0013-7227/01/$03.00/0
Printed in U.S.A.

The Journal of Clinical Endocrinology & Metabolism 86(12):5920–5924

The Importance of Adrenocortical Glucocorticoids for Adrenomedullary and Physiological Response to Stress: A Study in Isolated Glucocorticoid Deficiency

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Glucocorticoids are required for the normal functioning of chromaffin cells and their capacity to produce epinephrine. This was modeled in a unique clinical syndrome of isolated glucocorticoid deficiency due to unresponsiveness to ACTH. The working hypotheses were that in patients with isolated glucocorticoid deficiency, adrenomedullary epinephrine would be suppressed despite replacement therapy; that norepinephrine might show a compensatory response; and that the physiological response to stress would reflect these changes. Toward these hypotheses, patients with ACTH unresponsiveness on glucocorticoid replacement were subjected to three levels of acute stress: assumption of upright posture, cold pressor, and exercise. Their catecholamine and physiological response were monitored. Patients with isolated glucocorticoid deficiency of this study had severe adrenomedullary dysfunction, characterized by a minimal resting production of epinephrine (6 ± 2 pg/ml compared with 64 ± 22 pg/ml of the controls) and a minimal response to stress. A slight compensatory increase of norepinephrine was found in response to cold pressor test (754 ± 200 pg/ml compared with 431 ± 73 pg/ml of the control). The physiological response is characterized by low systolic blood pressure and high pulse rate in rest and mild stress and in a pressor response to exercise (diastolic 87 ± 5 mm Hg, compared with 73 ± 2 mm Hg of the control). It is concluded that intra-adrenal glucocorticoids are essential for epinephrine secretion, that norepinephrine may be compensatory, and that these result in a distinct physiological response. The implications of the pressor response to exercise, the declining pulse pressure, and the increased pulse response insinuate a lower physical fitness in patients with adrenal insufficiency. (*J Clin Endocrinol Metab* 86: 5920–5924, 2001)

THE ADRENOCORTICAL AND adrenomedullary systems are intimately linked both anatomically and functionally in the adrenal gland (1, 2). Glucocorticoids are required for the survival and maintenance of chromaffin cells, and their production of epinephrine (3, 4). The expression of phenylethanolamine *N*-methyltransferase (PNMT), the enzyme that converts norepinephrine to epinephrine, depends on glucocorticoids at the transcriptional level (5). The effect of impaired adrenal function on sympatho-adrenomedullary function has been studied previously in ACTH deficiency (6) and in mice (7) or humans (8) with 21-hydroxylase deficiency. ACTH deficiency resulted in reduced epinephrine levels and normal norepinephrine. Congenital adrenal hyperplasia compromises both the development and the functioning of the adrenomedullary system. Severe adrenomedullary dysfunction was characterized by alterations in chromaffin cell migration, development, structure, and catecholamine synthesis.

The complex endocrine syndrome of 21-hydroxylase deficiency includes glucocorticoid deficiency but also mineralocorticoid deficiency with compensatory increased activity of the renin-angiotensin system as well as excess androgens. Every single one and the totality of these components may affect the physiological response to stress. The present study took advantage of a unique clinical syndrome of isolated glucocorticoid deficiency due to unresponsiveness to ACTH. Undetectable levels of glucocorticoids and dehydroepiandrosterone but a normal renin-angiotensin system characterize this group of autosomal recessive diseases (9). Plasma ACTH is increased but the adrenal fasciculata and glomerulosa are unresponsive to ACTH (9). Unresponsiveness to ACTH may be due to defective ACTH receptors (10), a mutant WD-repeat of a regulatory protein, as is the case in Allgrove syndrome (11), or unknown cause (12).

The working hypotheses of the present study were that in patients with isolated glucocorticoid deficiency, adrenomedullary epinephrine would be suppressed despite replacement therapy, that norepinephrine might be compensatory, and that physiological response would reflect these changes. Toward these hypotheses, patients with isolated glucocorticoid deficiency were subjected to acute stress, and their physiological response and catecholamines were monitored. To avoid the confounding influence of circulating glucocorticoid deficiency, patients were studied while on glucocorticoid replacement therapy, and the set of stress tests was limited to acute effects, which do not involve glucocorticoid mediated response.

Patients and Methods

Patients

Six patients (ages 4–18 yr) with congenital isolated glucocorticoid deficiency due to ACTH unresponsiveness were the subjects of this

Abbreviation: PNMT, Phenylethanolamine *N*-methyltransferase.

study (Table 1). They suffered no other disease, nor did they take any drug therapy other than their hydrocortisone acetate replacement. These patients with undetectable serum cortisol and dehydroepiandrosterone and increased plasma ACTH were previously reported to have neonatal hypoglycemia (12), normal response to salt loss of the renin-angiotensin system, and no response of either glucocorticoid or mineralocorticoid to ACTH (9). They have intact ACTH receptors (10) and normal sequence of the Allgrove gene (Tiosano, D., and Z. Hochberg, unpublished observations). The age-matched control group was comprised of four siblings of the patients, shown previously to have normal serum cortisol and two volunteers with no other chronic disease or drug therapy. The Helsinki Committees of the Rambam Medical Center approved the protocol, and the Israel Ministry of Health and parents signed informed consent forms.

Stress tests

Patients received their regular morning cortisol dose, adjusted previously to deliver a peak serum cortisol of 400–600 nmol/liter at 2–4 h(13). Patients and controls were admitted 1 h before the test after a normal breakfast meal. An iv catheter was placed in a forearm vein for blood sampling and kept patent with a heparin lock. Then, patients were allowed to rest in a supine position for 45 min.

Subjects were subjected to three levels of consecutive stress tests in an escalating order with 20 min rest in between. All of the patients completed the three tests.

Test 1: Upright posture. After supine-resting, the patient stood up. Pulse and blood pressure readings were taken at 30 sec intervals. The test was considered concluded if the pulse increased by more than 20% and a blood sample was collected.

Test 2: Cold pressor test. After a 20-min rest, the subject dipped his hand in an ice-water bucket for as long as he could tolerate (1–2 min). Pulse and blood pressure were taken at 30 sec intervals. The test was considered concluded if the pulse increased by more than 30% and a blood sample was collected.

Test 3: Strenuous exercise. After upright-resting pulse and blood pressure were monitored, the patient ran up and down a staircase with 20 steps. Pulse and blood pressure were taken at 30 sec intervals. The test was considered concluded if the pulse increased by more than 40%, serum lactic acid increased more than 2.5 mol/liter, and a blood sample was collected.

Blood collection and assays

Samples of blood were drawn into 10-ml heparinized tubes, and plasma was separated at 4 C. Plasma was stored at −70 C until the assays were performed. Plasma concentrations of norepinephrine and epinephrine were determined by liquid chromatography with electrochemical detection after extraction by alumina adsorption (14).

TABLE 1. Clinical characteristics of six patients with isolated glucocorticoid deficiency and six control subjects

Subjects	Age (yr)	Sex	Pubertal stage	Body mass index (kg/m^2)
Patient no.				
1	13	F	5	23
2	7	M	1	16
3	4	M	1	17
4	10	F	3	20
5	18	M	5	21
6	14	M	5	25
Control no.				
1	15	M	5	30
2	7	M	1	16
3	10	F	2	23
4	13	M	4	22
5	7	F	1	17
6	14	F	5	27

Statistical analysis used the Wilcoxon (Mann-Whitney *U*) test for nonparametric small samples and two-way ANOVA. Differences were regarded significant with P less than 0.05.

Results

Neither physiological response nor catecholamine levels differed between male and female patients, nor did they correlate with age or body mass index.

Rest

Whereas resting plasma epinephrine levels were extremely low in the patients (6 ± 2 pg/ml) compared with controls (64 ± 22 pg/ml; $P < 0.05$) (Fig. 1), resting plasma norepinephrine levels were similar in both groups. Resting pulse and systolic and diastolic blood pressure of the patients and control subjects were similar (Fig. 2).

Assumption of upright posture

Plasma epinephrine remained extremely low during assumption of upright posture of the patients, and their plasma norepinephrine was insignificantly higher in the patients (585 ± 213 pg/ml) compared with controls (354 ± 33 pg/ml) (Fig. 1). After assumption of upright posture, the patients' pulse response (123 ± 7) was higher than that of the control

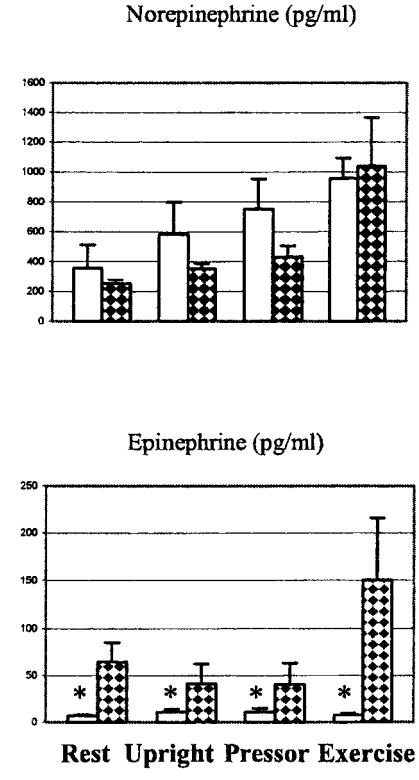

FIG. 1. Norepinephrine (*top*) and epinephrine (*bottom*) plasma levels in six patients with isolated glucocorticoid deficiency due to ACTH unresponsiveness (*white bars*) and in control subjects (*patterned bars*). Catecholamine levels were measured in rest and after three levels of stress: upright posturing, cold pressor test, and strenuous exercise. Mean ± SD; *, $P < 0.05$ of patients *vs.* the control group.

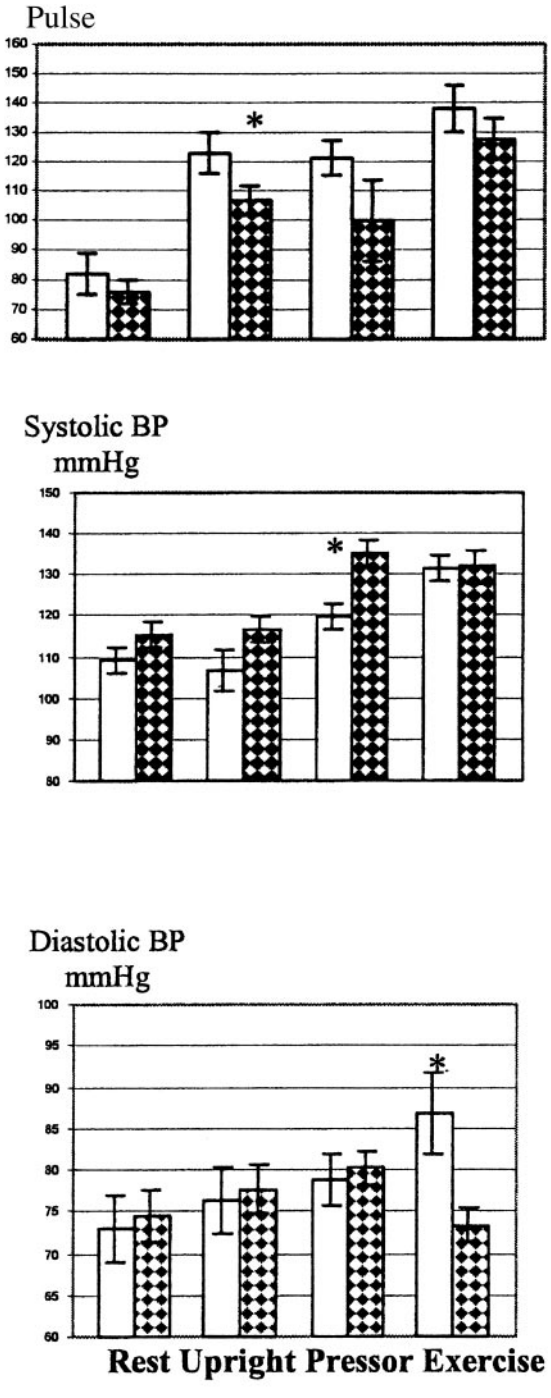

FIG. 2. Pulse (*top*), systolic (*middle*), and diastolic (*bottom*) blood pressure in six patients with isolated glucocorticoid deficiency due to ACTH unresponsiveness (*white bars*) and in control subjects (*patterned bars*). Measurements were taken in rest and after three levels of stress: upright posturing, cold pressor test, and strenuous exercise. Mean ± SD; *, $P < 0.05$ of patients *vs.* the control group.

subjects (107 ± 5; $P < 0.05$), and their systolic (107 ± 5 mm Hg), but not the diastolic, blood pressure was lower than that of the controls (117 ± 3 mm Hg; ANOVA, $P < 0.002$) (Fig. 2). The pulse increase (Δ) was 41 ± 3 in the patients and 31 ± 5 in control ($P < 0.05$). The Δ blood pressure was statistically insignificant.

Cold pressor test

Plasma epinephrine remained extremely low in the patients during a cold pressor test, and plasma norepinephrine was higher in the patients (754 ± 200 pg/ml) compared with control subjects (431 ± 73 pg/ml) (Fig. 1). In response to cold pressor test, the pulse was higher in the patients (121 ± 8) than the control subjects (100 ± 6; ANOVA, $P < 0.002$) and the patients' systolic (120 ± 3 mm Hg), but not the diastolic, blood pressure was lower than controls (135 ± 3 mm Hg; $P < 0.05$) (Fig. 2). The Δ pulse was 39 ± 6 in the patients and 24 ± 3 in controls ($P < 0.05$). The Δ blood pressure was 11 ± 2 / 6 ± 3 in the patients and 21 ± 4 / 3 ± 3 in controls ($P < 0.05$/NS).

Exercise

Adequacy of strenuous exercise was monitored by plasma lactate, required to be more than 2.5 mol/liter. Serum lactate was 4 ± 0.5 mol/liter in the patients and 5 ± 1.1 mol/liter in control subjects (NS). Plasma epinephrine remained extremely low in the patients after exercise, and plasma norepinephrine increased to a similar maximum in the patients compared with control subjects (Fig. 1). In response to exercise, the patients' pulse and systolic blood pressure peaked to a maximum similar to that of the control subjects. The patients' response was characterized by diastolic hypertension (87 ± 5 mm Hg), compared with that of control subjects (73 ± 2 mm Hg; $P < 0.05$) (Fig. 2). The Δ diastolic blood pressure was 14 ± 4 in the patients and −1 ± 2 in control ($P < 0.05$).

Compensatory norepinephrine response

To evaluate possible compensation by norepinephrine for epinephrine deficiency, the ratio of norepinephrine to epinephrine was calculated (Fig. 3). Compared with control ratios that ranged from 5 to 18, the mean resting ratio in the patients was 100. It increased insignificantly during assumption of upright posture to a mean 188, after a cold pressor test to a mean 399 ($P = 0.06$), and after exercise to a mean 389 ($P = 0.06$). Marginal significance may suggest compensatory increase of norepinephrine.

Discussion

Results of this study show that patients with isolated glucocorticoid deficiency have severe adrenomedullary dys-

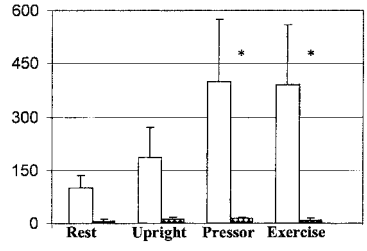

FIG. 3. Norepinephrine to epinephrine ratio in six patients with isolated glucocorticoid deficiency due to ACTH unresponsiveness (*white bars*) and in control subjects (*patterned bars*). Catecholamine levels were measured in rest and after three levels of stress: upright posturing, cold pressor test, and strenuous exercise. Mean ± SD; *, $P = 0.06$ of stress tests *vs.* resting levels.

function, characterized by a minimal resting production of epinephrine and a minimal response to stress. As a consequence, their physiological response to stress is impaired.

Ethical and practical consideration of a study in relatively young subjects instigated the experimental design. The timing of sampling was indicated by a change in pulse over resting pulse. In the case of the exercise test, a lactate threshold also confirmed it. In humans, epinephrine constitutes over 80% of adrenal catecholamine secretion (15), and epinephrine secretion increases under stressful conditions (16). To obviate potential impact of the glucocorticoid deficiency, the tests were conducted during glucocorticoid replacement therapy. Moreover, the design called for testing acute responses within minutes of short stressful events, which normally do not involve the much slower glucocorticoid response to stress.

Patients with isolated glucocorticoid deficiency due to ACTH unresponsiveness provide a unique opportunity to study *in vivo* and in humans the role of the adrenal cortex in medullary function. With isolated malfunction of their adrenal fasciculata and reticularis and normal glomerulosa (9), the consequent effects during glucocorticoid replacement therapy must be attributed to the two components of their distinct defect: unresponsiveness to ACTH and intra-adrenal glucocorticoid deficiency. Moreover, because this is a congenital defect, it also reflects on the developmental role of adrenal cortex on medullary organogenesis. Indeed, chromaffin precursor cells start migrating into the adrenal anlage in the sixth week of gestation and differentiate into mature chromaffin cells under the influence of adrenocortical steroids (17). The extremely low resting and stimulated plasma epinephrine levels in our patients with ACTH unresponsiveness must have been due to the lack of high intra-adrenal glucocorticoid concentrations at the time of the study and, thus, to defective PNMT expression or to a faulty medullary embryogenesis. Indeed, patients with acquired secondary adrenal insufficiency have also diminished basal epinephrine secretion, even with glucocorticoid-replacement therapy, but not to the same extent as the hereby reported subjects (18). Likewise, the defective medullary function in the current patients was much more severe than that reported previously in congenital adrenal hyperplasia (8). In that report, the extent of the hormonal deficiency correlated with the severity of adrenocortical dysfunction.

Compensatory increases in sympathetic-nerve activity and norepinephrine secretion have been suggested, although not proven, to occur in patients with Addison's disease (18) and in those who have undergone bilateral adrenalectomy (19). Whereas resting and stimulated norepinephrine levels in our patients with isolated glucocorticoid deficiency tended to be higher compared with control, these did not reached statistical significance against control subjects. Yet, when expressed as the ratio of norepinephrine to epinephrine, it is obvious that with increasing stress the ratio increases, up to a maximal response. In the normal adrenal medulla, there are two major types of vesicles: 1) large, round or elongated, epinephrine-containing vesicles of medium density with a particulate substructure; and 2) small, norepinephrine-containing vesicles of high density within large, lucent vacuoles. In patients with 21-hydroxylase deficiency, the epinephrine secretory vesicles were depleted, and the remaining vesicles were primarily norepinephrine-containing, high-density vesicles within large, lucent vacuoles (8). It is speculated, yet unproven, that compensatory norepinephrine increase in the patients with isolated glucocorticoid deficiency might be related to the severely defective PNMT activity and accumulation of its substrate. Plasma norepinephrine is derived from both the adrenal medulla and the sympathetic paraganglia. Compensation, observed in our patients, suggests that it may be an intra-adrenal effect. The issue of compensatory norepinephrine increase has been previously addressed in several studies. Medullary norepinephrine decreased in 21-hydroxylase deficient mice (7); it remained unchanged in hypopituitary hypocortisolemic patients (6) and in human 21-hydroxylase deficiency (8); and it increased in experimental animals with reduced glucocorticoid synthesis (20), in autoimmune Addison' patients (18), and after adrenalectomy (8, 19). None of these reports studied norepinephrine under stress stimulation. It was previously suggested that tyrosine hydroxylase is stimulated directly by ACTH (21). The high levels of norepinephrine in the current patients with ACTH unresponsiveness do not support such a direct effect.

The physiological impacts of medullary epinephrine deficiency and intact or slightly compensatory norepinephrine are quite distinct. These patients have a slightly lower than normal basal systolic blood pressure. During assumption of upright posture, a baroreceptor response maintains blood pressure through a sympathetic reflex in control subjects and only minimally lower in the patients. Yet, this was low enough to prompt an excessive pulse response. This is solely a norepinephrine effect. The differences between the groups widened at the cold pressor test. Systolic blood pressure was significantly lower in the patients and the pulse was significantly higher, indicating a role for the adrenomedullary epinephrine in the cold pressor test response.

It is noteworthy that in response to exercise, lactic acidemia and apparent (unmeasured) hypoxia, systolic pressure, and pulse rate responded normally. On the other hand, diastolic pressor response was evident in the patients, reflecting inadequate medullary epinephrine-mediated β-adrenergic vascular relaxation. Whereas hypoxic stress stimulates mostly the paraganglion norepinephrine, it also induces a depolarization and catecholamine release in dispersed chromaffin cells (22). It was recently shown that acute hypoxia differentially regulates adrenal tyrosine hydroxylase and PNMT mRNA expression in fetal sheep both before and after the development of adrenal innervation (23). After the development of adrenal innervation, however, the effect of acute hypoxia upon adrenal tyrosine hydroxylase and PNMT mRNA expression depend on neurogenic input, acting via nicotinic receptors.

This group of patients with ACTH unresponsiveness has been reported previously to have early and extreme postnatal clinical hypoglycemia (12). Whereas isolated glucocorticoid deficiency might contribute to neonatal hypoglycemia, the medullary underdevelopment and epinephrine deficiency might contribute to its severity.

It was shown previously that adrenocortical insufficiency is often characterized by changes in cardiac function and

blood pressure, which can be partly reestablished by glucocorticoid replacement therapy (24). We now show that the adrenal medulla is an essential player in the fine-tuning of the cardiovascular response to mild stress.

Acknowledgements

Received March 15, 2001. Accepted September 11, 2001.

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