Agouti-Related Protein Antagonizes Glucocorticoid Production Induced through Melanocortin 4 Receptor Activation in Bovine Adrenal Cells: A Possible Autocrine Control

MABROUKA DOGHMAN, PHILIPPE DELAGRANGE, ANTONINE BLONDET, MARIE-CLAUDE BERTHELON, PHILIPPE DURAND, DANIELLE NAVILLE, AND MARTINE BÉGEOT

Institut National de la Santé et de la Recherche Médicale Unité 418-Institut National de la Recherche Agronomique Unité Mixte de Recherche 1245 and Institut Fédératif de Recherche 62 Laennec (M.D., A.B., M.-C.B., P.Du., D.N., M.B.), Hôpital Debrousse and Claude Bernard University, Lyon 69322 Cedex 05, France; and Institut Recherches Internationales Servier (P.D.), Courbevoie 92415 Cedex, France

Agouti-related protein (Agrp), primarily expressed in the hypothalamus, is an endogenous antagonist of αMSH at the level of melanocortin 3 receptor (MC3-R) and MC4-R, but the adrenal gland represents the second major Agrp-expressing tissue. In adrenal fasciculata cells, the glucocorticoid secretion is under the control of ACTH, which binds specifically MC2-R, the only functional melanocortin receptor described in these cells to date. Nevertheless, using cultured bovine fasciculata adrenal cells, we report that Agrp has no antagonistic properties against ACTH at the level of MC2-R. In our studies, (Nle4, d-Phe7)-αMSH (NDP-αMSH) stimulated the production of cortisol in a dose-dependent manner, and these effects were abolished by Agrp or SHU9119, a synthetic antagonist of MC3-R and MC4-R. Using a more specific antagonist (JKC-363) and RT-PCR analysis, we can postulate that the effects of NDP-αMSH were mediated via MC4-R. These results are suggestive that adrenal glucocorticoid production could be regulated through MC4-R that may have some relevance in the physiology of adrenal cells. Moreover, Agrp might exert an autocrine control on adrenal cells because a protein with biological Agrp-like activity is secreted by these cells. This peptide could then modulate locally the functions of some peripheral tissues such as adrenals. (Endocrinology 145: 541–547, 2004)

A GOUTI-RELATED PROTEIN (Agrp) is an orexigenic neuropeptide expressed primarily in the arcuate nucleus of the hypothalamus. It plays a role in the control of feeding behavior (1, 2) and is a very potent antagonist of αMSH at the level of the melanocortin 3 receptor (MC3-R) and melanocortin 4 receptor (MC4-R) (3–5). The biological action of Agrp has been shown to reside in its C-terminal fragment (residues 83–132) (6). In addition to hypothalamus, Agrp is also known to be expressed in some peripheral tissues such as the adrenal gland of human and rodent and even to a lesser extent in the testis, lung, and kidney of the mouse (1, 2, 7).

The natural melanocortin ligands (αMSH, βMSH, γMSH, and ACTH), derived from the protein precursor proopiomelanocortin that is expressed in the hypothalamic arcuate nucleus and pituitary gland, bind to five receptor subtypes that exhibit distinct affinities for the different melanocortins. At the peripheral level, the MC1-R mediates hair and skin pigmentation (8). The MC2-R (ACTH receptor) is also a peripheral receptor mediating the regulatory effects of ACTH on steroidogenesis in the adrenal gland (9). The MC3-R and MC4-R are predominantly expressed in the brain, and both are involved in regulating energy metabolism and feeding behavior (10–12). It appears that MC4-R regulates food intake and energy homeostasis, whereas MC3-R regulates partitioning of nutrients into fat. Confirmation of the role of MC4-R in obesity came from the analysis of MC4-R knockout mice showing an obese phenotype (13). MC5-R has been found in the brain but is mainly considered to be a peripheral ubiquitous receptor that plays a role in the functions of exocrine glands (14). Until now, MC2-R is the only melanocortin receptor known to be coupled to glucocorticoid synthesis. However, it is conceivable that other melanocortin receptors may be involved as the presence of MC5-R mRNAs in zona glomerulosa and MC3-R and MC4-R mRNAs in total rat adrenal gland has been reported (15, 16).

Although Agrp is highly expressed in the adrenal cortex, its role in this tissue is still unknown. Because Agrp has no significant antagonistic properties toward the MC2-R, the question arises about the interaction of Agrp with another functional MC-R besides MC2-R in this tissue. In this article, we describe the antagonistic effects of Agrp on (NDP-αMSH-induced bovine adrenal cell cortisol production and demonstrate that they are mainly mediated through MC4-R. Moreover, we show evidence that a protein with Agrp-like activity is secreted by adrenal cells, suggesting a possible autocrine control of Agrp on adrenal steroidogenesis.

Materials and Methods

Materials

Penicillin, streptomycin, nystatin, fetal calf serum (FCS), trypsin, ITS (insulin 10 mg/liter⁻¹, transferrin 5.5 mg/liter⁻¹, and selenium 5 μg/liter⁻¹), DMEM F12 (DMEM/F12, 15 mm HEPES) were purchased from

Abbreviations: Agrp, Agouti-related protein; BAC, bovine adrenocortical cell; FCS, fetal calf serum; FG, familial glucocorticoid deficiency; ITS, insulin, transferrin, and selenium; MC3-R, melanocortin 3 receptor; MC4-R, melanocortin 4 receptor; NDP, (Nle4, d-Phe7).
In 19 May 2018

In Vitro (Cergy-Pontoise, France); [124]AMP from Beckman (Mar-

eseille, France), and [3H]cortisol from Amersham (Saclay, France); (1–24) ACTH, NDP-αMSH, SHU9119, and MTII from Bachem (Voisins-

Le-Bretonnais, France). Human Agrp (83–132)-NH2 and JKC-363 were purchased by RNA Phoenix Personal Services, Inc. (Kalsruhe, Germany). Oligonucleotides were prepared by Sigma Genosys (Cambridge, UK). Taq Polymerase (EuroblueTaq) were purchased from Eurobio (Les Ulis, France) and DNase from Invitrogen.

**Cell culture**

Bovine adrenocortical cells (BACs) were prepared as previously de-

scribed (17). Briefly, bovine adrenal glands were freed of fat, dissected, and cut with a microtome delivering slices of 0.5 mm. The first slice (capsule and glomerulosa cells) was not used, whereas the second and the third slices were used for preparation of fasciculata-reticularis cells. Dispersed cells were obtained by sequential treatment with trypsin (0.19% diluted in DMEM/F12 medium supplemented with gentamicin (20 μg/ml), nystatin (20 U/ml), penicillin (100 U/ml), streptomycin (0.1 mg/ml), and vancomycin (2 μg/ml)). The cells were harvested, washed and placed in medium composed of DMEM/F12 medium containing 5% of FCS. The next day the medium was changed and replaced by medium containing 1% ITS, ascorbic acid (10–4 M), and 2% FCS. On the second day of culture, the medium was changed and replaced by the same medium without serum.

Treatments were performed on the third day of culture in serum-free medium containing ITS, ascorbic acid, and bacitracin (0.1 μg/ml). Se-

creted cortisol was measured in the culture media at the end of the treatment by specific RIA as previously described (17).

In some experiments, an M3 cell line stably expressing the human MC2-R (clone 119) and human adrenocortical tumor cell line H295R have been used and cultured as previously described (18, 19).

**RT-PCR analysis**

To determine the presence of the MC3-R and MC4-R mRNA in the adrenal gland, total RNA was prepared from various tissues or cells: bovine fasciculata-reticularis adrenal tissue, primary cultured bovine fasciculata-reticularis adrenal cells, H295R cell line, and human adrenal tissue, using the Chomczynsky and Sacchi method (20). Human adult adrenals were obtained after organ removal from brain-dead patients for transplantation, with the approval of the ethical committee of the Hos-

pices Civiles de Lyon. Human total brain RNA (Clontech, Ozyme; Mon-

tigny-le-Bretonnais, France) was also used as positive control. Then 2 μg of each RNA was reverse transcribed using the Moloney murine leukemia virus reverse transcriptase (Invitrogen) according to the manu-

facturer’s protocol.

The primers used in the PCR for the MC3-R were designed to amplify a coding sequence common to both human and mouse MC3-R (GenBank accession nos.: NM_019888 and NM_008561) because the bovine se-

quence is not available. The corresponding oligonucleotides were

hMC3-R 5′-gacatgtgacattcgct-3′ and hMC3-R AS 5′-gtgtagcagatgtc-

cagg-3′.

The PCR was also carried out using oligonucleotides MC4-R S 5′-

gaggttctttctgtctgg-3′ and MC4-R AS 5′-gaacatgtgacattcgct-3′. They have been chosen to recognize the reported bovine, mouse and human MC4-R sequences (GenBank accession nos. AF266221, NM_005612, NM_016977, respectively).

PCR was performed with 35 cycles of reaction, one cycle correspond-

ing to denaturation at 94 C for 55 sec, annealing at 54.9 C for 50 sec, and extension at 72 C for 1 min using MC3-R primers and to denaturation for 60 sec at 94 C, annealing for 60 sec at 49 C, and extension for 90 sec at 72 C using MC4-R primers. The PCR products were expected to be 493 and 503 bp in length for MC3-R and MC4-R, respectively. To rule out the possibility that PCR products could result from the amplification of genomic DNA contaminating the RNA samples, total RNA from bovine adrenal cells was submitted to RT-PCR after a DNase treatment accord-

ing to the manufacturer’s protocol. The sequence of the resulting DNA fragments obtained by using bovine adrenal RNA was verified (17 sequencing kit, Amersham).

**Agrp-like protein biological activity assay**

To measure the Agrp-like biological activity in medium conditioned by cultured bovine adrenal cells, a model of human normal MC4-R ECFP fusion protein stably expressed in HEK293 cell line was used (21). In this model, Agrp antagonized the stimulatory effect of NDP-αMSH on in-

tracellular cAMP production. A dose-response curve (calibration curve) was established by treating these cells with 10–9 to 10–7 M NDP-αMSH in the presence of increasing concentrations of Agrp, and intracellular cAMP production was measured by RIA. For this assay, cells were plated in 12-well dishes and washed once with serum-free medium. The cells were incubated for 5 min at 37 C in media containing 1 mM 3-isobutyl-1-methyl-xanthine and treated for 20 min with 10–9 M NDP-αMSH and Agrp. Ice-cold 60% ethanol (500 μl/well) was added to both stop the reaction and precipitate cellular protein. Cells in each well were scraped, transferred to a 1.5-ml tube and centrifuged for 5 min at 12,000 × g. The supernatant was evaporated and the pellet was dissolved in 1 ml RIA buffer. These cells were also treated with 24-h medium conditioned by cultured control bovine adrenal fasciculata cells and the Agrp calibration curve was used to measure the concentration of the Agrp-like protein present in this medium.

**Statistical analysis**

Each experiment was performed at least three times, and each experi-

mental point was determined in triplicate or quadruplicate. Results are expressed as fold stimulation over each corresponding control to correct for differences in absolute hormone secretion between experi-

ments. All data are presented as mean ± SEM, and statistical differences were determined by two-way ANOVA followed by post hoc testing with Fisher’s least significant difference test to determine individual differences.

**Results**

**Absence of effect of Agrp on ACTH-induced cortisol production**

To investigate whether Agrp could modify ACTH-induced cortisol production, primary bovine cultured adre-

nal cells were treated with increasing concentrations of ACTH (10–15 M to 10–9 M) for 2 h, with or without 10–9 M Agrp (83–132)-NH2. No effect of 10–7 M Agrp on ACTH-

induced cortisol production was observed, whatever the ACTH concentration (Fig. 1). In addition, the same results were obtained on ACTH-induced intracellular cAMP produc-

duction in M3 cell line stably expressing the human MC2-R (our unpublished results). All these results showed that Agrp could not antagonize ACTH effects at the level of MC2-R.

**Effect of Agrp on NDP-αMSH-induced cortisol production**

Bovine cultured adrenal cells were treated for 2 h with increasing concentrations of NDP-αMSH, a more potent and enzyme resistant analog than natural αMSH (22). Cortisol production was measured after a 2-h cell treatment. NDP-

αMSH stimulated cortisol secretion in a dose-dependent manner (Fig. 2A). Indeed, we observed an increase of cortisol release by 5-fold over control using 10–8 M NDP-αMSH, and the maximal stimulation was obtained for 10–8 M NDP-

αMSH (7- to 8-fold over control cells). The effect of αMSH, the endogenous melanocortin receptor ligand, on cortisol release was also evaluated. As expected, αMSH stimulated the cortisol release but was less efficient than NDP-αMSH, a sign-

ificant stimulation being only observed from 10–8 M (Fig. 2A).

We next studied the effects of Agrp (83–132)-NH2 on basal or 10–9 M NDP-αMSH-stimulated cortisol secretion from bovine adrenal cells (Fig. 2B). Agrp alone did not affect cortisol release. However, it decreased the stimulatory effect of NDP-αMSH-induced cortisol production in a dose-depen-

dent manner. The stimulation was completely abolished by 10–8 M Agrp.
Role of MC4-R on NDP-αMSH-stimulated cortisol release

To identify and characterize more precisely the mechanism involved in the effects of NDP-αMSH and Agrp on cultured bovine adrenal cells, we used the nonselective synthetic agonist (MTII) and antagonist (SHU9119) of MC3-R/MC4-R. The results presented in Fig. 3A show that 10^{-7} M MTII increased cortisol secretion (5-fold over the control) and the effects of either MTII or NDP-αMSH were completely antagonized by 10^{-7} M Agrp. In addition, the 10^{-9} M NDP-αMSH effect was also abolished by 10^{-7} M SHU9119. Agrp and SHU9119 used alone at the same doses had no effect on this release.

Then we compared the effects of SHU9119 with those of JKC-363, a more selective MC4-R antagonist, during a 2-h NDP-αMSH treatment of bovine adrenal cells (Fig. 3B). JKC-363 (10^{-7} M) decreased NDP-αMSH-induced cortisol secretion by approximately 65% (Fig. 3B) whereas a total inhibition was obtained with 10^{-7} M SHU9119 (Fig. 3A). However, the potencies of each antagonist, when tested on 10^{-7} M NDP-αMSH-induced intracellular cAMP production by HEK293 cell line stably expressing only the MC4-R, were equivalent to those obtained with bovine adrenal cells: 70% inhibition using 10^{-7} M JKC-363, compared with 97% using 10^{-7} M SHU9119.
Expression of MC4-R mRNA in adrenocortical tissues

RT-PCR analysis using primers specific for bovine, human, and mouse MC4-R mRNAs and sequencing of the PCR products demonstrated that mRNAs encoding MC4-R were expressed both in cultured bovine adrenal cells and fresh bovine adrenocortical tissue. MC4-R mRNAs were also present in human adrenal tissue and H295R cell line (Fig. 4). However, using MC3-R primers, we did not detect MC3-R mRNA in human adrenal tissue, whereas an expression was observed in H295R cell line. A similar study for bovine adrenal tissue and cells was not possible because the primers did not recognize the bovine MC3-R sequence when using bovine genomic DNA. RT-PCR analysis of human total brain mRNA represented a positive control expressing both MC3-R and MC4-R.

Effect of a pretreatment with 10^{-9} M NDP-αMSH on the ACTH responsiveness of BACs

To investigate the effect of NDP-αMSH on ACTH-induced cortisol production, cells were pretreated without (control) or with 10^{-9} M NDP-αMSH for 24 h. At the end of the treatment, the incubation medium was removed and replaced by fresh medium containing ACTH (10^{-12} M or 10^{-10} M), and the cortisol production was measured after 2 h. Interestingly, we observed that the ACTH responsiveness of cells was enhanced when cells were pretreated with 10^{-9} M NDP-αMSH (Table 1). Indeed, pretreatment with 10^{-9} M NDP-αMSH significantly increased the 10^{-12} M ACTH-induced cortisol response (167.2 ± 9.8%; P < 0.002, n = 3). Even though this effect was less marked, it still remains significant at 10^{-10} M ACTH (122.9 ± 11.3%; P < 0.001, n = 3).

Secretion of an Agrp-like biological activity by adrenal cells

As reported in several others species, Agrp mRNA is expressed in bovine cultured fasciculata adrenal cells and adrenocortical tissue (data not shown). Therefore, we determined whether Agrp or a related molecule was secreted in the culture medium of bovine adrenal cells. For that purpose, we used a model of cells stably expressing the human MC4-R developed in our group. These cells responded to 10^{-9} M NDP-αMSH by raising their secretion of intracellular cAMP, and this stimulation was inhibited in a dose-dependent man-
mouse and rat adrenal cortex (1, 7) which raises the question of its role in this particular steroidogenic tissue. Agrp or its active (83–132) C-terminal fragment have been shown to strongly antagonize αMSH at the level of MC3-R and MC4-R in hypothalamus, resulting in an increase in appetite and food intake (5). Agrp is only a weak antagonist of MC5-R, does not bind to the MC1-R (2, 23), and has no antagonistic effect at the level of MC2-R as previously reported using clones stably expressing MC2-R (23).

In these studies, we confirmed that Agrp has no significant antagonistic properties toward ACTH on MC2-R in cultured fasciculata-reticularis bovine adrenal cells. However, glucocorticoid steroidogenesis is stimulated by ACTH-induced activation of MC2-R, which is the unique melanocortin receptor whose expression has been clearly reported in the fasciculata reticularis adrenal cells. The presence of MC5-R mRNA has been reported only in glomerulosa cells (15) and more recently MC3-R and MC4-R mRNAs in total rat adrenal gland by RT-PCR analysis (16).

αMSH, γMSH, and ACTH are the three major melanocortins that bind and activate MC-1, MC3, MC4, and MC5-R, whereas the only high-affinity natural ligand for the MC2-R is ACTH. In the literature, there are several evidences that proopiocorticotin-derived peptides other than ACTH could act on adrenal growth or steroidogenesis (24, 25). This could be explained by the presence of high-affinity binding sites for Lys-γ3MSH as reported in rat adrenal cortex (26), suggesting the existence of another MC-R besides MC2-R.

In this study, we observed that both NDP-αMSH and αMSH stimulated in a dose-dependent manner the acute cortisol secretion of cultured bovine adrenal cortical cells. NDP-αMSH was active at a relatively low concentration (10−9 M) with a maximal effect at 10−8 M. Baird et al. (27) also described a stimulatory effect on cortisol production by human fetal adrenal cells cultured during 48 h but only in the presence of a pharmacological concentration (3 × 10−5 M) of αMSH. In a recent article, it was reported that 10−7 M αMSH stimulated by 2-fold the corticosterone production of dispersed rat glomerulosa cells (16).

More importantly, we report that Agrp (83–132)-NH2 was able to completely abolish the NDP-αMSH-induced cortisol secretion of cultured BACs without any effect on basal cortisol release. Agrp has been described in the brain as an antagonist of αMSH at the level of MC3-R and MC4-R (2). In the present work, the induction of cortisol by NDP-αMSH was blunted by SHU9119, a synthetic antagonist of both MC3-R and MC4-R, as well as by Agrp. These results indicate that the effects of NDP-αMSH (or αMSH) should be mediated by MC4-R and/or MC3-R on adrenal cells.

The major role of MC4-R in NDP-αMSH-induced cortisol secretion by bovine adrenal cells was confirmed by using JKC-363, an MC4-R antagonist, that is more selective than SHU9119, with a 100-fold higher affinity for MC4-R than for MC3-R (28). Nevertheless, a residual cortisol production was observed even in the presence of a relatively high concentration of JKC-363 (10−7 M), which could be explained by a partial agonist effect of this molecule. In fact, treatment of a HEK 293 cell line stably expressing human MC4-R (21) with JKC-363 alone resulted to a slight increase in basal intracellular cAMP production.

**Discussion**

Agrp is primarily expressed in the hypothalamus, but relatively high expression has also been reported in human, bovine adrenal, and human total brain RNA (HTB) as a positive control. PCR-C and RT-C represent the PCR and reverse transcription reaction control, respectively. Markers of weight correspond to the 1-kb ladder (Invitrogen). The size of the expected RT-PCR products are 492 bp for MC3-R and 503 bp for MC4-R.

**TABLE 1.** Effect of a pretreatment with 10−9 M NDP-αMSH on responsiveness to ACTH by bovine adrenal cells

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Cortisol (ng/106 cells · 2 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10−12 M ACTH</td>
</tr>
<tr>
<td>Control</td>
<td>572.7 ± 8.61</td>
</tr>
<tr>
<td>10−9 M NDP-αMSH</td>
<td>929.6 ± 52.6</td>
</tr>
</tbody>
</table>

On the third day of culture, cells were pretreated in absence (control) or presence of 10−9 M NDP-αMSH during 24 h. At the end of the treatment, the incubation medium was removed and replaced by fresh medium containing ACTH (10−12 and 10−10 M), and the cortisol production was measured after a 2-h treatment. This table corresponds to a representative experiment and shows absolute hormone secretion values. Two other separate experiments gave similar results.

**Fig. 4.** RT-PCR analysis of MC4-R (A) and MC3-R (B) gene expression in various tissues: bovine fasciculata-reticularis adrenal tissue (FR), cultured fasciculata-reticularis bovine adrenal cells (two different preparations BAC 1 and 2), human adrenal tissue (HAT), H295R cell line, and human total brain RNA (HTB) as a positive control. PCR-C and RT-C represent the PCR and reverse transcription reaction control, respectively. Markers of weight correspond to the 1-kb ladder (Invitrogen). The size of the expected RT-PCR products are 492 bp for MC3-R and 503 bp for MC4-R.

**TABLE 1.** Effect of a pretreatment with 10−9 M NDP-αMSH on responsiveness to ACTH by bovine adrenal cells
Both MC4-R and MC3-R mRNAs have been detected by RT-PCR in total adrenal tissue of chicken (29) and rat (16) without indication of their cellular localization. We showed by RT-PCR that MC4-R mRNAs are expressed in cultured bovine fasciculata-reticularis adrenal cells and bovine adrenal tissue. This could be extended to fresh human adrenal tissues and H295R, a human adrenal tumor cell line. On the other hand, there is no evidence of the presence of MC3-R mRNAs in human adrenal tissue except in tumoral H295R cells and studies using bovine adrenal tissue are not conclusive.

Our data suggest that although αMSH, via MC4-R activation, induces a modest cortisol production, compared with ACTH-induced level, it may have some physiological or clinical relevance. In particular, it could explain why some patients with the familial glucocorticoid deficiency (FGD), carrying an inactivating mutation of MC2-R gene, exhibit clinical signs more lately than other patients as reported by several authors (30, 31). There is no clear correlation between phenotype and genotype among FGD patients, which suggests an adaptive response of the adrenal gland to the ACTH resistance. The late appearance of FGD symptoms is often associated with the highest basal plasma cortisol levels (even if they remained lower than in normal individuals), whatever the nature of the mutation of MC2-R. This raises the question of the existence of an alternative receptor, notably MC4-R, that may be activated by ACTH because the plasma level is often very high and differs strongly among FGD patients (30). Moreover, the population of MC4-R could probably vary with individuals. Furthermore, our results show that the cortisol production induced by physiological concentrations of ACTH is enhanced by a prior activation of MC4-R with NDP-αMSH, and this action might be necessary for a maximal response of adrenocortical cells to ACTH. These results could be linked to previous reports showing that chronic exposure to glucocorticoids potentiate the subsequent trophic effects of ACTH on adrenal steroidogenesis in several species (32–35). These effects are exerted through an enhancement of ACTH receptor mRNA levels and cholesterol side chain cleavage activity (36, 37).

Another important question concerns the inhibitory control by Agrp on NDP-αMSH-induced cortisol release by adrenal cells because we showed that Agrp mRNAs were expressed in these cells. Herein we report that an active Agrp-like product is secreted in the 24-h cultured bovine adrenal cells conditioned medium. The concentration of this secreted factor is equivalent to that required for Agrp to inhibit by 50% the NDP-αMSH-induced cortisol release by bovine adrenal cells. This suggests that Agrp, released from adrenal cells, could modulate the effects of αMSH on adrenal steroidogenesis through a local autocrine control. This is an important feature to take into account in the case of obesity considering that Katsuki et al. (38) demonstrated an elevated circulating concentration of αMSH that was significantly correlated with insulin resistance during obesity. In another study, the same authors (39) showed that plasma levels of Agrp were increased in obese subjects and that these levels were correlated with body mass index, fasting insulin levels, and plasma levels of αMSH.

To conclude, our data support the concept that adrenal steroidogenesis could be modulated by the melanocortin αMSH through activation of MC4-R and that Agrp could be involved in an autocrine regulatory mechanism on MC4-R signaling not only in brain but also in peripheral tissues such as the adrenal gland.

Acknowledgments

We thank Frédéric Volland for his valuable help with adrenal collection and W. E. Rainey (Dallas, TX) for his generous gift of H295R. We are grateful to Dr. Ann Clark for reviewing the English manuscript.

Received May 16, 2003. Accepted October 20, 2003.

Address all correspondence and requests for reprints to: Martine Bégeot, Institut National de la Santé et de la Recherche Médicale Unité 418-Institut National de la Recherche Agronomique Unité Mixte de Recherche 1245, Hôpital Debrousse, 29 rue Soeur Bouvier, 69322, Lyon Cedex 05, France. E-mail: begeot@lyon.inserm.fr.

M.D. was recipient of a fellowship from IRIS (Institut de Recherches Internationales Servier).

References

Endocrinology is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.