Structure and Proteolysis of the Growth Hormone Receptor on Rat Hepatocytes

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ABSTRACT: ^{125}I-Labeled human growth hormone is isolated in high molecular weight (M_r) (300 000, 220 000, and 130 000) and low molecular weight complexes on rat hepatocytes after affinity labeling. The time-dependent formation of low molecular weight complexes occurred at the expense of the higher molecular weight species and was inhibited by low temperature or inhibitors of serine proteinases. Exposure to reducing conditions induced loss of M_r 300 000 and 220 000 species and augmented the amount of M_r 130 000 complexes. The molecular weight of growth hormone (22 000) suggests that binding had occurred with species of M_r 280 000, 200 000, and 100 000. Two-dimensional gel electrophoresis demonstrated that the 100 000-dalton receptor subunit is contained in both the 280 000- and 200 000-dalton species. Reduction of interchain disulfide bonds in the growth hormone receptor did not alter its elution from gel filtration columns, but intact, high molecular weight receptor constituents were separated from lower molecular weight degradation products. Digestion of affinity-labeled growth hormone–receptor complexes with neuraminidase increased the mobility of receptor constituents on sodium dodecyl sulfate–polyacrylamide gel electrophoresis. These observations show that the growth hormone receptor is degraded by hepatic serine proteinases to low molecular weight degradation products which can be separated from intact receptor by gel filtration. Intact hormone–receptor complexes are aggregates of 100 000-dalton sialoglycoprotein subunits held together by interchain disulfide bonds and by noncovalent forces.

A first step in growth hormone action is binding to specific receptors on the surface of target cells (Posner et al., 1974; Kelly et al., 1974; Herington et al., 1976; Posner, 1976; Ranke et al., 1976; Donner et al., 1978, 1980; Barrazone et al., 1980; Donner, 1980; Fagin et al., 1980; Murphy et al., 1983). In various cells, such interactions are transduced into changes of cell metabolism, growth, or differentiation (Isaksson et al., 1985). Since it is assumed that receptor structure and function are related, considerable effort has been expended toward characterizing the growth hormone receptor (Wallis, 1980; Hughes & Friesen, 1985).

Affinity labeling is one method used to study the structure of peptide hormone receptors (Jacobs et al., 1979, 1980; Pilch & Czech, 1979, 1980; Johnson et al., 1981; Linsley et al., 1979, 1981; Massague & Czech, 1982). In this procedure, radioactively labeled hormones are covalently coupled to membrane receptors using organic cross-linking reagents. The products of such reactions can be characterized by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE)[1] and autoradiography. We have used this method to show that ^{125}I-labeled human growth hormone is incorporated into somatogenic complexes of M_r 300 000, 220 000, and 130 000 on rat hepatocytes (Donner, 1983; Yamada & Donner, 1984). The molecular weight of growth hormone (22 000) suggests that the hormone had bound to species of M_r 280 000, 200 000, and 100 000. Varying amounts of less predominant, lower molecular weight complexes were also typically observed in these studies. Some of these lower molecular weight complexes resulted from cross-linking of growth hormone to itself or to albumin (Donner, 1983); however, the significance of the remaining species was undetermined. One explanation for the observation of low molecular weight complexes is that they result from hormone binding to growth hormone receptor constituents. Another explanation is that they are products of receptor proteolysis.

One goal of this study was to determine if the growth hormone receptor is labile and if lower molecular weight complexes isolated after affinity labeling are products of receptor proteolysis. A second aim was to study the relationship of intact receptor components to one another to better understand the structure of the receptor for growth hormone.

Experimental Procedures

Materials

Female Sprague-Dawley rats (150–200 g) from Charles River Breeding Laboratories were fed Purina Laboratory Chow ad libitum. Neuraminidase (180 units/mg) was from Worthington Biochemical Co. Ovine prolactin (38.9 IU/mg) was from Sigma. The sources of other materials were described previously (Donner, 1983).

Methods

Purified human growth hormone (hGH) was isolated by Dr. Brij Saxena (Cornell University Medical College) (Saxena & Henneman, 1966). Monomeric hGH and bGH were labeled with ^{125}I as described (Donner, 1983). Each labeled hormone (specific activity 75–125 Ci/g) was greater than 95% pre-

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[1] Abbreviations: hGH, human growth hormone; bGH, bovine growth hormone; ^{125}I-hGH, iodine-125-labeled human growth hormone; ^{125}I-bGH, iodine-125-labeled bovine growth hormone; HBSS, Hank's balanced salt solution; M_r, apparent molecular weight; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; DTT, dithiothreitol; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); Tris, tris(hydroxymethyl)-aminomethane; EDTA, ethylenediaminetetraacetic acid; PMSF, phenylmethanesulfonyl fluoride; BSA, bovine serum albumin.

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cipitable in 5% w/v trichloroacetic acid. Hepatocytes were isolated by the method of Berry and Friend (1969).

The binding of ^{125}I-bGH to hepatocytes was assayed by centrifugation (Donner et al., 1978). Radioactive uptake in the presence of ^{125}I-bGH is defined as total binding. Nonspecific binding is defined as the uptake of ^{125}I-bGH in the presence of unlabeled bGH (1 μM). Specific binding is the difference between total and nonspecific binding. In binding experiments, each point is the mean of triplicate determinations. Binding and cross-linking experiments have been replicated 3 times with results similar to those shown.

Affinity Labeling. Cells from female animals were used in this study as somatogenic receptors are affinity labeled with higher efficiency on hepatocytes from female than from male rats (Yamada & Donner, 1984). In most experiments, ^{125}I-hGH, which binds to somatogenic and lactogenic receptors, was used in preference to ^{125}I-bGH, which binds only to somatogenic sites, because the former hormone may be cross-linked to receptors with greater efficiency (Yamada & Donner, 1984, 1985). To affinity label somatogenic receptors, hepatocytes (10^6 cells/mL) were incubated with ^{125}I-bGH (5 nM) or with ^{125}I-hGH (5 nM) in the presence of prolactin (1 μM, to inhibit binding to lactogenic receptors) in HBSS/1% bovine serum albumin at 23 °C for 60 min. The incubate was centrifuged (1500*g*, 3 min), and the supernatant was aspirated from the resultant cell pellet which was resuspended in ice-cold HBSS and recentrifuged. This process was repeated twice to remove albumin and unbound hormone from the hepatocytes. The hepatocytes were suspended in HBSS at 10 °C, and freshly prepared disuccinimidyl suberate in dimethyl sulfoxide was added to a final concentration of 1 mM. After 15 min, the reaction was terminated by addition of 10 volumes of ice-cold 10 mM Tris, 1 mM EDTA, and 40 mM *N*-ethylmaleimide, pH 7.4. The mixture was centrifuged and suspended in ice-cold 0.25 M sucrose/40 mM *N*-ethylmaleimide. The hepatocytes were disrupted in a Dounce homogenizer with a tight-fitting pestle (30 strokes) and drawn through a 22-gauge needle (10 times) until light microscopy verified that virtually none of the cells remained intact. The homogenate was centrifuged at 800*g* for 10 min, and the supernatant was centrifuged at 80000*g* for 15 min to yield a crude membrane pellet which contained covalently bound ^{125}I-labeled hormone. This pellet was boiled for 5 min in solubilization medium containing 3% sodium dodecyl sulfate, 10% glycerol, 6 M urea, 0.001% bromphenol blue, 60 mM Tris-HCl, and 40 mM *N*-ethylmaleimide. Insoluble components were separated from solubilized membrane by centrifugation at 80000*g* for 15 min at room temperature.

In experiments characterizing receptor proteolysis, ^{125}I-labeled hormone was covalently coupled to receptors as described above. Cross-linking was terminated by extensively washing cells with ice-cold HBSS prior to isolation of crude, affinity-labeled membranes which were stored in liquid nitrogen. Membranes were subsequently incubated for various times in the absence or presence of inhibitors of proteolysis, and the integrity of ^{125}I-labeled hormone–receptor complexes was assayed by SDS–PAGE.

Digestion of Affinity-Labeled Receptors with Neuraminidase. ^{125}I-hGH was covalently coupled to somatogenic receptors on hepatocytes from female rats as described above. Crude membranes (1 mg/mL) containing affinity-labeled receptors were digested with neuraminidase (0.5 unit/mL) for 2 h at 37 °C in HBSS, pH 7.4 (Figure 8), or in 100 mM sodium acetate buffer, pH 5.0, with identical results. Control membranes were affinity labeled and then incubated in the absence of neuraminidase as described above. Membranes were sedimented by centrifugation (185000*g*, 15 min) and suspended in SDS–PAGE buffer prior to electrophoresis and autoradiography as described below.

Gel Filtration. Membranes containing affinity-labeled receptors were shaken in 50 mM Tris-HCl buffer/1% Triton X-100 (pH 7.4) for 60 min at 4 °C and centrifuged (100000*g*, 60 min). Part of the supernatant was applied to a 1 × 100 cm column of Sepharose 6B equilibrated with 50 mM Tris-HCl/0.1% Triton X-100 (pH 7.4) (Figure 5). Alternatively, hormone-receptor complexes were reduced with 50 mM DTT (15 min, 4 °C) and applied to the same column equilibrated and developed with 50 mM Tris-HCl, 0.1% Triton X-100, and 0.5 mM DTT, pH 7.4 (Figure 6). The void volume (V_0) of the column (38.5 mL) was measured with blue dextran. The column volume (V_t = 74.5 mL) was measured with DTNB. One-milliliter fractions were assayed for ^{125}I in a γ counter. Protein in fractions containing soluble receptor was precipitated with 5% trichloroacetic acid and centrifuged (1000*g*, 20 min). Protein precipitates were neutralized with 0.1 N sodium hydroxide and solubilized in extraction medium in preparation for gel electrophoresis.

Electrophoresis and Autoradiography. Samples were analyzed on 1.5-mm linear gradient gels of 4–20% acrylamide [acrylamide:bis(acrylamide) ratio of 100:1] at 25-mA constant current for 4–5 h using the discontinuous buffer system described by Laemmli (1970). For two-dimensional electrophoresis, the first dimension was conducted in a tube of 5% acrylamide [acrylamide:bis(acrylamide) ratio of 100:1] with a 3% stack in the absence of reductant. After electrophoresis, each tube gel was incubated in 0.125 M Tris-HCl (pH 6.8) containing 1% SDS and 100 mM DTT for 30 min at room temperature and then for an additional 10 min at 80 °C. The tube gel was connected to a 5–15% gradient polyacrylamide gel [acrylamide:bis(acrylamide) ratio of 37.5:1] with 1% agarose. Electrophoresis in the second dimension was conducted overnight at 5 mA. Gels were stained for 1 h in 0.5% Coomassie Blue R dissolved in 25% isopropyl alcohol and 7% acetic acid and dried before autoradiography. The molecular weight standards run in parallel with experimental samples were as follows: myosin (M_r 200 000), β-galactosidase (M_r 116 250), phosphorylase *b* (M_r 92 500), bovine serum albumin (M_r 66 200), ovalbumin (M_r 45 000), carbonic anhydrase (M_r 31 000), and soybean trypsin inhibitor (M_r 21 000). The positions of these standards are shown to the left of each autoradiograph. The apparent molecular weights of cross-linked complexes are to the right of each autoradiograph. Autoradiography was carried out in cassettes at −80 °C with Kodak X-Omat XAR-2 film using Dupont Cronex Lightning Plus enhancing screens. Lead plates ($^1/_8$th in. thick) between cassettes prevented fogging of films. The proportion of radioactivity that fractionated into intact or degraded growth hormone-receptor complexes was quantitated by densitometry of autoradiographs with a Helena Laboratories Quick Scan Jr. densitometer.

Results

One explanation for the observation of low molecular weight complexes in affinity labeling experiments is that the growth hormone receptor is labile and can be degraded. To test for proteolysis, membranes containing affinity-labeled receptors were incubated at 25 °C for various times to permit the formation of degradation products. In samples subsequently reduced with dithiothreitol, M_r 63 000 complexes accumulated (Figure 1, lanes a–e). Thus, after 0 and 60 min of aging, the proportions of M_r 130 000 to M_r 63 000 complexes were 2:1

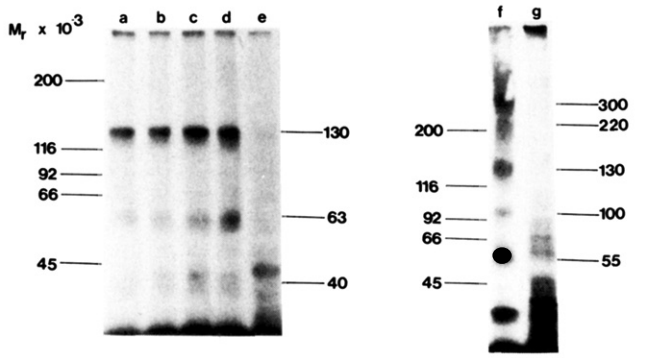

FIGURE 1: Proteolysis of the affinity-labeled growth hormone receptor. Growth hormone receptors were affinity labeled after incubation of hepatocytes with ^{125}I-hGH (5 nM) + prolactin (1 μM) (lanes a–d and f) or ^{125}I-hGH (5 nM) + hGH (1 μM) (lanes e and g). Crude membranes from these cells were then incubated in HBSS at 25 °C for 0 (lane a), 15 (lane b), 30 (lane c), or 60 min (lanes d–g) before solubilization into SDS-containing medium. Equal amounts of radioactive label that was (lanes a–e) or was not (lanes f and g) reduced with dithiothreitol were fractionated by SDS–PAGE.

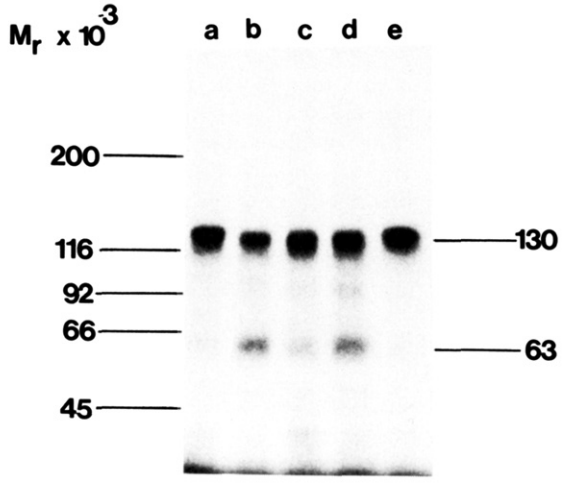

FIGURE 2: Effects of enzyme inhibitors on receptor proteolysis. Growth hormone receptors were affinity labeled after incubation of hepatocytes with ^{125}I-hGH (5 nM) and prolactin (1 μM). Membranes from these cells were incubated for 60 min at 4 °C (lane a) or 25 °C (lanes b–e) in the absence or presence of inhibitors of proteolysis: lane b, no addition; lane c, 10 mM PMSF; lane d, 0.14 mg/mL soybean trypsin inhibitor; lane e, 50 μg/mL leupeptin. Equal amounts of radioactive label from these membranes were reduced with dithiothreitol and fractionated by SDS–PAGE.

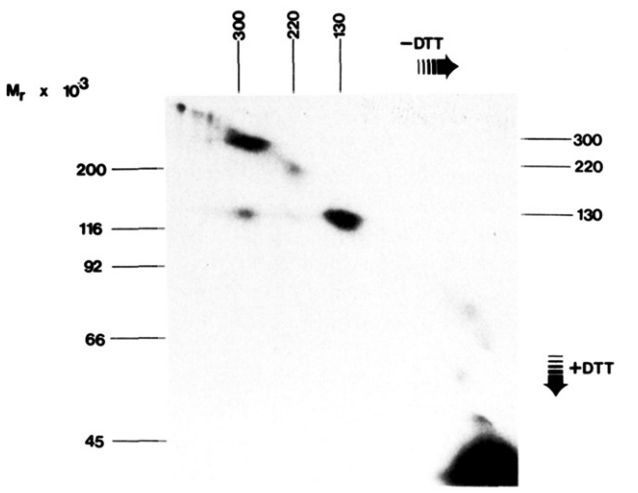

FIGURE 3: Two-dimensional gel electrophoresis of growth hormone receptors. Growth hormone receptors were affinity labeled after incubation of hepatocytes with ^{125}I-hGH (5 nM) and prolactin (1 μM). Two-dimensional gel electrophoresis was then conducted as described under Methods.

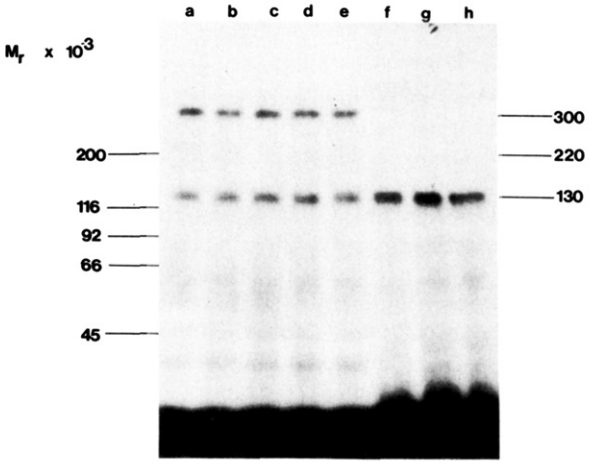

FIGURE 4: Effect of dithiothreitol on the growth hormone receptor. Growth hormone receptors were affinity labeled after incubation of hepatocytes with ^{125}I-hGH (5 nM) and prolactin (1 μM). After extraction into SDS-containing medium, samples were boiled for 5 min in the presence of the following concentrations of dithiothreitol: lane a, 0; lane b, 0.01 mM; lane c, 0.05 mM; lane d, 0.2 mM; lane e, 1 mM; lane f, 5 mM; lane g, 20 mM; lane h, 100 mM. Equal amounts of radioactive label from each incubate were fractionated by SDS–PAGE.

and 1.3:1, respectively. In some incubates, a less prominent degradation product of M_r 40 000 was also observed. In nonreduced samples, complexes of M_r 100 000 and 55 000 were detected (lanes f and g) which also accumulated with time (not shown). Isolation of the 63 000-dalton species and the other degraded complexes was inhibited by unlabeled growth hormone, suggesting that these resulted from specific interaction of ^{125}I- hGH with receptors. The formation of the 63 000-dalton species was also inhibited by conducting incubations at low temperature or by PMSF or leupeptin (Figure 2). Soybean trypsin inhibitor was less effective in preventing formation of the 63 000-dalton complex than other inhibitors of serine proteases.

Our results suggest that growth hormone is incorporated into nondegraded complexes of M_r 300 000, 220 000, and 130 000 on rat hepatocytes. The proportions of the M_r 300 000 and 220 000 complexes to the M_r 130 000 complex varied considerably from one cell preparation to another [(1.2 ± 0.6):(0.24 ± 0.09):1, respectively; n = 2]. Exposure to reductant results in loss of the 300 000- and 220 000-dalton complexes with an increase in the amount of 130 000-dalton complexes isolated by SDS–PAGE. However, analysis by one-dimensional gel electrophoresis is insufficient to determine if 130 000-dalton species are constituents of 300 000- and 220 000-dalton complexes. In order to address this issue, affinity-labeled growth hormone receptors were fractionated by two-dimensional SDS-PAGE (Figure 3). The first dimension, conducted in the absence of reductant, separated the M_r 300 000, 220 000, and 130 000 species. Exposure to dithiothreitol in the second dimension resulted in isolation of a 130 000-dalton complex derived from the 300 000- and 220 000-dalton complexes.

The relationship of intact growth hormone–receptor complexes to one another was further characterized by systematically varying the concentration of reductant to which they were exposed (Figure 4). Since the amount of M_r 220 000 complexes varies from one hepatocyte preparation to another, it was possible to select a cell preparation that contained very little of this species. Under this condition, changes in the amount of the M_r 220 000 moiety would be easily observed

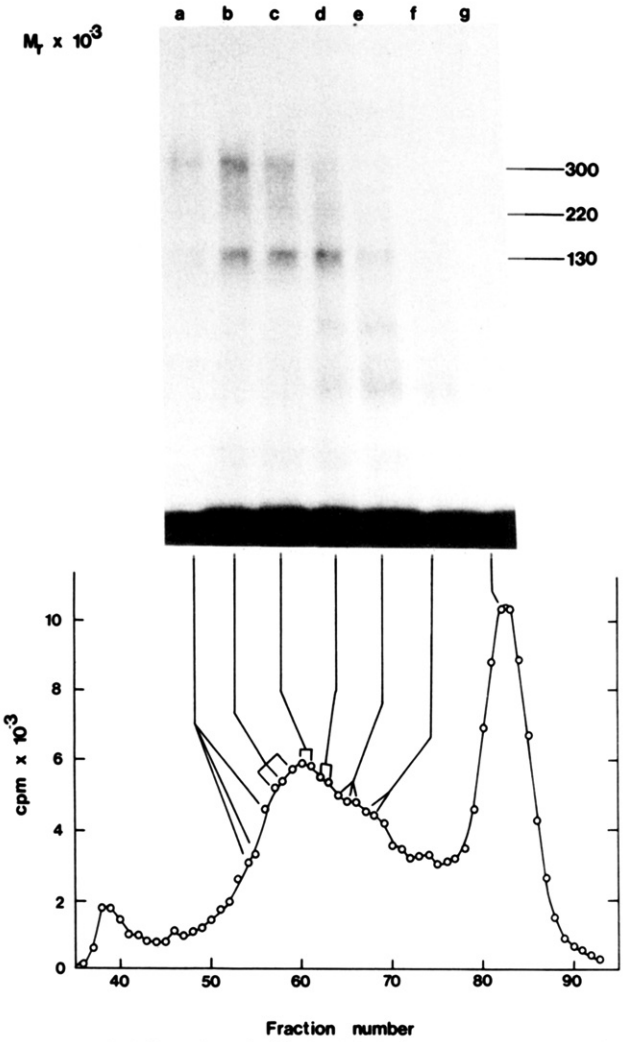

FIGURE 5: Gel filtration of affinity-labeled receptor not exposed to reductant. Growth hormone receptors were affinity labeled after incubation of hepatocytes with ^{125}I-hGH (5 nM) and prolactin (1 μM). Complexes solubilized from crude membranes isolated from these cells were fractionated on a column of Sepharose 6B as detailed under Methods. Fractions (1 mL) from the column were assayed for ^{125}I (bottom) or combined (as indicated in the figure) and fractionated by SDS–PAGE under nonreducing conditions (top).

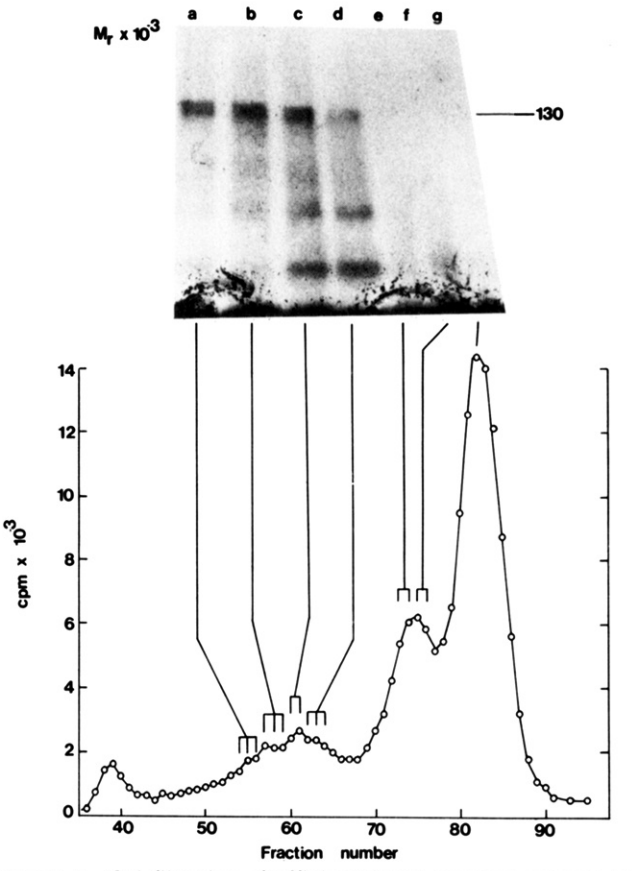

FIGURE 6: Gel filtration of affinity-labeled receptors exposed to reductant. Growth hormone receptors were affinity labeled after incubation of hepatocytes with ^{125}I-hGH (5 nM) and prolactin (1 μM). Hormone–receptor complexes solubilized from crude membranes isolated from these cells were reduced (Methods) and fractionated sequentially by gel filtration and SDS–PAGE under reducing conditions.

if it were produced on reduction of the M_r 300 000 complex. Low concentrations of dithiothreitol did not release detectable amounts of M_r 220 000 from M_r 300 000 complexes. Under fully reducing conditions, 100 000-dalton subunits were the sole intact receptor constituent released from higher molecular weight complexes. Thus, monomeric M_r 100 000 subunits are retained in higher molecular weight complexes by interchain disulfide bonds.

The isolation of 300 000-, 220 000-, and 130 000-dalton complexes from cells not exposed to reductant suggests that all of the components of somatogenic receptors may not be retained in aggregates by covalent (disulfide) bonds. Therefore, experiments were conducted to determine if noncovalent forces could also maintain the structure of somatogenic receptors. Membranes containing affinity-labeled growth hormone receptors were solubilized and fractionated on a column of Sepharose 6B (Figure 5). Radioactive label from the column was isolated in a broad peak centered around fraction 60 (K_{av} = 0.384, M_r 560 000) and in a second peak at fraction 82, corresponding to the elution of ^{125}I-hGH. SDS–PAGE showed that growth hormone-receptor complexes of 130 000 daltons eluted with complexes of M_r 300 000 and 220 000 in fractions 54–64; 130 000-dalton complexes and receptor degradation products of M_r 100 000 and 55 000 (Figures 1 and 2) were in fractions 64–68.

Affinity-labeled complexes were also fractionated by gel filtration after reduction of disulfide bonds with dithiothreitol (Figure 6). Radioactive label was eluted in the same fractions (54–64) that contained higher molecular weight complexes in unreduced samples. However, SDS–PAGE demonstrated that reduction had eliminated all M_r 300 000 and 220 000 species in these fractions. Therefore, 130 000-dalton complexes exhibited chromatographic behavior indistinguishable from those of the species from which they were derived. As observed under nonreducing conditions, specific degradation products of M_r 63 000 and 40 000, that were not detected incubates containing protease inhibitors, were in the tail of the peak containing growth hormone receptors. In reduced samples, fractions 71–90 contained modified growth hormone released from complexes into which it had not been covalently bound. In summary, elution from gel filtration columns suggests that growth hormone receptor constituents aggregate and assume properties of high molecular weight species in detergent solution.

To determine if membrane glycoproteins affect growth hormone–receptor interactions, hepatocytes were digested with neuraminidase which reduced specific hormone binding (not shown) and inhibited the formation of specific growth hormone–receptor complexes of M_r 300 000, 220 000, and 130 000 (Figure 7). That the growth hormone receptor itself is a glycoprotein was demonstrated by digesting affinity-labeled complexes with neuraminidase. Such treatment increased the

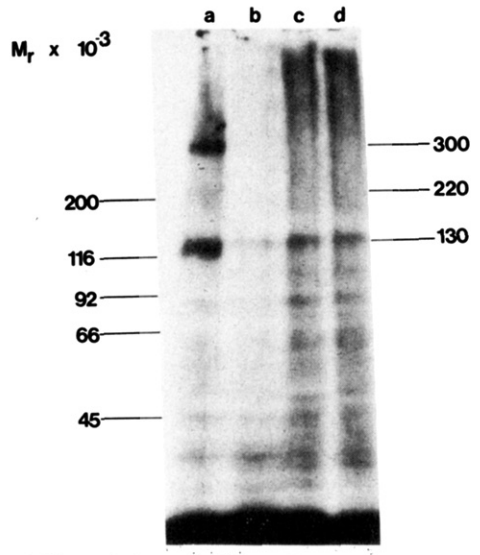

FIGURE 7: Effect of glycosidases on affinity labeling of the growth hormone receptor. Hepatocytes were incubated in HBSS (lanes a and b) or HBSS containing 50 μg/mL neuraminidase (lanes c and d) for 20 min at 25 °C. The cells were washed with HBSS and resuspended in HBSS/1% BSA containing 5 nM ^{125}I-bGH (lanes a and c) or ^{125}I-bGH + bGH (1 μM) (lanes b and d). After a 60-min incubation at 25 °C, bound hormone was covalently coupled to membrane receptors. Equal amounts of radioactive label from these cells were fractionated by SDS–PAGE.

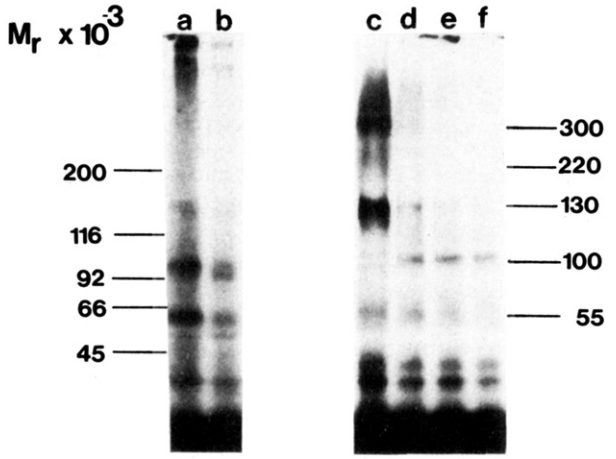

FIGURE 8: Digestion of affinity-labeled receptors with neuraminidase and the time course of receptor proteolysis at 37 °C. Crude membranes containing affinity-labeled somatogenic receptors were incubated for 120 min at 37 °C in the absence (lane a) or presence (lane b) of neuraminidase. In a separate experiment, crude membranes that contained affinity-labeled receptors were incubated in HBSS at 37 °C for 0 (lane c), 30 (lane d), 60 (lane e), or 120 (lane f) min. Equal amounts of radioactive label from membranes not exposed to reductant were fractionated by SDS–PAGE.

mobility of intact (M_r 130 000) and degraded (M_r 100 000) receptor components on SDS–PAGE (Figure 8, lanes a and b).

In the previous experiment, incubation of affinity-labeled receptors at 37 °C for 2 h in the absence or presence of neuraminidase resulted in complete loss of M_r 300 000 and 220 000 complexes, isolation of low levels of M_r 130 000 complexes, and recovery of substantial amounts of growth hormone in M_r 100 000 degradation products (Figure 8, lanes a and b). The time course of receptor proteolysis at 37 °C showed that within 1 h M_r 300 000 and 220 000 complexes were no longer apparent and the amount of M_r 130 000 complexes was substantially diminished with the concomitant isolation of M_r 100 000 degradation products (Figure 8, lanes c–f). Thus, the conditions necessary for neuraminidase digestion also produced substantial receptor proteolysis.

Discussion

Attempts to identify subunits of the growth hormone receptor have yielded perversely variable results. In affinity labeling experiments with rat hepatocytes (Donner, 1983; Yamada & Donner, 1984), rat adipocytes (Carter-Su et al., 1984; Gorin & Goodman, 1984), and human IM-9 lymphocytes (Hughes et al., 1983), ^{125}I-hGH bound to high molecular weight species of 280 000, 200 000, and 100 000. All of these receptor components were not identified in each type of cell, however, and when present, appeared so in varying proportions relative to one another and to less abundant lower molecular weight species of undetermined significance. In contrast, predominant lower molecular weight (50 000–67 000) constituents of growth hormone receptors are observed in pregnant rat liver (Hughes & Friesen, 1983), rabbit mammary gland (Hughes & Friesen, 1983), and in liver from pregnant (Tsushima et al., 1982) or nonpregnant rabbits (Haeuptle et al., 1983). The 300 000-dalton growth hormone receptor purified from pregnant rabbit liver is reported to contain a 75 000–80 000-dalton subunit (Waters & Friesen, 1979). The isolation of apparently distinct structural forms of growth hormone receptors from various sources, the pleiotropic actions of growth hormone (Isaksson et al., 1985), and the distinguishable binding and biological properties of hormone variants and fragments (Hughes & Friesen, 1985) have led to the proposition that more than a single type of receptor may exist for growth hormone.

In this study, ^{125}I-hGH was incorporated into covalent complexes of M_r 300 000, 220 000, and 130 000 on hepatocytes from female rats. Less predominant complexes of M_r 100 000 and 55 000 (isolated as M_r 63 000 and 40 000 species in reduced samples) were also present. In an experiment in which affinity-labeled membranes were permitted to age, the amount of the lower molecular weight species increased with time. The accumulation of lower molecular weight complexes was prevented by inhibitors of serine proteinases or by low temperature. The effects of time, temperature, and enzyme inhibitors on the recovery of various lower molecular weight species from affinity labeling demonstrate that the growth hormone receptor is labile and degraded by hepatic proteases.

Receptors are sensitive to proteolysis, and it can be difficult to distinguish receptor subunits from degradation products. For example, in leaky cells or in membranes contaminated with lysosomal proteases, the β subunit of the insulin receptor is degraded to a 45 000-dalton fragment (Massague et al., 1981). Thus, while it has been proposed that the insulin receptor contains a subunit of M_r 40 000 (Yip & Moule, 1983), it is generally accepted that the receptor is a highly conserved heterotetrameric structure that contains only two types of intact subunits of M_r 130 000 and 90 000 (Massague & Czech, 1982). The demonstrated lability of the growth hormone receptor leads to the proposal that the structure of this moiety may be more highly conserved than has been previously recognized and that proteolysis can account for many apparent structural variances.

The insulin receptor contains interchain disulfide bonds that hold α subunits to one another or to β subunits (Massague & Czech, 1982). The distinct classes of disulfide bonds in the insulin receptor are differentially labile to reductant. For this reason, exposure to various concentrations of dithiothreitol releases partially reduced components (for example, α–β complexes) from the intact receptor (Massague & Czech, 1982). Careful characterization of the relationship of intact

to partially reduced receptor complexes was useful in demonstrating the presence of a β subunit and for defining the subunit structure of the insulin receptor (Massague & Czech, 1982).

The relationship of the nondegraded M_r 300000, 220000, and 130000 growth hormone receptor components to one another has been investigated by systematic exposure of affinity-labeled membranes to various concentrations of reductant. Reducing conditions diminished the amount of the 300000- and 220000-dalton species and augmented the amount of 130000-dalton complexes in membranes. It was not possible to trap partially reduced intermediates, implying that the disulfide bonds that retain receptor subunits in larger aggregates are equally sensitive to reduction and that a receptor subunit not labeled by growth hormone probably does not exist. The 100000-dalton receptor subunit, the smallest nondegraded receptor component identified under fully reducing conditions, is contained in the 300000- and 220000-dalton growth hormone-receptor complexes. These observations lead us to propose that the growth hormone receptor is an aggregate of M_r 100000 subunits. The varying amounts of 300000-, 220000-, and 130000-dalton complexes in hepatic (Donner, 1983; Yamada & Donner, 1984), adipose (Carter-Su et al., 1984; Gorin & Goodman, 1984), and lymphatic (Hughes & Friesen, 1983) cells may indicate that the common 100000-dalton receptor subunit can be aggregated differently in these tissues and may also result from the sensitivity of receptor components to proteolysis.

Reduction of interchain disulfide bonds did not affect the elution of growth hormone receptor constituents from gel filtration columns. This suggests that noncovalent forces as well as covalent bonds may play a role in stabilizing the structure of the growth hormone receptor. An important byproduct of these observations is the demonstration that gel filtration was able to resolve intact, higher molecular weight receptor components from lower molecular weight species now identified as degradation products. In this study, it is also demonstrated that the growth hormone receptor is a sialoglycoprotein (Figure 8; Waters & Friesen, 1979; Tsushima et al., 1980; Asakawa et al., 1986) and that growth hormone-receptor interactions are affected by membrane glycoproteins. These observations are important to understanding the structure and purification of the growth hormone receptor. Partial purification may be effected on chromatography media that specifically retains glycoproteins. After affinity purification, gel filtration could resolve intact receptor from copurified degradation products.

Registry No. hGH, 9002-72-6; serine proteinase, 37259-58-8; neuraminidase, 9001-67-6.

REFERENCES

Asakawa, K., Hedo, J. A., McElduff, A., Rouiller, D. G., Waters, M., & Gorden, P. (1986) *Biochem. J. 238*, 379–386.

Barrazone, P., Lesniak, M. A., Gorden, P., Van Obberghen, E., Carpentier, J.-L., & Orci, L. (1980) *J. Cell Biol. 87*, 360–369.

Berry, M. N., & Friend, D. S. (1969) *J. Cell Biol. 43*, 506–520.

Carter-Su, C., Schwartz, J. & Kikuchi, G. (1984) *J. Biol. Chem. 259*, 1099–1104.

Donner, D. B. (1983) *J. Biol. Chem. 258*, 2736–2743.

Donner, D. B., Nakayama, K., Tani, S., Lutz, U., & Sonenberg, M. (1978) *J. Biol. Chem. 253*, 6717–6723.

Fagin, K. D., Lackey, S. L., Reagan, C. R., & DiGirolamo, M. (1980) *Endocrinology (Baltimore) 107*, 608–615.

Gorin, E., & Goodman, H. M. (1984) *Endocrinology (Baltimore) 114*, 1279–1286.

Haeuptle, M.-T., Aubert, M. L., Djiane, J., & Kraehenbuhl, J.-P. (1983) *J. Biol. Chem. 258*, 305–314.

Herington, A. C., Phillips, L. S., & Daughaday, W. H. (1976) *Metab., Clin. Exp. 25*, 341–353.

Hughes, J. P., & Friesen, H. G. (1985) *Annu. Rev. Physiol. 47*, 469–482.

Hughes, J. P., Simpson, J. S. A., & Friesen, H. G. (1983) *Endocrinology (Baltimore) 112*, 1980–1985.

Isaksson, O. G. P., Eden, S., & Jansson, J.-O. (1985) *Annu. Rev. Physiol. 47*, 483–499.

Jacobs, S., Hazum, E., Shecter, Y., & Cuatrecasas, P. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 4918–4921.

Jacobs, S., Hazum, E., & Cuatrecasas, P. (1980) *J. Biol. Chem. 255*, 6937–6940.

Johnson, G. L., MacAndrew, V. I., & Pilch, P. F. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 875–878.

Kelly, P. A., Posner, B. I., Tsushima, T., & Friesen, H. G. (1974) *Endocrinology (Philadelphia) 95*, 532–539.

Laemmli, U. K. (1970) *Nature (London) 227*, 680–685.

Linsley, P. S., Blifeld, C., Wrann, M., & Fox, C. F. (1979) *Nature (London) 278*, 745–748.

Linsley, P. S., Das, M., & Fox, C. F. (1981) *Recept. Recognit., Ser. B 11*, 89–114.

Massague, J., & Czech, M. P. (1982) *J. Biol. Chem. 257*, 5038–5045.

Massague, J., & Czech, M. P. (1982) *J. Biol. Chem. 257*, 6729–6738.

Massague, J., Pilch, P., & Czech, M. P. (1981) *J. Biol. Chem. 256*, 3182–3190.

Murphy, L. J., Vrhovsek, E., & Lazarus, L. (1983) *Endocrinology (Baltimore) 113*, 750–757.

Pilch, P. F., & Czech, M. P. (1979) *J. Biol. Chem. 254*, 3375–3381.

Pilch, P. F., & Czech, M. P. (1980) *J. Biol. Chem. 255*, 1722–1731.

Posner, B. I. (1976) *Endocrinology (Philadelphia) 98*, 645–654.

Posner, B. I., Kelly, P. A., Shiu, P. C., & Friesen, H. G. (1974) *Endocrinology (Philadelphia) 95*, 521–531.

Ranke, M. B., Stanley, C. A., Rodbard, D., Baker, L., Bongiovanni, A., & Parks, J. S. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 847–851.

Saxena, B. B., & Henneman, P. H. (1966) *Biochem. J. 100*, 711–717.

Tsushima, T., Sasaki, N., Imai, Y., Matsuzaki, F., & Friesen, H. G. (1980) *Biochem. J. 187*, 479–492.

Tsushima, T., Murakami, H., Wakai, K., Isozaki, O., Sato, Y., & Shizume, K. (1982) *FEBS Lett. 147*, 49–53.

Wallis, M. (1980) in *Cellular Receptors for Hormones and Neurotransmitters* (Schulster, D., & Levitski, A., Eds.) Wiley, New York.

Waters, M. J., & Freisen, H. G. (1979) *J. Biol. Chem. 254*, 6815–6825.

Yamada, K., & Donner, D. B. (1984) *Biochem. J. 220*, 361–369.

Yamada, K., & Donner, D. B. (1985) *Biochem. J. 228*, 383–390.

Yip, C. C., & Moule, M. L. (1983) *Diabetes 32*, 760–767.