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Ectopic and Abnormal Hormone Receptors in Adrenal Cushing's Syndrome*

ANDRÉ LACROIX, NINA N′DIAYE, JOHANNE TREMBLAY, AND PAVEL HAMET

Division of Endocrinology, Department of Medicine, Research Center, Hôtel-Dieu du Centre Hospitalier de l'Université de Montréal (CHUM), Montréal, Québec, Canada H2W 1T8

ABSTRACT

The mechanism by which cortisol is produced in adrenal Cushing's syndrome, when ACTH is suppressed, was previously unknown and was referred to as being "autonomous." More recently, several investigators have shown that some cortisol and other steroid-producing adrenal tumors or hyperplasias are under the control of ectopic (or aberrant, illicit, inappropriate) membrane hormone receptors. These include ectopic receptors for gastric inhibitory polypeptide (GIP), β-adrenergic agonists, or LH/hCG; a similar outcome can result from altered activity of eutopic receptors, such as those for vasopressin (V1-AVPR), serotonin (5-HT_4), or possibly leptin. The presence of aberrant receptors places adrenal cells under stimulation by a trophic factor not negatively regulated by glucocorticoids, leading to increased steroidogenesis and possibly to the proliferative phenotype. The molecular mechanisms responsible for the abnormal expression and function of membrane hormone receptors are still largely unknown. Identification of the presence of these illicit receptors can eventually lead to new pharmacological therapies as alternatives to adrenalectomy, now demonstrated by the long-term control of ectopic β-AR- and LH/hCGR-dependent Cushing's syndrome by propanolol and leuprolide acetate. Further studies will potentially identify a larger diversity of hormone receptors capable of coupling to G proteins, adenylyl cyclase, and steroidogenesis in functional adrenal tumors and probably in other endocrine and nonendocrine tumors. (*Endocrine Reviews* **22:** 75–110, 2001)

I. Introduction

ENDOGENOUS Cushing's syndrome (CS) is characterized by clinical symptoms and signs resulting from chronic exposure to increased secretion of glucocorticoids (GCs) and other steroids by the adrenal cortex (1–3). Most frequently, endogenous CS is ACTH dependent, arising from excess ACTH production by pituitary corticotrope adenoma (Cushing's disease) or from an extrapituitary tumor secreting POMC and ACTH (ectopic ACTH syndrome); rarely, a CRH-secreting tumor causes excessive ACTH production from the pituitary (ectopic CRH syndrome). Less frequently, CS is ACTH independent, as it results from excess secretion of cortisol by benign and malignant adrenocortical tumors or hyperplasias (1–4). Rare cases of ectopic cortisol production from ovarian tumors that led to ACTH-independent CS have been described (5). Lastly, cortisol hypersensitivity with variable increases in GC receptor numbers has been proposed to explain the clinical features of CS in two patients with low or dysregulated cortisol and ACTH levels and no exposure to exogenous GC (6, 7).

The mechanisms by which cortisol is produced in adrenal CS, when ACTH is suppressed, were previously unknown and referred to as being "autonomous." Studies by several groups have now shown that some of the cortisol-producing

Address reprint requests to: André Lacroix, M.D., Division of Endocrinology, Research Center, Hôtel-Dieu du CHUM, 3840 St. Urbain Street, Montréal, Québec, Canada H2W 1T8. E-mail: andre.lacroix@umontreal.ca

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adrenal tumors or hyperplasias may actually be under the control of ectopic (or aberrant, illicit, inappropriate) hormone membrane receptors (8–10). After a brief overview of the regulation of normal adrenocortical function by its main trophic hormones and of the etiologies of adrenal CS, the present review will focus on *in vitro* and *in vivo* findings, identifying abnormalities of expression or function of receptors for various hormones in primary adrenal CS. The mechanisms regulating tissue-specific expression of eutopic membrane receptors in the normal adrenal cortex and the potential molecular alterations leading to the ectopic expression of hormone receptors in adrenocortical tumors and hyperplasias will also be discussed. The identification of abnormal membrane hormone receptors in adrenal CS has now opened the field of new therapeutic strategies to control hypercortisolism by interfering with ligand binding to these receptors and will also be presented.

II. Hormonal Regulation of the Normal Adrenal Cortex

The normal regulation of adrenocortical function has been the subject of recent reviews (11, 12) and, hence, will be discussed only briefly here. An important site of regulation of the hypothalamic-pituitary-adrenal axis (HPA) is located in neurons of the medial parvocellular part of the hypothalamic paraventricular nucleus (PVN) where CRH and arginine vasopressin (AVP) are produced and travel along their axons to the median eminence to be released in the hypophyseal portal blood system (13, 14). The binding of CRH or AVP to its respective specific receptors CRH-R1 (15) and AVP V3R (16) on corticotrophs of the anterior lobe stimulates the synthesis and maturation of POMC, leading to ACTH secretion (17). Vasoactive intestinal polypeptide (VIP) and pituitary adenylate cyclase activating peptide (PACAP), which are also produced in hypothalamic neurons, enhance CRH and ACTH release (18, 19). CRH secretion can be stimulated in the PVN by α_1-adrenoreceptor agonists, serotonin ($5\text{-}HT_{1A}$) receptor agonists, muscarinic and nicotinic receptor agonists of acetylcholine, histamine, and γ-aminobutyric acid ($GABA_A$), whereas it is inhibited by $GABA_B$ agonists (14). CRH release is also stimulated by angiotensin II (Ang-II), neuropeptide Y (NPY), cholecystokinin (CCK), and gastrin-releasing peptide, or suppressed by atrial natriuretic peptide (ANP), substance P, somatostatin, and nitric oxide (NO) (14). Several cytokines, including interleukin-1 (IL-1), tumor necrosis factor α (TNF-α), and IL-6, stimulate CRH, possibly through the production of prostaglandins in brain vascular endothelium (20). ACTH secretion can also be modulated by paracrine/autocrine interactions, as corticotroph cells have been shown to express CRH, which can effectively stimulate ACTH release (21).

ACTH binds to its G protein-coupled membrane melanocortin type 2 receptor (22, 23) to elicit short-term (acute) and long-term (chronic) specific responses, as illustrated in Fig. 1 (24, 25). Activation of the adenylyl cyclase (AC)/cAMP/cAMP-dependent protein kinase (PKA) pathway leads to the phosphorylation of proteins that regulate the early and late steps of steroidogenesis (26, 27). ACTH rapidly (within a few minutes) promotes the mobilization and transfer of free cholesterol to the inner mitochondrial membrane (27). Cloning of the steroidogenic acute regulatory (StAR) protein (28), the subsequent finding of mutations in the StAR gene responsible for the steroid deficiency disease, lipoid adrenal congenital hyperplasia (29, 30), as well as the knockout of this gene in the mouse (31) have identified this ACTH-inducible protein as a key modulator of cholesterol transport into mitochondria. A second protein involved in this process is the peripheral-type benzodiazepine receptor (PBR), which completes the final step of cholesterol delivery to CYP11A1 ($P450_{scc}$) for transformation into pregnenolone (32, 33). ACTH also up-regulates the immediate early genes c-*fos* and c-*jun* via the PKA pathway (25, 34, 35). A positive feedback loop for the long-term effects of ACTH is established by the hormone up-regulating its own receptor (36, 37).

The chronic effects of ACTH require several hours and involve transcriptional and/or posttranscriptional regulation of most genes coding for steroidogenic enzymes, such as CYP11A1, 3β-hydroxysteroid dehydrogenase II (3β-HSD), CYP 17 ($P450_{c17}$), CYP21A2 ($P450_{c21}$), and CYP11B1 ($P450_{c11}$) (24, 26, 38). This long-term regulation is complex, as no clear correlation exists between mRNA and protein levels of steroidogenic enzymes *in vivo* (25).

Many ACTH effects are mediated by specific transcription factors (TFs), including orphan nuclear receptors such as nur77 (also called NGFI-B) (39) or steroidogenic factor 1 (SF-1) (40, 41). Indeed, stressful stimuli induce SF-1 and nur77 transcription in corticotrophs and in the adrenal cortex (39, 42). Nur77 and SF-1 both modulate the expression of steroidogenic enzyme genes in the adrenal cortex, nur77 being activated by dephosphorylation and SF-1 by putative PKA-dependent phosphorylation (41, 43, 44).

As an example, SF-1 is involved in the regulation of CYP YP11A (45–48) and CYP17 (49, 50), where it has been postulated to play a role in constitutive and cAMP-regulated expression. The analysis of the promoter regions of these genes has led to the identification of cAMP-responsive sequences (CRS) and TFs that bind them or synergize cAMP-dependent transcription; general TFs, as cAMP response element (CRE)-binding (CREB) protein and the homeodomain protein Pbx1, both bind CRS and drive cAMP-dependent expression of steroidogenic genes (51–55). Another ubiquitous TF, Sp1, was shown to regulate basal and cAMP-dependent expression of the CYP11A gene (56). Recent data have suggested that SF-1 is able to mediate cAMP-induced transcription of the CYP17 gene: the proximal CRS (CRS2: −80 to −40) has been identified as a SF-1 binding site (57); moreover, a dominant negative mutation preventing SF-1 binding suppresses cAMP-regulated expression of a reporter gene (58). The coactivator CREB-binding protein (CBP/p300) has been proposed to integrate the effects of TFs such as SF-1, Sp1, CREB, and probably Pbx1 for the regulation of CYP11A and CYP17 genes (59, 60). Moreover, nur77 and nurr1 (nur-related factor 1) positively regulate POMC expression in the pituitary (61, 62). SF-1 up-regulates StAR expression and activity (63). Knockout nur77−/− mice demonstrate no remarkable phenotype (64), suggesting that other members of the nur family play redundant roles, perhaps in humans as well. In contrast, SF-1 appears to be essential for the devel-

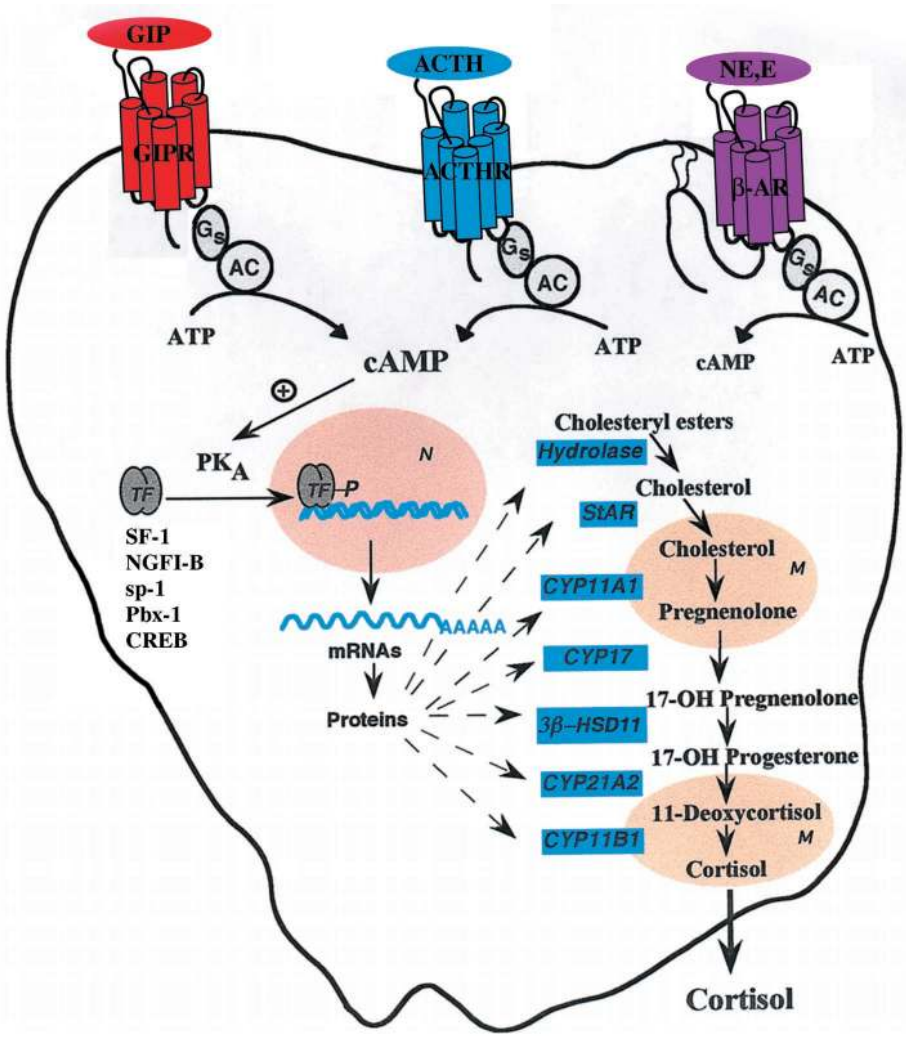

FIG. 1. Regulation of steroidogenesis by ectopic hormone receptors in fasciculata cells of adrenal CS. ACTH is the physiological modulator of steroidogenesis in the adrenal cortex. Binding to its receptor (ACTHR) activates AC and leads to cAMP production with cAMP-dependent protein kinase (PKA) activation and phosphorylation of specific TFs (SF-1, NGFI-B, Sp-1, Pbx-1, CREB) that regulate free cholesterol availability and steroidogenic enzymes expression. ACTH also regulates the early steps of steroid synthesis by the direct activation of CYP enzymes. The ectopic expression of membrane hormone receptors functionally coupled to steroidogenesis confers inappropriate sensitivity to adrenocortical cells either to GIP, to catecholamines (E, NE), or to other hormones (LH/hCG, TSH, etc. . .). These ectopic or abnormal receptors probably regulate steroidogenesis in adrenal CS by mimicking the cellular events triggered by ACTHR activation. N, Nucleus; M, mitochondria; E, epinephrine; NE, norepinephrine. [Modified with permission from N. N'Diaye *et al.*: *Horm Metab Res* 30: 440–446, 1998 (10). © Georg Thieme Verlag.]

opment and survival of steroidogenic organs, as SF-1–/– mice lack adrenal glands and gonads and exhibit male-to-female sex reversal of their genitalia (65, 66).

Increasing evidence indicates that adrenocortical steroidogenesis is modulated not solely by ACTH but also by multiple circulating and local peptide hormones, neuropeptides, neurotransmitters, ions, and cytokines (11, 67–70). Both *in vivo* and *in vitro* studies have clearly demonstrated that AVP stimulates aldosterone and cortisol secretion in bovine adrenals (71, 72); in rat cells, AVP stimulates aldosterone but not corticosterone secretion (73, 74). However, it stimulates aldosterone (250%) and cortisol (60–260%) secretion from normal human adrenals *in vitro* (75–77) via activation of V1-AVP receptors (V1-AVPR) localized mainly in compact cells of the zona reticularis and, to a lesser extent, in the zona glomerulosa (ZG) and fasciculata (68, 74, 78, 79). V2-AVPR were not detected initially in human adrenal cortex tissues (68), but were identified recently by RT-PCR studies (79); their stimulation by DDAVP does not modulate steroidogenesis (79, 80). V3-AVPR (or V1bR) are not detected in the normal human adrenal cortex (79), but are expressed in rat and human chromaffin cells (68, 77, 81), where AVP can stimulate catecholamine release from the adrenal medulla. Thus, AVP could exert significant direct effects on adrenal cortex function, both in endocrine and paracrine modes, but its physiological role has not yet been clearly established. However, in patients with congenital central diabetes insipidus, there is no evidence for clinically significant decreased cortisol secretion (82, 83).

Catecholamines have also been shown to stimulate cortisol and aldosterone secretion *in vitro* in bovine, pig, and fowl via β1-adrenoreceptors (11, 84, 85), but this does not appear to

occur in human adrenocortical cells (86). Serotonin (5-HT) is another neurotransmitter that may play a role in the control of steroidogenesis (87). 5-HT is able to directly trigger cortisol and aldosterone release, as demonstrated *in vitro*, in rat, frog, and human adrenal cells (87–89) but also, indirectly, by stimulating adrenal blood flow (90). The receptor subtype involved in these adrenal effects is still controversial in the rat, but was determined to be 5-HT4 receptor (5-HT_4R) in frogs and humans (88, 89). The 5-HT_4R is positively coupled to the cAMP and calcium pathways. *In vivo*, 5-HT4 agonists such as cisapride or zacopride induce an increase in aldosterone but not in cortisol secretion in humans (91, 92). Possible paracrine control of steroidogenesis by 5-HT can be proposed since its presence has been demonstrated in human perivascular mast cells and in chromaffin cells of the frog, rat, and mouse adrenals (93–95). Central 5-HT is known to enhance ACTH release from the pituitary and to activate the systemic renin-angiotensin system (RAS) to stimulate aldosterone secretion. However, no study has established whether these secretory responses can occur within the adrenal gland *in vivo*.

VIP and PACAP have been shown to play a paracrine role in the secretory activity of the adrenal cortex in the rat, human, and cow, as they are synthesized by adrenomedullary chromaffin cells (18). VIP stimulates aldosterone release from ZG through the activation of selective VIP receptors (VIPR2/VIPR3), whereas it stimulates cortisol secretion moderately through the nonspecific activation of ACTH receptor (ACTHR) (96–98). VIP/PACAP-induced adrenal steroidogenesis can also be enhanced by an indirect mechanism: indeed, both stimulate catecholamine secretion from adrenal chromaffin cells (99, 100), which in turn elicit a β-adrenoreceptor-mediated aldosterone release (101, 102). Moreover, cortisol secretion can be raised by increasing the intraadrenal blood flow as it is stimulated by VIP and PACAP (103, 104).

Ang-II, the biologically active peptide of the RAS, and potassium ion are the major regulators of aldosterone synthesis and secretion (2). A decrease in potassium balance activates the RAS, leading to Ang-II, and then to aldosterone release. Ang-II mediates its effect on steroidogenesis via AT1 receptors (AT1R), which are coupled to phospholipases C and A_2 (PLC, PLA_2). It has been demonstrated that Ang-II inhibits the expression of P450c17 at the transcriptional level in ovine adrenocortical cells (105). Moreover, it augments the expression of StAR protein (106). In the rat, Ang-II enhances the transcription of AT1R and P450 aldo synthase (CYP 11B2) *in vivo* and *in vitro* (107, 108). However, Ang-II seems to inhibit AT1R expression in bovine and human fasciculata cells (109, 110). The presence of a local RAS in the adrenal cortex suggests that Ang-II can regulate aldosterone production in a paracrine fashion (111) (for review see Refs. 112 and 113). Inhibitory signals contribute to maintain aldosterone homeostasis. Dopamine and somatostatin blunt Ang-II-induced aldosterone production (114, 115). The natriuretic peptides ANP and C-type natriuretic peptide (CNP), which are present in the circulation but are also expressed in the adrenal medulla, have been demonstrated to exert an inhibitory action on aldosterone release *in vitro* (116, 117). ANP also inhibits ACTH and Ang-II-induced cortisol production by decreasing the level of StAR expression (118). Other neuropeptides regulate the steroidogenic function of the adrenal cortex by acting both at the central and adrenal levels, as endothelin 1 (ET-1) (119, 120) and NPY (121, 122) enhance cortisol and aldosterone release.

Recent attention has been drawn to leptin as a negative regulator of the HPA axis. Acute injection of leptin in humans (123) and mice (124) counteracts fasting-induced activation of the HPA axis. This effect is proposed to be driven by a direct action of the peptide, both at the hypothalamic and adrenal levels (125). Leptin and its receptor, Ob-R, are expressed in the pituitary (126, 127) and in human, rat, and mouse adrenal glands (128–130). Moreover, the adrenal is embedded in adipose tissue, the physiological source of leptin, which acts at the transcriptional level to prevent the stress-induced stimulation of CRH and CYP17 mRNAs in the hypothalamus and adrenal, respectively (131–133). Other studies have shown opposite effects of leptin on the pituitary where CRH (known to suppress appetite and food intake) and ACTH levels are stimulated, leading to cortisol secretion (134, 135). These discrepancies may arise from anatomic and functional differences in CRH neurons in the PVN where leptin might have inhibitory effects on some and stimulatory effects on other populations of cells. Leptin is induced by GCs (136, 137), resulting in higher plasma levels in CS patients (138, 139).

The integrity of adult adrenal size is maintained by a continuous process of cell division in the ZG and centripetal migration and differentiation into fasciculata cells (140). Chronic stimulation by ACTH induces a phenotypic change of glomerulosa cells into fasciculata cells (141) whereas GCs inhibit this differentiation process namely by reducing P450scc expression (142–144); it was proposed that GCs may play a role in the functional zonation of the adrenal cortex (11). Indeed, high levels of GC (as high as in the inner adrenal cortex owing to centripetal blood flow) were shown to inhibit the 18-hydroxylation step in ACTH-treated cultures of human fetal adrenals, thus decreasing 18-OH-deoxycorticosterone (DOC) and aldosterone levels (11). In contrast to GC, ACTH can lead *in vivo* to hypertrophy and hyperplasia of the adrenal cortex, a process that is reversible. Paradoxically, it seems to harbor inhibitory effects on cell proliferation *in vitro*. A trophic effect is observed after a 2-h exposure to ACTH. This is correlated with a PKA-dependent increase of c-Jun and c-Fos expression (145, 146). After 24 h of stimulation, c-Myc expression is decreased, and inhibition of cell growth is observed (145, 147). Recent data suggest a cAMP-independent proliferation-promoting effect of ACTH (148, 149). Indeed, ACTH was shown to stimulate the mitogen-activated protein (MAP)-kinase pathway *in vivo* and *in vitro*, leading to the accumulation of c-Fos, c-Jun, and c-Myc (147, 150). Ang-II is another peptidic hormone that can also activate the MAP-kinase cascade in adrenal cells in a PKC-dependent mechanism (146, 151). *In vivo*, a chronic stimulation with Ang-II induces ZG hypertrophy. ET-1 also augments cell proliferation in the ZG *in vitro* and *in vivo* by interacting with its ET_A receptor, which is specifically expressed in the ZG (119). Chronic treatment with VIP exerts a moderate hyperplasia of ZG *in vivo* (152, 153). Somatostatin exerts direct antiproliferative effects on the ZG *in vivo* (115). It can also antagonize the mitogenic action of Ang-II. ACTH stimulates the autocrine production of growth factors (GFs)

such as insulin-like growth factor I (IGF-I), IGF-II, and transforming growth factor-β1 (TGF-β1), which regulate the trophic and steroidogenic functions of the adrenal cortex *in vivo* (11, 154). IGF-I and IGF-II have mitogenic effects. IGF-II is more highly expressed in fetal than in adult adrenals (155). In addition, it is highly expressed in hormonally active adrenocortical carcinomas but not in benign tumors, which suggests an important role in tumor acquisition or progression (156, 157). In bovine cells, IGF-I and TGF-β1 exert opposite effects on adrenocortical function by inhibiting the expression of specific adrenal genes; IGF-I enhances the transcription level of ACTH-R, StAR, and specific steroidogenic enzymes, whereas TGF-β1 inhibits it (158). TGF-β1 is thought to play a role in human fetal adrenal remodeling, as it inhibits fetal zone cell proliferation and promotes apoptosis *in vitro* (159, 160). However, this has not been demonstrated *in vivo*.

III. Primary Adrenal Cushing's Syndrome (CS)

The incidence of CS has not been determined with great precision. The increasing frequency of subclinical cortisol-secreting adrenal lesions, identified during the evaluation of adrenal incidentalomas, renders precise estimation of the true incidence even more difficult. The incidence of clinical CS secondary to unilateral adrenal adenoma is approximately two cases per million per year (161); this estimate is close to that of 1.7 per million per year for adrenocortical carcinoma, where clinically significant hormonal secretion occurs in 30–60% of cases, including clinical hypercortisolism, in approximately half of the hormonally active cases (162–164). Since pituitary Cushing's disease is approximately 3-fold more frequent than primary adrenal disease, its incidence would be close to five to six cases per million per year. When clinically detectable ectopic ACTH secretion is also taken into account, the overall incidence of endogenous CS would reach approximately 10 cases per million per year.

Primary adrenal etiologies account for 15–20% of endogenous CS in adults and are secondary to unilateral tumors in 90–98% of cases (1, 2, 163); in contrast, in prepubertal children, primary adrenal causes are responsible for almost 65% of CS. In adults, some case series have suggested that adenomas and carcinomas are equally responsible for adrenal CS, whereas in other series, adenomas were responsible for up to 80% of cases (165, 166). Cortisol-secreting adrenal carcinomas are 3–4 times more frequent than adrenal adenomas in children. For unclear reasons, adrenal tumors are more frequent in females than in males with a ratio of 4:1 for adenomas and 2:1 for carcinomas (161–164).

Less than 10% of ACTH-independent CS can be secondary to bilateral adrenal lesions, and their pathophysiology is diverse. Primary pigmented nodular adrenocortical disease (PPNAD) or micronodular adrenal dysplasia can be familial, associated with other tumors such as myxomas, schwannomas, pigmented cutaneous lesions, and peripheral endocrine tumors (Carney's complex), and linked to unknown genes on chromosome 2 or to mutations of protein kinase A Type 1-α located on chromosome 17 (167–169, 169a). In PPNAD, the overall size of the adrenal gland is usually not enlarged, but is occupied by several small black or brown nodules spread in an otherwise atrophic cortex. High synaptophysin expression in PPNAD nodules suggests a neuroendocrine phenotype of these cells (170). A paradoxical increase in cortisol production is often found in these patients during Liddle's dexamethasone suppression test (171). In McCune-Albright syndrome, activating mutations of $G_{s\alpha}$ occur in some adrenal cells in a mosaic pattern during early embryogenesis and lead to the formation of adrenal nodules, in which constitutive activation of AC and the steroidogenic cascade produce increased cortisol secretion with ACTH suppression; the internodular adrenal cortex, where the $G_{s\alpha}$ mutation is not present, becomes atrophic (172, 173).

ACTH-independent bilateral macronodular adrenal hyperplasia (AIMAH) is a rare cause of CS, as it is estimated to represent less than 1% of all endogenous cases of this syndrome (1–4). In a review by Lieberman *et al.* (174) in 1994, only 24 published cases had been identified, but several other cases and series have been reported since then (175–178). AIMAH has been described by various terms, including massive macronodular adrenocortical disease (MMAD), autonomous macronodular adrenal hyperplasia (AMAH), ACTH-independent massive bilateral adrenal disease (AIMBAD), and "giant" or "huge" macronodular adrenal disease (175). The clinical syndrome becomes evident during the patient's fifth or sixth decade and has a relatively even gender distribution when compared with Cushing's disease or unilateral adrenal tumors, which are more prevalent in women. Most cases have been sporadic, but a few familial cases have been reported as well (179–182). An activating R201S mutation of $G_{s\alpha}$ was found in the AIMAH tissues of a patient without any other features of McCune-Albright syndrome (183).

IV. Initial *in Vitro* Evidence of Ectopic Adrenal Membrane Hormone Receptors

The concept of ectopic adrenal membrane receptor expression was proposed initially by Robert Ney and his collaborators in 1971 (8, 9, 184). In studying the role of AC in mediating the effects of ACTH in rat adrenal steroidogenesis, only ACTH was capable of stimulating AC in normal cortex membrane preparations; however, in corticosterone-producing rat adrenocortical carcinoma 494, they demonstrated that AC was stimulated by hormones other than ACTH, such as epinephrine, norepinephrine, and TSH (8). Catecholamine effects on AC were induced by β-, but not by α-, adrenergic agonists. Further studies (Table 1) indicated that AC from this tumor was also stimulated by FSH, LH, and slightly by PGE_1) (184), but not by glucagon, insulin, vasopressin, PTH, or calcitonin. Propranolol was able to block the effects of catecholamines but not of other hormones on AC. The illicit hormones exerted no additive or synergistic actions, suggesting that the tumor possessed multiple specific receptors which activated a common AC (Fig. 1). The presence of ectopic and functional β-adrenergic receptors was also confirmed by other groups (185, 186); high-affinity β-adrenergic binding sites and AC stimulation were observed in rat adrenocortical carcinoma 494 membranes, but not in normal

TABLE 1. Initial *in vitro* studies of abnormal hormone receptors in adrenocortical tumors

Tissues	Abnormal receptors	References
Rat adrenal carcinoma 494	AC stimulation by epinephrine (E), norepinephrine (NE), TSH, LH, FSH, PGE_1	(8,184)
	β-AR binding	(185)
	cGMP stimulation by α-adrenergic agonists	(188,189)
Y1 mouse tumor cell line	AC stimulated only by ACTH	(187)
Human cortisol-secreting adrenal adenomas and carcinomas	AC stimulated by TSH	(9)
Human steroid-secreting adrenal carcinoma	AC and steroid stimulation by FSH, LH, GH, human placental lactogen, and PRL; inhibition by insulin	(190)
Human cortisol-producing adenomas	AC stimulation by NE, E, TSH, LH, and Ang-II	(191)
Human primary nodular hyperplasia	AC stimulation by glucagon	(191)
Human adrenal carcinoma	AC not stimulated by any hormone	(191)
Human cortisol-producing adenomas	β-AR binding and stimulation of cortisol secretion	(192)
Human cortisol-producing carcinomas	β-AR binding and AC stimulation; AC stimulation by TSH in one tumor	(193)
Human adenomas and carcinomas	AC not stimulated by any hormone	(194)
Human androgen-secreting adenomas	LH/hCGR binding and stimulation of androgen secretion	(195,196)
Human cortisol-producing adenoma	Type I, IL-1R expression and stimulation of cortisol secretion by IL-1	(198)
Human cortisol-secreting adrenal carcinoma	LH/hCGR by immunohistochemistry and *in situ* hybridization	(197)

adrenal membranes (185). A direct effect on steroidogenesis could not be verified in these initial studies, as AC was not efficiently coupled to steroidogenesis in rat adrenal carcinoma 494 (186). The aberrant response of AC to various hormones is not a universal phenomenon, as the AC of the Y1 mouse adrenocortical tumor cell line was found to be stimulated by ACTH, but not by epinephrine, PTH, insulin, glucagon, TSH, or PGE_1 (187). The presence of ectopic α-adrenergic receptors stimulating guanylate cyclase and cGMP production was also demonstrated in rat adrenal carcinoma 494 (188, 189).

Hingshaw and Ney (9) studied AC activity in three cortisol-secreting adenomas and one androgen-secreting carcinoma removed from patients with CS or virilization. AC stimulation was induced by TSH and ACTH, but not by epinephrine, LH, or glucagon in the androgen-secreting carcinoma; in only one of three adenomas, AC was stimulated slightly only by TSH and ACTH. They concluded that "at present the physiological significance of these aberrant tumor responses is uncertain, and their relationship to tumor function has to remain speculative. However it is possible that, in certain cases, the autonomous behavior of endocrine tumors may be more apparent than real, and that this behavior is the result of stimulation of the tumor by hormones other than the appropriate ones for the parent gland." (9).

Other *in vitro* studies have further supported the functional coupling of several, most frequently G protein-linked, membrane hormone receptors to steroidogenesis in some human adrenocortical benign and malignant tumors (Table 1). Millington *et al.* (190) investigated the effects of various hormones on the secretion of steroids in a human feminizing adenocarcinoma secreting mostly estrogens and androgens, but also some GC. AC activity was stimulated more by PRL, human placental lactogen, LH, and FSH preparations than by ACTH; insulin inhibited AC slightly, while TSH was without effect. In tumor explant culture, estrone and estradiol secretion was stimulated by PRL, insulin, and ACTH, but little by LH or GH. Androstenedione secretion was augmented by LH, GH, PRL, and ACTH. The synthesis of 11-hydroxycorticosteroids was stimulated by LH, GH, and PRL, but very little by ACTH. It must be stressed that hormone preparations available at that time were not pure and that contamination was quite possible. Matsukura *et al.* (191) studied AC activity in human cortisol-secreting adrenal tissues from adenomas, adenocarcinoma, and primary nodular hyperplasia (AIMAH), compared with normal adrenals and bilateral hyperplasias from pituitary Cushing's disease. In normal tissues, only ACTH and PGE_1 stimulated AC activity; in most adenomas, AC activity was increased by norepinephrine, in some by epinephrine, and in a few by TSH, LH, or Ang-II. In a case of AIMAH, AC was stimulated by glucagon and ACTH only. No stimulation of AC was found in adrenal carcinoma tissue. Hirata *et al.* (192) demonstrated the presence of high-affinity β-adrenergic binding sites in two of three cortisol-secreting adenomas, but not in the normal adrenal cortex or in one case of aldosterone-producing adenoma; furthermore, epinephrine stimulated cortisol secretion in cultured tumor cells from one of the patients with an adenoma, and Katz *et al.* (193) studied six human adrenal carcinomas with diversified steroidogenic activities and compared them with the normal adrenal cortex from three individuals; AC was stimulated by β-adrenergic agonists in four of six tumors but not in normal tissues. In one tumor examined for other hormone responses, AC was also stimulated by TSH, but not by glucagon or hCG. In two cases, membranes from metastatic adrenocortical cancer were compared with the primary tumor and had lost stimulation of AC by epinephrine or ACTH. Specific high-affinity β-adrenergic binding sites were detected only in tumors in which AC was stimulated by β-adrenergic agonists. In contrast, Saez *et al.* (194) did not find any AC responsiveness to norepinephrine, glucagon, and TSH in crude adrenal membranes from 11 patients with adenomas and carcinomas.

The aberrant expression of LH/hCG receptors was also previously reported *in vitro* in androgen-secreting adrenal adenomas (195, 196). Testosterone production was stimulated by hCG and ACTH in adrenal adenoma cells in culture, while only ACTH but not hCG was able to stimulate secre-

tion of cortisol, testosterone, and other steroids from the adjacent normal adrenal cortex (195); binding studies performed on cell membranes from hCG-responsive adrenal adenoma demonstrated high-affinity (0.14 nM) binding capacity (198 fmol/g). A preliminary report of the presence of LH/hCG receptor in a cortisol-secreting adrenocortical carcinoma was presented recently (197).

Willenberg *et al.* (198) investigated the adrenal adenoma of a 62-yr-old woman who presented CS with no particular clinical characteristics; striking lymphocytic infiltration of the adenoma was identified at histology. In contrast to normal control human adrenals or other cortisol-secreting adenomas or carcinomas, immunostaining revealed CD45 and CD68-positive macrophage-like cells in this patient's adenoma, and these cells are a major source of IL-1. Type I IL-1 receptor, which is not a seven-transmembrane G-coupled-receptor, was also found to be aberrantly expressed in the adenoma, by *in situ* hybridization and RT-PCR, but not in the normal adrenal cortex or other tumors. In cells dispersed from the adenoma, cortisol secretion was stimulated 2.6-fold by IL-1β, but poorly by ACTH (198); in normal adrenocortical cells or other cortisol-secreting adenomas, cortisol secretion was increased by approximately 1.5-fold during incubation with IL-1β. Since infiltration of mononuclear cells occurs in 15% of adrenal tumors, it will be of interest to further explore the prevalence of abnormal cytokine receptor expression in adrenal hyperplasias and tumors.

V. *In Vivo* Demonstration of the Functionality of Ectopic or Abnormal Membrane Hormone Receptors

The proposed concept of ectopic hormone receptors had been demonstrated *in vitro* only, until it found a clinical manifestation of its significance, *in vivo,* with the description of food-dependent CS (199); this resulted from ectopic adrenal expression of the receptor for a gastrointestinal hormone called gastric inhibitory polypeptide or GIP (200, 201).

A. Food- and GIP-dependent CS

Hamet *et al.* (199) were the first to identify "food-dependent" cortisol production in a 41-yr-old male patient presenting with CS secondary to a unilateral adrenal adenoma and periodic hormonogenesis. Plasma cortisol was consistently low in the morning or during fasting, but increased to abnormal levels after meals; food-induced elevations of plasma cortisol were not suppressed by high oral doses of dexamethasone. AC activity in the resected adrenal adenoma membrane preparation was stimulated 27% by ACTH and 62% by vasopressin, but not by FSH, glucagon, or Ang-II; the effects of various gastrointestinal hormones were not examined in this case. Another female patient with CS secondary to an adrenal adenoma had been previously reported to have "persistent diurnal cortisol secretory rhythm" (202); the low fasting plasma cortisol levels in the morning increased during the day at the presumed, but not indicated, meal times, suggesting that this patient also had food-dependent CS.

Two patients with bilateral AIMAH and food-dependent cortisol production were studied in detail a few years later and allowed to clarify the pathophysiology of this syndrome (200, 201). The first patient, a 48-yr-old French-Canadian woman, presented with typical symptoms of CS, which had become manifest during the previous 2–3 yr (200). Initial investigation revealed low plasma cortisol levels, fasting in the morning, and higher levels during the day, whereas plasma ACTH was always suppressed. The suspicion that cortisol production was regulated by a gastrointestinal hormone came from the observation that plasma cortisol was stimulated by oral administration of glucose or by lipid-rich or protein-rich meals, but not by intravenous glucose. In addition, somatostatin pretreatment inhibited the cortisol-stimulatory effect of oral glucose. A review of the various secretagogues of gastrointestinal hormones indicated that only GIP and the glucagon-like peptides (GLPs) were stimulated significantly by oral glucose and lipids, and to a lesser extent by proteins. Plasma cortisol levels were correlated with plasma GIP concentrations during the various test meals. *In vivo* GIP infusion, to reproduce physiological postprandial concentrations, augmented cortisol production in the patient, but not in four normal controls. In the patient, plasma cortisol was stimulated by the administration of ACTH but not by CRH, glucagon, insulin-induced hypoglycemia, pentagastrin, or AVP. The presence of GIP receptors (GIPRs) in adrenal tissues was supported by adrenal imaging after the injection of [^{123}I]-GIP *in vivo* (200). The incubation of dispersed adrenal cells *in vitro* confirmed GIP-mediated cortisol secretion in the patient's cells, whereas no cortisol response to GIP was found in normal adult or fetal adrenal cells or in other cortisol- or aldosterone-secreting adenomas (200); there was no stimulation of cortisol production in the patient's adrenal cells after *in vitro* incubation with secretin, CCK, VIP, substance P, bombesin, calcitonin gene-related peptide, glucagon, vasopressin, ANP, CRH, TRH, GHRH, neurotensin, or neurokinin A. It was thus concluded that food-dependent cortisol secretion resulted from the abnormal responsiveness of adrenal cells to the physiological secretion of GIP; "illicit" or ectopic GIPR expression on adrenal cells (Figs. 1 and 2) presumably were the basis for this new etiology of CS (200).

The second patient, a 49 yr-old French woman, had been followed for approximately 5 yr for CS and AIMAH (201). Unusual fluctuations of plasma cortisol were noted, and the patient was treated with cortisol biosynthesis inhibitors. After the preliminary report on the first case of GIP-dependent CS (203), the potential food-dependent nature of plasma cortisol secretion was also explored in this patient. Fasting plasma cortisol was low in the morning and increased after mixed meals, oral glucose, lipid-rich meals, and protein-rich meals, but not after intravenous glucose (201). Subcutaneous octreotide administration blocked the oral glucose effect on plasma cortisol. Plasma GIP levels were closely correlated with plasma cortisol levels during these various tests. Intravenous infusion of GIP produced an elevation of plasma cortisol levels in this patient, but not in four normal subjects pretreated with dexamethasone. Here again, plasma cortisol did not rise after *in vivo* administration of lysine-vasopressin (LVP), glucagon, insulin, or pentagastrin, but was stimulated by ACTH. Chronic octreotide administration, up to 100 μg three times daily resulted in a temporary improvement of the clinical syndrome and a return of urinary free cortisol levels

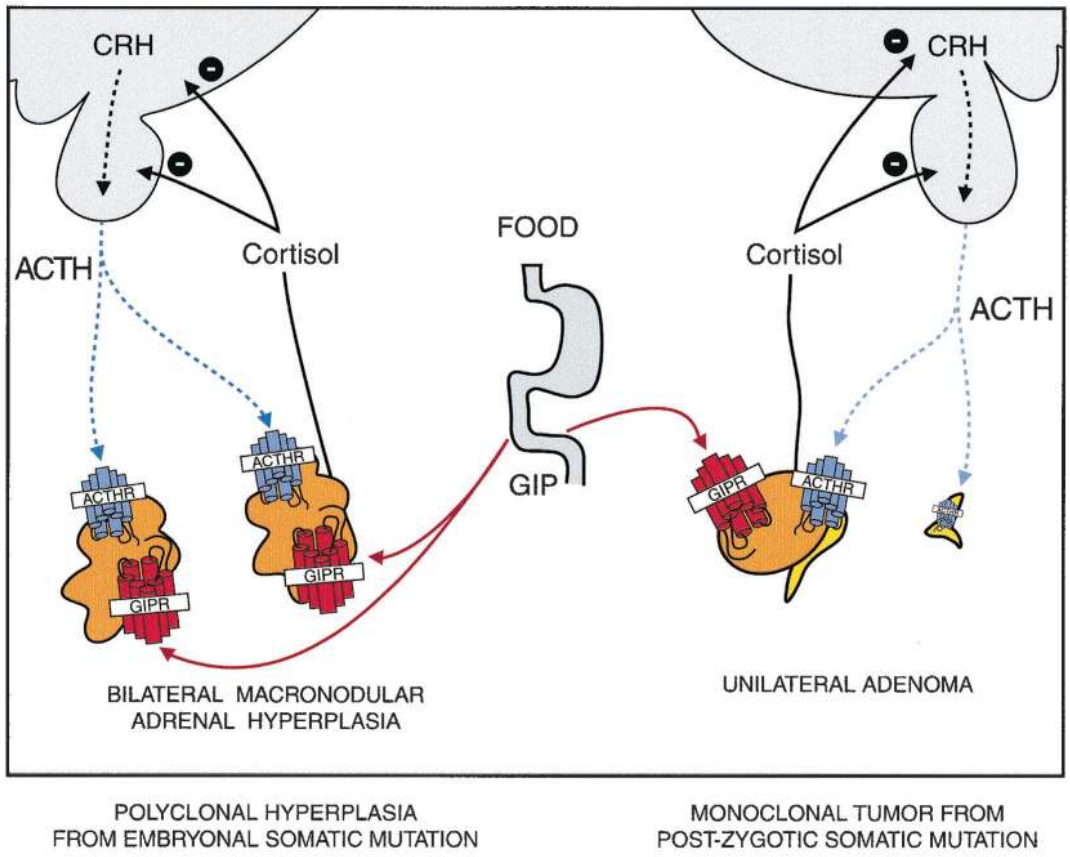

FIG. 2. HPA axis in GIP-dependent CS. The ectopic adrenal expression of the GIPR (in *red*) has been identified both in bilateral macronodular adrenal hyperplasia (*left* of figure) or in unilateral adrenal adenomas (*right side* of figure). After food ingestion, GIP is released in physiological concentrations by K cells from the duodenum and small intestine and binds to the ectopic adrenal GIPR; this results in postprandial supraphysiological increases of plasma cortisol (*full black lines*) which exerts its negative feedback on CRH and ACTH synthesis. In the absence of food ingestion (low plasma GIP levels), the suppressed levels of plasma ACTH (*dashed blue lines*) leads to decreased occupation of the ACTHR (decreased expression) and low fasting levels of plasma cortisol. A somatic postzygotic mutation occurring in a single cell leading to GIPR expression would eventually result in the growth of a GIP-dependent monoclonal unilateral cortisol-secreting adenoma with adjacent and contralateral adrenal cortex atrophy (*right side* of figure). A somatic mutation occurring during early embryonal life and responsible for ectopic GIPR expression in the progenitor cells of the adrenal cortex (polyclonal) would be responsible for the long-term development of nonfamilial GIP-dependent bilateral macronodular adrenal hyperplasia and CS (*left hand side* of figure).

to the upper limit of normal. However, there was an eventual escape from octreotide after 5 months of therapy, requiring bilateral adrenalectomy (204, 205).

Food- or GIP-dependent CS has now been identified in 13 patients with AIMAH (139, 200, 201, 205–208a) and in seven with unilateral adenoma (199, 205, 208a–213), as summarized in Table 2. At pathological examination, no distinctive features were reported, compared with non-GIP-dependent cortisol-secreting adenomas or bilateral macronodular hyperplasia, except in one case (207). This patient was described in a preliminary report to have facial pigmented spots, a blue nevus on one leg, lipofuscin pigments in bilateral adrenal macronodules, and a periadrenal schwannoma suggestive of Carney's complex without any family history; a full description has not yet been published, but *in vitro* studies clearly confirmed GIP-induced stimulation of cortisol secretion by adrenal cells (205). In two cases of AIMAH, the patient initially presented with a unilateral lesion and developed contralateral enlargement only later in time (206, 208a). Except for three patients [the first patient described with food-dependent CS but not proven to be GIP-dependent (199) and two recent ones with AIMAH (GIPR overexpression not yet confirmed)], all other patients are females; adrenal CS is more frequent in females (161), but it remains to be seen whether an even higher female frequency will be found in GIP-dependent CS and what molecular mechanism underlies this sex distribution. Average age at the time of diagnosis may be somewhat greater in patients with AIMAH than in patients with unilateral adrenal adenoma (Table 2) (174, 175); the youngest patient with a unilateral adenoma was only 15 yr old. In GIP-dependent CS, chronic GIP-induced hypercortisolism eventually leads to suppression of CRH and ACTH; this suppression, coupled with low GIP levels in the fasting state, is responsible for the decreased plasma cortisol levels, which can be accompanied by symptoms of relative cortisol insufficiency (201, 209). However, in certain patients (Table 2), fasting plasma cortisol levels were not particularly low, indicating that GIP-dependent CS should not be excluded without performing a test meal (139, 206); this finding could indicate that subpopulations of adrenal cells in the

TABLE 2. Summary of cases of food- and GIP-dependent adrenal Cushing's syndrome[a]

Sex	Age (yr)	Lowest fasting plasma cortisol (nmol/liter)	Cortisol stimulation by GIP		GIP receptor overexpression	Treatment with octreotide	References
			In vivo	*In vitro*			
Bilateral macronodular adrenal hyperplasia							
F	48	138	6.0-fold	8.4-fold	Yes	ND	(200,219)
F	49	124	3.7-fold	1.5-fold	Yes	Partial improvement for 5 months	(201,205)
F	45	149	ND	1.4-fold	Yes	ND	(205,207)
F	33	279	2.5-fold	ND	Yes	ND	(206)
F	60	140	ND	2.1-fold	Yes	Partial improvement for 5 months	(208)
F	43	414	ND	ND	Yes	ND	(208a)
F	40	122	ND	ND	Yes	ND	(208a)
F	57	338	2.4-fold	ND	Yes	ND	(208a)
F	36	556	ND	1.6-fold[b]	ND	ND	(139)
F	35	190	1.6-fold	1.7-fold	Yes	ND	(208a)
F	54	198	ND	ND	Yes	ND	(208a)
M	49	420	ND	ND	ND	ND	(208a)
M	34	140	ND	ND	ND	ND	C. Siame-Mourot and J. P. Cappoen[c]
Unilateral adrenal adenoma							
M	41	132	ND	ND	ND	ND	(199)
F	47	4	7.8-fold	7.5-fold	Yes	Partial improvement during 3.5 months	(209)
F	32	121	ND	10-fold	Yes	ND	(210)
F	43	140	4.5-fold	7.7-fold	Yes	Very transient improvement	(205,212)
F	33	66	ND	2.1-fold	Yes	ND	(211)
F	15	20	ND	15-fold	Yes	ND	(208a)
F	41	114	ND	5.5-fold	Yes	ND	(213)

ND, not done.
[a] In all cases, the food-dependent stimulation of cortisol secretion was clearly demonstrated.
[b] Also stimulated by leptin.
[c] Unpublished observations.

tumor or hyperplasia have lost their GIP dependency and are secreting cortisol under different mechanisms, or that more than one abnormal receptor regulating cortisol production are expressed in these cells. In one patient with food-dependent AIMAH but in whom fasting plasma cortisol was relatively elevated, Pralong *et al.* (139) reported that, in addition to GIP, leptin also aberrantly stimulated cortisol secretion in dispersed adrenal cells; thus, the potential presence of more than one abnormal receptor may modify the phenotypic appearance. The potential presence of ectopic GLP-1 receptors has been excluded to date by the lack of stimulation of cortisol production after GLP-1 administration, either *in vivo* or *in vitro* (139, 206, 210). In one patient with GIP-dependent AIMAH, plasma ACTH and cortisol responses to CRH were still preserved, presumably because the intermittent food-dependent stimulation of cortisol had not yet completely suppressed the HPA axis (208). In a female patient with hirsutism and a unilateral adenoma, both adrenal androgens and cortisol were found to be stimulated by food intake *in vivo* and GIP *in vitro* (213); hypercortisolism was modest and ACTH was not fully suppressed.

The abnormal adrenal regulation of cortisol production by GIP suggested that this aberrant adrenal sensitivity to GIP was secondary either to ectopic expression or activating mutation of GIPR, not normally expressed or functional in adrenal cortical tissues. Cloning of GIPR cDNA from rat (214), hamster (215), and later human (216–218) sources allowed these hypotheses to be investigated.

De Herder *et al.* (209) used *in situ* hybridization to demonstrate abundant GIPR mRNA in adrenal adenoma cells from their patient with GIP-dependent CS; this signal was not present in the adenoma from a patient with non-food-dependent CS, but was not examined in the normal adrenal cortex in this initial study. Using RT-PCR amplification, N'Diaye *et al.* (219) demonstrated pronounced adrenal GIPR overexpression in adrenal adenoma or hyperplastic tissues from GIP-dependent CS compared with the normal human pancreas, normal adult or fetal adrenal cortex, or non-GIP-dependent adrenal CS tissues. A small amount of GIPR mRNA was detected in normal fetal and adult adrenal tissues after at least 35 cycles of amplification and hybridization with the labeled cDNA but was not coupled efficiently to steroidogenesis. Sequence analysis of the full-length cDNA of normal and GIP-dependent adrenal tissues revealed no mutation of GIPR in the affected adrenal tissues (219); similar proportions of isoforms lacking exons 4 and 9 were identified in normal and GIP-dependent adrenals. Chabre *et al.* (210) confirmed the presence of the same overexpressed GIPR isoforms in a GIP-dependent adenoma by RT-PCR and sequencing; no GIPR bands could be detected in the atrophic adrenal cortex adjacent to the tumor or in normal adult adrenals, but only ethidium bromide staining was used. The ACTHR was found to be expressed at a lower level in GIP-dependent adenoma compared with normal tissues (210); this may be secondary to the chronic suppression of endogenous ACTH, which is known to up-regulate ACTHR expression (36, 37). If the relative suppression of ACTHR in GIP-dependent adrenal tissues is confirmed in further stud-

ies, this would indicate that GIP cannot substitute for ACTH in inducing the expression of ACTHR; it must be noted, however, that plasma GIP levels are only elevated transiently postprandially, which is different from conditions where ACTH is elevated chronically. GIPR overexpression was confirmed in other cases (Table 2) of GIP-dependent adrenal macronodular hyperplasias (205, 206, 208, 208a) and adenomas (205, 208a–210, 213) and was not demonstrated in non-GIP-dependent CS adrenal tissues (205, 210, 213, 219) or the human adrenocortical carcinoma cell line H295 (211). GIPR overexpression was detected, even in the early stages of adrenal hyperplasia (206). The small amount of GIPR mRNA sometimes found in normal fetal or adult adrenal tissues after amplification was not efficiently coupled to steroidogenesis (219) and may reflect a low number of GIPR in endothelial cells (214) rather than in adrenocortical cells. Thus, the concept of functional ectopic receptors remains valid in explaining the pathophysiology of GIP-dependent CS (Figs. 1 and 2).

It has been reported that the *in vitro* cortisol-stimulating effects of GIP are coupled to an increase of cAMP, but not of IP_3 production (205, 210). In studying GIP-dependent adrenal cells in primary culture, GIPR down-regulation by its own ligand has been demonstrated, as assessed by the induction of steroidogenic enzyme expression, cortisol secretion, or GIPR mRNA levels by *in situ* hybridization and RT-PCR studies (205, 220). By stimulating steroidogenic enzyme activity, ACTH pretreatment of cells increased the GIP-induced cortisol response but did not appear to modify GIPR expression directly (205).

Stimulation of thymidine incorporation into newly synthesized DNA by GIP was observed in primary cultures of adrenal cells from GIP-dependent CS, but not in normal cells (210). Activation of p42-p44 MAP kinases was observed after treatment of pathological cells with GIP (210). Depending on the cell culture conditions used, ACTH can be shown to inhibit or stimulate markers of cell proliferation in adrenal cells. In the studies by Lebrethon *et al.* (205), under conditions where ACTH inhibited thymidine incorporation in normal and GIP-dependent adrenal cells, GIP was also found to suppress DNA synthesis only in GIP-dependent, and not in normal adrenal cells. Such results suggest that GIP is possibly capable of regulating cell proliferation, in addition to steroidogenesis, in these tissues; however, cell growth stimulation by GIP has not yet been clearly demonstrated.

It should be stressed that food-induced cortisol secretion has been reported in some non-GIP-dependent CS. Bercovici *et al.* (221) described a patient with pituitary Cushing's disease in whom ACTH and cortisol were increased strikingly after mixed meals. ACTH secretion was stimulated by protein-rich meals, but not by oral glucose or lipid-rich meals. Intravenous infusion of amino acids was capable of inducing this response, while octreotide administration did not modify urinary cortisol levels. It was concluded that the pituitary corticotroph adenoma of this patient retained the capacity that normal corticotroph cells have to enhance their release of ACTH after protein ingestion. It has been shown very clearly that, in normal individuals, mixed meals produce an increase in ACTH release and in plasma cortisol levels; this is more evident at lunchtime than after breakfast, when the diurnal peak of ACTH and cortisol may mask the response (222–224). This stimulation is of hypothalamic-pituitary origin and is abolished by dexamethasone administration (225). It is believed that the effect may be secondary to the heightened serotonin production and related to tryptophan content in the meal (224). α-Adrenergic agonists can also increase postprandial stimulation of ACTH (226).

B. Vasopressin-responsive CS

A large proportion of pituitary corticotroph adenomas have been shown to augment their ACTH release after LVP administration, resulting in increased plasma cortisol levels (227, 228). In contrast, in adrenal CS, where ACTH is suppressed, it is expected that plasma cortisol should not increase after LVP administration (229). However, abnormal adrenal stimulation of cortisol secretion in response to exogenous AVP or LVP administration has been described in canine (230) and human ACTH-independent CS, secondary to unilateral adrenal adenomas, carcinomas, or AIMAH (Table 3).

In comparing the response of plasma ACTH and 11-hydroxycorticosteroids to insulin-induced hypoglycemia and LVP infusion in 10 patients with CS of various etiologies, Demura *et al.* (231) noted an unexpected increase in plasma cortisol after LVP in two of two patients with an adrenal adenoma, while ACTH remained suppressed. Makino *et al.* (232) described a 51-yr-old male with AIMAH in whom a combined LVP-CRH test elevated plasma cortisol levels, without any detectable rises in plasma ACTH. Itagaki *et al.* (233) studied a 53-yr-old woman with CS and AIMAH in whom plasma ACTH was undetectable basally and remained so after a metyrapone test or after intramuscular injection of 10 IU LVP; surprisingly, plasma cortisol increased 2.2-fold, and aldosterone increased 3.1-fold, after LVP administration. After bilateral adrenalectomy, dispersed adrenal cells from this patient augmented cortisol production 2-fold when incubated with LVP, while there was no stimulation in cells from another cortisol-secreting adenoma. Since plasma cortisol was not suppressed by the administration of a 1.2-liter water load, the role of endogenous vasopressin in regulating cortisol secretion by the tumor was considered to be uncertain by the authors.

Horiba *et al.* (234) reported two male Japanese patients with bilateral macronodular adrenal hyperplasia and clinical CS in whom im injection of 10 IU LVP increased plasma cortisol 2.3- to 2.6-fold, while plasma ACTH remained undetectable; there were no ACTH or cortisol responses to CRH or dexamethasone. Upon pathological examination, the glands were replaced by macronodules composed of compact and clear cells, but there were some regions of cortical internodular atrophy. In dispersed adrenal cells from both patients, LVP stimulated cortisol secretion (2.8- to 3.2-fold) more efficiently than ACTH. In seven other patients with CS and unilateral adenoma, LVP injection resulted in small increases of plasma cortisol, varying between 9.8 and 25.3%. In four normal subjects pretreated with 2 mg dexamethasone at bedtime and 0.5 mg on the morning of the test, LVP injection elevated plasma cortisol 1.6- to 1.8-fold (up to 45 nmol/liter from basal levels of 20.9 nmol/liter). An exaggerated 2.6-fold

TABLE 3. *In vivo* and *in vitro* studies of abnormal hormone receptors other than GIPR in adrenal tumors or hyperplasia

Tissues	Abnormal receptor	References
Vasopressin-responsive, cortisol-secreting adrenal tumors and AIMAH	Steroidogenesis overstimulated by LVP or AVP; variable eutopic expression of V1 vasopressin receptor	(80,182,229,231,232,234, 235,237–240)
	One familial case of AIMAH responsive to vasopressin	(182)
Vasopressin-responsive preclinical AIMAH	Increased secretion of cortisol after vasopressin administration	(242)
Catecholamine-dependent AIMAH and CS	Steroidogenesis stimulated by β-adrenergic agonists and inhibited by propranolol	(86,240)
LH-dependent CS and AIMAH	Cortisol secretion stimulated by hCG, LH, and GnRH; hypercortisolism normalized by leuprolide acetate	(251)
	Stimulation of cortisol secretion by LH in preclinical AIMAH	(242)
Transient CS during pregnancies; no permanent adrenal lesions	Stimulation of 17-OH-corticosteroids by hCG	(262)
Human virilizing adenoma	LH/hCG receptor binding; *in vivo* and *in vitro* stimulation of androgens by hCG	(195,264,266–268)
Serotonin-responsive CS with AIMAH	Cortisol secretion stimulated by 5-HT_4R agonists	(251,272)
	Cortisol secretion increased by 5-HT_4R agonists in preclinical AIMAH	(242)
Estrogen-stimulated bilateral nodular adrenal hyperplasia	Transient hypercortisolism during three pregnancies; *in vitro* stimulation of cortisol secretion by estrogens	(273)
PPNAD nodules	Paradoxical stimulation of cortisol by dexamethasone; increased GC receptors in nodules	(171,274)

rise in plasma cortisol after 10 IU of LVP was also reported in a patient with a unilateral cortisol-secreting adenoma and mild ACTH-independent CS (235). Intracellular calcium flux in dispersed tumor cells was stimulated by AVP and inhibited by a V1-AVPR antagonist. Using RT-PCR amplification, the V1-AVPR signal was stronger in the cortisol-secreting tumor than in the normal gland; there was a faint V2-AVPR signal in normal and tumoral adrenal tissues, and no V3-AVPR in either.

A 36-yr-old female American patient with CS and AIMAH presented an unusual association with orthostatic hypotension (80). Exogenous AVP, but not desmopressin, triggered large elevations of plasma cortisol (3.4-fold) and aldosterone (67-fold) levels. During upright posture and hypotension, cortisol and aldosterone secretion increased, despite the suppression of ACTH and renin levels. AVP, which normally rises during upright posture and even further in orthostatic hypotension, remained below the limit of assay detection, until the correction of hypercortisolism. Under dexamethasone suppression, plasma cortisol, aldosterone, and androgens were elevated by exogenous AVP in the patient, but not in the controls. Cells freshly dispersed from the diffuse adrenal hyperplasia displayed higher cortisol stimulation (4.2-fold) during incubation with AVP than normal adrenal cells (1.3-fold); the cortisol response was mediated by V1-AVPR, as shown by the effects of V1 antagonists and the lack of effect of V2 agonists. The presence of V1-AVPR was supported by binding studies, intracellular Ca^{2+} flux studies, and RT-PCR amplification of mRNA for all three AVPR. The binding studies revealed a similar V1-AVPR affinity (2.63 nM) in AIMAH adrenal cells, compared with membranes from human glomerulosa-rich normal adrenal cells or myometrium (236). The ED_{50} of AVP on $[Ca^{2+}]_i$ was similar in the adrenal cells of the patient (0.9 nM) compared with glomerulosa-rich cells (1.4 nM) from normal adrenals (76). Interestingly, CRH administration stimulated cortisol *in vivo* but not *in vitro* without any stimulation of ACTH; it is possible that CRH increased the adrenal production of vasopressin (68) and cortisol in a paracrine manner. Alteration of the V1-receptor-effector system was not limited to the adrenal tissues of this patient, as there was also an abnormal, prolonged vascular vasoconstrictive response to AVP, compared with the arterioles of normal or hypertensive subjects. The persistence of decreased stimulation of plasma vasopressin and endothelin levels during postural hypotension, several months after correction of the hypercortisolism, also raised the possibility of an exaggerated V1-AVPR signal at the hypothalamic level in this patient. The causal relationship between abnormal V1-AVPR-mediated-responses and postural hypotension remains uncertain (80). Another male Japanese patient with AIMAH and CS was found to have a 1.8-fold increase in plasma cortisol after LVP injection (237); food intake, GIP infusion, octreotide, and CRH were without effects. Removal of the large bilateral macronodular adrenals showed no areas of internodular atrophy; LVP stimulated cortisol production in cells freshly dispersed from a macronodule. Stimulation of plasma cortisol by administration of 0.2 IU AVP was noted in a Japanese man with AIMAH and coincident multiple adenomatous polyps and colon cancer (238); a point mutation of the *APC* gene was revealed in the colon cancer but not in the adrenal nodules.

In a retrospective study of 26 patients with CS secondary to unilateral cortisol-secreting tumors, Arnaldi *et al.* (79) observed an increase of plasma cortisol greater than 30 ng/ml after LVP testing in 27% of cases (five adenomas and two carcinomas). Quantitative RT-PCR assay of V1-AVPR showed that the levels of message were similar in 20 cortisol-secreting adenomas, compared with three normal adult adrenals; the levels were lower in 19 adrenocortical carcinomas, but there was a large overlap with adrenal adenomas. The normal adrenal glands and the majority of tumors also expressed low amounts of V2-AVPR, but no V3-AVPR. Only six of the patients for whom adrenal tumor material was available had undergone LVP testing; responders had somewhat higher V1-AVPR concentrations in their tumors than nonresponders, but the levels were not higher than in normal

adrenal tissues. In one patient with an *in vivo* cortisol response (~1.6-fold) to LVP, the AVP-induced cortisol secretion (2-fold) of perifused adrenal cells was inhibited by V1-AVPR antagonists.

The demonstration of an exaggerated cortisol response to pharmacological levels of exogenous vasopressin does not constitute direct evidence that fluctuations of endogenous AVP levels are the main regulator of steroidogenesis in these patients. This was illustrated in a male patient with AIMAH who was shown to have increased plasma cortisol in response to upright posture and administration of 10 IU AVP (86); however, the modulation of endogenous AVP levels by water dilution or hypertonic saline infusion did not modify plasma cortisol levels. In addition, *in vivo* administration of a V1-AVPR antagonist inhibited the response of cortisol to exogenous AVP, but not to upright posture. In fact, this patient was found to have ectopic β-adrenergic receptors (see *Section V.C.*) in his adrenal tissues; it is believed that pharmacological AVP levels stimulated catecholamine release, including from the adrenal medulla (68), and then mediated cortisol release in this case. Further support comes from the fact that there was no evidence of V1-AVPR in his adrenal tissues (N. N'Diaye and A. Lacroix, unpublished observation).

Daidoh *et al.* (239) studied a 49-yr-old man with very large bilateral AIMAH and severe CS; intravenous injection of small amounts of AVP (0.3 IU) increased plasma cortisol 3.7-fold without any detectable rise in ACTH. Similarly, insulin-induced hypoglycemia elevated plasma AVP and cortisol without any increase in plasma ACTH; catecholamine effects were not studied however. Upright posture augmented plasma AVP and cortisol. Oral administration of the V1-AVPR antagonist OPC-21268 for 8 days decreased urinary free cortisol levels, but potential spontaneous fluctuations of cortisol secretion were not evaluated for long periods. It was further shown, in dispersed adrenal cells, that AVP stimulated cortisol secretion in AIMAH cells but not in normal control cells, and that this effect was inhibited by OPC-21268; GIP was without effects on AIMAH cells, but catecholamine and insulin were not tested directly. We recently studied a 50-yr-old American woman with CS and AIMAH in whom plasma cortisol was stimulated by upright posture (1.7-fold) and exogenous AVP (3.4-fold), but not by dDVAP (240). In this patient, we were able to demonstrate that plasma cortisol was inhibited by water loading (24% decrease), and elevated during hypertonic saline infusion (1.7-fold). This patient was also found to have abnormal responses to β-adrenergic receptor agonists (see *Section V.C.*), in addition to the abnormal V1-AVPR response in her adrenals. These last two cases represent the first demonstrations of fluctuations in plasma cortisol levels in parallel with small physiological changes in endogenous vasopressin levels. All the previously reported cases of cortisol stimulation by lysine- (231–235, 237) or arginine-vasopressin (80) were related to exogenous pharmacological amounts. In these last two patients, as in another patient (80), plasma vasopressin was found to be suppressed to undetectable levels basally and showed only a very modest increase upon potent physiological stimulation. This may be due to the suppressive effects of hypercortisolism on vasopressin gene expression (241). It has also been postulated that abnormal V1-AVPR may modify vasopressin production via a short loop regulation mechanism in hypothalamic nuclei (80).

An abnormal increase of plasma cortisol in response to vasopressin administration was also noted in patients with preclinical bilateral macronodular adrenal hyperplasia (242). Recently, an exaggerated plasma cortisol response to LVP was seen in a 67-yr-old woman with CS and bilateral macronodular adrenal hyperplasia, whose brother had died after bilateral adrenalectomy for CS and AIMAH (182); the precise nature of the abnormal hormone receptor implicated is unknown, but this constitutes the first demonstration of abnormal hormone responsiveness in familial AIMAH.

Since V1-AVPR are present in the normal adrenal cortex and modulate modest effects of vasopressin on steroidogenesis, the exaggerated steroidogenic responses to vasopressin in these patients would be secondary to the abnormal function of an "eutopic" receptor-effector system, rather than to the presence of an ectopic receptor. V1-AVPR mRNA levels were found to be expressed either at higher (235) or similar (79, 80) levels, compared with normal control adrenal tissues. The binding affinity and dose response of intracellular calcium flux for V1-AVPR noted in the adrenal tissues of a patient with AIMAH (80) were not different from those reported in other normal tissues. Thus, no evidence of ectopic receptor or gross overexpression of the eutopic V1-AVPR has been presented to date; the molecular mechanisms leading to the abnormal response of V1-AVPR or its effector system, which would increase the response to AVP, remain to be elucidated.

Recently, V3-AVPR were shown to be expressed ectopically in a series of bronchial carcinoids secreting ACTH (243). A large proportion of patients with Cushing's disease, but not normal individuals, secrete ACTH in response to DDAVP (244, 245). V3-AVPR were found to be overexpressed in corticotroph adenomas (229); as DDAVP can also bind in part to V3-AVPR, this may explain the effects of DDAVP on ACTH release in Cushing's disease. Thus, stimulation of cortisol levels after vasopressin administration in CS cannot directly distinguish between pituitary corticotroph adenoma, ACTH-independent primary adrenal tumor or hyperplasia, or relatively well differentiated carcinoid tumors producing ACTH.

C. Catecholamine-dependent CS

Catecholamines are known to modulate HPA activity. Activation of α_1-adrenoreceptors in the PVN leads to CRH release with increased plasma levels of ACTH and cortisol (14). Administration of β_1- or β_2-adrenergic agonists or antagonists has no effect on ACTH or cortisol secretion (246). Peripherally administered α_1-adrenoreceptor agonists fail to activate the HPA, as the blood-brain barrier prevents their access to the PVN. Direct adrenal stimulatory or inhibitory effects of catecholamines on GC or mineralocorticoid secretion have been noted in several species, but are limited to aldosterone secretion in humans, where cortisol secretion is unaffected (11).

As discussed in *Section IV*, the abnormal presence of β-adrenergic receptors or the activation of AC activity by cat-

echolamines has been reported *in vitro* in several cases of human adrenal tumors associated with CS (191–193); no evidence of such receptors has been found in the normal adrenal cortex. However, the clinical expression of this abnormality was appreciated only recently in two patients. A 56-yr-old French-Canadian man with AIMAH and CS (86) was shown to have ACTH-independent overproduction of cortisol and aldosterone during elevations of endogenous catecholamines level (upright posture, insulin-induced hypoglycemia, and EKG stress test). Augmented plasma cortisol during upright posture was decreased after pretreatment with the β-adrenergic antagonist, propranolol; in contrast, this did not occur after inhibition of the RAS system with captopril or losartan, or of AVP with a V1-AVPR antagonist. Isoproterenol infusion stimulated cortisol (2.1-fold) and aldosterone (2.2-fold) secretion in the patient, but not in normal subjects, in whom ACTH had been suppressed by dexamethasone. Plasma cortisol was not influenced by mixed meals, or administration of TRH, GnRH, glucagon, or cisapride; as discussed previously, a late increase of cortisol after AVP administration was believed to result from stimulation of release of adrenomedullary catecholamines. High-affinity binding sites compatible with β_1-adrenergic receptor (β_1-AR) or β_2-AR were found in the adrenal tissues of the patient, but not in the controls. They were efficiently coupled to steroidogenesis (Fig. 1), as shown by AC stimulation with isoproterenol *in vitro* and catecholamine-induced steroidogenesis *in vivo* (86). Further molecular studies are needed to properly characterize the β-adrenergic receptor subtype expressed in hyperplastic adrenal tissues and to determine whether or not it is mutated.

Another 50-yr-old American woman with CS and AIMAH (240) was found to have abnormal responses to catecholamines in addition to an exaggerated response to AVP (described previously in *Section V.B.*). In this patient, plasma cortisol had risen after upright posture (1.7-fold) and exogenous AVP (3.4-fold), but also after insulin-induced hypoglycemia (2.7-fold), while ACTH remained suppressed. Infusion of isoproterenol for 30 min increased plasma cortisol from 323 to 630 nmol/liter, which returned rapidly to baseline when the infusion was discontinued. Pretreatment of the patient with the angiotensin receptor type-1 antagonist losartan did not prevent the elevation of plasma cortisol during upright posture. There were no increases of plasma cortisol after mixed meals, GnRH, TRH, glucagon, or cisapride. It was concluded that cortisol secretion was mediated by the abnormal presence and function of β-adrenergic and V1-AVPR, and medical therapy with the β-blocker propranolol was proposed to the patient; she did not tolerate this medication well and elected to undergo surgery in her home city (tissues not available).

D. LH-dependent CS

The LH/hCG receptor (LH/hCGR) normally activates AC and PLC to stimulate gonadal steroidogenesis (247). The receptor is mainly expressed in gonadal tissues, but also in other tissues, including the uterus, fallopian tubes, placenta, brain, hypothalamus, and prostate (248); recently, the presence of LH/hCGR was identified in the zona reticularis of the human adrenal (249) by immunohistochemistry and *in situ* hybridization. hCG stimulates DHEAS secretion in human fetal adrenal cells (250).

A 63-yr-old French-Canadian woman was studied for CS and AIMAH (251). Retrospectively, she described having gained between 18–22 kg during each of four full-term pregnancies, with Cushingoid fat distribution, but without high blood pressure, purple skin striae, or hirsutism. Her weight returned rapidly to baseline after delivery with symptoms of lack of appetite, nausea, and fatigue, which subsided within 2–3 months. Chronic hypercortisolism became clinically manifest only 10 yr after menopause (Fig. 3). Cortisol production was increased by the *in vivo* administration of GnRH, hCG, and recombinant human LH (hLH). Plasma free testosterone and estradiol were also augmented by hLH administration. Abnormal stimulation of cortisol, free testosterone, and DHEAS production was also evoked in this patient by oral intake of cisapride and metoclopramide, two 5-HT_4R agonists (251). Administration of the long-acting GnRH analog leuprolide acetate initially increased LH and FSH secretion, which was paralleled by a rise in cortisol secretion; however, this was followed within 10 days by suppression of endogenous LH and FSH levels and normalization of cortisol production. Stimulation of cortisol by hCG and recombinant hLH, but not by FSH, suggests that a functional adrenocortical LH/hCGR was coupled to steroidogenesis (Fig. 3); the lack of stimulation by GnRH, when LH levels were suppressed by chronic administration of leuprolide acetate, excludes an adrenal GnRH receptor. Studies of normal adult controls did not indicate any coupling of LH/hCGR to adrenal synthesis of cortisol or DHEAS. Abnormal stimulation of plasma cortisol after GnRH and LH administration was also found in one woman with bilateral macronodular adrenal hyperplasia and normal urinary cortisol levels, which did not suppress normally with dexamethasone (242). This suggests that diverse ectopic hormone receptors can be present in preclinical bilateral macronodular adrenal hyperplasia.

Pregnancy is relatively rare in women with CS, as only about 100 cases have been summarized in recent reviews (252–254). GC and androgen excess induce suppression of the pituitary-gonadal axis, causing oligomenorrhea or amenorrhea in 75% of woman of reproductive age affected by CS (1–3). In women in whom CS was associated with pregnancy (252), the etiology was more often secondary to an adrenal adenoma (44%) or carcinoma (17%) than to pituitary corticotroph adenoma (29%) or ectopic ACTH secretion (4%). Hypercortisolism is often responsible for high rates of abortion, premature labor, stillbirths, and perinatal deaths (252). In some cases of pregnancy and CS secondary to adrenal adenomas (255–257) or large bilateral macronodular adrenal hyperplasia (258), the clinical syndrome regressed after abortion or delivery. The syndrome was identified in a few cases only after exacerbation of the hypercortisolism during a subsequent pregnancy (259). When these patients were tested after delivery, biochemical evidence of residual abnormal cortisol secretion was still present, despite substantial improvement, and was fully corrected only after surgical removal of the adenoma. In the case of a woman with a large AIMAH reported by Calodney *et al.* (258), clinical CS oc-

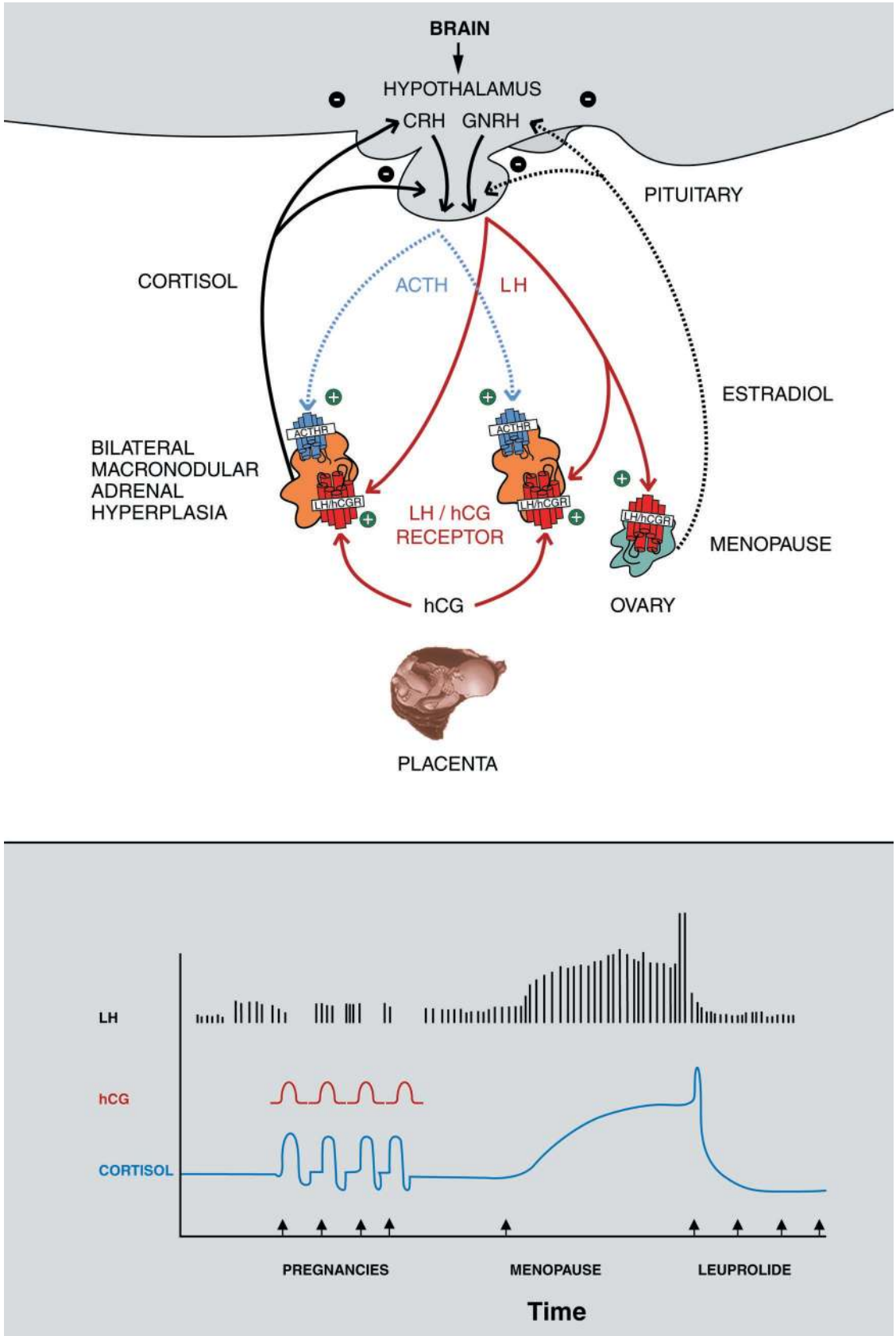

FIG. 3. HPA axis in CS secondary to the ectopic adrenal expression of LH/hCGR. Adrenal expression of the LH/hCGR in the adrenal cortex is illustrated. Occupation of this receptor either by hCG of placental origin or by LH of pituitary origin induces cortisol secretion, which exerts negative feedback inhibition on CRH and ACTH production (*upper panel*). The development of bilateral adrenal hyperplasia and hypercor-

curred only during the sixth pregnancy; the hypercortisolism was not suppressible with dexamethasone, but rather displayed paradoxical increases in urinary steroid secretion. Basal urinary 17-ketogenic and 17-ketosteroids returned to normal basal levels during the next 2 yr with the disappearance of clinical CS; however, the diurnal rhythm of plasma cortisol remained abnormal, as was dexamethasone suppressibility, leading to bilateral adrenalectomy. There was no evidence of pituitary CS in this patient, but ACTH assays were not available, and no metyrapone test was performed.

Transient corticotropin-independent CS during pregnancy with complete resolution after spontaneous abortion or delivery was described in two patients with mild bilateral adrenal hyperplasia (260, 261) or unknown adrenal pathology (262). A paradoxical increase in cortisol excretion during dexamethasone administration in pregnancy completely returned to normal after delivery (261). One patient developed severe biochemical and clinical evidence of CS during each of three pregnancies, and ACTH or cortisol levels were not stimulated by vasopressin administration (260); there was a transient period of hypocortisolism after each delivery, followed by complete clinical regression. In one case, short-term hCG administration elevated urinary 17-hydroxycorticosteroid levels, while sequential estrogen and progestogen administration had no effect (262).

It is thus possible that some of these patients with transient CS during pregnancy, or in whom hypercortisolism increased during pregnancy, also expressed ectopic LH receptors in their adrenal adenomas or in their adrenal cortex. Specific testing of the regulation of steroidogenesis with LH or estrogens in future cases of transient CS during pregnancy will help in elucidating the pathophysiology. It must be pointed out that spontaneous remission of CS after delivery has also been reported in a patient with ACTH-dependent CS of probable pituitary origin (263); the mechanisms involved in this regression have not been elucidated.

E. LH-dependent adrenal androgen-secreting tumors

Although the regulation of cortisol secretion by LH in adrenal CS was demonstrated only recently *in vivo*, there have been several reports indicating that the regulation of steroidogenesis in some rare, pure, androgen-secreting tumors was stimulated by hCG or GnRH (195, 264, 265). As plasma LH was found to be relatively suppressed in some of these patients, the role of endogenous LH in maintaining androgen production may be uncertain. In some cases, suppression of endogenous LH levels by administration of estrogens (266, 267) or by ACTH stimulation of GC (268) inhibited androgen production. In other cases, estrogens were unsuccessful in depressing androgen production (264, 269, 270). It has been suggested that gonadal cells localized in the adrenals could explain this phenomenon; however, clear evidence of adrenal origin of the tumors was identified in certain cases (195).

F. Serotonin-responsive CS

5-HT is produced by intraadrenal mast cells in humans and can regulate corticosteroid production via a paracrine mechanism (87, 271); these effects are mediated by the 5-HT_4R subtype, which is expressed mainly in adrenal ZG but also in zona fasciculata cells (89, 91). 5-HT_4R agonists are potent stimulators of aldosterone secretion in humans; they are weak stimulators of cortisol secretion by human adrenocortical cells *in vitro*, but not of plasma cortisol in normal subjects (87).

In the patient with LH-dependent CS (251), cisapride and metoclopramide, two 5-HT_4R agonists, produced 4.8- and 2.6-fold peak elevations, respectively, in plasma cortisol 120 min after their oral administration. Plasma corticotropin levels remained undetectable during cisapride and metoclopramide testing. Stimulation of plasma cortisol in this patient after treatment with cisapride and metoclopramide was proportional to their respective affinity for 5-HT_4R (87); no such response to cisapride was found in five other patients with bilateral adrenal hyperplasia, 11 with unilateral adenoma, and one with carcinoma and CS (240). A patient with CS and AIMAH was found to increase plasma cortisol in response to cisapride as well as to LVP and CRH, despite suppression of ACTH (272). Recent observations in patients with bilateral macronodular adrenal hyperplasia and preclinical hypercortisolism also documented marked stimulation of cortisol secretion upon cisapride administration (242).

The exaggerated cortisol responses to cisapride in these patients could be secondary to the increased zona fasciculata expression or abnormal function of an "eutopic" 5-HT_4R-effector system, rather than to the presence of an ectopic receptor. The presence of a 5-HT_4R has been detected by RT-PCR in the adrenal tissues of one of these patients and was similar to that found in normal adrenal cortex; however, full receptor sequencing and adrenal zone distribution have not been performed (272).

G. Steroid-responsive CS

Caticha *et al.* (273) described a 33-yr-old woman who developed transient and reversible clinical and biochemical signs of ACTH-independent CS during three pregnancies and during intake of oral contraceptives. Her adrenal histology was described as being compatible with primary nodular dysplasia, but there were no comments on pigmentation of her adrenal nodules; there was also no family history of adrenal disease and no other features of Carney's complex. Paradoxical increases in cortisol production were noted during oral dexamethasone suppression tests. Dose-responsive stimulation of cortisol secretion occurred after bilateral adrenalectomy when the cells were exposed to estradiol; the *in vitro* addition of dexamethasone was not reported, nor were the effects of antiestrogens.

Paradoxical increases in plasma cortisol and urinary free

tisolism is produced transiently and reversibly due to occupation of the receptor by hCG during pregnancies; delivery is followed by a transient period of hypocortisolism (*upper* and *lower panels*). At the time of menopause, a sustained elevation of LH levels follows a decrease in ovarian estrogen production and results in a progressive increase of bilateral adrenal hyperplasia and hypercortisolism. Administration of long-acting leuprolide acetate initially induced transient stimulation of LH and cortisol, followed by long-term suppression of LH and restoration of normal cortisol production.

cortisol were observed during the last 2 days of classical Liddle's 4-day low- and high-dose oral dexamethasone tests in patients with PPNAD with or without Carney's complex (171). We found no evidence of ectopic membrane hormone receptors in two patients with PPNAD, who showed an increase in cortisol secretion during prolonged dexamethasone administration; GC receptors appeared to be highly expressed by immunohistochemistry in PPNAD micronodules, compared with the adjacent internodular atrophic adrenal or to the normal control adrenal cortex (274). Similar paradoxical elevations of cortisol production during dexamethasone have been reported in several cases of CS during pregnancy (258, 261).

H. Other abnormal hormone responses in adrenal CS

Hashimoto *et al.* (275) described a 51-yr-old male with large bilateral AIMAH in whom plasma cortisol increased during insulin-induced hypoglycemia, while ACTH, measured by RIA, remained at undetectable levels (<10 pg/ml); *in vitro*, dispersed adrenal cells stimulated cortisol secretion with ACTH, but not with insulin, catecholamines, vasopressin, or Ang-II. A very similar patient with AIMAH studied by the same group (232) also displayed elevated plasma cortisol during insulin-induced hypoglycemia and combined LVP-CRH tests while plasma ACTH remained undetectable; *in vitro* studies were not performed in this case. It remained unclear whether insulin itself, a factor increased during hypoglycemia, or subdetectable rises in plasma ACTH were responsible for the regulation of cortisol secretion in these cases.

Leptin synthesis is stimulated by GC (136), and leptin receptors are expressed in normal adrenals as well as in adrenocortical adenomas and carcinomas (128, 276). Plasma leptin has been found to be elevated in patients with CS. The leptin receptor is expressed in the adrenal cortex, where leptin normally inhibits cortisol secretion. Leptin negatively regulates the HPA, both at the pituitary level, where it suppresses CRH secretion, and the adrenal level, where it depresses steroidogenesis (124, 130, 276). Pralong *et al.* (139) recently reported a 36-yr-old woman with AIMAH and CS in whom a mixed meal heightened plasma cortisol levels, and this effect was decreased by octreotide pretreatment. GIP was not infused, but GIP stimulated cortisol secretion *in vitro*. Leptin (single dose of 100 nM) increased cortisol secretion *in vitro*, whereas in normal adrenal tissues, it normally suppresses this parameter. Plasma leptin levels were elevated in this patient with CS but did not increase after meals. GIPR or leptin receptor were not measured directly. Thus, this case raises the possibility of paradoxical stimulation of steroidogenesis by leptin in some cases of AIMAH, but more detailed studies are required in other similar cases to confirm this possibility.

VI. Investigation Strategy

A. Initial clinical screening protocol

A protocol has been developed to screen patients with adrenal CS for the presence of ectopic/abnormal adrenal hormone receptors (277); the strategy is based on monitoring plasma levels of steroids during various tests that transiently modulate the levels of ligands for potentially abnormal receptors. The protocol includes serial measurements of plasma ACTH, cortisol, and other steroids or hormones as indicated (aldosterone, free testosterone, DHAS, and estradiol) at 30- to 60-min intervals for 2–3 h during the course of various tests performed after an overnight fast and in a supine posture for at least 1 h. Initial screening includes a posture test performed in a 2-h supine position, followed by a 2-h ambulatory period (to evaluate potential modulation by Ang-II, vasopressin, catecholamines, ANP, etc.); this is followed by a standard mixed meal (to evaluate the response of gastrointestinal hormones) and then by the administration of 250 μg ACTH 1–24 iv, which serves as a reference test. On another day, the administration of 100 μg GnRH iv (modulation by FSH, LH, GnRH) is followed by 200 μg TRH iv (modulation by TSH, PRL, TRH). Responses to 1 mg glucagon iv, 10 IU AVP im, and 10 mg cisapride orally (a serotonin 5-HT_4R agonist; this is now replaced by 10 mg metoclopramide as cisapride was withdrawn from the market) are tested sequentially on the third day. A change of less than 25% plasma cortisol is arbitrarily defined as no response, a 25–49% change is defined as a partial response, and a change of 50% or greater is considered a positive response. If a partial or positive cortisol response is found, the test is repeated to verify its consistency and to determine whether other steroids, such as aldosterone, DHAS, testosterone, and estradiol, are also modified. At the same time, fluctuations of potentially interesting ligand hormones (*i.e.*, catecholamines, vasopressin, renin/Ang-II, and ANP during a posture test) are measured. If a prolonged response to a test masks the evaluation of the following test, it is repeated separately.

B. Further characterization of abnormal hormone receptors

After initial screening, other tests can be performed to confirm the responses or to elucidate which hormone is implicated (Fig. 4). For example, if cortisol stimulation by upright posture is found, the inverse effect, *i.e.*, suppression by assuming a supine posture after ambulation, is verified. The respective contributions of vasopressin, catecholamines, and Ang-II or ANP modifications need to be distinguished. An exaggerated cortisol response to pharmacological levels of exogenous vasopressin is followed by evaluation of whether physiological fluctuations of endogenous vasopressin would modify plasma cortisol levels. An increase of plasma vasopressin during an upright posture test should parallel the elevation of plasma cortisol levels. Endogenous plasma AVP levels can be modulated by a 20 cc/kg water load, followed by infusion of NaCl 3% at 0.1 cc/kg/min for 120 min. The expected result would be an initial suppression of AVP and cortisol during water loading, followed by an increase of AVP and cortisol levels. To determine whether the vasopressin receptor involved in this response is a V1, V2, or V3 receptor type, 2.5 μg desmopressin, a preferential V2 receptor agonist, is administered subcutaneously (80, 86); the absence of a response to desmopressin would suggest a V1 or V1b/V3 receptor-mediated response. Pretreatment with a specific oral V1 receptor antagonist (SR 49049) has been used

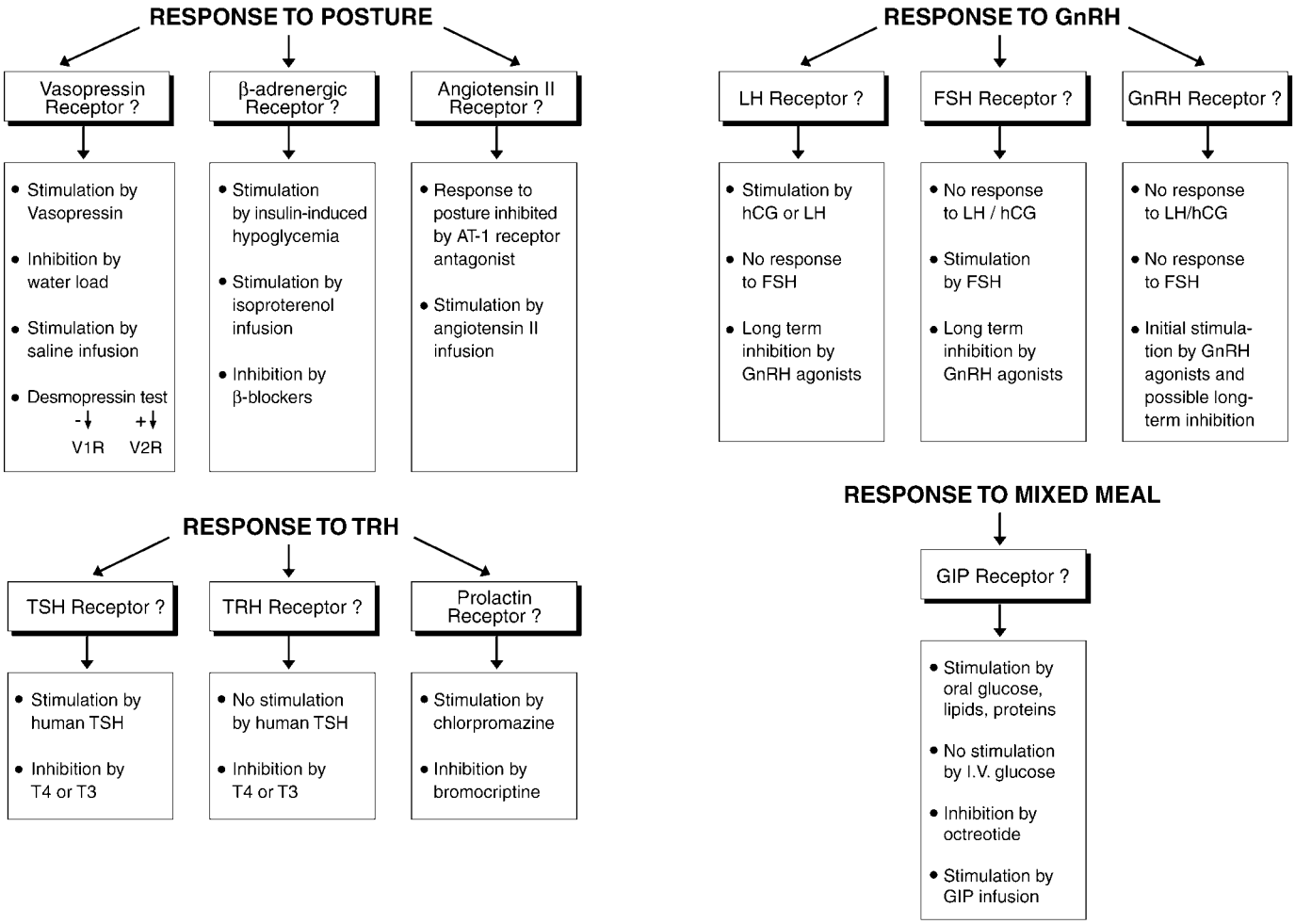

FIG. 4. Outline of the investigation protocol to further characterize abnormal adrenal hormone receptors when a positive response is identified during initial screening. Plasma cortisol levels are monitored during the various tests. [Reproduced with permission from A. Lacroix *et al*.: *The Endocrinologist* 9:9–15, 1999 (277). © Lippincott Williams & Wilkins.]

to demonstrate *in vivo* the involvement of the V1 receptor in this response (86). In case of no response to exogenous AVP, the role of Ang-II is assessed by repeating the posture test after administration of an AT1R antagonist or by direct infusion of Ang-II. If a catecholamine response is suspected, endogenous catecholamine stimulation is produced by insulin-induced hypoglycemia, and, if positive, by isoproterenol infusion (86). An attempt to block the response and to treat the patient with a β-blocker would be conducted if the stimulation of cortisol production is reproduced.

In the case of a response to a mixed meal, confirmation and identification of a specific gastrointestinal hormone involvement are based on evaluation of the effects of carbohydrates, proteins, or lipids on cortisol secretion. Patients ingest, at 3-h intervals, 75 g oral glucose, an isocaloric protein-rich meal, or a lipid-rich meal (200, 206). Plasma cortisol, ACTH, GIP, and insulin levels are measured at regular intervals during these tests. The absence of a cortisol response to the administration of 25 g glucose iv, or when 100 μg octreotide is administered sc 60 min before repeating the oral 75 g glucose challenge, confirms the role of a gastrointestinal hormone (200, 206). As only GIP and GLP-1 respond well to oral glucose, lipids, and partially to proteins, human GIP is infused at a rate of 0.6 μg/kg/min during the administration of 150 cc/h of 10% glucose and compared with the response to GLP-1 infused at a rate of 0.75 pmol/kg/min, also under 10% glucose (200, 206). The pattern of response to the various secretagogues would be different in the presence of abnormal receptors for gastrointestinal hormones other than GIP. Various candidate hormones would then be infused to confirm the identity of the steroidogenesis modulator.

Stimulation of cortisol production after GnRH administration could result from the abnormal adrenocortical presence of receptors for LH/hCG, FSH, or GnRH itself. The cortisol response after the administration on different days, of hCG 10,000 U im, purified human FSH 150–300 U im, and recombinant LH 300 U iv can be compared (251). A response to GnRH coupled to an absence of response to FSH, LH, and hCG would suggest an ectopic GnRH receptor; various analogs and antagonists of this receptor are available for testing the hypothesis. In addition, the response to an acute dose of GnRH should persist despite the suppression of endogenous gonadotropins by the administration of supraphysiological doses of gonadal steroids or the use of long-acting GnRH

analogs. In the presence of an ectopic LH/hCGR, a response to exogenous hCG or LH, but not to exogenous FSH, should be evident; the response to acute GnRH administration should disappear when the LH response is abolished by exogenous gonadal steroids or after the chronic administration of long-acting GnRH analogs. Therapy with long-acting GnRH analogs should produce eventual suppression of the endogenous LH ligand and normalize cortisol production, as demonstrated recently by our group in one such patient (251). In the presence of an ectopic FSH receptor, there should be no response to hCG or LH, but cortisol production should be increased after the administration of purified FSH. Here again, long-acting GnRH analogs should suppress the biologically active ligand and correct the hypercortisolism.

Stimulation of cortisol synthesis after TRH administration has not yet been reported. However, AC stimulation by TSH has been demonstrated in adrenocortical adenomas *in vitro* (9). Thus, a response to TRH would suggest the possibility of an ectopic receptor either for TSH, TRH, or PRL. The PRL receptor does not belong to the family of G-coupled seven-transmembrane receptors, which could mimic the ACTHR and activate AC. However, adrenocortical stimulation by PRL has been described *in vitro* (190), and the presence of this receptor in adrenal tumors has been confirmed (278). Elevation of endogenous PRL levels after a chlorpromazine test and its inhibition by a bromocriptine test would easily clarify the role of endogenous PRL. The potential presence of an ectopic TSH receptor would be assessed directly by the administration of purified human TSH and by inhibiting endogenous TSH production with exogenous T_4. The lack of an ectopic TRH receptor would be confirmed by disappearance of the cortisol response when the response of TSH to TRH has been suppressed by T_4 administration.

The *in vitro* response of AC to glucagon has been demonstrated previously in a cortisol-secreting adenoma (190), but a clinical case has not yet been reported. If a response to 1 mg of exogenous glucagon is found, it would be necessary to show that fluctuations of endogenous glucagon levels during insulin-induced hypoglycemia, fasting, or oral administration of glucose correlate well with fluctuations of cortisol levels.

The oral administration of 10 mg cisapride, a 5-HT_4R agonist, is expected to induce a large increase in aldosterone, but not in cortisol levels in normal individuals (91). If a cortisol response to cisapride is found, a response to other 5-HT_4R agonists such as zacopride or metoclopramide should be seen, but not to specific 5-HT-1,2,3 agonists. Although some specific 5-HT_4R antagonists are currently under investigation, their availability is limited, but they should become very valuable in confirming the role of this abnormally expressed receptor.

C. Systematic clinical screening for ectopic/abnormal hormone receptors

There has been only one report to date of the systematic clinical screening of patients with adrenal CS for the presence of diverse abnormal hormone receptors (240). In that study, 20 consecutive patients with adrenal CS secondary to either bilateral macronodular adrenal hyperplasia (n = 6), unilateral adenoma (n = 13), or carcinoma (n = 1) were tested for evidence of an abnormal hormone receptor. All six patients with AIMAH had a positive response to at least one test, in addition to ACTH 1–24: two patients, to the mixed meal (GIP-dependent); one patient, to GnRH (LH/hCGR) and cisapride (5-HT_4R); and three patients, to the upright posture and vasopressin (1 β-AR, 1 V1-AVPR, 1 β-AR, and V1-AVPR). In patients with unilateral adenoma, only one patient had a positive response to upright posture, while three partial responses to either mixed meals, vasopressin, or posture were also noted but were not further characterized. In the patient with adrenocortical carcinoma or in two patients with micronodular adrenal dysplasia (274), plasma cortisol was not modified by any of the tests. Initial experience suggests that the adrenal expression of various ectopic or abnormal hormone receptors is frequently implicated in the pathophysiology of bilateral macronodular adrenal hyperplasia (240), but less frequently in unilateral adenoma (79). It must be noted that the initial protocol used to date did not screen for many other G protein-coupled membrane receptors, such as those for PTH, calcitonin, acetylcholine, dopamine, opiates, prostaglandins, etc; it may thus become pertinent to investigate these other potential abnormal receptors in the future.

VII. Molecular Mechanisms of Ectopic/Abnormal Hormone Receptors

A. Tissue-specific expression and regulation of membrane hormone receptors

The hormonal regulation of adrenal cortex development and function requires the tissue-specific expression of hormone receptors. This implies the existence of fine-tuned mechanisms of regulation that involve *cis*-acting regulatory elements (promoters) and *trans*-acting factors (TFs) for these receptors. It is thus pertinent to briefly review which factors regulate the appropriate tissue-specific expression of the membrane hormone receptors of interest in adrenal CS before considering which molecular mechanisms could be responsible for their abnormal adrenal expression and function.

The ACTH MC-2 receptor gene, localized on human chromosome 18 (18p11.2), is highly expressed in the adrenal cortex and, at lower levels, in fat tissue and skin (22, 279, 280). The proximal promoter region (~1,000 bp) of the human ACTHR (hACTHR) gene is responsible for the basal transcriptional rate and contains several potential regulatory elements: one SP1 element, four AP1 elements, seven CRE (cAMP-responsive element)-like regulatory elements, and three SF-1-like elements (SF-35, SF-209, and SF-98) (23, 281, 282). Both SF-35 and SF-98 sites were shown to be essential for the cAMP regulation of ACTHR transcription. Although absolutely required, SF-1 is not sufficient for ACTHR expression in the adrenals, since it is not expressed in gonads, whereas both Leydig and ovarian cells express SF-1 (41). The well known up-regulation of the receptor by its own ligand (36, 37, 283–285) is probably mediated by one of the CREs. The same regulatory elements are present in the proximal promoter of the mouse ACTHR, except for CREs, which have

been changed for GRE (GC-responsive element) sites (286). A negative regulatory region (silencer), located between –1,236 and –908 from the transcription start site, prevents expression of the receptor in heterologous systems or in non-SF-1-containing cell sites (286). This suggests that other factors are needed for the receptor to be expressed properly.

β-Adrenergic receptors (β-AR) are subject to extremely tight regulation. In addition to short-term regulatory phosphorylation of receptor proteins, their gene expression is also regulated. Cloning of the 5′-flanking region of human β_1-AR (chromosome 10q24–26) revealed several potential thyroid response elements (TRE), GREs, and CREs (287). These putative response elements support the pathophysiological evidence that thyroid and GC hormones regulate β_1-AR by affecting receptor expression (287, 288). Hyper- and hypothyroidism have been associated with increases or decreases in β_1-AR number and activity. Thus, the presence of TRE in the 5′-flanking region of β_1-AR is consistent with these clinical conditions (289, 290). β_2-AR (chromosome 5q32) expression is up-regulated by GC in various tissues and is due to a direct increase in the rate of its transcription (291, 292). This is probably mediated by GRE, as demonstrated for hamster β_2-AR (293). In contrast, β_1-AR is down-regulated by GC. The stability of β_1-AR mRNA is not influenced by GC, but nuclear run-on assays have revealed that down-regulation is due to a decline in the relative transcription rate of the receptor (294). Homologous desensitization of β-AR has been observed for the three receptor types, β_1-, β_2-, and β_3-AR (291, 295, 296). This is compatible with the presence of CRE in the promoters of both β_1- and β_2-AR (287). Moreover, β-adrenergic stimulation causes not only down-regulation of β-AR but also loss of coupling to G_s/AC effectors (297). *In vivo* investigations of GC effects on β-agonist-induced down-regulation of β_1- and β_2-AR have shown that GC can prevent down-regulation of β_2-AR number and mRNA at the transcriptional level; the TF CREB may be involved (294).

LH/hCGR have also been reported in the human adrenal zona reticularis (249), although they are more highly expressed in gonadal tissues. The LH/CGR is one of the largest seven-transmembrane receptors (683 amino acids) as it harbors an unusually long extracellular ligand-binding domain (247). This receptor is encoded by two genes: gene I isolated from a lymphocyte library, and gene II isolated from a placental library (298). The four copies of hLHR genes are localized on chromosome 2p16–21 loci. The two proximal 5′-untranslated regions have been well characterized (299–301) and differ by several base changes and a 6-bp deletion in the coding region (+55 to +60). The transcription initiation site is localized at position –176 bp for both promoter regions. Additional upstream transcription start sites have been identified in human testicular and choriocarcinoma JAR cells. These data suggest that tissue-selective LHR promoter utilization and gene (I or II) expression may underlie the specific pattern of LHR expression. TATA and CAAT-like boxes have been identified in human, but not in mouse and rat, promoters; the human promoter contains one CRE, seven AP1 sites, and one half-ERE site. Three negative control regions (NCRs), when complexed with proteins of JEG-3 cell nuclear extracts, disable the proximal promoter activity (300); these regions might be very important in nongonadal tissues.

The actions of vasopressin are mediated by three G protein-coupled membrane receptor subtypes. V2 receptors are expressed almost exclusively in renal collecting ducts to promote water permeability via activation of G_s and AC (302, 303). VIa (or VI) receptors are expressed in blood vessels, where they promote vasoconstriction (304), and in the liver, where they promote glycogenolysis (305), while VIb (or V3) receptors are located mainly in the anterior pituitary, but also in the adrenal medulla. VIa and Vlb receptors are coupled to various pertussis toxin-sensitive G proteins and activate PLA_2, PLC, and PLD through activation of ligand-gated calcium channels (306). VIa receptors are also present in the adrenal cortex where they are involved in steroid secretion (see *Section II*). Dexamethasone increases the expression of VIa receptors in the rat liver and forebrain (307, 308). The elevation of mRNA levels precedes the rise in binding activity, suggesting a transcriptional effect. Isolation and analysis of the 5′-regulatory region of the rat VIa receptor have demonstrated that *trans*-acting factors such as CREB, AP-2, and GR are involved in the expression of the receptor gene (309, 310). At the protein level, GC have been shown to produce an early decrease in binding site density, followed later by an increase, which becomes more prevalent with time. Perhaps GC initially affects the stability of receptor protein or that of mRNA levels (307, 311). GC can also negatively regulate the stimulated expression of V1a receptor by a mechanism not involving GR-binding to DNA (310). Furthermore, it has been reported that GC amplify the vasopressin-induced transduction signal (increased IP accumulation in the presence of dexamethasone) (312, 313). This mechanism of regulation was demonstrated for the V1b receptor in the anterior pituitary where prolonged exposure to dexamethasone decreased the number of receptors, while increasing their coupling efficiency. Potentiation was found to be due, in part, to an increase in the guanylyl nucleotide-binding protein, Gq (314). The effect of GC on adrenal VIa receptors has not been studied.

The recent cloning of rat (214), hamster (215), and human GIPR (216–218) has revealed that it is a member of the secretin-VIP family of receptors. This gene is proposed to be involved in the pathogenesis of diabetes as GIPR knockout mice displayed glucose intolerance with impaired insulin secretion (218a). The human GIPR gene is localized on the chromosome 19q13.3 locus and consists of 14 exons; it is expressed in several tissues, including the rat brain, fat, gut, vascular endothelium, and adrenals (214, 315, 316). *In situ* hybridization studies indicate that the GIPR is localized in the inner layers of the rat adrenal cortex (214); GIP is also able to stimulate AC and corticosterone synthesis in the rat adrenal cortex (317). In humans, the tissue distribution of GIPR mRNA has not yet been examined extensively, but has been discovered in the pancreas and brain, but not in spleen (210). Several splice variants of the receptor have been described in the human pancreas, of which one with a 27-amino acid insertion in the cytoplasmic tail is functional (216, 219). The rat GIPR has been shown to be desensitized by its own ligand *in vivo* and *in vitro* (318). The rat 5′-flanking region of the receptor gene has recently been cloned (319); it is a TATA-less promoter harboring one CRE, an octamer-binding site, three Sp1 sites, and an initiator element (319). Distal negative con-

trol sequences, not yet clearly identified, seem to confer cell-specific regulation of GIPR expression (319). The human GIPR promoter, however, has not yet been characterized.

5-HT_4R-mediated stimulation of corticosteroid secretion is the only known endocrine effect mediated by this receptor. Activation of 5-HT_4R augments AC activity and elevates cAMP. Homologous desensitization has been postulated to occur via a specific receptor kinase. Splice variants of 5-HT_4R have been detected in several human tissues, and their tissue distribution suggests some degree of tissue specificity (320). These splice variants differ in their capacity to trigger the signal transduction cascade after receptor activation.

B. Potential mechanisms of ectopic or abnormal hormone receptors

The molecular mechanisms responsible for the ectopic or abnormal expression and function of membrane receptors in adrenal CS have not yet been identified. In fact, the important question of regulation of the tissue-specific expression of genes is raised by this new pathophysiology of adrenal CS. Several hypothesis can be proposed, however (Fig. 5). A gene rearrangement could potentially lead to adrenocortical-specific, inappropriate expression of a hormone receptor gene. Examples of this mechanism in endocrine tumors include rearrangements described in subsets of parathyroid adenomas (321), in GC-remediable aldosteronism (322), and in papillary carcinoma of the thyroid (323). The PTH promoter has been found to be recombined with the cyclin D gene, giving rise to the prad-1 oncogene (321). The aldosterone synthase gene has been shown to be fused with the 11β-hydroxylase promoter, resulting in ectopic production of aldosterone in zona fasciculata (322). In 25% of human papillary carcinomas (up to 62% after exposure to Chernobyl irradiation), a chromosomal break fuses the intracellular tyrosine kinase domain of the growth factors receptor RET to one of at least eight new promoters including H4, ELE1, R1α, NTRK1, RFG, and other genes (324, 325); this results in constitutive dimerization and activation of the tyrosine kinase of RET, bypassing the requirement for ligand binding. None of the ectopic hormone receptors identified to date in adrenal CS is located on the same chromosome as the ACTHR promoter; gross gene rearrangements have not been reported to date. More discrete mutations in the promoter regions of the membrane hormone receptor could also greatly increase the expression of a receptor normally expressed at such a low level that it would not play a significant role in steroidogenesis. A point mutation in the promoter region of the hormone receptor could generate an appropriate binding site for an adrenocortical-specific TF/co-activator complex, leading to ectopic expression (Fig. 5A).

Another mechanism by which altered expression of hormone receptors could be achieved would involve abnormalities in TFs, their coactivators, or corepressors (Fig. 5B). Indeed, excessive activity or mutations of TFs can induce

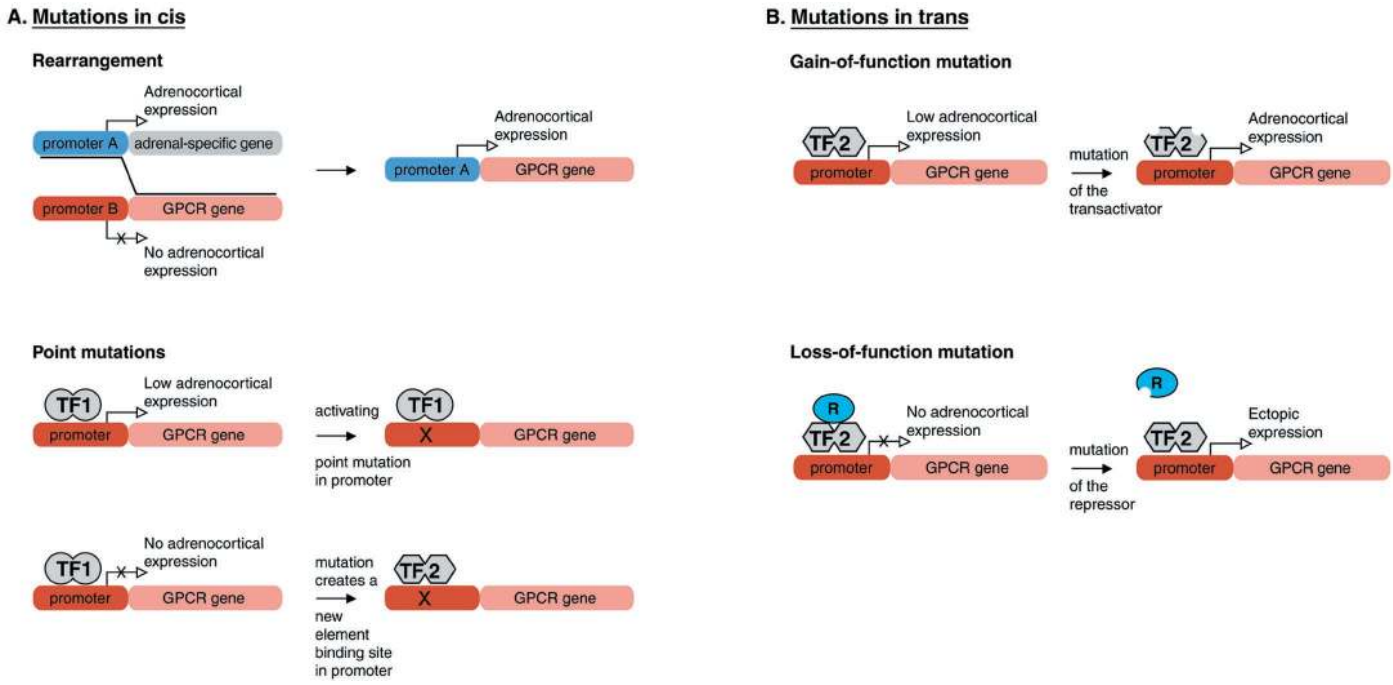

FIG. 5. The potential molecular mechanisms leading to ectopic expression of G protein-coupled receptor (GPCR) in adrenal CS may include mutations in *cis*-acting regulatory regions (A) or in *trans*-acting factors (B). A, Gene rearrangement could fuse a specific GPCR gene that is not expressed in adrenals with an adrenocortical-specific promoter, resulting in ectopic expression of the receptor in the adrenal cortex. More discrete mutations in the GPCR promoter could also lead to abnormal expression. Thus, a point mutation could enhance the transcription of a receptor that is normally weakly expressed in the adrenals and not coupled to steroidogenesis; a point mutation in the promoter of a GPCR gene that is not expressed in the adrenals could generate a new binding site for an adrenocortical-specific TF leading to ectopic expression. B, A gain-of-function mutation of an adrenocortical-specific activator could induce overexpression of an adrenal receptor; the ectopic adrenal expression of a transcription factor (TF) or of its coactivator (not shown) could also result in the ectopic expression of a GPCR. A loss-of-function mutation of a repressor (R) or corepressor (not shown) that prevents the GPCR adrenal expression could result in ectopic expression.

overexpression or a lack of gene expression (61, 326). A gain-of-function mutation could affect an activator that specifically regulates gene expression in the adrenal cortex, or the loss of a repressor could induce ectopic gene expression. In the case of overexpression of a single receptor, any of these hypotheses is plausible. In contrast, when more than one ectopic adrenal receptor is present, mutation in a factor regulating cell differentiation or in a TF common for the different receptors involved may be more likely. It is improbable that acquired mutations of several unrelated promoters would occur simultaneously.

Another interesting hypothesis emerges from a recent study by Kero *et al.* (327), who observed that transgenic mice expressing bLHβ-CTP (a chimeric protein of β-subunit fragments of bovine LH and hCG) in their pituitary develop adrenal CS in addition to polycystic ovaries and ovarian tumors after chronically elevated serum LH levels. It was shown that this resulted from ectopic expression of LH/CG receptors in the adrenal cortex, not detectable or functional in control mice. Since this induction is abolished by gonadectomy, it was proposed that elevated estrogens and PRL levels were responsible for inducing the illicit expression of the LH/CG receptor in the adrenal cortex. This observation would thus raise the possibility that the "ectopic expression" of a receptor may not require a mutation of *cis*- or *trans*-acting regulators, but may result from exaggerated stimulation of a gene that is normally silent.

The presence of abnormal membrane hormone receptors in unilateral cortisol-secreting adenomas may arise from the monoclonal expansion of a primary adrenocortical cell that acquired a somatic mutation, leading to the abnormal expression and function of that receptor at the postzygotic stage of adrenal cortex development (Fig. 2); most studies confirm the monoclonal composition of human adrenocortical tumors (157, 163). In patients with ectopic membrane hormone receptors in bilateral macronodular adrenal hyperplasia, the mutational event must have occurred very early during embryogenesis so that every cortical cell of both adrenals would be affected by the defect, which is polyclonal. There have been rare reports of familial AIMAH (179–182), and in only one case, an abnormal response to LVP was demonstrated in one sibling (182); thus, abnormalities of receptor expression in these syndromes may frequently be the consequence of somatic mutations but, in some cases, could also be germline mutations. In the case of a very early mutational event resulting in AIMAH, the abnormal expression could affect diverse tissues so that polymorph aberrant manifestations would be expected. This was the case in the patient with vasopressin-dependent CS (80) who also displayed an abnormal vascular response to AVP and decreased hypothalamic AVP release during postural hypotension. One patient with GIP-dependent CS and AIMAH suffered from psychiatric dysfunctions that persisted even after correction of the hypercortisolism (200); since GIPR have been shown to be expressed in the brain (214), it is possible that the brain GIPR is also altered. The McCune-Albright syndrome is an example of a somatic mutation occurring during embryogenesis and leading to defects in the adrenal cortex as well as in several other tissues (172); it is still unclear how a somatic mutation could affect all cells in a polyclonal mode in one case (ectopic hormone receptors in AIMAH and CS) and result in a mosaic or oligoclonal pattern of distribution in another case (McCune-Albright syndrome).

The majority of ectopic or abnormal hormone receptors in adrenocortical tumors or hyperplasias (8–10) belong to the G protein-coupled receptor superfamily. Studies of the second messengers implicated in ectopic/abnormal hormone receptors in adrenal CS suggest that they regulate steroidogenesis by mimicking the cellular events triggered normally by ACTHR activation (Fig. 1). It is thus expected that only ectopic hormone receptors capable of coupling efficiently to the intracellular signaling systems present in adrenocortical cells (*i.e.*, those for ACTH, V1-AVP, etc.) will be able to regulate steroidogenesis aberrantly. Certain receptors may be involved more frequently than others, however, if they share more characteristics of the promoters or TFs essential for adrenal cell type-specific tissue expression. It is thus noteworthy that GIPR and the β-AR are expressed normally and are functional in the adrenal fasciculata cells of rodents; it will be interesting to compare the structures of promoters and TFs between species. The LH/hCGR is expressed in the human adrenocortical reticularis during embryonic life; it remains to be seen which events render its expression possible in the fasciculata in adrenal CS (251). Similarly, the 5-HT_4R is usually very efficiently coupled to aldosterone synthesis in the human glomerulosa and already possesses some tropism for fasciculata cells; increased functional coupling to cortisol secretion may require only the inactivation of a relative silencer in fasciculata cells.

C. Role of ectopic hormone receptors in adrenocortical cell proliferation

What is the role of abnormal hormone receptors in altered cell growth and tumorigenesis? One could postulate that the primary event is a mutation resulting in aberrant adrenal expression of the receptor, leading to increased proliferation and eventually to increased hormone production. Alternatively, it can be proposed that the primary event is an unknown proliferative one resulting in cell dedifferentiation with resultant expression of "embryonal" type genes, including one or several hormone receptors. In either hypothesis, it is clear that a relatively long time period is necessary before phenotypic expression of the abnormal hormone receptor becomes evident. This is particularly true for AIMAH, as several decades are necessary before the hyperplasia and hyperfunction become clinically manifest. This may be secondary to the transient occupation of the receptor by the ligand, as illustrated by the cases of GIP (Fig. 2) and LH-dependent (Fig. 3) CS. In GIP-dependent CS, the adrenal tissues are stimulated only briefly but repeatedly after each food ingestion; in LH/hCG-dependent CS, the hyperplasia and hyperfunction occurred only after intense and prolonged exposure to the ligands, *i.e.*, during pregnancy for hCG, or after menopause for LH. Reversal of the hyperplasia between pregnancies would favor the hypothesis that the ectopic receptor is a primary event rather than one that is secondary to another proliferative event; however, this awaits clear demonstration of adenoma or AIMAH regression after complete blockade of the ectopic receptor. There is

also indication, based on cases of preclinical cortisol production in bilateral macronodular disease, that steroidogenesis can be relatively inefficient, despite significant proliferation. This suggests poor steroidogenic enzyme activities in the adrenal lesions or that the low expression of abnormal receptors is better coupled to proliferative signals than to hormone synthesis.

The elucidation of this question requires better understanding of the factors regulating normal adrenal gland development. Knowledge of the ontogeny of steroidogenic tissues (adrenals and gonads) was provided by the identification of tissue-specific TFs (328, 329). Indeed, by using SF-1 as a marker, it became possible to trace steroidogenic cells back to the earliest stage of differentiation (330). Investigation of the spatiotemporal expression of SF-1 revealed the existence of the adreno-genital primordium (AGP) which is composed of a SF-1-immunoreactive single cell population (for review see Ref. 331). This structure lies between the coelomic epithelia of the urogenital bridge and the dorsal aorta. The AGP then gives rise to adrenocortical and gonadal primordia, which both express SF-1. Studies in SF-1 knockout mice have shown that the earliest stages of urogenital ridge development occur normally; however, regression of the adrenals and gonads is observed as soon as gonadal sexual differentiation takes place (66). These results suggest a complex cascade of transcriptional events for establishment of the endocrine axis. The adrenocortical primordium gives rise to the adrenal cortex that differentiates into three zonae (glomerulosa, fasciculata, and reticularis). The adrenal medulla is composed of neural cells (SF-1-negative cells) that have migrated from a dorsal root ganglion to the adrenal primordium. In adult mice, SF-1 is expressed in adrenocortical, testicular Leydig, ovarian theca, and granulosa cells, and, at a lower level, in spleen and pituitary gonadotropes (reviewed in Ref. 41).

DAX-1 (dosage-sensitive sex reversal, AHC critical region on the X chromosome, gene 1) is another steroidogenic-specific TF involved in the adrenal cortex and gonads, as demonstrated by disorders due to DAX-1 mutations (332–334). DAX-1 belongs to the orphan nuclear receptor superfamily as does NGFI-B and SF-1 and it acts as a transcriptional repressor. However, the protein is atypical since it possesses no DNA-binding domain, suggesting possible interactions with other TFs. Indeed, DAX-1 has an expression profile similar to that of SF-1, suggesting a functional correlation between these two proteins (335–337). Moreover, SF-1 was shown to be a critical regulator of DAX-1 expression, as functional SF-1-binding sites have been identified in the promoter region of DAX-1 gene (338, 339). Another transcriptional repressor has been shown to play a role in adrenal development. Initially designated as an essential actor throughout nephrogenesis (340, 341), the Wilm's tumor suppressor gene (WT1) has recently been implicated in adrenogenesis (342). Unlike SF-1, WT1 expression is not detectable during adrenal cortex formation (343, 344) but is in the developing kidney and urogenital system. Taken together, these results suggest, first, that the WT1 gene may be expressed in a very transitory manner in adrenocortical precursor cells, and second, that WT1 activity may be required at early steps of adrenal development, probably in the AGP stage. Functional interactions between SF-1, DAX-1, and WT1 have been demonstrated for transcriptional regulation of the Mullerian inhibiting substance (MIS) sex-specific gene *in vitro* (344). Such combinational regulation may occur for the expression of genes determining the fate of the AGP. It should be interesting to determine whether any alterations in SF-1, DAX-1, or WT1 could be present, particularly in cases of AIMAH with ectopic membrane hormone receptors.

The concept of abnormal G protein-coupled receptors and/or postreceptor events leading to increased cAMP and proliferation is now well established (Table 4, reviewed in Refs. 345–347), especially in somatotroph and thyroid cells (348, 349). Stimulation of G-protein-coupled receptors, alone or in association with tyrosine kinase receptors, is known to evoke powerful mitogenic signals via G protein-mediated activation of ras (346). Thus, altered activity at any step of the transduction signal cascade may predispose to tumor formation. Transgenic mice with thyroid-specific expression of adenosine A2 receptor (which activates AC via G_s protein) develop thyroid hyperplasia and severe hyperthyroidism (350), clearly demonstrating that *in vivo* constitutive activation of the cAMP cascade in thyroid cells is sufficient to stimulate autonomous hyperfunction and uncontrolled cell proliferation. There are many examples of hormone receptor mutations involved in endocrine pathologies (Table 4). Some include somatic or germline constitutive mutational activation of the TSH receptor, resulting in hyperfunctionning thyroid adenomas and hyperplasias (351, 352); familial male precocious puberty (characterized by Leydig cell hyperplasia and testosterone production) is due to constitutive activation of LH/hCGR (353). At the G protein level, the mosaic-activating mutation of $G_{s\alpha}$ leads to McCune-Albright syndrome (172); activating mutations of inhibitory $G_{\alpha i}$ protein (Gip) have been identified in some, but not all, adrenocortical and ovarian tumors (355), and $G_{s\alpha}$ overexpression has been shown in insulinomas and other endocrine tumors (356). There are examples of transgenic mice with cardiac overexpression of β_2-AR or $G_{s\alpha}$ that display enhanced cardiac function and develop myocardial fibrosis (357). However, it must be stressed that cAMP is not mitogenic in all cell types. Counterregulatory mechanisms are initiated in response to persistently elevated cAMP levels. This was the case for transgenic mice expressing gsp in pancreatic β-cells (358) where inhibitors of phosphodiesterases were required to obtain high cAMP levels and enhanced insulin secretion. In the Y1 mouse adrenocortical cell line transfected with β_2-AR, ectopic receptors have been found to be efficiently coupled to steroidogenesis, but cell growth has not been studied (359).

We hypothesize that the ectopic expression of any G protein-coupled receptor could induce the stimulation of adrenal cells by trophic factors lacking regulatory negative feedback by cortisol. This stimulus may lead to increased function and confer a proliferative advantage. The event provides a gain of function to adrenocortical cells; thus, this category of mechanism, *i.e.*, the abnormal tissue-specific expression of membrane hormone receptors should be added to the list of abnormalities of hormone receptors implicated in certain human diseases (Table 4). GIP has been shown to stimulate the cAMP production and DNA synthesis in GIP-dependent, cortisol-secreting adenoma cells in a manner similar to

TABLE 4. Examples of human diseases resulting from gain-of-function mutations of membrane hormone receptor-effector systems

Receptor	Disease
I. Membrane Hormone Receptors	
a. Activating mutations of G protein-coupled hormone receptors	
TSHR: somatic	Toxic thyroid adenomas (351)
TSHR: germline	Familial neonatal hyperthyroidism (352)
LHR: germline	Testotoxicosis (353)
CaR: germline	Familial hypoparathyroidism (394)
PTH/PTHrPR: germline	Jansen metaphysical chondrodysplasia (395)
TSHR mutation with hCG affinity	Transient hyperthyroidism during pregnancy (396)
b. Illicit activation of a normal receptor	
TSHR activated by thyroid stimulating immunoglobulins	Grave's disease (397)
TSHR activated by high concentrations of hCG	Hyperthyroidism in choriocarcinoma (397)
Insulin receptor activation by IGF-I or IGF-II	Hypoglycemia of malignancy (398)
c. Ectopic G-protein coupled hormone receptors	
GIPR in adrenal adenomas or AIMAH	Food-dependent Cushing's syndrome (see Table 2)
LH/hCGR in adrenal AIMAH or adenomas	Postmenopausal and transient Cushing's syndrome during pregnancy; virilization (see Table 3)
β-AR in adrenal AIMAH or adenomas	Cushing's syndrome (see Table 3)
Glucagon receptor in pheochromocytomas	Pheochromocytomas (367,368)
TRH receptor in GH-secreting pituitary adenomas	Acromegaly (370–375)
d. Increased activity of eutopic G-protein coupled receptor-effector systems	
V1-AVP receptor in adrenal AIMAH and adenomas	Cushing's syndrome (see Table 3)
5-HT_4R in adrenal AIMAH	Cushing's syndrome (see Table 3)
e. Other membrane receptors	
Mutations of RET oncongene: germline	MEN 2A, MEN 2B, FMTC (323)
Mutations of RET oncogene: somatic	Medullary carcinoma of thyroid; rarely pheochromocytomas (323)
RET rearrangements	Papillary thyroid carcinomas (323)
EGFR, CSF-1 receptor	Various malignancies (399)
IL-1R	Adrenal Cushing's syndrome adenoma (198)
II. G Proteins	
$G_s\alpha$: somatic; mosaic in embryo	McCune-Albright syndrome (172,173)
$G_s\alpha$: somatic mutation	Acromegaly (355,400)
$G_s\alpha$: somatic mutation	Toxic thyroid nodules (355,401)
$G_s\alpha$: somatic overexpression	Insulinomas (356)
$G_{i2}\alpha$: somatic mutation	Ovarian and adrenal tumors (355)
$G_s\alpha$: germline	Mixed testotoxicosis with pseudohypoparathyroidism type Ia (402)
Gβ3: germline	Essential hypertension (354)

ACTH (210). Hormone-stimulated LH/hCGR can act as an adrenocortical tumor promoter when ectopically expressed in the adrenal cortex of gonadectomized mice transgenic for the inhibin α-subunit promoter/simian virus 40 T-antigen fusion gene (360); it remains to be seen whether the expression of ectopic adrenocortical receptors, in the absence of other oncogenic events, is sufficient for adrenal overgrowth. Future animal models such as transgenic mice expressing ectopic membrane hormone receptors in the adrenal cortex will be informative in this regard. This is already supported by the demonstration of bilateral adrenal hyperplasia and CS in the mice transgenic for bLHβ-CTP with ectopic adrenal expression of LH/CGR (327).

What is the cell of origin in which the receptor is expressed abnormally? Based on the profile of steroids produced, it appears that it can occur in well differentiated cells of the fasciculata/reticularis (pure cortisol- or mixed cortisol-/androgen-secreting adenoma), and in cells from the reticularis (pure androgen-secreting adenoma); the three classes of adrenal steroids are sometimes secreted in macronodular hyperplasia, suggesting that all zonae are affected. It remains to be seen whether some cases of unilateral adenomas or bilateral hyperplasia in primary hyperaldosteronism can also be secondary to ectopic hormone receptors.

No constitutive activating mutations of the ACTHR have yet been found in adrenocortical neoplasms or hyperplasias (361). Recent studies suggested that ACTHR could act as a tumor suppressor gene in adrenal tumorigenesis (362) in a way similar to p53, which is involved in many tumor types, including adrenocortical tumors (363). Loss of heterozygosity of the ACTHR gene was shown to be associated with high malignancy or the absence of secretion in a subset of human adrenocortical tumors. Furthermore, lower expression of ACTHR was found in adrenocortical carcinomas compared with adrenocortical adenomas from patients with CS (364, 365). ACTH is known to be a differentiating factor with low potential for promotion of cell proliferation, as demonstrated by *in vitro* experiments. It has thus been speculated that a defect in the ACTHR signal cascade could result in dedifferentiation and increased cell proliferation (362). Obviously, much work remains to be done to better understand the mechanisms underlying tumorigenesis of the adrenal cortex.

VIII. Ectopic/Abnormal Hormone Membrane Receptors in Nonadrenocortical Tumors

The ectopic or abnormal expression of membrane hormone receptors is not limited to endocrine tumors of the adrenal cortex; an extensive review of their abnormal expression in nonadrenal cortex tissues is beyond the objective of this article, but a few examples will be cited. The aberrant stimulation of AC in other human endocrine tumors has been explored initially by Robert Ney and colleagues (366). AC stimulation was induced by glucagon in three of nine pheochromocytomas and in two of three parathyroid adenomas, by LH and TSH in a thyroid follicular carcinoma, and by ACTH in a pituitary chromophobe adenoma (9, 366). The AC of normal adrenal medulla was not stimulated by glucagon, suggesting the ectopic expression of glucagon receptor in pheochromocytoma (9, 367). This finding served, for a prolonged period of time, as a diagnostic provocative test, particularly in periodically secreting pheochromocytomas. With the advent of more sensitive catecholamine determinations, the glucagon provocative test was rarely used, and this may have contributed to the paucity of molecular characterization of the receptor in pheochromocytomas (368). We do not know whether the glucagon receptor structure in pheochromocytomas is normal, or what regulates its expression. The presence of glucagon receptor in pheochromocytomas remains of clinical relevance as glucagon is commonly used in premedication for endoscopic and radiological investigation of the digestive system, and inadvertent crisis still occurs after its administration in unsuspected pheochromocytomas (369).

Matsukura *et al.* (370) found aberrant AC stimulation in four GH-secreting pituitary adenomas by TRH (two of four), GnRH (two of four), norepinephrine (three of four), dopamine (one of four), glucagon (one of three), or PGE_1 (four of four); in one ACTH-secreting pituitary adenoma, AC was stimulated by GnRH, norepinephrine, and glucagon, but not by TRH. The paradoxical stimulation of GH or ACTH after the GnRH or TRH tests *in vivo* in patients before surgery correlated well with the AC stimulation *in vitro*. The AC of two ectopic ACTH secreting tumors (gastric carcinoid and malignant thymoma) was also stimulated by TRH, GnRH, norepinephrine, epinephrine, serotonin, and PGE_1 (370).

The frequently observed paradoxical increase in GH in acromegalic patients after administration of TRH, or in a lesser proportion, of GnRH (371–373) and the AC stimulation found *in vitro* (370) suggested the presence of ectopic TRH or GnRH receptors in GH-secreting pituitary tumors. The expression of TRH receptors type 1 has been confirmed in GH-secreting adenomas (374), where the structure of the receptor does not appear to be mutated (375); the TRHR-1 is normally expressed in rat somatotroph cells (376), and it is unknown whether the abnormal response of GH in acromegaly results from ectopic expression of one of the TRH receptors, or rather from abnormal coupling of this receptor to GH secretion in adenoma cells. In a preliminary report, the paradoxical increase in GH following oral glucose in acromegaly was found to result from aberrant GH-tumor response to GIP (376a); this would suggest that ectopic GIPR could also occur in acromegaly. Epidermal growth factor (EGF) receptor is overexpressed in several types of human cancers including aggressive GH-secreting tumors (377).

As a corollary to the ectopic expression of LH/hCGR in the adrenal cortex, the stimulation of androgen secretion in patients with ovarian arrhenoblastomas, after administration of ACTH, and their suppression by dexamethasone indicate the ectopic expression of ACTHR in some of those tumors (378, 379).

In a sporadic human medullary thyroid carcinoma (MTC), Matsakura *et al.* (380) found that the AC was activated by TRH, glucagon, epinephrine, norepinephrine, and serotonin, but not by TSH, ACTH, or PRL. A large number of studies have now evaluated the expression and function of hormone and growth factor receptors in MTC (381, 382). It is problematic to distinguish which of the receptors identified are indeed ectopic, as frequently, the search for their expression in normal C cells has not been performed. Mutations of the normally C cell-expressed RET protooncogene (eutopic receptor) are present in almost all cases of genetic forms of familial MTC and MEN-2 (multiple endocrine neoplasia, type 2), and in a proportion of sporadic MTC cases (somatic), and play a crucial role in initiation of C cell proliferation (323). Clearly, other receptors contribute to the development and progression of MTC, *e.g.*, the trk family, neurotrophin receptors, where the type trkB is reduced, while trkC expression is increased during the progression of the disease (381). Some of these proliferative-related receptors are expressed also in normal thyroid; this appears to be the case for transforming growth factor-α (TGF-α and EGF), as well as for their common EGF receptor (382). However, EGF binding protein, particularly EGFBP-2 and -3, are detected only in MTC (382). Rat MTC cell line 6/23 also expresses GLP-1 receptor, VIP receptor, and PACAP receptor (383); in addition, several splice variants of PACAP were expressed in 6/23 cell line. The GLP-1 receptor expression is responsible for glucagon effect on calcitonin secretion via cAMP stimulation (384). Additional receptors in which ectopic or increased expression may be related to the progression of the disease include progesterone receptors, which are focally detected in all studied cases of MTC without the concurrent presence of estrogen receptors (385). Expression of gastrointestinal hormones and their receptors, particularly those of CCK-B/gastrin, also received attention in MTC. Thus, CCK-B/gastrin receptors were detected in all biopsy specimens, while they were not found in normal thyroid tissues or in other thyroid tumors such as follicular adenoma, papillary carcinoma, or anaplastic carcinoma (386). Therefore, the presence of CCK-B/gastrin receptor in MTC may have clinical implications. Much attention has been paid, over the last decade, to somatostatin receptor expression in MTC and many other tumor types. The genomic structure and transcription regulation of the various types of somatostatin receptors are now better understood in MTC (387).

Many other receptors have been described, during the last decade, as being expressed in the adrenal medulla tumors without apparent clinical evidence of their ectopic activities. Such is the case for the ANP receptor and its effect on catecholamine release in human pheochromocytoma (388). Most of the receptors studied more recently have, at least, a potential relevance for control of proliferation. Thus, IGF-II

itself is produced and released by the adrenal and is accompanied by the presence of IGF-II R in pheochromocytomas (389). As the ectopic expression of Src homology 2 (SH2) and SH3-containing oncogenic adaptor protein v-Crk in PC12 cells results in EGF-inducible neuronal differentiation, v-Crk was studied and demonstrated able to regulate the strength of a tyrosine kinase signal that leads to prolonged activation of Ras and MAP kinase, respectively (390). Pheochromocytoma shares the expression of several genes with MTC; one example is TGFα gene and its receptor EGFR (391). Both of these tumors express these receptors *in vivo* and *in vitro,* and it has been suggested that TGFα is involved in the regulation of tumor cell growth. Since the signaling pathway from the TrkA receptor via the MAP kinase is not altered in PC12 cells, it has been proposed that p300 could play a pivotal role in triggering the antimitogenic effect of NGF and neuronal differentiation (392).

Since all cells are regulated in their function and proliferation by a series of hormone and growth factors that signal the cells via membrane receptors, it appears quite plausible that several other examples of ectopic or abnormal membrane receptors will be identified in various hyperplasias and tumors in diverse endocrine and nonendocrine human tissues.

IX. An Opportunity for New Pharmacological Therapeutic Strategies

The identification of ectopic or abnormal adrenal hormone receptors in cortisol-secreting hyperplasias or tumors provides new opportunities to use specific pharmacological therapies as alternatives to adrenalectomy. This was initially suggested by the short-term improvement of hypercortisolism when T_3 was administered briefly to a patient before resection of an adrenal adenoma in which AC was stimulated by TSH (9); however, these data were very preliminary and could not clearly distinguish whether the changes in cortisol levels reflected spontaneous fluctuations of cortisol secretion or were truly the result of endogenous TSH suppression.

Pharmacological blockade of postprandial GIP release with octreotide was attempted in a few patients with GIP-dependent CS as an alternative to surgery (Table 2; Refs. 201, 208, and 209). During the first months of subcutaneous octreotide administration before each meal, clinical and biological improvements were documented, but long-term treatment proved to be ineffective. It is presumed that the escape of octreotide efficacy was secondary to down-regulation of somatostatin receptors in GIP-secreting intestinal cells. Thus, adrenalectomy remains the long-term treatment of choice for this syndrome until specific GIPR antagonists become available. Short-term use of the oral V1-AVPR antagonist OPC-21268 for 8 days decreased urinary free cortisol levels in a patient with vasopressin-responsive AIMAH and CS (239).

In the patient with catecholamine-dependent CS and bilateral AIMAH (86), initial treatment with propranolol up to 320 mg daily was able to considerably reduce cortisol secretion; however, urinary cortisol levels remained approximately twice the upper limit of normal, and it was decided to remove one of the two very large adrenals surgically. It then became possible, upon restoration of propranolol administration, to completely normalize cortisol production. Interestingly, the control of hypercortisolism was followed by a decreased requirement in the dosage of the β-blocker from 320 mg to 20 mg of propranolol daily, as higher doses were causing adrenal insufficiency. GC are known to stimulate β_2-AR transcription (292) via GRE located in promoters of the target genes (293). The normalization of cortisol levels may have decreased β-AR density, which would explain the lower requirement for the antagonist. Propranolol therapy did not reduce the size of the remaining adrenal even after 3 yr of follow-up; however, the minimal dose of propranolol necessary to maintain normal cortisol production was administered, without blocking the receptors completely. This constituted the first example of long-term pharmacological blockade of an ectopic adrenal membrane hormone receptor.

TABLE 5. Potential pharmacological therapy for abnormal hormone receptors in adrenocortical tumors

Abnormal receptor	Therapy
GIPR	Somatostatin or GIPR antagonist
β-AR	β-blockers
TSHR	L-T_4
V1-AVPR	V1-AVPR antagonist
Angiotensin-II R	AT-1 R antagonist
LH/hCGR	GnRH analogs
5-HT_4R	5-HT_4R antagonists

In the patient with LH/hCG-dependent AIMAH and CS, the suppression of endogenous LH levels with chronic, long-acting leuprolide acetate controlled the hypercortisolism (Fig. 3) and avoided bilateral adrenalectomy (251). Leuprolide acetate, a long-acting GnRH agonist, initially stimulated gonadotropin release, which increased cortisol production for 1 week; this was followed by suppression of endogenous LH levels and normalization of cortisol production. Despite complete suppression of endogenous LH levels, the patient did not present cortisol insufficiency. It is possible that basal cortisol production was maintained by serotonin stimulation, since there was also evidence of abnormal 5-HT_4R function in the same adrenals. The absence of regression of bilateral adrenal hyperplasia, despite chronic suppression of endogenous LH, indicates that its size was maintained by abnormal function of 5-HT_4R, or that aberrant receptors regulate steroidogenesis but not cell proliferation. It will be interesting to study the effects of a specific 5-HT_4R antagonist in this patient when it becomes available. A GnRH analog has previously been used successfully in long-term suppression of testosterone-secreting ovarian tumor (393).

Further studies will probably identify a larger diversity of hormone receptor abnormalities and should eventually allow the use of new pharmacological tools to inhibit either the production of endogenous ligands or block the receptors with appropriate specific antagonists (Table 5). Since it is also possible to detect the presence of ectopic/abnormal hormone receptors at the stage of preclinical steroid hormone production (242), it will be of great interest to investigate whether the progression of adrenal tumors or hyperplasias can be prevented by these new pharmacological approaches.

X. Summary and Conclusions

Taken together, the results of *in vitro* and *in vivo* studies indicate that a wide diversity of abnormal adrenocortical membrane hormone receptors can be present in adrenal CS. These may include ectopic hormone receptors, such as those for GIP, β-adrenergic agonists, LH/hCG, or other receptors capable of coupling to G proteins, AC, and steroidogenesis. There is evidence that the IL-1R, which do not belong to the seven-transmembrane receptor family and do not use the same signaling pathway as the ACTHR, may also become coupled to steroidogenesis. A similar outcome may result from increased or altered activity of eutopic receptors, such as those for vasopressin (V1-AVPR), or 5-HT. The presence of ectopic or abnormal receptors places adrenal cells under stimulation of a trophic factor that is not under the main regulatory negative feedback exerted by GC. This constitutes an unregulated new trophic stimulus, which leads to increased function and possibly to hyperplasia and proliferative advantage. The molecular mechanisms responsible for the ectopic expression of hormone receptors or to increased activation of the signaling cascade and steroidogenesis are still largely unknown. Characterization of the pathophysiology of adrenal hyperplasias or tumors can eventually lead to diverse pharmacological therapies as alternatives to adrenalectomy; this has now been illustrated by the short-term improvement of hypercortisolism with T_3 in TSH-dependent adrenal cortisol-secreting adenoma (9), with octreotide in GIP-dependent CS (201, 208, 209), and by the long-term control of ectopic β-AR and LH/hCGR by propranolol (86) and leuprolide acetate, respectively (251). Further studies will probably identify a larger diversity of hormone receptor abnormalities in adrenal and other endocrine and nonendocrine tissues. Elucidation of the molecular mechanisms leading to abnormal hormone receptor expression will probably contribute to our understanding of the regulation of tissue-specific expression of genes.

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Studio Congressi
Via Francesco Ferrara, 40
00191 Rome, Italy
Telephone: 39-06-329-0250, 39-06-329-0239
Fax: 39-06-3630-6897
E-Mail: sc.congressi@agora.stm.it
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