Molecular Characterization of Growth Hormone Secretagogue Receptors

Andrew D. Howard, PhD,
Scott D. Feighner, PhD, Roy G. Smith, PhD,
and Lex H. T. Van der Ploeg, PhD

CONTENTS

INTRODUCTION
OCYTE EXPRESSION CLONING
G-PROTEIN ADMINISTRATION RESTORES GHS-R COUPLING IN XENOPUS OOCYTES
GHS-R CLONING
GHS-R GENE STRUCTURE
PHARMACOLOGICAL CHARACTERIZATION
GHS-R CHROMOSOMAL LOCATION
GHS-R EXPRESSION
GHS-R RELATIONSHIP TO OTHER GPC-RS
GHS-R RESIDUES INVOLVED IN LIGAND BINDING
FUTURE DIRECTIONS
ACKNOWLEDGMENTS
REFERENCES

INTRODUCTION

The synthetic hexapeptide growth hormone releasing peptide 6 (GHRP-6) mediates growth hormone (GH) release from primary pituitary cells through a distinct mechanism from that controlled by growth hormone releasing hormone (GHRH) or somatostatin (1–3). Biochemical and pharmacological evidence supports the notion that GHRP-6 and the nonpeptide growth hormone secretagogues (GHSs) act through the same receptor. Numerous attempts to characterize the GHRP or GHS receptors (GHS-Rs) biochemically were frustrated by a low GHS-R abundance. The development of procedures for high-specific-activity [35S] radiolabeling of the nonpeptide GHS MK-0677 in conjunction with

From: Human Growth Hormone: Research and Clinical Practice
Edited by: R. G. Smith and M. O. Thorner © Humana Press Inc., Totowa, NJ

69
improved receptor preparation procedures led to the identification of a GHS-R binding site (4,5). The GHS-R bound $[^{35}\text{S}]$-MK-0677 with high affinity, and the rank order of potency of diverse peptide and nonpeptide ligands for $[^{35}\text{S}]$-MK-0677 displacement correlated with their in vivo GH secretory activity. Based on its binding characteristics the authors assumed that the GHS-R was a G protein-coupled receptor (GPC-R) found in low abundance in the anterior pituitary and hypothalamus. This data facilitated the development of a strategy to clone the GHS-R (Fig. 1). The assay for identification of the GHS-R relied on the knowledge that GHS-R activation leads to G protein-mediated activation of phosphoinositol-specific phospholipase C (PI-PLC) and subsequent calcium mobilization.

**OOCYTE EXPRESSION CLONING**

cDNAs encoding several low abundance cell membrane receptors have been isolated by functional expression either in *Xenopus* oocytes or in mammalian cells using specific assays to detect receptor-ligand interactions. These assays varied from radioligand binding to detection of intracellular calcium mobilization or secretion of a particular hormone (6,7). Cloning of the GHS-R was hampered by the relative paucity of biochemical information on the receptor protein, because of its low abundance (6 fmol/mg membrane protein), and the requirement to use primary pituitary tissue as a source for mRNA or protein since cell lines expressing the receptor were lacking. GHS-R cloning required the development of a sensitive and robust high-throughput screening assay. The ability to functionally express the GHS-R in *Xenopus* oocytes, injected with swine pituitary poly