Super hormones

Raymond W. Ruddon

The glycoprotein hormones are a group of evolutionarily conserved hormones involved in the regulation of reproduction and metabolism. They are present in species as diverse as eels and humans. This family of hormones includes the gonadotropins follicle-stimulating hormone (FSH) and luteinizing hormone (LH) as well as thyroid-stimulating hormone (TSH)—all produced in the pituitary gland—and chorionic gonadotropin (CG), produced in the placentas of horses, higher apes, and humans. All of them are heterodimers, consisting of an α-subunit (~15 kDa) and a β-subunit (20–25 kDa), held together by tight hydrophobic bonds. Formation of the heterodimer is necessary for full biological activity. All of these hormones share, within a given species, a common α-subunit and a β-subunit that is specific for each hormone[1].

Because these hormones have important therapeutic potential for the treatment of fertility problems, thyroid diseases, and cancer, investigators have for some time been trying to understand how to enhance receptor binding activity and in vivo biological activity[2–6]. This work has provided information on how chemical manipulations of the subunits affect receptor binding affinity, signal transduction (in the case of glycoprotein hormones, stimulation of adenylyl cyclase), and in vivo pharmacokinetics. Although the nature of the glycoprotein hormone–receptor contacts that lead to receptor activation have been modeled[6–7], the precise structural determinants of hormone–receptor interactions have not yet been defined. However, several modifications of the oligosaccharides have been made that increase in vivo bioactivity[2–5]. These increases in activity are based mostly on increases in plasma half-life due to decreased rates of clearance.

Up until now, there have been no reports indicating a way to produce a glycoprotein hormone with both increased receptor binding activity and in vivo bioactivity. In this issue, Szkudlinski et al.[8] have generated by site-directed mutagenesis a series of "super-active analogs" of the human glycoprotein hormones human TSH and human CG. The design of these analogs was based on the observation that the common α-subunit, though highly conserved, differs among species in a domain containing amino acid residues 11 to 21. In lower species, from eels up through Old World monkeys, there are basic amino acids lysine or arginine at positions 11, 13, 16, and 20, whereas in orangutans, chimpanzees, and humans, these positions are occupied by threonine, glutamine, proline, and glutamine, respectively.

On the basis of previous observations that the 11–21 region is not involved in α–β contact sites (from the crystal structure[9,10] and antibody reactivity[6]) and that a loop of the α-subunit containing amino acids 16–23 appears to be involved in receptor binding[7], Szkudlinski et al. hypothesized that by substituting basic amino acids for neutral ones in this region, a weak, hydrogen bond-type, hormone–receptor interaction would be replaced by a stronger electrostatic interaction with acidic amino acids of the receptor. Thus, they produced hormone heterodimers containing these mutant α-subunits substituted by lysine at positions 13, 16, and 20 or 13, 14, 16, and 20. These mutant α-subunits were then co-expressed with the appropriate β-subunit in Chinese hamster ovary (CHO) cells.

Not only were receptor binding affinity and cyclic AMP formation increased in cell culture models for both human TSH and human CG activity, but also in vivo potency and efficacy were increased for TSH analogs in a mouse model for TSH-induced secretion of thyroid hormone. This is the first clear demonstration of both in vitro and in vivo increased potency and efficacy not based on changes in pharmacokinetics. For example, six hours after intraperitoneal injection, serum TSH levels in mice were reported to be similar for wild-type human TSH and the modified analogs. The significance of these results lies in the fact that glycoprotein hormones of increased potency and efficacy could improve current treatments of ovulatory dysfunction and other fertility problems as well as the treatment of certain malignant neoplasms. Moreover, studies of such hormone analogs with high receptor affinity may allow more definitive structural analyses of hormone–receptor interactions.

The reasons why hormones with decreasing potency and efficacy would evolve over time are puzzling. Szkudlinski et al. speculate that the decreased potency is related to the decreased need for gonadotropic activity due to the slower reproductive "turnover" requirements of higher species. In the case of TSH, the attenuated bioactivity may be due to the greater need to conserve iodine for thyroid hormone synthesis during long periods of fasting in the nomadic life that early hominids had to endure. While these are possible explanations, others must be considered. One of these is the relevance of the models used to test the bioactivity of human hormones. For example, all of the α–β heterodimers in this study were produced in CHO cells. The composition and structure of the oligosaccharides of the glycoproteins are likely to differ from that of human pituitary or placental cells. Asparagine-linked oligosaccharides of the glycoprotein hormones significantly affect their folding and assembly[11] as well as their ability to transduce a receptor-mediated signal[12]. Furthermore, receptor binding activity of TSH analogs was assessed using porcine receptors or with CHO cells transfected with the human TSH receptor.

These systems may not perfectly reflect the structure or membrane orientation of human receptors in vivo. In the case of the human CG analogs, the increase in potency of the most active ones for receptor binding or progesterone production was 4-fold or less, whereas the increased potency of the most active human TSH analogs for receptor binding and cyclic AMP production was almost 40-fold. This suggests that introduction of basic amino acids into the α-subunit has dissimilar effects among the glycoprotein hormones.

These reservations aside, the data of Szkudlinski et al. strongly suggest that such manipulation of the human glycoprotein hormones could lead to the production of more active analogs with markedly increased therapeutic potential. Perhaps the advent of super hormone drugs is not far off.

Raymond W. Ruddon is director at the Eppley Institute for Research in Cancer and Allied Diseases, University of Nebraska Medical Center, Omaha NE 68198-6805. He is currently on sabbatical at the Corporate Office of Science and Technology, Johnson & Johnson, 410 George Street, New Brunswick, NJ 08901 (Tel: 908 524 2567; Fax: 908 524 2549).

1. Pierce, J.G. and Parsons, T.F. 1981. *Annu. Rev. Biochem.* **50**:465–495.
2. Fares, F.A. et al. 1992. *Proc. Natl. Acad Sci. USA.* **89**:4304–4308.
3. LaPolt, P.S. et al. 1992. *Endocrinology* **131**:2514–2520.
4. Joshi, L. et al. 1995. *Endocrinology* **136**:3839–3848.
5. Grossmann, M. et al. 1995. *Mol. Endocrinol.* **9**:948–958.
6. Moyle, W.R. et al. 1995. *J. Biol. Chem.* **270**:20020–20031.
7. Jiang, X. et al. 1995. *Structure* **3**:1341–1353.
8. Szkudlinski, M.W., et al. 1996. *Nature Biotechnology* **14**:1257–1263.
9. Lapthorn, A.J. et al. 1994. *Nature* **369**:455–461.
10. Wu, H., Lutbader, J.W., Liu, Y., Canfield, R.E., and Hendrickson, W.A. 1994. *Structure* **2**:545–558.
11. Feng, W., Huth, J.R., Norton, S.E., and Ruddon, R.W. 1995. *Endocrinology* **136**:52–61.
12. Szkudlinski, M.W., Thotakura, N.R., and Weintraub, B.D. 1995. *Proc. Natl. Acad. Sci.USA.* **92**:9062–9066.