THE MEMBRANE AS TRANSDUCER IN PEPTIDE HORMONE ACTION

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With peptide hormones, as with other ligands acting at the cell surface, the problem remains, "How does a hormonal or chemical message get translated into biochemical and biological effects?" As a working hypothesis the problem may be dissociated kinetically into a series of steps. The first step likely involves recognition and binding of the hormone to a site or sites on the plasma membrane. This is accompanied or followed by a conformational change in membrane components. These events occur within seconds or fractions of a second. There is then, within minutes, a rapid activation of one or more enzymes with the possible formation of a "second messenger" or chemical which mediates subsequent biochemical reactions. These events most likely occur within the plasma membrane. Subsequent biochemical reactions, which occur within hours, may be intracellular as well as intramembranous. The late expression of the foregoing biochemical and biophysical reactions is a biological effect and may be apparent within hours or days.

Although there is a large literature developing on the subcellular site of action of peptide hormones, this discussion will be limited to the interaction of growth hormone and the plasma membrane and will not be concerned with other possible subcellular sites of action. More specifically, we shall be concerned with the problem of whether hormone binding is coupled to membrane enzyme activation. Is a selective enzyme activation or triggering involved or is there a general membrane conformational change induced? What other mechanisms may be involved? To gain some insight into this problem we have attempted to correlate binding of growth hormone with membrane conformational and enzymatic changes produced by the hormone.

To our knowledge there are no explicit data on the binding of growth hormone to isolated plasma membranes. In our studies (1) with plasma membranes isolated by modifications (2, 3) of the Neville (4) and Ray (5) procedure from the livers of hypophysectomized rats, we have noted binding of ^{125}I growth hormone to plasma membrane fractions. When the latter were further fractionated in low ionic strength buffers by centrifugation on stepped sucrose gradients, three membrane subfractions were obtained (2, 3). The membrane subfraction which was most dense, appearing at the interface between densities of 1.1612 and 1.2296 g/1, bound radioactive growth hormone. Carrier native growth hormone competed with the labeled growth hormone while carrier insulin did not. This peak was enhanced in 5′ nucleotidase activity and was least contaminated with mitochondria as assayed by measuring cytochrome oxidase activity. Electron microscopy revealed only membrane sheets to be present. The membrane peak of intermediate density, between 1.0381 and 1.1612 g/1, was more effective in binding insulin

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than growth hormone. Carrier growth hormone competed less effectively with ^{125}I growth hormone in binding to this intermediate peak.

As in the case of human erythrocyte membranes interacting with human growth hormone (6, 7), bovine growth hormone produced conformational changes in the proteins of liver plasma membranes obtained from hypophysectomized rats. Circular dichroism measurements showed changes in secondary structure following administration of the hormone to hypophysectomized rats (8) or *in vitro* incubation of the hormone with isolated liver membranes (9, 10). These changes were dependent on growth hormone concentration (9, 10). Liver membranes showed decreased emission of fluorescence and a shift of the emission peak from 333 to 338 nm in the presence of bovine growth hormone (10^{-7} to 10^{-17} M), consistent with a change in tertiary structure of membrane proteins. Both circular dichroism and spectrofluorescence changes were noted as quickly as measurements could be made, i.e., in about 15 seconds. There were no changes in optical density between 700 and 200 nm to suggest changes in light scattering or change in particle size. Denatured bovine growth hormone or bovine serum albumin were without effect on these membranes. The membrane response to bovine growth hormone was destroyed by sonication, suggesting need for some biological organization. Membranes treated with phospholipase A_2 were similarly unresponsive, suggesting a primary or secondary role for phospholipids in the response to growth hormone.

Subsequent to the immediate events of binding of hormone to membranes and membrane protein conformational changes, growth hormone has produced changes in membrane enzyme activity. After incubation of isolated liver plasma membranes with growth hormone there was increased ATPase (11) and NADase (12) activity which occurred within minutes. Denatured growth hormone was without effect.

Hypophysectomy, which removes growth hormone as well as other hormones, increased total liver membrane ATPase and decreased 5′ nucleotidase (8). Treatment of hypophysectomized rats with growth hormone decreased the specific activities in liver membranes of total and Mg^{2+} –dependent ATPases and increased the ratio of Na^+, K^+ –ATPase/total ATPase as well as 5′ nucleotidase (8). Bovine growth hormone treatment of hypophysectomized rats increased the liver plasma membrane phosphatidylserine concentration (8). These effects were observed within one day. It is difficult to relate these experiments with the whole organism and attendant metabolic changes due to growth hormone and other hormones to *in vitro* incubations of liver membrane and growth hormone.

With evidence at hand to indicate that growth hormone can bind to isolated liver plasma membranes, produce general membrane protein conformational changes, and effect activation of some membrane bound enzymes, it is important to describe the intramembranous events which relate these immediate and rapid effects. Toward this end we have employed several fluorescent probes. Initially, 7, 12-dimethylbenzanthracene (DMBA) has been added to isolated liver membranes and we have found (10) using fluorescence and ^3H–DMBA that this polycyclic hydrocarbon is quantitatively taken up by the membrane. When growth hormone was added directly to a suspension of liver membranes with DMBA, there was a decrease in both the intrinsic fluorescence of the protein as well as the extrinsic fluorescence of DMBA (10). Since there was no associated change in light scattering it would appear that there is a general membrane conformational change involving protein and lipid phases.

An attempt has been made to follow conformational changes in specific membrane proteins to complement the previously noted stimulation by growth hormone of membrane enzyme activity. For this purpose, we have prepared (13) a series of 1, N^6 –etheno

derivatives of adenosine by recently reported procedures (14, 15). Thus, 1, N^6 etheno-adenosine triphosphate (ϵ–ATP) was an effective substrate for isolated liver membrane Mg^{2+} –ATPase (11). As an extrinsic probe in membranes it yielded an emission peak, 395 nm, separate from the intrinsic peak at 333 nm with excitation at 282 nm (11). When growth hormone in concentrations as low as 10^{-15} M was added to the liver membranes with ϵ-ATP there was a decrease in the extrinsic fluorescent peak (11). Denatured growth hormone was without effect in this system (11). There was a smaller response in this system using 1,N^6 –ethenoadenosine diphosphate (ϵ–ADP) and no response with the etheno derivatives of adenosine monophosphate, cyclic adenosine monophosphate, or adenosine (11). Growth hormone also produced an increase in polarization of the extrinsic fluorescence (11) and a decrease in the fluorescent lifetime (16) of ϵ–ATP in liver membranes. With the relationship of Perrin (17) in mind, the increased polarization is consistent with a decrease in the observed fluorescent lifetime and cannot be wholly accounted for by a change in rotational relaxation time. The latter may change as a consequence of change in microviscosity of the membrane. This is a difficult parameter to establish with confidence.

An isolated membrane preparation in buffer with growth hormone is significantly removed from the physiological circumstance. In addition to the likely changes in the membrane associated with its isolation, there is likely to be the loss of other substances or cofactors which influence the response of the membrane to peptide hormones. One such category of cofactors includes the nucleotides. Guanosine triphosphate (18) and other nucleoside triphosphates (19), for example, have profound effects on membrane adenyl cyclase activity. We have confirmed and extended these observations (20). In addition to adenyl cyclase (20), we have noted increased activity of liver membrane NADase (2) and ATPase (11) in response to added GTP. The increase in these enzyme activities appears to be the consequence of a general membrane conformational change as revealed by changes in spectrofluorescence (2). The maximum change in fluorescence (2) occurred at approximately the same concentration of GTP where maximum stimulation in ATPase (11) and NADase (2) was produced by this nucleotide. Maximum stimulation of adenyl cyclase by GTP occurred at different concentrations of the latter dependent on substrate, ATP, concentration (20). Moreover, the S-shaped curve of decrease in fluorescence with increase of GTP concentration suggests a cooperative effect. Although the changes in enzyme activity and membrane conformation appear to be related, the mechanism of this effect of GTP on liver membranes is not clear. There was radioactivity associated with membrane fractions after incubation with ^{14}C–GTP and this was displaced by carrier GTP but not ATP (3). More than 95% of the GTP was rapidly metabolized to GDP and guanosine (3). Whether the membrane protein conformational change is a direct effect of GTP or a metabolic product, phorphorylation of membrane proteins or other proteins or other mechanisms has yet to be elucidated.

In conclusion, with the additional evidence we have adduced, it would appear that growth hormone binds to liver membranes with concomitant conformational changes in the liver membrane. There are changes in both the protein and lipid phase of the membrane. Moreover, some data suggest conformational changes in a specific enzyme. It would appear that the conformational changes may be of a cooperative nature. These changes are followed by changes in several enzyme activities. The conformational changes and enzyme activation produced by growth hormone are enhanced by cofactors like GTP by a mechanism not yet understood.

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