Growth hormone (GH) secretion is regulated by indirect negative feedback mechanisms. To address whether GH has direct actions on pituitary cells, lipid signaling in GH4ZR7 somatomammotroph cells was examined. GH (EC_{50} = 5 nM) stimulated diacylglycerol (DAG) and ceramide formation in parallel by over 10-fold within 15 min and persisting for >3 h. GH-induced DAG/ceramide formation was blocked by pertussis toxin (PTX) implicating G_{i3}/G_{o} proteins and was potentiated 1.5-fold by activation of G_{i3}/G_{o}-coupled dopamine-D2S receptors, which had no effect alone. Following PTX pretreatment, only PTX-resistant G_{i3}, not G_{o} or G_{i2}, rescued GH-induced DAG/ceramide signaling. GH-induced DAG/ceramide formation was also blocked in cells expressing G_{b_{2}} blocker GRK-ct. In GH4ZR7 cells, GH induced phosphorylation of JAK2 and STAT5, which was blocked by PTX and mimicked by ceramide analogue C2-ceramide or sphingomyelinase treatment to increase endogenous ceramide. We conclude that in GH4 pituitary cells, GH induces formation of DAG/ceramide via a novel G_{i3}/G_{b_{2}}-dependent pathway. This novel pathway suggests a mechanism for autocrine feedback regulation by GH of pituitary function.

Pituitary somatotrophs synthesize and secrete GH, which acts at the liver and other tissues to stimulate IGF formation, promoting somatic growth throughout the body (1, 2). Secretion of GH is stimulated by hypothalamic GH-releasing hormone and inhibited by the hypothalamic tetradecapeptide somatostatin and by IGF. In addition, somatostatin agonists (e.g. octreotide) and dopamine-D2 agonists (e.g. bromocriptine) are used clinically to treat acromegaly (a syndrome produced by hypersecretion of GH) and to inhibit somatomammotroph growth and GH production (3). Negative feedback via autocrine actions of GH at the pituitary has been postulated (4) but is yet to be clearly demonstrated.

The GH receptor is a member of the type I cytokine receptor superfamily, related to PRL and erythropoietin receptors that homodimerize to initiate signaling (5). The GH receptor signals through the JAK2 tyrosine kinase-signal transducer and activator of transcription 5 (STAT5) transcription factor pathway to induce gene expression (6–9). Phosphorylation on residue Tyr-694 by JAK2 is obligatory for STAT5 activation (10). The two STAT5 variants, STAT5a and STAT5b, have 90% identical protein sequences and are independently regulated and activated in various cell types (11). Studies using STAT5a or STAT5b knockout mice have demonstrated that STAT5b, but not STAT5a, is required for GH-induced regulation of IGF1 and sex-specific steroidogenic enzymes in liver (11–13). While STAT5 activation is implicated in many GH actions, other signaling pathways not involving STAT5 appear to be recruited for GH-induced stimulation of other pathways including MAPK phosphorylation and phosphatidylinositol 3′-kinase or protein kinase C activation (14, 15) in a cell type-dependent manner (16).

Ceramide is a novel second messenger implicated in regulation of cell differentiation, proliferation, inflammation, and apoptosis (17–19). Ceramide plays an important role in signaling of a subgroup of cytokine receptors that includes tumor necrosis factor and interleukin-1 receptors (5, 15, 20, 21). However, the coupling of the GH/PRL-related family of receptors to ceramide has not been reported. We therefore examined whether GH might influence ceramide formation in pituitary cells as part of an autocrine feedback pathway and whether dopamine-D2 agonists would influence GH action.

Rat pituitary tumor GH4C1 cells synthesize and secrete PRL and GH and provide an excellent model of pituitary somatomammotrophs used for over 30 years (22). In this report we have identified a novel induction of DAG and ceramide formation by GH that is blocked by PTX, implicating the involvement of G_{i3}/G_{o} proteins (23). The contribution of specific G_{i3}/G_{o} subunits to GH autocrine signaling pathways was addressed using PTX-insensitive mutants of G_{i3}/G_{o} subunits transfected into GH4ZR7 pituitary cells (GH4C1 cells transfected with the dopamine-D2S receptor (24, 25)). In PTX-insensitive G protein mutants, the carboxyl-terminal ribosyl-acceptor cysteine was changed to a nonacceptor serine. The Cys-to-Ser mutation is a structurally conservative change, and the mutant G proteins remain functional following PTX pretreatment (26–28). The role of G_{b_{2}} subunits was evaluated by using the carboxyl-terminal domain of G protein-coupled receptor kinase (GRK-ct), a selective G_{b_{2}} scavenger (29). In GH4ZR7 cells, dopamine-D2S receptor activation potentiated GH-induced DAG and ceramide formation. We have identified G_{i3} and G_{b_{2}} as crucial for both GH-induced ceramide formation and dopamine-D2-induced potentiation of the GH response.
Materials and Methods

Materials—Apomorphine, dopamine, Staphylococcus aureus SMase, PTX, 1,2-dioleoyl-rac-glycerol (C18:1(n-9)), DAG, puromycin, and all other chemicals, standards, and salts were purchased from Sigma. Human GH (iodination grade) and Escherichia coli DAG kinase (13 units/mg protein) were from Calbiochem (San Diego, CA). Sera, media, and Geneticin (G418) were obtained from Invitrogen, Inc. [32P]ATP and [α-32P]CTP (>3000 Ci/mmol) were from Amersham Biosciences. Thin-layer chromatography (TLC) plates (0.25 mm thick) were purchased from Whatman. All reagents were supplied by BDH. Plasmids pY3 and pCMV-LacZ II were obtained from the American Type Culture Collection (Manassas, VA). The cDNAs encoding wild-type rat Goα1, Goα21, Goα3, and Goα5 were generously provided by Dr. Randall Reed, Johns Hopkins University, Baltimore, MD. Phospho-STAT5 (Tyr-694) antibody, phosphoprotein α STAT3 (Tyr-705) and phosphoprotein α p44/42 MAPK antibody kits were purchased from New England Biolabs (Mississauga, Ontario, Canada).

Plasmid Construction—As previously described (27), PTX-insensitive Goα5 mutants were generated by point mutation of rat cDNAs (30) encoding Goα2 and Goα3 subunits at cysteine 351 (352 for Goα2). The TGT (cysteine codon) was mutated to TCT (serine) and confirmed by Sanger dideoxynucleotide sequencing. The mutant Goα2 and Goα3 cDNAs were FLAG-tagged at the initiator ATG codon, and the cDNAs were subcloned in Kpn1/EcoRI-cut pcDNA3 (Invitrogen) to generate Goα2-PTX, Goα3-PTX, and Goα5-PTX. The carboxyl-terminal domain of OK-GRK2 cDNA (31), beginning from Thr-495, was tagged at the amino-terminal with RGS-His6, and the His-GRK-ct fragment was cloned into pcDNA3 to produce the GRK-ct construct.

Co-transfection—GH4ZR7 cells and derivative clones were maintained in Ham’s F10 medium with 8% fetal bovine serum (FBS) at 37 °C, 5% CO2, 95% air at 37 °C, grown to 80% confluency, and placed in serum-free F10 medium for 16 h. For PTX treatment, the cells were treated with 10 ng/ml PTX for 16 h prior to experimentation. Cells grown in serum-free F10 medium and treated with imental compounds at 37 °C as indicated. Following incubations, the cells were twice rinsed with ice-cold PBS and lysed extracted (32). After centrifugation at 500 × g for 1 min at 4 °C, the supernatants were aspirated and the cells were lysed with 0.5 ml of chloroform/methanol/HCl (20:40:1, v/v/v), and sonicated in 5-s intervals × 6 on ice. Cells were then centrifuged at 14,000 × g for 15 min at 4 °C. The upper aqueous layer was discarded, and the lower lipid-containing layer was transferred to a 1-ml glass solvent mixture of chloroform/acetone/methanol/acetic acid/water (10:4:3:2:1, v/v/v/v/v). Since DAG kinase can use ceramide or DAG as substrates, [32P]ceramide-phosphate represented ceramide production and [32P]phosphatic acid represented DAG production. The TLC plates were exposed to phosphor screens for 18 h, and [32P]ceramide-phosphate and [32P]phosphatic acid were quantified using the Molecular Dynamics System ImageQuaNt computer software. Results are expressed as percentage of control.

Western Blot Analysis—Cells were treated as described above. Cell pellets were frozen on dry ice/ethanol and stored at −80 °C. Samples were sonicated 10-15 s, heated at 95 °C for 5 min, and centrifuged, and 40 μl/sample loaded onto SDS-PAGE gel and electrophoresed to poly-vinylidene difluoride membrane. The membrane was blocked (1 h, room temperature), probed with primary antibody (1:1000, overnight, 4 °C), washed in TBST (10 mM Tris-HCl, pH 8, 150 mM NaCl, and 0.05% Tween 20) and incubated with horseradish peroxidase-conjugated secondary antibodies (1:2000) and horseradish peroxidase-conjugated antibody antibody (1:1000) to detect biotinylated protein markers (2 h at room temperature). The blot was then washed, incubated with Lumi GLO (1 min), and exposed to x-ray film. Exposures in the linear range (gray scale) were scanned and quantified using the UnScanIt program (Silk Scientific Inc., Orem, Utah).

Statistical Analysis—The data were analyzed by repeated measure using analysis of variance for each set of experiments. Differences of p < 0.05 were considered statistically significant.

Results

Concentration- and Time-dependent Increase in DAG/Ceramide Formation Induced by GH—The acute action of GH on endogenous DAG and ceramide levels in GH4ZR7 pituitary cells was assessed by the DAG kinase assay. The cells were washed to remove extracellular (secreted) GH and assayed in serum-free medium. GH induced a 10-fold increase in both DAG and ceramide production in a concentration-dependent manner from 10−10 to 10−7 M at 20 min with an EC50 of 5 × 10−7 M (Fig. 1). Addition of exogenous SMase (0.1 units/ml) was included as a positive control to demonstrate the hydrolysis of endogenous SM to form ceramide. The phosphorylated DAG and ceramide species co-migrated with the respective standards, confirming the identity of the products. GH (10−7 M) robustly increased both DAG and ceramide production in parallel, which was maximal within 15 min and declined but remained significantly elevated at 3 h (Fig. 2). Low levels of GH are secreted by GH4C1 cells at a rate of 0.2 ng/ml/min or 10−11 mol/liter/min (36), sufficient to reach a threshold concentration (10−9 M) for DAG/ceramide formation in 1.5 h following initiation of treatments (see “Materials and Methods”). However, GH is also metabolized, hence the actual GH concentration under culture conditions may be lower and did not appear to interfere with actions of exogenous GH.

PTX Blocks GH-induced Ceramide Production in GH4ZR7 Cells—We recently showed that in Balb/c-3T3 fibroblasts, activation of the D2S receptor induces DAG and ceramide formation that is blocked by PTX, which inactivates Gβ/γ proteins.2 Cells were pretreated with or without 10 ng/ml PTX for 16 h, a concentration that blocks Gβ/γ-mediated signaling in these cells (22). PTX treatment blocked GH-induced DAG and ceramide formation, thus implicating Gβ/γ proteins (Fig. 3). By contrast, PTX or dopamine-D2 agonist apomorphine (10−6 M) alone did not alter DAG or ceramide formation. Importantly, Liu, G., Robillard, L., Banhahsemi, B., and Albert, P. R., in press.
GH-induced Diacylglycerol and Ceramide Formation

**Fig. 1.** Concentration-dependent GH-induced DAG and ceramide formation in GH4ZR7 cells. GH4ZR7 cells were treated with 10^{-10} to 10^{-4} μM GH for 20 min. Lipids were extracted from cells, and [32P]phosphatidic acid and [32P]ceramide-phosphate were separated from other [32P]-containing lipids by TLC to assay DAG and ceramide content, respectively. A representative image of [32P]phosphatidic acid and [32P]ceramide-phosphate is shown above. Below, the data are expressed as mean ± S.E. from three independent experiments. *, p < 0.05; **, p < 0.01; and ***, p < 0.001. B, blank; C, control; Std, standard.

**Fig. 2.** Sustained GH-induced increases in DAG and ceramide production in GH4ZR cell lines. GH4ZR cell lines were treated with 10^{-7} M GH for 15 min and 3 h. Lipids were extracted from cells and separated as described under "Material and Methods", and a representative image is shown. Below, the quantified data from three independent experiments are expressed as mean ± S.E. *, p < 0.05; ***, p < 0.01. B, blank; C, control; Std, standard.

PTX treatment did not change the level of specific 125I-GH binding sites measured in crude membranes from GH4ZR7 cells. Specific 125I-GH binding was 118 ± 45 fmol/mg in GH4ZR cells (mean ± S.E., n = 3), and binding in PTX-treated cells was 104 ± 8% of control binding, indicating that blockade of GH-induced ceramide by PTX was not due to loss of receptor sites.

**Fig. 3.** GH, but not apomorphine, induces PTX-sensitive DAG and ceramide production. GH4ZR7 cells were treated with 10^{-7} M GH or 10^{-6} M apomorphine with or without PTX pretreatment or treated with SMase 0.1 unit/ml, and a representative image of labeled DAG and ceramide products of the DAG kinase assay is shown. Below, the data are expressed as mean ± S.E. from three independent experiments. *, p < 0.05; ***, p < 0.001. A, apomorphine; B, blank; C, control; G, GH; P, PTX; S, SMase, or combinations as indicated.
As a selective Gβγ scavenger (29), the carboxyl-terminal domain of G protein-coupled receptor kinase (GRK-ct) was used to examine the role of Gβγ subunits in signaling to ceramide formation. We have transfected GRK-ct into GH4ZR7 cells and identified expression of GRK-ct by Western blot (25). Neither apomorphine nor apomorphine/GH induced DAG or ceramide formation in GRK-ct cells (Fig. 9). This suggests that Gβγ subunits are necessary for ceramide formation induced by the combination of apomorphine and GH.

GH and Ceramide Enhance JAK2/STAT5 Phosphorylation in GH4ZR7 Cells—Based on the results above, we examined the influence of GH, apomorphine, PTX, and ceramide on well-known and potential downstream pathways of the GH receptor including phosphorylation of JAK2, STAT5 (Fig. 10), STAT3 or MAPK. In GH4ZR7 cells, GH alone increased phosphorylation of JAK2 (100% increase over basal) and STAT5 (40% increase), which was more strongly enhanced with both apomorphine and GH (160% increase over basal for phospho-JAK2, 90% increase for phospho-STAT5). Treatment with a ceramide analogue (C2-ceramide) or SMase (to increase endogenous ceramide) also increased JAK2 phosphorylation by 90 and 150%, and STAT5 phosphorylation by 60 and 90%, respectively. Interestingly, PTX-blocked apomorphine/GH-induced STAT5 phosphorylation by 50%, further supporting a role for the PTX-sensitive ceramide pathway in GH-induced STAT5 phosphorylation in

FIG. 4. Potentiation of GH-induced DAG and ceramide formation by dopamine-D2 receptor activation. GH4ZR7 cells were treated with 10⁻⁷ M GH, 10⁻⁶ M apomorphine, both, or 0.1 unit/ml SMase for 20 min. Above, a representative image of [³²P]phosphatidic acid and [³²P]ceramide-phosphate is shown. Below, the data are expressed as mean ± S.E. from three independent experiments. *, p < 0.05; **, p < 0.03; and ***, p < 0.01. A, apomorphine; B, blank; C, control; G, GH; S, sphingomyelinase; Std, standard; or combinations as indicated.

FIG. 5. Both GH- and apomorphine/GH-induced DAG and ceramide formation is blocked by PTX pretreatment. GH4ZR7 cells were treated as described in previous figures, and representative image of labeled DAG and ceramide products is shown above, and below averages of three experiments (mean ± S.E.), *, p < 0.03 and **, p < 0.01, compared with control. A: apomorphine; B: blank; C: control; G: GH; P: PTX; S: sphingomyelinase; Std: standard; or as indicated.

FIG. 6. GH- or apomorphine/GH-induced DAG and ceramide formation is not rescued by Gβγ-PTX. GH4ZR7 cells expressing PTX-insensitive GoGα2 were treated as indicated in previous figures. Above is a representative image of DAG and ceramide product. Below, data are expressed as mean ± S.E. of three independent experiments. *, p < 0.05; **, p < 0.03.

FIG. 7. Apomorphine/GH-induced DAG and ceramide formation is rescued by GoGα3-PTX. GH4ZR7 cells expressing PTX-insensitive GoGα3 cDNA were treated for 20 min with 10⁻⁶ M apomorphine or apomorphine and 10⁻⁷ M GH without or with PTX pretreatment (10 ng/ml, 16 h). Abbreviations are as in previous figures. Above is a representative image of labeled DAG and ceramide products, and below averaged data are expressed as mean ± S.E. *, p < 0.03 and **, p < 0.01.
with 10 previous figures. Labeled DAG and ceramide products is shown. Abbreviations are as in with PTX pretreatment (10 ng/ml, 16 h). A representative image of labeled DAG and ceramide products is shown. Abbreviations are as in previous figures.

**DISCUSSION**

**GH-induced Coupling to G Proteins and Lipid Signaling—** Our results indicate that GH induces a G protein-dependent increase in lipid metabolism to generate DAG and ceramide in GH$_4$ cells. Previous studies in pre-adipocyte Ob1771 cells (37, 38) and in pancreatic β-cells (39) have shown that GH induces DAG formation via activation of PC-PLC. By analogy, GH may activate PLC in GH$_4$ cells to induce DAG formation. Both DAG and ceramide formation were induced in parallel, suggesting interconversion between these lipids possibly via SM synthase, which can convert DAG into ceramide, leading to depletion of SM (40, 41). Alternately, DAG can activate acidic SMase to generate ceramide (42, 43). Interconversion of DAG to ceramide would account for the identical Ga$_3$ and Gβγ dependencies of GH-mediated lipid formation.

The actions of GH in GH$_4$ cells were sensitive to PTX pretreatment, indicating a role for G$_i$, G$_s$, proteins. Upon activation, GH receptors dimerize, associate with JAK2, and recruit a family of negative regulators, the SOCS (suppressors of cytokine signaling) proteins (44, 45). Coupling of the GH receptor to PTX-sensitive G proteins is relatively unexplored, and potential interactions of GH receptors or associated proteins such as SOCS proteins with G proteins remain elusive. There is some evidence that GH-like receptors interact with G proteins. In Nb2 cells, Gαo proteins labeled by PTX-mediated ADP ribosylation were cross-linked to the PRL receptor using a 16-Å cross-linking agent, but not cross-linkers with shorter molecular lengths, consistent with a direct physical interaction (46). In addition, some PTX-sensitive GH-induced responses have been reported. For example, GH-induced PC-PLC activation in Ob1771 preadipocytes (37, 38) and GH-mediated DAG formation and mitogenesis in pancreatic β-cells (39) are PTX-sensitive actions. Similarly, activation of the homologous PRL receptor in Nb2 lymphoma cells enhances PTX labeling of G$_i$ proteins (suggesting activation) and induces PTX-sensitive mitogenesis (47–49). Taken together, these results are consistent with coupling of the GH receptor to PTX-sensitive G$_i$ proteins to activate PLC thereby generating DAG, which can be converted to ceramide.

Although coupled to G$_i$, G$_s$, proteins, GH signaled differently from the G$_i$/G$_s$-coupled dopamine D2 receptor to induce PTX-sensitive DAG and ceramide formation since apomorphine alone had no effect. Nevertheless there was an interaction between GH and D2 signaling since apomorphine potentiated GH-induced lipid signaling and JAK2/STAT5 activation. Furthermore, GH- and apomorphine/GH-induced DAG and cera-
mide formation were both rescued by Goα3-PTX and blocked by
GRK-ct, suggesting a crucial role for Goα3/Gβγ for both recep-
tors. The dopamine-D2 receptor utilizes Gβ3 to mediate activa-
tion of potassium channels in pituitary cells (50) via binding of
Gβγ to the GIRK potassium channel (51), and is likely to couple to
Goα3/Gβγ in GH4 pituitary cells. The mechanism by which GH
receptors couple to Gβ3 remains to be elucidated, but GH recep-
tors appear to interact with Gβ proteins differenty from
Gα-coupled heptahetical receptors (such as adenosine or D2S
receptors). In adipocytes, GH prevented coupling of adenosine
receptor-mediated inhibition of cAMP and activation of phos-
phatidylinositol-specific-PLC and blocked PTX-induced ADP-
ribosylation (52, 53). GH may induce relocalization of Goα units,
prevent their coupling to adenyl cyclase (53, 54), and allow effi-
cient coupling to DAG/ceramide formation. Since sites of
ceramide synthesis display discrete subcellular localization
(55), differences in the localization of D2S- and GH-receptor
coupling might account for their differing effectiveness to in-
duce ceramide formation in GH4 pituitary cells. 

A Novel Pathway for GH-induced JAK2/STAT5 Activation—
Here we show that C2- ceramide and sphingomyelin induce
JAK2/STAT5 activation in GH4 cells, suggesting a link be-
tween GH-induced changes in DAG/ceramide and the classical
GH-receptor-mediated JAK2/STAT pathway. Consistent with
our results, sphingomyelin was shown to increase ceramide
levels and was found to activate JAK2 and STAT1/3 in cultured
human fibroblasts (56). Importantly, as observed for GH-medi-
ated ceramide formation, GH-induced JAK2/STAT5 activation
was enhanced by apomorphine and was partially blocked by
PTX, suggesting that both G protein-dependent and independent
pathways lead to JAK2/STAT5 activation in these cells. Thus Gβ3-mediated ceramide signaling regulates GH-induced
JAK2/STAT5 activation. 

In addition to regulating JAK2/STAT5, GH-induced cerami-
de formation may activate other signaling cascades (19). Both
GH (16, 57) and ceramide (19, 20) have been shown to activate
the MAPK cascade in other cell types, but we observed no
induction of p42/44-MAPK by either GH or ceramide in GH4
cells. Ceramide regulates other pathways including the SAPK/
JNK cascade, and several proapoptotic pathways, but the roles
of these pathways in GH4 cells is not known.

GH-mediated Autocrine Regulation of Pituitary Cells—
Multiple negative feedback pathways regulate GH secretion at
the level of the hypothalamus and pituitary. At the level of the
hypothalamus, GH inhibits GH-releasing hormone synthesis
and enhances somatostatin release, resulting in decreased GH
secretion at the pituitary (58–60). GH-induced IGF formation
is believed to be the primary negative feedback pathway to
inhibit GH synthesis in somatotrophs (1, 2). In addition, Gβγ-
coupled dopamine-D2 and somatostatin receptors also inhibit
GH secretion and somatomammotroph growth (3). It is tempt-
ing to speculate that GH may negatively regulate its own
secretion; however, evidence for a non-IGF-mediated autocrine
pituitary feedback by GH is indirect (4, 61, 62). The GH recep-
tor is expressed in rat and human anterior pituitary and binds
and internalizes radiolabeled GH, suggesting a role for GH to
regulate its secretion from the pituitary (63–66). However, the
signaling of the GH receptor in pituitary cells has not been
investigated. Our finding of a novel G protein-mediated action
of GH to induce DAG/ceramide as well as JAK2/STAT5 activa-
tion in GH4 cells suggests a role for GH in regulation of pitui-
tary function. GH4 cells are a pituitary cell strain that has
provided an important model of somatotrophs that synthesize
and secrete levels of GH that are sufficient to mediate auto-
ocrine GH-induced actions (22). Interestingly, C2-ceramide has
been shown to inhibit GH secretion from rat anterior pituitary
50. Lledo, P. M., Homburger, V., Bockaert, J., and Vincent, J. D. (1992) Neuron 8, 455–463
51. Clapham, D. E., and Neer, E. J. (1997) Annu. Rev. Pharmacol. Toxicol. 37, 167–203
52. Roupas, P., Chou, S. Y., Towns, R. J., and Kostyo, J. L. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 1691–1695
53. Doris, R. A., Kilgour, E., Houslay, M. D., and Vernon, R. G. (1998) J. Endocrinol. 158, 295–303
54. Yip, R. G., and Goodman, H. M. (1999) Endocrinology 140, 1219–1227
55. Hannun, Y. A., and Obeid, L. M. (2002) J. Biol. Chem. 277, 25847–25850
56. Maziere, C., Conte, M. A., and Maziere, J. C. (2001) PERS Lett. 507, 163–168
57. Yarwood, S. J., Sale, E. M., Sale, G. J., Houslay, M. D., Kilgour, E., and Anderson, N. G. (1999) J. Biol. Chem. 274, 8662–8668
58. Peng, X. D., Park, S., Gadela, M. E., Coschigano, K. T., Kopchick, J. J., Frohman, L. A., and Kineman, R. D. (2001) Endocrinology 142, 1117–1123
59. Kamegai, J., Unterman, T. G., Frohman, L. A., and Kineman, R. D. (1998) Endocrinology 138, 3554–3560
60. Zheng, H., Bailey, A., Jiang, M. H., Honda, K., Chen, H. Y., Trumbauer, M. E., Van der Ploeg, L. H., Schaeffer, J. M., Leng, G., and Smith, R. G. (1997) Mol. Endocrinol. 11, 1709–1717
61. Nakamoto, J. M., Gertner, J. M., Press, C. M., Hintz, R. L., Rosenfeld, R. G., and Genel, M. (1986) J. Clin. Endocrinol. Metab. 62, 822–826
62. Ross, R. J., Borgez, F., Grossman, A., Smith, R., Ngahfong, L., Rees, L. H., Savage, M. O., and Besser, G. M. (1987) Clin. Endocrinol. 26, 117–123
63. Mertani, H. C., Peuchoux, C., Garcia-Caballeras, T., Waters, M. J., and Morel, G. (1995) J. Clin. Endocrinol. Metab. 80, 3361–3367
64. Mertani, H. C., Waters, M. J., Jambeau, R., Gossard, F., and Morel, G. (1994) Neuroendocrinology 59, 483–494
65. Harvey, S., Baumbach, W. R., Sadeghi, H., and Sanders, E. J. (1993) Endocrinology 133, 1125–1130
66. Fraser, R. A., Siminoski, K., and Harvey, S. (1991) J. Endocrinol. 128, R9–R11
67. Negishi, T., Chik, C. L., and Ho, A. K. (1999) Endocrinology 140, 5691–5697