. The lack of an effect of HGH on erythrocyte membranes washed with distilled H_2O suggests that some organization of the membrane is necessary for this interaction. Human erythrocyte membranes have been reported (1,2) to have significantly different morphology in distilled water than in hypotonic salt solution. Bovine red cell membranes have less structure in distilled water and proteins may actually be solubilized by distilled water (5). Thus, the structure of the erythrocyte membrane stabilized by ions is necessary for interaction with HGH.

Since in these experiments (Fig. 1) contribution to the ellipticity by the membranes at 222 mμ is approximately -12,000 and that of HGH -330 deg cm^2/decimole, the change in ellipticity most likely reflects a change in the membrane and not in HGH. There is, however, an associated change in HGH inasmuch as the near U-V CD spectrum is that of HGH with no significant contribution from the erythrocyte membranes. The near U-V CD changes are consistent with alterations in the transitions of the aromatic and disulfide residues of HGH (7) .

The nature of the changes in optical activity of the membranes is of interest. It is not likely the result of "distortions in circular dichroism patterns of particulate (or membranous) systems" (3,8) due to light scattering and absorption flattening effects. No changes in light scattering as reflected in phase microscopy, OD_{700} or spectroscopy from OD_{700} to OD_{350} have been noted. Nor have we noted any red shift of the $n\text{-}\pi^*$ transition at 222 mμ or the crossover wave length either in erythrocyte membranes with HGH or alone, as previously noted (4). If the ellipticity at 222 mμ originates solely from an α-helix an approximate helical con-

tent of 30% of membranes decreases to about 22% under the influence of HGH.

At 4.8×10^{-11} M HGH and approximately 6.0×10^{6} erythrocyte membranes per ml there may be as few as 160 molecules of HGH per erythrocyte membrane. To obtain the effect observed suggests a cooperative mechanism.

ACKNOWLEDGEMENTS

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