Plasma Immunoreactive Gamma Melanotropin in Patients with Idiopathic Hyperaldosteronism, Aldosterone-producing Adenomas, and Essential Hypertension

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Abstract

A non-ACTH aldosterone-stimulating factor(s) has been implicated in the pathogenesis of idiopathic hyperaldosteronism (IHA). Although this factor has not been fully characterized, some evidence suggests that it may be related to a pro-γ-melanotropin (pro-γ-MSH), derived from the NH_2-terminal region of pro-opiomelanocortin. In the present study, plasma immunoreactive (IR-) γ-MSH levels at 0800 h in patients with IHA were evaluated (90±17 fmol/ml; range: 13–173 fmol/ml) and found to be significantly higher ($P < 0.05$) than those in subjects with aldosterone-producing adenomas (33±8 fmol/ml), essential hypertension (33±6 fmol/ml), and normotensive controls (19±2 fmol/ml). Seven of nine IHA subjects had circulating IR-γ-MSH levels above the normal range (>35 fmol/ml). In plasmas sampled at 1200 h, IR-γ-MSH was significantly higher in patients with IHA (95±26 fmol/ml) and adenomas (63±23 fmol/ml), as compared with essential hypertensives (31±6 fmol/ml) and normotensives (19±3 fmol/ml). Mean plasma IR-ACTH, plasma cortisol, and urinary cortisol levels did not differ significantly between any of these groups. In order to evaluate the effect of a pro-γ-MSH in vitro, adrenal adenoma tissue was obtained from two patients, one with elevated IR-γ-MSH (61 fmol/ml) and a second with low IR-γ-MSH (12 fmol/ml). Aldosterone secretion by dispersed adenoma cells from the former, but not the latter, underwent a fourfold dose-dependent (10^{-14}–10^{-9} M) increase in response to human Lys-γ3-MSH. These data suggest that a pro-γ-MSH may be implicated as a pathogenic factor in a subset of patients with primary aldosteronism, particularly among those differentially diagnosed as having IHA.

Introduction

There is mounting evidence that a non-ACTH pituitary factor(s) may be involved in the regulation of aldosterone secretion. Major support for this hypothesis comes from investigations of idiopathic hyperaldosteronism (IHA),[1] a variant in primary aldosteronism (1, 2). Patients with IHA have increased levels of a circulating glycoprotein termed aldosterone-stimulating factor (ASF) (3–5). This finding may explain the bilateral adrenal hyperplasia and increased aldosterone production associated with IHA. Although the characterization of ASF is incomplete, it has been localized to the pituitary by immunohistochemical staining (6). A number of pituitary polypeptide candidates have been evaluated for ASF-like activity, including several that are derived from pro-opiomelanocortin (POMC): α-melanotropin, melanocyte-stimulating hormone (α-MSH) (7, 8), β-MSH/β-lipotropin (9–12), and β-endorphin (13, 14). Interest has also focused on the glycosylated NH_2-terminal segment of POMC, which includes the γ-MSH sequence (15), and which can be cleaved at several sites to generate a small group of pro-γ-MSHs. Various of these pro-γ-MSHs have been shown to stimulate aldosterone secretion by dispersed aldosteronoma cells (16); to potentiate aldosterone secretion in vitro (17) and in vivo (18); and to exhibit hyperplastic and hypertrophic effects on the adrenal cortex (19). The present study was undertaken to evaluate the circulating levels of immunoreactive- (IR-) γ-MSH in patients with IHA, aldosterone-producing adenomas (APA), and essential hypertension (EH), as compared with normotensive controls (NC).

Methods

Subjects. Four groups of subjects were studied: IHA (total/females—9/5; × age—51 yr; × weight—188 lb); APA (5/2; 49 yr; 154 lb); EH (9/6; 49 yr; 173 lb); NC (7/4; 32 yr; 144 lb).

All hypertensive patients underwent a 7-d protocol in a metabolic unit before diagnosis. Medications influencing the renin-aldosterone or pituitary-adrenal systems, including diuretics, adrenergic-blockers, vasodilators, and estrogens, were discontinued for at least 4 wk preceding admission. Where required, prazosin (1–10 mg/d) (20) was the primary antihypertensive therapy. After admission to the metabolic unit, patients were given a fixed isocaloric diet containing 128 meq Na^+ and 80 meq K^+/d. After one to two days of electrolyte equilibration, a 24-h urine sample was collected for measurement of aldosterone secretion rate (ASR), cortisol, tetrahydroaldosterone, electrolytes, and

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1. *Abbreviations used in this paper:* AI, angiotensin I; APA, aldosterone-producing adenoma; ASF, aldosterone-stimulating factor; ASR, aldosterone secretion rate; EH, essential hypertension; IHA, idiopathic hyperaldosteronism; IR, immunoreactive; KRBGA, Krebs-Ringer bicarbonate/glucose/albumin buffer; MSH, melanotropin, melanocyte-stimulating hormone; NC, normotensive controls; POMC, pro-opiomelanocortin; PRA, plasma renin activity.

creatinine. After 8 h of recumbancy, plasma was collected at 0800 h while the patient was supine and again at 1200 h after 4 h of upright posture. On a subsequent day plasma was collected (1100 h) at 3 h after upright posture and furosemide (80 mg, oral). Additional diagnostic testing included an adrenal iodocholesterol scan (NP-59), computerized axial tomography of the abdomen (21, 22), and selective adrenal vein catheterization, if necessary.

The diagnosis of hypertension was established by two or more diastolic blood pressures > 90 mmHg, or systolic blood pressures > 140 mmHg (23). The diagnosis of IHA was established by the following criteria: hypertension; spontaneous hypokalemia; suppressed plasma renin activity (PRA) (<1.5 ng angiotensin I [AI]/ml per h); elevated ASR (>150 ml/d); postural aldosterone increment 25% above the supine level; plasma 18-hydroxycorticosterone level <100 ng/dl at 0800 h (24); nonlateralizing selective adrenal vein aldosterone sampling; and symmetric adrenal morphology on abdominal computerized axial tomographic scanning.

Analytical methods. Cortisol, 18-hydroxycorticosterone, aldosterone, and PRA were measured by standard assay techniques (24–27). To assess ASR, [^{3}H]aldosterone was injected and the standard activity of urinary metabolites was calculated after derivitization and chromatography, as previously described (28).

IR-γ-MSH was determined by direct radioimmunoassay (RIA) on 50-μl aliquots of plasma. The procedure has been described previously for assay of IR-γ-MSH in rat plasma (29), but differed here in the elimination of the SepPak extraction step. This was deemed feasible, because dilutions of human plasma samples consistently exhibited displacement in parallel with assay standards. The antiserum is directed against the COOH-terminal end of γ-MSH and shows no significant cross-reactivity with α- or β-MSH, human β-lipotropin, ACTH, or β-endorphin (29). Standards and radioiodinated tracer (29) were prepared from synthetic human Lys-γ3-MSH (30) (courtesy of Dr. N. Ling, The Salk Institute, La Jolla, CA). The detection limit of the assay was 0.3 fmol of standard, and 50% tracer displacement occurred in the presence of 10 fmol. The in-run and between-run coefficients of assay variation were 3.1 and 8.8%, respectively. Our established upper limit of the normal range for human plasma IR-γ-MSH in this assay was 35 fmol/ml (n = 45), in close agreement with that reported by another group (31).

IR-ACTH was measured by RIA essentially as described by Nicholson et al. (32) on unextracted aliquots (20–50 μl) of plasma using a commercial antiserum (IgG Corp., Nashville, TN) of high affinity. The antiserum did not cross-react significantly with α-, β-, or pro-γ-MSHs, CLIP, β-lipotropin, or β-endorphin. Human ACTH(1–39) (Bachem, Inc., Torrance, CA) was used for standards and tracer, and radioiodination was accomplished using Iodogen (29). In our hands, the detection limit of the assay was 0.06 fmol ACTH, with 50% tracer displacement at ~1.0 fmol. For both IR-γ-MSH and IR-ACTH determinations, the samples were run in duplicate or triplicate. Randomly selected samples were run at three dilutions to confirm parallel displacement in the assays.

Dispersion and incubation of adrenal cells. Tissue was obtained from two APA patients undergoing unilateral adrenalectomy. Approximately 1 g of tissue from each tumor was grossly dissected and minced. Cell dispersion was then carried out as follows. Each mince was incubated separately for 50 min at 37°C in Krebs-Ringer bicarbonate/glucose/albumin buffer (KRBGA) (140 mM Na^+, 4.9 mM K^+, 2 mg/ml glucose, and 40 mg/ml bovine serum albumin; pH 7.40) containing 3.7 mg/ml collagenase (Worthington Biochemicals, Freehold, NJ) and 50 μg/ml DNase I (Sigma Chemical Co., St. Louis, MO). Tissue fragments were then disrupted by gentle trituration, filtered through a fine platinum mesh, and centrifuged (10 min, 100 g at 4°C). The pellets were resuspended and washed in KRBGA thrice in this manner. Final resuspension and incubation were carried out in KRBGA supplemented with 0.2 mg/ml L-glutamine and 1.34% (vol/vol) each of Eagle's basal and modified nonessential amino acid media (Gibco Laboratories, Grand Island, NY). Incubations were in polypropylene vials for 2 h at 37°C under an atmosphere of 95% O_2/5% CO_2. Each vial contained 1.9 ml of suspension (6.0×10^4 cells) and 0.1 ml of test solution. The latter consisted of ACTH(1–24) (Organon of Canada, Ltd., Ontario, Canada) or synthetic human Lys-γ3-MSH, which was reconstituted just before addition with peptide diluent (0.15 M NaCl containing 1 mg/ml bovine serum albumin, adjusted to pH 2.3 with 1 N HCl). After incubation, the cells were centrifuged and the medium was removed for determination of aldosterone.

Statistical analysis. Where appropriate, results are expressed as x±SE. Multiple comparisons were carried out by analysis of variance using Dunnett's modified t test (33, 34). Comparisons between two means were performed using unpaired t test. A nonparametric Krushall-Wallis one-way analysis of variance by ranks and Mann-Whitney U test for two independent samples was also used for statistical inferences. Rejection of the null hypothesis was at a probability of $P < 0.05$.

Results

Blood pressures were comparable among the three hypertensive groups, while the potassium levels were low in IHA and APA as compared with normal levels in EH (Table I). All patients with primary aldosteronism (IHA and APA) had suppressed upright and postfurosemide PRA (<1.5 ng AI/ml per h) and elevated ASR (>150 μg/d). To distinguish IHA from APA, at least three of the following differential diagnostic criteria were applied (Table II): (*a*) postural plasma aldosterone increment > 25% above the corresponding supine value (35, 36) in IHA (n = 7 of 9) but not in APA (1/5); (*b*) plasma 18-hydroxycorticosterone <100 ng/dl (24) in IHA (8/9) but not in APA (1/5); (*c*) bilateral uptake on adrenal iodocholesterol scan (37, 38) in IHA (9/9) but not in APA (0/5); and (*d*) symmetrical adrenal vein aldosterone sampling (39, 40) in IHA (5/5) but not in APA (1/4). Four APA patients underwent surgery, which confirmed the diagnosis. The fifth APA patient refused surgery.

In assessing the pituitary-adrenal axis, neither the concentration of plasma IR-ACTH nor plasma cortisol (Table I) differed significantly between any of the groups, either at 0800 or 1200 h. Urinary-free cortisol levels were also unremarkable (Table I). However, in IHA, the mean plasma IR-γ-MSH concentration (90±17 fmol/ml; range: 13–173 fmol/ml) was significantly elevated ($P < 0.05$) at 0800 h (supine), as compared with the mean levels in EH (33±6 fmol/ml), APA (33±8 fmol/ml), and NC (19±2 fmol/ml) (Fig. 1). At 1200 h (upright), the mean plasma IR-γ-MSH was significantly higher in both IHA (95±26 fmol/ml; range: 6–202 fmol/ml) and APA (63±23 fmol/ml; range: 5–131 fmol/ml), as compared with EH (31±6 fmol/ml) and NC (19±3 fmol/ml) (Fig. 1). Two IHA subjects with very high IR-γ-MSH levels (252 and 112 fmol/ml) had evidence of pituitary disease; in one case, a chromophobe pituitary adenoma, and in the other an enlarged sella turcica for which further diagnostic evaluation was declined. Of the nine EH patients, five had IR-γ-MSH levels within the normal range (<35 fmol/ml). Among the EH patients with elevated plasma IR-γ-MSH levels, two of these (57 and 51 fmol/ml at 0800 h) also had low PRA (0.1 ng AI/ml per h upright and postfurosemide) and relatively high ASR (123 and 133 μg/d).

Three of five APA patients had normal IR-γ-MSH levels, while two patients had 1200 h IR-γ-MSH concentrations > 100 fmol/ml. In order to evaluate the potency of a pro-γ-MSH as an aldosterone secretogogue in vitro, an adrenal tumor from one of the latter APA subjects (patient B1: IR-γ-MSH, 131 fmol/ml; aldosterone, 56 ng/dl), as well as another tumor from an APA patient (B2) with low plasma IR-γ-MSH

Table I. Diagnostic Data By Subject Group

Index	Limits of normal range	EH	APA	IHA
Systolic BP (*mmHg*)		157±8.8 (9)	156±8.3 (5)	158±8.9 (9)
Diastolic BP (*mmHg*)		95±2.2 (9)	99±5.0 (5)	97±3.8 (9)
K^+ (meq/liter)	3.6–5.0	3.8±0.2 (9)	3.1±0.1 (5)*	3.1±0.1 (9)*
PRR (*ng AI/ml per h*)				
0800 h, supine	0.6–1.2	1.0±0.3 (9)	0.2±0.1 (5)*	0.3±0.1 (9)*
1200 h, upright	1.2–2.2	2.0±0.7 (9)	0.4±0.2 (5)*	0.5±0.2 (9)*
Postfurosemide	2.5–6.3	4.5±1.9 (7)	0.3±0.2 (3)*	0.5±0.2 (9)*
Plasma aldosterone (*ng/dl*)				
0800 h, supine	4–11	7±0.8 (8)	33±9.4 (5)*	17±2.2 (9)*
1200 h, upright	10–33	24±4.8 (8)	28±10.2 (5)	28±5.8 (9)
Postfurosemide	30–50	22±4.6 (8)	34±9.0 (4)	24±6.4 (6)
ASR (*μg/d*)	50–150	137±22.3 (8)	282±49.2 (5)*	256±26.3 (9)*
Plasma cortisol (*μg/dl*)				
0800 h, supine	4–22	14±1.3 (9)	25±4.2 (3)	13±1.6 (9)
1200 h, upright	4–18	10±1.1 (8)	22±4.4 (3)	12±2.4 (9)
UFC (*μg/d*)	15–50	28±8.4 (8)	44±6.8 (4)	41±6.1 (9)
18-Hydroxycorticosterone (*μg/dl*)				
0800 h, supine	15–50	20±4.7 (9)	130±45.5 (5)*	46±7.7 (9)*
1200 h, upright	15–50	53±11.5 (9)	114±33.2 (5)*	59±16.7 (9)
Postfurosemide	15–50	37±6.0 (6)	143±52.5 (4)*	63±15.4 (5)
Plasma IR-ACTH (*fmol/ml*)				
0800 h, supine	<9.9	8.6±0.9 (9)	7.6±1.8 (5)	7.9±0.7 (9)
1200 h, upright	<10.8	8.0±0.7 (9)	8.0±1.1 (5)	7.4±0.7 (9)
Plasma IR-γ-MSH (*fmol/ml*)				
0800 h, supine	<29	33±6.5 (9)	33±7.7 (5)	90±17.1 (9)*
1200 h, upright	<35	31±6.4 (9)	63±22.7 (5)*	95±26.1 (9)*

Values are $\bar{x}$±SE (*n*). UFC, urinary free cortisol; BP, blood pressure; AI, angiotensin I. Numbers in parentheses indicate number of subjects.
*$P < 0.05$ vs. EH.

(12 fmol/ml) and aldosterone (10 ng/dl), was obtained at adrenalectomy. Incubation of the dispersed adenoma cells with human Lys-γ3-MSH (10^{-14}–10^{-9} M) produced a dose-dependent increase (approximately fourfold) in aldosterone secretion by cells from B1 but not by those from B2 (Fig. 2). Both sets of cells were viable by the criterion of Trypan blue exclusion.

Discussion

The results of this study demonstrate that circulating IR-γ-MSH is significantly increased in a major subgroup of patients with IHA, as compared with the mean levels observed in APA, EH, and NC. Six of nine IHA patients, two with evidence of pituitary disease, had plasma IR-γ-MSH concentrations > 80 fmol/ml, well above the upper limit of our normal range.

In contrast, Gullner et al. (41) failed to observe elevated IR-γ-MSH in either of the two IHA subjects they tested. Their negative findings may reflect the small sample size, since two IHA patients in the present study also exhibited normal IR-γ-MSH levels. Alternatively, the differing results may be a function of differences in the γ-MSH antisera employed. To illustrate, Gullner and colleagues (41) reported concentrations of IR-γ-MSH approximately equimolar with those of IR-ACTH in the plasma of their subjects. Although this would not be entirely unexpected, since both polypeptides are generated by cleavage from the same pituitary prohormone—POMC—we and others (42) have found the molar concentrations of circulating IR-γ-MSH to be severalfold higher than IR-ACTH. Physiologically, this could be explained by differing rates of metabolic clearance for ACTH and pro-γ-MSH, as in the rat (43). There have been no reports that any circulating pro-γ-MSH is derived from a non-POMC precursor.

The coincidental elevations of plasma IR-γ-MSH and aldosterone in IHA can be interpreted in several ways. First, they may be epiphenomena with no causal relationship. Although this cannot be ruled out, there is evidence that in certain settings pro-γ-MSHs can modulate aldosterone secretion. For example, pro-γ-MSHs potentiate the effect of ACTH on aldosterone secretion by the rat adrenal cortex in vitro (17) and in vivo (18), and we have previously demonstrated specific, high-affinity receptors for a pro-γ-MSH on the plasma membrane of the rat adrenal cortex (44). Moreover, a substantial,

Table II. Diagnostic Data for Subjects with Idiopathic Hyperaldosteronism

Patient	D.J.	T.A.	B.C.	G.D.	E.G.	E.S.	H.W.	R.H.	R.J.
Gender	F	F	F	F	F	M	M	M	M
Age	34	53	56	46	60	70	58	50	32
BP (*mmHg*)	180/110	130/90	150/100	140/90	160/90	160/100	130/84	220/120	150/86
K^+ (*meq/liter*)	3.1	3.0	3.2	3.2	3.1	3.2	3.5	3.1	2.6
PRA (*ng AI/ml per h*)									
0800 h, supine	0.5	0.5	0.9	0.1	0.2	0.1	0.1	0.6	0.1
1200 h, upright	0.7	0.6	1.5	0.1	0.2	0.1	0.1	0.9	0.1
Postfurosemide	1.2	0.6	1.2	0.1	0.2	0.1	0.1	1.3	0.1
Aldosterone (*ng/dl*)									
0800 h, supine	25	17	9	22	10	9	16	22	28
1200 h, upright	52	11	29	51	17	18	28	52	12
ASR (*μg/d*)	206	267	231	344	159	241	187	430	236
Cortisol (*μg/dl*)									
0800 h, supine	20	12	21	10	13	9	6	16	9
1200 h, upright	27	11	14	10	8	7	8	23	4
18-Hydroxycorticosterone (*ng/dl*)									
0800 h, supine	72	57	67	52	29	15	16	31	79
1200 h, upright	180	39	15	95	45	24	17	35	81
IR-ACTH (*fmol/ml*)									
0800 h, supine	11.2	4.6	8.1	5.9	10.1	6.4	9.5	6.8	8.7
1200 h, upright	10.0	4.2	7.9	3.9	9.1	6.1	9.0	9.7	7.0
IR-γ-MSH (*fmol/ml*)									
0800 h, supine	173	88	91	164	87	29	108	13	55
1200 h, upright	202	91	71	252	62	6	112	20	36

Limits of the normal range for each of the assays appear in Table I. All patients in this group had bilateral uptake on adrenal iodocholesterol scan and symmetric adrenal morphology by abdominal computed axial tomography. Of the five subjects who underwent adrenal venous sampling (D.J., T.A., H.W., R.H., R.J.), all had nonlateralizing aldosterone levels. BP, blood pressure; M/F, male/female; AI, angiotensin I.

independent tropic effect on aldosterone secretion from dispersed adrenal adenoma cells has been demonstrated in this study and elsewhere (45, 46) using synthetic human Lys-γ3-MSH, and by Chretien and colleagues (16, 46) with a human pro-γ-MSH. Although glucocorticoid biosynthesis is also stimulated by this hormone, there is some evidence (18, 45) that at physiologically relevant concentrations, pro-γ-MSHs are selectively more potent as aldosterone secretogogues. This may explain why cortisol levels are unremarkable in the IHA subjects. It is also noteworthy that based on data from compensatory adrenal hypertrophy studies in the rat, Lowry et al. (19) have invoked an adrenal hypertrophic role for pro-γ-MSH, while another group has reported that infusion of an

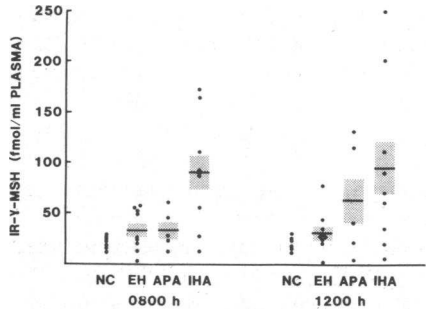

Figure 1. Plasma IR-γ-MSH levels at 0800 h (supine) and 1200 h (upright) in IHA, APA, EH, and NC. Crossbars and shaded regions represent group x±SE.

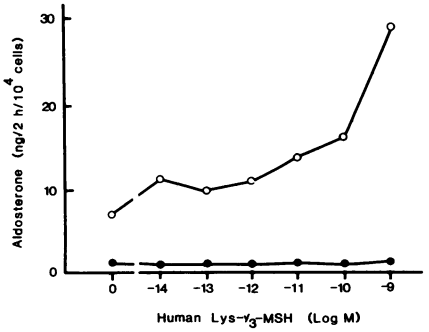

Figure 2. Aldosterone response to graded doses of synthetic human Lys-γ3-MSH by dispersed adrenal adenoma cells from two subjects, one with high plasma IR-γ-MSH and aldosterone levels (patient B1; open circles), and a second with low values (patient B2; closed circles).

anti-γ-MSH antiserum into spontaneously hypertensive and WKY rats blocks the positive effect of ACTH on aldosterone secretion (47).

Even if a causal relationship should exist between increased pro-γ-MSH and aldosterone in IHA, it could be argued that the pituitary response is secondary to an augmented aldosterone production mediated by some other secretogogue. Specific mineralocorticoid receptors have been reported in the pituitary (48, 49), but their role is unknown. However, we have found no significant effect of chronic aldosterone administration on pituitary or circulating pro-γ-MSH in the rat (Pedersen, R. C., and A. C. Brownie, unpublished observations). Moreover, the normal IR-γ-MSH levels in two of our subjects with IHA and three of five with APA suggest it is unlikely that elevated pro-γ-MSH is a secondary response to hyperaldosteronism.

Sen and colleagues (50, 51) have partially characterized an ASF which has several characteristics in common with the major human pro-γ-MSH. For example, both are pituitary glycoproteins, and the mean plasma concentrations of their ASF in normotensives and patients with IHA (31 and 71 fmol/ml, respectively) (52) are remarkably similar to the values for pro-γ-MSH reported here. Both appear to mediate their effects by a mechanism(s) that is independent of cAMP (44, 53). Carey et al. (52) have reported that an antiserum to ASF does not cross-react with synthetic human Lys-γ3-MSH, but data concerning the converse—the affinity of an anti-γ-MSH antibody for ASF—are not available.

There is a disparity between the molecular weights reported by Sen et al. for ASF (~26,000) (6) and the major form of human pro-γ-MSH (~12,000). However, from preliminary data obtained using size exclusion HPLC (Pedersen, R. C., and A. C. Brownie, unpublished observations), we believe that much of the increased plasma IR-γ-MSH in our IHA subjects may be of higher molecular weight than the predominant pro-γ-MSH (54, 55) observed in normotensive controls. This high molecular weight material, perhaps reflecting incompletely processed forms of POMC, appears to cross-react poorly with the ACTH antiserum, a fact which may explain why IHA plasma IR-ACTH values are within the normal range, while both IR-γ-MSH (this study) and IR-β-endorphin levels (56) are elevated. An intermediate in POMC processing (22,000-mol wt ACTH; ACTH biosynthetic intermediate), consisting of the NH_2-terminal region of POMC and ACTH still intact, is reported to be a potent agonist and synergist of aldosterone secretion in vitro (57).

Increased ASF levels have been reported not only in IHA but also in low renin EH. Two EH patients in the present study had high IR-γ-MSH levels (>50 fmol/ml) and low renin hypertension. The PRA levels in these patients were suppressed (<0.1 ng AI/ml per h upright and postfurosemide), but their aldosterone secretion rates were relatively high (122 and 133 μg/d). Previous studies (4) have demonstrated that subjects with low renin EH, like IHA, have augmented aldosterone secretogogue responses and elevated levels of ASF by bioassay. Except for the level of aldosterone production, low renin EH cannot be differentiated from IHA, and it has been postulated (58, 59) that these diseases are part of a continuum rather than distinct entities. If this is true, and if pro-γ-MSH is etiologic, it is possible that excess pro-γ-MSH results first in low renin, normal aldosterone EH, and later evolves to IHA as aldosterone production increases.

Recently, it has been suggested (60) that IHA may be an abnormality of the pars intermedia. In normal adults this region of the pituitary is usually difficult to discern, but it can be identified by histochemical staining (61). In man, less information is available concerning POMC processing and secretory control from cells in this region as compared with corticotrophs in the anterior pituitary, but studies in other species have demonstrated substantial differences (62). For example, there is evidence that the release of POMC-derived products from the intermediate zone may be under serotonergic (stimulatory) and dopaminergic (inhibitory) control, and is not responsive to feedback inhibition by glucocorticoids. It is noteworthy, therefore, that cyproheptadine, a serotonin antagonist, lowers aldosterone production in IHA but not in APA or NC (63). Conversely, in a patient with IHA, administration of levodopa reportedly reversed the clinical manifestations of the disorder, including the elevated levels of urinary and plasma ASF (64). In the study by Gullner et al. (41) cited above, dexamethasone paradoxically failed to suppress the levels of IR-γ-MSH in their IHA subjects. In view of the possibility that pro-γ-MSH could be of pars intermedia origin, or even nonpituitary (see, for example, references 65–67), the exact source of the elevated plasma IR-γ-MSH observed in some subjects of this study would be of considerable interest.

In conclusion, we have found that circulating plasma IR-γ-MSH levels are significantly elevated in a major subset of patients with IHA. Two of the IHA patients with the highest levels had pituitary abnormalities, which raises the possibility of an associated pituitary disorder. Among the EH subjects tested, the two with increased IR-γ-MSH levels also had low renin hypertension and inappropriately high-normal aldosterone secretion rates. Although florinef suppression was not attempted, it is possible that these two patients had indeterminant or evolving hyperaldosteronism (2, 68, 69). Together with accumulated data demonstrating that pro-γ-MSHs can stimulate or potentiate aldosterone secretion under certain circumstances, these findings suggest the possibility that pro-γ-MSH may be a significant etiological factor in some forms of hyperaldosteronism.

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