

## Brief Report

## PROPRANOLOL THERAPY FOR ECTOPIC $\beta$ -ADRENERGIC RECEPTORS IN ADRENAL CUSHING'S SYNDROME

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**M**OST patients with corticotropin-independent Cushing's syndrome have an adrenal adenoma or carcinoma,<sup>1</sup> but a few have bilateral adrenal hyperplasia. Three patients with Cushing's syndrome and corticotropin-independent bilateral adrenal hyperplasia<sup>2-4</sup> and two patients with adrenal adenomas<sup>5,6</sup> in whom food stimulated cortisol secretion have been described; the abnormal adrenal tissues in these patients aberrantly overexpressed receptors for gastric inhibitory polypeptide.<sup>6,7</sup> We describe a patient with Cushing's syndrome and corticotropin-independent bilateral adrenal hyperplasia in whom endogenous catecholamines, acting through an ectopic adrenal  $\beta$ -adrenergic receptor, stimulated cortisol secretion; the hyperadrenocorticism was inhibited by  $\beta$ -blockade.

### CASE REPORT

A 56-year-old man presented with a left ileofemoral thrombophlebitis, an 8-kg weight gain, decreased libido, and sleep disturbance. Abdominal computed tomography revealed unsuspected bilateral macronodular adrenal hyperplasia (right adrenal gland, 5.2 by 4.0 by 6.0 cm; left adrenal gland, 6.5 by 6.0 by 8.5 cm). Physical examination revealed hypertension and abdominal obesity, but no cervicodorsal fat pads, abdominal striae, or muscle weakness. Urinary cortisol excretion was 711 and 1070  $\mu$ g (1963 and 2953 nmol) per 24 hours (normal, 18 to 119  $\mu$ g [50 to 330 nmol] per 24 hours) on two separate days. The patient's plasma corticotropin concentration was 4.3 pg per milliliter (0.86 pmol per liter; normal, 10 to 55 pg per milliliter [2 to 11 pmol per liter]) at 8 a.m. The plasma cortisol concentration was 26.2  $\mu$ g per deciliter (723 nmol per liter) at 8 a.m. and 18.1  $\mu$ g per deciliter (499 nmol per liter) on the morning after the administration of 2 mg of dexamethasone at midnight. The plasma aldosterone concentration, measured while the patient was supine, was 5.5 ng per deciliter (153 nmol per liter; normal, 1.0 to 16.0 ng per deciliter [27.7 to 443.8 nmol per liter]), plasma renin activity was 0.43 ng per milliliter per hour (0.12 ng per liter per second; nor-

mal, 0.50 to 1.58 ng per milliliter per hour [0.14 to 0.44 ng per liter per second]), and the plasma potassium concentration was 3.7 mmol per liter.

### METHODS

#### Clinical Studies

The studies were approved by the institutional review committee, and written informed consent was obtained from all subjects. Plasma corticotropin, cortisol, and aldosterone were measured after an overnight fast and every 30 to 60 minutes for up to 3 hours after changes in posture, intake of food or water, and the administration of several hormones and drugs.

#### Assays

Plasma cortisol was measured by an immunofluorometric assay (Technicon Immuno I, Bayer, Tarrytown, N.Y.), corticotropin by an immunoradiometric assay (Allegro, Nichols Diagnostics, San Juan Capistrano, Calif.), and aldosterone, renin, and vasopressin by a radioimmunoassay.

#### $\beta$ -Adrenergic-Receptor Binding and Adenylyl Cyclase Assays

Membranes from adrenocortical tissues from the patient and from three patients who underwent adrenalectomy for Cushing's disease or radical nephrectomy were prepared as described previously.<sup>8</sup> The binding of 2400 pM <sup>125</sup>I-labeled cyanopindolol (a nonselective  $\beta$ -adrenergic antagonist) was measured in triplicate with 15  $\mu$ g of membrane protein in the presence or absence of 10  $\mu$ M alprenolol (to detect nonspecific binding). For experiments analyzing competitive binding, tubes containing 50 pM <sup>125</sup>I-labeled cyanopindolol and 0 to 100  $\mu$ M alprenolol, CGP-12177A (a selective  $\beta_3$ -adrenergic agonist), propranolol, or pindolol were incubated for 90 minutes at room temperature followed by filtration with ice-cold 25 mM TRIS-hydrochloric acid (pH 7.4) over Whatman GF/C filters (Whatman, Clifton, N.J.). Data were analyzed by nonlinear least-squares regression analysis (Scatfit program).

Adenylyl cyclase activity<sup>9</sup> was measured with 5  $\mu$ g of membrane protein in a 50- $\mu$ l solution containing 120  $\mu$ M ATP, 1  $\mu$ Ci [ $\alpha$ -<sup>32</sup>P]ATP, 100  $\mu$ M cyclic adenosine monophosphate (cAMP), 53  $\mu$ M guanosine triphosphate, 2.8 mM phosphoenolpyruvate, 0.2 U of pyruvate kinase, 1 U of myokinase, 30 mM TRIS-hydrochloric acid (pH 7.4), 2 mM magnesium chloride, 0.8 mM EDTA, and 0.1 mM isobutylmethylxanthine in the absence or presence of 1 nM to 0.1 mM isoproterenol, 100  $\mu$ M forskolin, or 10 mM sodium fluoride for 15 minutes at 37°C. Reactions were terminated by the addition of an ice-cold solution containing 0.4 mM ATP, 0.3 mM cAMP, and 20,000 cpm of [<sup>3</sup>H]cAMP; cAMP was separated by sequential chromatography and quantitated by ligand scintigraphy.

### RESULTS

#### In Vivo Studies

Stimulation tests were performed to identify a modulator of cortisol production in the patient. A change from supine to upright posture increased plasma cortisol concentrations (Table 1 and Fig. 1), heart rate, and plasma aldosterone concentrations (from 2.8 to 20.5 ng per deciliter [79 to 569 pmol per liter]) but had no effect on plasma corticotropin concentrations or plasma renin activity. During insulin-induced hypoglycemia, the plasma cortisol concentration doubled (Table 1), but the corticotropin concentration remained below 4 pg per milliliter (0.8 pmol per liter).

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**TABLE 1.** MODULATION OF CORTISOL SECRETION IN A PATIENT WITH CUSHING'S SYNDROME AND CORTICOTROPIN-INDEPENDENT MACRONODULAR ADRENAL HYPERPLASIA.\*

TEST OR CHALLENGE†	BASAL PLASMA CORTISOL CONCENTRATIONS	PEAK PLASMA CORTISOL CONCENTRATIONS DURING TEST	CHANGE (PEAK AS A % OF BASAL)
	μg/dl		
Upright posture‡	17.1, 19.5, 21.3	30.3, 32.2, 33.5	177, 165, 157
Supine posture‡	23.8, 30.3, 32.2	19.5, 20.9, 20.5	82, 69, 64
Mixed meal	24.7	20.9	85
Cosyntropin, 250 μg IV	18.5	60.2	325
Gonadotropin-releasing hormone, 100 μg IV	48.6	37.2	77
Thyrotropin-releasing hormone, 200 μg IV	48.4	28.9	60
Corticotropin-releasing hormone, 1 μg/kg IV	23.8	23.9	100
Glucagon, 1 mg IV	26.5	23.1	87
Arginine vasopressin, 10 U IM	23.1	31.4	136
Arginine vasopressin, 10 U IM during dexamethasone infusion, 4 mg IV§	17.8	38.3	215
Desmopressin, 2.5 μg SC	20.8	21.3	102
Insulin, 0.25 U IV	25.7	50.6	197
Cisapride, 10 mg orally	24.7	26.7	108
Dexamethasone, 4 mg IV	25.2	25.7	102

\*To convert values for plasma cortisol to nanomoles per liter, multiply by 27.59.

†All but the posture tests were performed while the patient was supine, which resulted in a progressive decline in plasma cortisol concentrations in the absence of any stimulation.

‡The results of three tests are shown. IV denotes intravenously, IM intramuscularly, and SC subcutaneously.

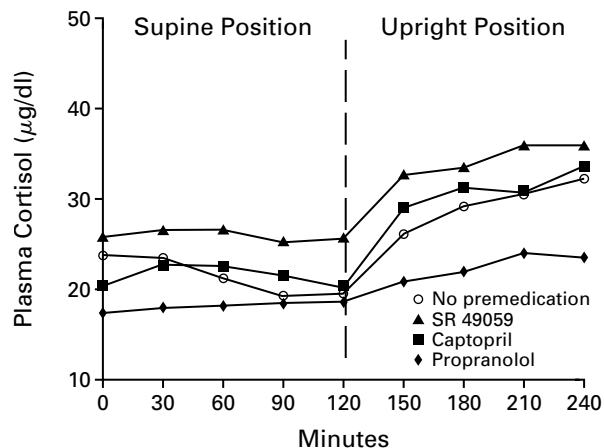
§Vasopressin was injected three hours after an infusion of dexamethasone (1 mg per hour) was initiated. Dexamethasone was administered to suppress potential corticotropin secretion after the administration of vasopressin.

The plasma cortisol concentration also increased after the administration of arginine vasopressin (Table 1) but not after desmopressin; the plasma corticotropin concentration did not change after the administration of arginine vasopressin. Plasma cortisol concentrations did not change after an oral water load (20 ml per kilogram of body weight), which inhibited the secretion of vasopressin. During a two-hour infusion of 3 percent sodium chloride, plasma vasopressin concentrations increased, but plasma cortisol concentrations did not. Oral administration of the V<sub>1</sub>-vasopressin-receptor antagonist SR 49059, with the patient in the supine position, inhibited the increase in plasma cortisol concentrations stimulated by arginine vasopressin, but did not block the increase in plasma cortisol concentrations stimulated by having the patient stand (Fig. 1). Oral administration of the angiotensin-converting-enzyme inhibitor captopril (50 mg) also did not block this posture-stimulated increase in plasma cortisol (Fig. 1) and aldosterone concentrations.

In contrast, pretreatment with the β-adrenergic-antagonist propranolol did block the increase in plas-

ma cortisol concentrations stimulated by having the patient stand (Fig. 1). During a treadmill stress test, the plasma cortisol concentration increased from 18.8 to 29.3 μg per deciliter (519 to 811 nmol per liter), plasma aldosterone increased from 3.2 to 14.4 ng per milliliter (89 to 401 pmol per liter), plasma epinephrine increased from 0.57 to 15.71 pg per deciliter (311 to 8574 pmol per liter), and plasma norepinephrine increased from 3.74 to 67.22 pg per deciliter (2.21 to 39.73 nmol per liter), with no changes in plasma corticotropin or renin values. An infusion of isoproterenol increased plasma cortisol (Fig. 2) and aldosterone concentrations (5.8 to 12.6 ng per deciliter [161 to 349 pmol per liter]) by factors of 2.1 and 2.2, respectively. In contrast, plasma cortisol concentrations did not change during isoproterenol infusion in two normal subjects in whom corticotropin secretion was suppressed by dexamethasone.

During these studies, urinary cortisol excretion ranged from 356 to 991 μg (983 to 2736 nmol) per 24 hours; the higher values coincided either with the administration of cosyntropin or with endogenous or exogenous stimulation of catecholamine se-

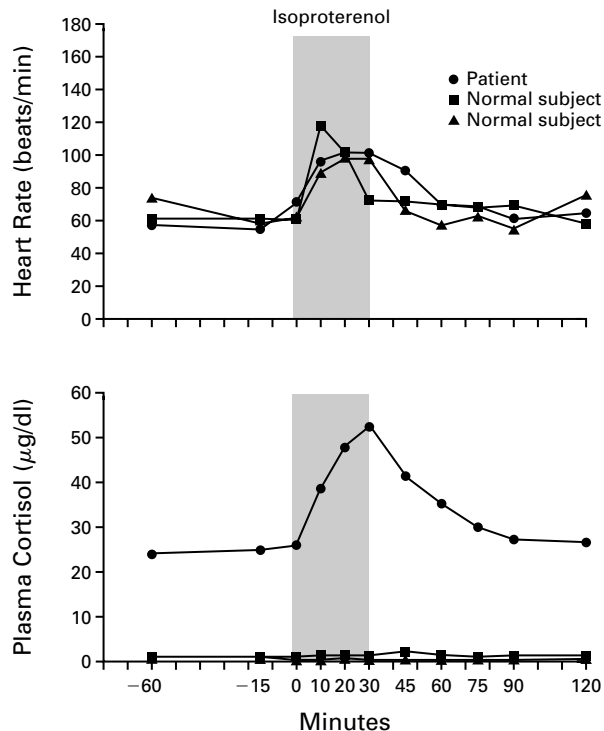


**Figure 1.** Effects of Posture on Plasma Cortisol Concentrations in a Patient with Cushing's Syndrome and Bilateral Macronodular Adrenal Hyperplasia.

Plasma cortisol was measured at the indicated time points when the patient was supine and upright. The tests were performed on different days after an overnight fast; the patient was supine for at least 120 minutes before standing, either without premedication, after the oral administration of 600 mg of the  $V_1$ -vasopressin-receptor antagonist SR 49059, after the oral administration of 50 mg of captopril, or after treatment with 80 mg of propranolol orally every 6 hours for 36 hours. To convert values for plasma cortisol to nanomoles per liter, multiply by 27.59.

cretion, such as is caused by insulin-induced hypoglycemia, petrosal-sinus sampling, treadmill stress testing, or isoproterenol infusion. Short-term and long-term (35 days) treatment with propranolol decreased the patient's urinary cortisol excretion to 222 to 425  $\mu\text{g}$  (612 to 1173 nmol) per 24 hours. Because cortisol excretion remained two to three times normal, the larger left adrenal gland was removed. Propranolol was stopped three days before surgery, after which urinary cortisol excretion increased rapidly to 5670  $\mu\text{g}$  (15,645 nmol) per 24 hours. The value was 3024  $\mu\text{g}$  (8345 nmol) per 24 hours four days after adrenalectomy. Propranolol therapy was resumed, after which urinary cortisol excretion decreased rapidly (Fig. 3).

Because of low urinary cortisol values, the daily dose of propranolol was progressively reduced from 320 mg to 20 mg. During this period, the patient's blood pressure was normal, his plasma cortisol concentrations ranged from 3.4 to 6.8  $\mu\text{g}$  per deciliter (95 to 189 nmol per liter) with no diurnal variation, and he had fatigue in the morning. Insulin-induced hypoglycemia increased the plasma cortisol concentration from 5.3 to 15.9  $\mu\text{g}$  per deciliter (147 to 438 nmol per liter) while the plasma corticotropin concentration remained low. Long-term treatment with 20 mg of propranolol daily and 5 mg of hydrocortisone at 6 a.m. daily beginning in mid-August 1996

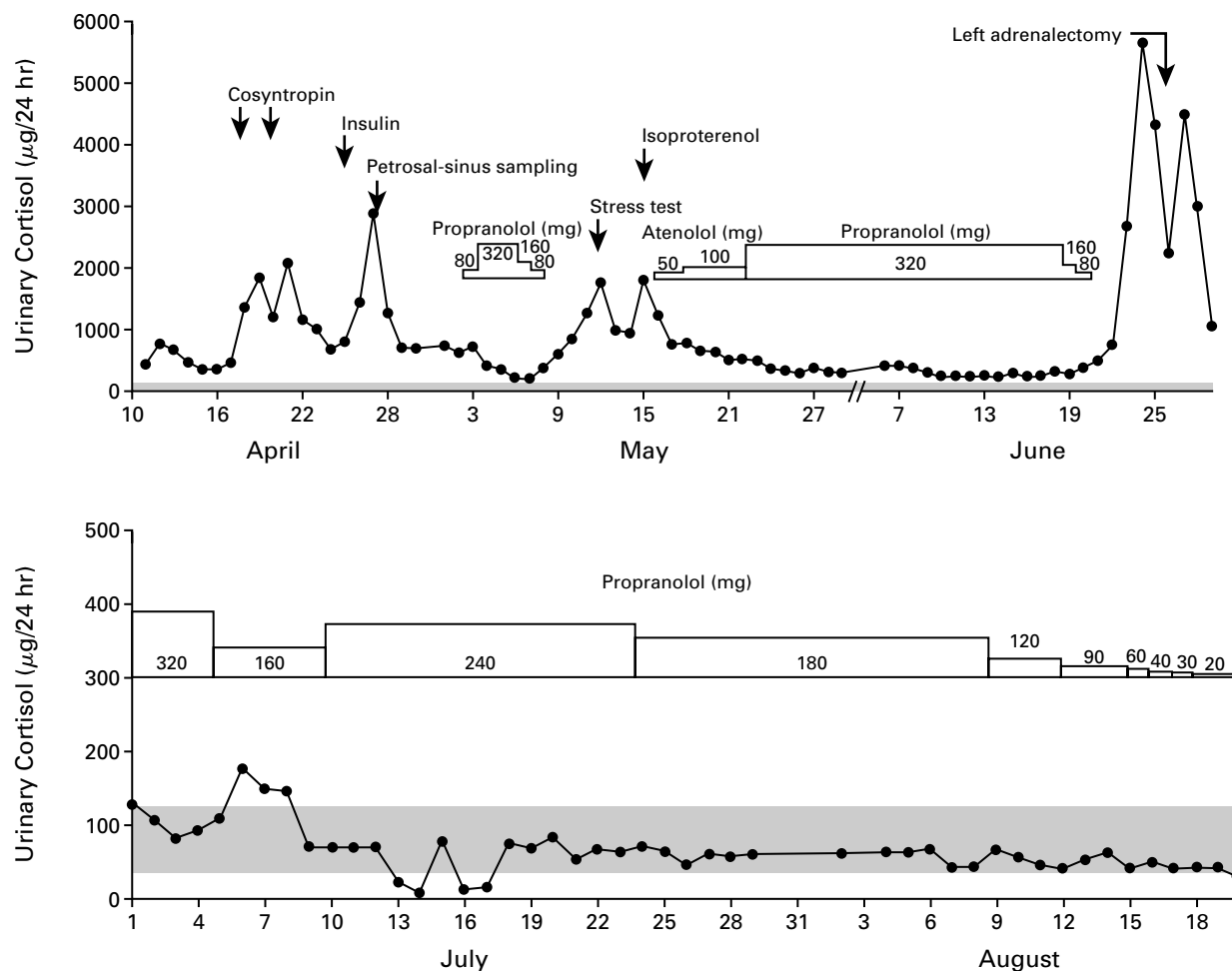


**Figure 2.** Changes in the Heart Rate and Plasma Cortisol Concentrations during the Infusion of Isoproterenol in a Patient with Cushing's Syndrome and Corticotropin-Independent Bilateral Macronodular Adrenal Hyperplasia and Two Normal Subjects.

The values were obtained while the subjects were supine. The normal subjects had received 0.5 mg of dexamethasone orally every 6 hours for 48 hours to inhibit the secretion of corticotropin. Isoproterenol was given intravenously in a dose of 20 ng per kilogram of body weight per minute (shaded area) for 30 minutes; in the patient, the dose was decreased to 10 ng per kilogram per minute during the last 10 minutes because of an increase in blood pressure. To convert values for plasma cortisol to nanomoles per liter, multiply by 27.59.

improved the patient's feeling of well-being and resulted in normal urinary cortisol excretion (74 to 109  $\mu\text{g}$  [205 to 303 nmol] per 24 hours).

Twelve months later, slight elevations in blood pressure led to an increase in the dose of propranolol to 30 mg twice daily. Seventeen months after adrenalectomy, urinary cortisol excretion increased to 155 to 163  $\mu\text{g}$  (428 to 451 nmol) per 24 hours, and hydrocortisone and propranolol were discontinued. The corticotropin-independent stimulation of plasma cortisol induced by having the patient stand and by insulin-induced hypoglycemia was still present. Urinary cortisol excretion decreased to normal after the dose of propranolol was increased to 120 mg twice daily. Abdominal computed tomography performed 6 and 18 months after adrenalectomy revealed no change in the size of the right adrenal gland.



**Figure 3.** Urinary Cortisol Excretion in a Patient with Cushing's Syndrome and Bilateral Macronodular Adrenal Hyperplasia during the Initial Studies and Treatment (Upper Panel) and after Left Adrenalectomy (Lower Panel).

The daily doses of  $\beta$ -adrenergic antagonists are shown; the shaded areas indicate normal urinary cortisol values (18 to 119  $\mu\text{g}$  per 24 hours). To convert values for urinary cortisol to nanomoles per day, multiply by 2.759.

### In Vitro Studies

At adrenalectomy, the left adrenal gland measured 13.5 by 8.0 by 4.5 cm, weighed 204 g, and was characterized entirely by macronodular adrenal hyperplasia. No internodular atrophy was detected on histologic examination.

### $\beta$ -Adrenergic-Receptor Binding

Studies of the patient's adrenal membranes revealed saturable binding of  $^{125}\text{I}$ -labeled cyanopindolol, with a mean ( $\pm\text{SE}$ )  $K_d$  of  $211 \pm 21$  pM and a maximal binding capacity of  $104 \pm 32$  fmol per milligram of protein. Competitive binding studies revealed distinct high-affinity and low-affinity binding sites. About 20 percent of all sites were high-affinity sites (approximately 21 fmol per milligram of protein); the affinities ( $K_d$  for alprenolol, 2.0 nM;  $K_d$  for CGP-12177A, 3.8 nM;  $K_d$  for propranolol, 12.0

nM; and  $K_d$  for pindolol, 18.6 nM) are similar to those reported for the  $\beta_1$ -adrenergic and  $\beta_2$ -adrenergic receptors, but not the  $\beta_3$ -adrenergic receptors.<sup>10-13</sup> The low-affinity sites ( $K_d$ , 2060 to 34,410 nM) did not correspond to any known adrenergic receptor. Although saturable binding was present in adrenal tissue from one control patient with Cushing's disease ( $K_d$ , 182 pM; maximal binding capacity, 75 fmol per milligram of protein), competitive binding studies with antagonists revealed only one class of low-affinity sites in the three control samples of adrenal glands, which were not compatible with any  $\beta$ -adrenergic receptor.

### Adrenal-Membrane Adenylyl Cyclase Activity

Isoproterenol stimulated adenylyl cyclase activity in a dose-dependent fashion in adrenal membranes from the patient (basal, 25.2 pmol per minute per

milligram of protein; after 100  $\mu\text{M}$  isoproterenol, 52.6 pmol per minute per milligram of protein), but not from a patient with Cushing's disease (basal, 17.1 pmol per minute per milligram of protein; after 100  $\mu\text{M}$  isoproterenol, 18.8 pmol per minute per milligram of protein). Sodium fluoride or forskolin stimulated adenylyl cyclase activity to the same extent in adrenal membranes from both patients.

#### DISCUSSION

The corticotropin-independent stimulation of cortisol produced by upright posture, insulin-induced hypoglycemia, and stress in this patient led to the recognition that cortisol secretion was stimulated by the activation of  $\beta$ -adrenergic receptors. We found a striking correlation between increased cortisol secretion and situations in which endogenous catecholamines are increased, such as insulin-induced hypoglycemia, petrosal-sinus sampling, and treadmill stress testing, suggesting that catecholamines caused the patient's hyperadrenocorticism. This theory was supported by the finding of a reduced plasma cortisol response to upright posture during propranolol treatment and the increase in plasma cortisol during isoproterenol infusion. The actions of catecholamines are mediated by  $\alpha$ -adrenergic and  $\beta$ -adrenergic receptors. The absence of an effect of isoproterenol infusion on cortisol secretion in normal subjects receiving dexamethasone indicates that  $\beta$ -adrenergic receptors are not normally coupled to cortisol secretion. Functional  $\beta$ -adrenergic receptors have been described in adrenal adenomas or carcinomas in vitro, but not in normal human adrenal cortex.<sup>14-18</sup> We detected high-affinity binding sites compatible with  $\beta_2$ -adrenergic or  $\beta_1$ -adrenergic receptors<sup>10-13</sup> in adrenal tissue from our patient, but not from controls; its effective coupling to G proteins and adenylyl cyclase was demonstrated by the stimulation of cAMP in vitro and steroidogenesis in vivo. It is not known whether the structure of this ectopic  $\beta$ -adrenergic receptor is normal or mutated.

Adrenocortical tumors have been shown in vitro to have ectopic receptors for several hormones,<sup>14,16,19,20</sup> but their role in stimulating adrenal hormone secretion in vivo has rarely been examined. In contrast, activation of overexpressed adrenal receptors for gastric inhibitory polypeptide can cause Cushing's syndrome.<sup>2-7</sup> Corticotropin-independent stimulation of cortisol secretion after administration of vasopressin was found in this patient and in other patients with adrenal Cushing's syndrome<sup>21-23</sup>; this process is mediated by a  $V_1$ -vasopressin receptor. However, the absence of changes in plasma cortisol concentrations during changes in endogenous vasopressin secretion and the inability to block the plasma cortisol response to upright posture with an antagonist of  $V_1$ -vasopressin receptor demonstrate that vasopressin was not an important regulator of steroidogenesis in

this patient. The bilateral nature of the adrenal hyperplasia in patients with Cushing's syndrome regulated by gastric inhibitory polypeptide<sup>2-4</sup> and vasopressin<sup>21,23</sup> or in this patient suggests that the respective putative mutations occurred during early embryogenesis; somatic mutations would be expected to cause solitary adenomas.<sup>5,6,22</sup>

The demonstration that catecholamines were the main modulators of cortisol secretion led us to treat the patient with propranolol. It proved effective; the need for a progressive decrease in the dose could have been due to its inverse agonist activity,<sup>24</sup> which eventually decreased the number of receptors. The expression of  $\beta_2$ -adrenergic receptors is transcriptionally stimulated by glucocorticoids<sup>25,26</sup> by means of glucocorticoid hormone-response elements in their promoter regions.<sup>27,28</sup> Control of hyperadrenocorticism may have decreased the number of  $\beta$ -adrenergic receptors and the requirement for propranolol. The size of the adrenal glands was not decreased by propranolol, but such an effect may require more complete blockade.

In conclusion, we propose that ectopic expression of  $\beta$ -adrenergic receptors in both adrenal cortices in this patient led to catecholamine-modulated nodular hyperplasia, hypersecretion of cortisol and aldosterone, and feedback suppression of the corticotropin-adrenal and renin-angiotensin axes. These findings lend support to the broader hypothesis that corticotropin-independent adrenal hyperplasia or tumors may be due to diverse ectopic hormone receptors, and this may lead to new pharmacologic therapies.

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