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ORIGINAL ARTICLE

The acute effect of a mineralocorticoid receptor agonist on corticotrope secretion in Addison's disease

R. Berardelli[1] · I. Karamouzis[2] · V. D'Angelo[1] · B. Fussotto[1] · M. A. Minetto[2] · E. Ghigo[2] · R. Giordano[3] · E. Arvat[1]

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Abstract
Purpose Mineralocorticoid receptors (MR) in the hippocampus display an important role in the control of hypothalamic–pituitary–adrenal (HPA) axis, mediating the "proactive" feedback of glucocorticoids (GC). Fludrocortisone (FC), a potent MR agonist, has been shown to decrease HPA activity through a hippocampal mechanism. Since it has been demonstrated that FC shows a significant inhibition of the HPA axis response to hCRH stimulus in normal subjects, also at doses usually administered as replacement therapy in patients with Addison's disease, an FC effect at MRs in human pituitary or a GR-pituitary agonism stronger than believed until now has been postulated.
Methods Ten patients affected by autoimmune Addison's disease received: (1) placebo p.o. + placebo i.v., (2) hydrocortisone (H) 10 mg p.o. + placebo i.v., (3) FC 0.1 mg p.o. + placebo i.v., (4) FC 0.1 mg and H 10 mg p.o. + placebo i.v. to verify a possible GR FC-mediated effect that might display a repercussion on the GC-replacement therapy.
Results H reduced ACTH ($p < 0.01$) and increased cortisol levels ($p < 0.01$) with respect to the placebo session, while FC did not affect either ACTH or cortisol levels compared to placebo, and higher ACTH and lower cortisol levels ($p < 0.03$ and $p < 0.01$) were observed compared with the H session; furthermore the co-administration of FC + H showed ACTH and cortisol profiles similar to that observed during H alone.
Conclusions Our study showed a lack of FC effect on corticotrope secretion in Addison's disease, thus making unlikely the hypothesis of its GR pituitary agonism and the risk of glucocorticoid excess in primary adrenal insufficiency.

Keywords Fludrocortisone · MR · GR · HPA axis · Adrenal cortex

Introduction

The hypothalamic–pituitary–adrenal (HPA) axis is mainly regulated by the neurohormones corticotrophin-releasing hormone (CRH) and arginine vasopressin (AVP), which, in turn, are under the influence of several neurotransmitters and neuropeptides [1, 2]. The HPA axis activity is controlled by a negative feedback determined by the interaction of glucocorticoids with the hippocampus, hypothalamus and pituitary gland [3–6]. Glucocorticoids exert their effect via two receptor types: the glucocorticoid (GRs) and mineralocorticoid receptors (MRs) [3, 4, 7–9]. The GRs have a low affinity for glucocorticoids, are widely distributed throughout the brain and the periphery [4, 10] and are saturated at high glucocorticoid concentrations, playing a role primarily in the stress response mediating the "reactive" feedback [4, 11]. The MRs are predominantly

R. Berardelli and I. Karamouzis contributed equally as first authors.

✉ R. Berardelli
rita.berardelli@unito.it

[1] Division of Oncological Endocrinology, Department of Medical Sciences, Azienda Ospedaliera Città della Salute e della Scienza, Via Genova 3, 10126 Turin, Italy

[2] Division of Endocrinology, Diabetology and Metabolism, Department of Medical Sciences, Azienda Ospedaliera Città della Salute e della Scienza, Turin, Italy

[3] Department of Clinical and Biological Sciences, University of Turin, Turin, Italy

located in the limbic structures, with the hippocampus being the main localization site [4, 12, 13]. At this site, the 11b-hydroxysteroid dehydrogenase type 1, which regenerates active glucocorticoids from the circulating inert 11-keto steroids, is widely expressed, while the type 2, which rapidly inactivates glucocorticoids, is non-detectable [11]. In the hippocampus, the MRs bind glucocorticoids with an affinity ten times greater than the GRs and are largely saturated, about 50–70 %, in the presence of low glucocorticoid levels [4, 12]. Thus, MRs are predominantly occupied during the nadir of the circadian rhythm regulating the "proactive" feedback, which controls the basal HPA activity [4]. The modulation of the basal HPA activity is carried out by a tonic inhibition mediated by GABAergic projections to the paraventricular hypothalamic nucleus [14]. In humans, MRs antagonists enhance the axis [5, 14, 15] not only during the nadir but also during the peak of the circadian rhythm, suggesting an involvement of MRs even during this phase [5, 6, 14–21]. Moreover, the MRs antagonists amplify ACTH, cortisol and DHEA response to both CRH and AVP stimulations, indicating that the stimulatory effect of MRs blockade is probably mediated by concomitant increase of both CRH/AVP [5, 20, 22]. In addition, both spontaneous and hCRH-stimulated HPA activity is amplified during the quiescent phase of the circadian rhythm by chronic treatment with MRs antagonists [20], while the stimulatory effect of canrenoate is abolished by pre-treatment with the benzodiazepine alprazolam [23].

Complementary studies have also shown that the administration of an MRs agonist as fludrocortisone (FC), an agent able to cross the blood brain barrier [24], exerts an inhibitory effect on the HPA axis. Indeed, the FC administration at supraphysiological doses reduces the ACTH and 11-deoxycortisol concentrations increased by a pre-treatment with metyrapone and the physiological cortisol levels during the quiescence-nadir of the circadian rhythm [25–27]. These effects are supposed to reflect the FC binding to hippocampal MRs. In our previous study, we have evaluated the acute effects of different FC doses on both basal and hCRH-stimulated HPA activity during the quiescent phase of the circadian rhythm in normal subjects, demonstrating that FC inhibited the HPA response to hCRH [28]. This inhibitory effect was detectable not only after high pharmacological doses of the MRs agonist, but also after those usually administered as replacement therapy in patients affected by Addison's disease [28]. Therefore, it is possible to assume that the inhibitory effect exerted by FC on the HPA axis response to hCRH is mediated at lower levels than hippocampus, providing indirect evidence of a possible existence of MRs in human pituitary, as demonstrated in animals [29, 30]. Alternatively, the hypothesis that FC may inhibit hCRH-induced corticotrope secretion through a pituitary GR agonism can be taken into account, as it has been suggested by previous pharmacological and "in vitro" studies that the FC can be a GR agonist much more powerful than thought until now [25–27, 31].

Based on these premises, the aim of the study was to verify the effect of FC, at a dose commonly used in the clinical practice, on the activity of the HPA axis in patients with Addison's disease.

Subjects and methods

Subjects

Ten patients affected by autoimmune Addison's disease (5 men and 5 women, span disease 11.1 ± 2.9 years, age 44.2 ± 2.8 years, BMI 22.6 ± 0.9 kg/m^2 mean ± SEM) were studied. All the subjects were screened to exclude acute physical illness or any acute or prior psychiatric disorder by physical examination, routine laboratory tests, urinary analysis, serum pregnancy test and structured interview. None of the subjects had history of alcohol, substance dependence, recent stress events or was under lactation.

They had been free of any drug known to influence HPA axis for at least 3 months before the study, except for their daily replacement therapy with hydrocortisone (H), a total of 20 mg p.o. divided into three daily administrations (10 + 5 + 5 mg), and fludrocortisone (FC) p.o. 0.1 mg/die (unchanged from a period of at least 6 months before the study). The last dose of H and FC date back to the day before the study. Substitution treatment appeared appropriate since the values of the electrolytes, at the randomization, were 142.7 ± 0.5 mmol/l for Na and 4.1 ± 0.2 mmol/l for K; the levels of the PRA were 3.9 ± 1.1 ng/ml/h and the values were included within their range. The study protocol had been approved by an independent, local ethical committee and written informed consent was obtained from all subjects.

Drugs

Fludrocortisone tablets (0.1 mg) were purchased from E.R. Squib and Sons Ltd (Uxbridge, England) and hydrocortisone tablets (10 mg) purchased from Sanofi Aventis (France), both from the local pharmacy by each patient.

Study design

All subjects were randomly assigned to receive each of the following test sessions: (1) placebo p.o (at 8.00 h) + placebo i.v. (500 ml saline from 8.00 am to 13.00 pm), (2) H 10 mg p.o (at 8.00 h) + placebo i.v., (3) FC 0.1 mg p.o. (at 8.00 h) + placebo i.v., (4) FC 0.1 mg and H 10 mg p.o. (at 8.00 h) + placebo i.v. after an overnight fasting. The tests started at 8.00 h,

30 min after an indwelling catheter had been placed into the antecubital vein of the forearm that was maintained patent until the end of the study by the slow infusion of isotonic saline. Blood samples were taken every 20 min from 8.00 to 13.00 h. The tests were consecutively performed with at least 3 weeks washout. The levels of ACTH and cortisol were analyzed at each time point. The substances were administered in a single-blind fashion.

Hormone and biochemical measurements

Blood samples were centrifuged immediately after collection and plasma and serum samples were frozen at −20 °C until assay. Plasma ACTH levels (pg/ml; 1 pg/ml = 0.22 pmol/L) were measured in duplicate by an immunoradiometric assay (IRMA, DiaSorin, Italy). The sensitivity of the assay was 1 pg/ml. The range of inter- and intra-assay coefficients of variations was 5.1 and 6.9 %, respectively. Serum cortisol levels (μg/dl; 1 μg/dl = 27.5 nmol/L) were measured in duplicate by a radioimmunoassay (RIA, Immunotech, France). The sensitivity of the assay was 0.36 μg/dl. The range of inter- and intra-assay coefficients of variations was 9.2 and 5.8 %, respectively.

The levels of plasma renin activity (PRA, ng/mL/h) were measured by RIA of angiotensin I (Beckman Coulter), after its generation in plasma samples as a result of enzymatic cleavage of the substrate of renin, angiotensinogen, in the presence of ACE-inhibitor. The immunologic assay of angiotensin I is a method of competition radioimmunoassay using coated tubes polyclonal antibodies. The analytical sensitivity and functional is 0.07 and 0.2 ng/ml/h, respectively. The range of inter- and intra-assay coefficients of variations was 11.3 and 20.9 %, respectively.

Serum Na and K (mmol/l) were measured using an ion-selective electrode (cobas ISE module) that was fully automated.

Statistical analysis

Hormonal responses are expressed as mean, standard error of the mean (SEM), and relative 95 % confidence interval (95 % C.I.) of either absolute values. For each subject, the differences between placebo p.o. + placebo i.v., H p.o + placebo i.v., FC p.o. + placebo i.v. and H + FC p.o. + placebo i.v. at each time point were compared by means of nonparametric Wilcoxon test. Differences with a p value < 0.05 were considered to be statistically significant. SPSS (Statistical Package for the Social Science), version 15.0, was used for the analysis.

Results

Placebo p.o. + placebo i.v. session ACTH and cortisol levels remained stable during the 4 h of hormonal evaluation (Fig. 1).

H p.o + placebo i.v. session H significantly reduced ACTH levels from the time point 80 min to the end of the session, while cortisol increased after 40 min, reaching a peak at 80 min with a subsequent slow decline ($p < 0.05$, $p < 0.05$; Fig. 1); during the H session, a significant reduction in ACTH levels from the time point 100 min and an increase in cortisol levels from 40 min compared with the Placebo p.o. + placebo i.v. session were observed ($p < 0.01$, $p < 0.01$; Fig. 1). Similar data expressed as $AUCs_{0–300}$ for both hormones were detected ($p < 0.01$, $p < 0.01$; Fig. 2).

FC p.o. + placebo i.v. session: FC did not affect either ACTH or cortisol levels and no differences were observed compared with the placebo p.o. + placebo i.v. session, while significant higher ACTH and lower cortisol levels ($p < 0.03$, $p < 0.01$; Fig. 1) were observed compared with the H p.o + placebo i.v. session starting from the time point 100 and 40 min respectively, as well as for $AUCs_{0–300}$ ($p < 0.03$, $p < 0.01$; Fig. 2).

H + FC p.o. + placebo i.v. session: ACTH levels were significantly reduced from the time point 80 min and cortisol levels increased from 40 min ($p < 0.05$, $p < 0.05$; Fig. 1); no differences were observed compared with H p.o + placebo i.v. session for both hormones, while significant differences were observed compared with FC p.o. + placebo

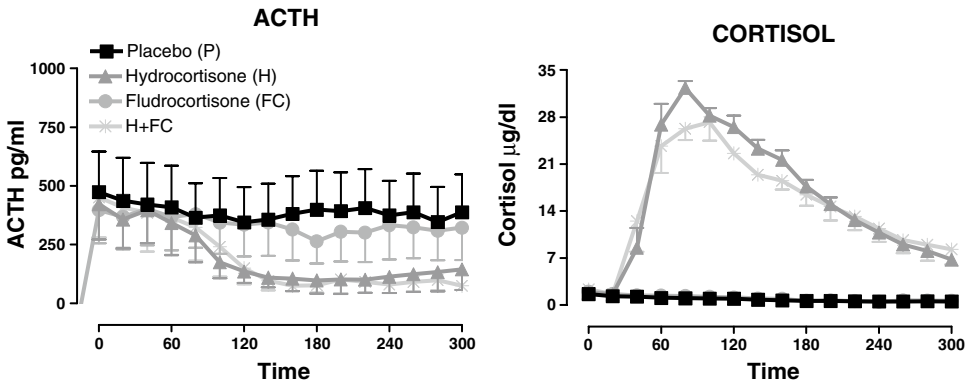

Fig. 1 ACTH and cortisol levels during placebo, hydrocortisone (H), fludrocortisone (FC) and H + FC administration

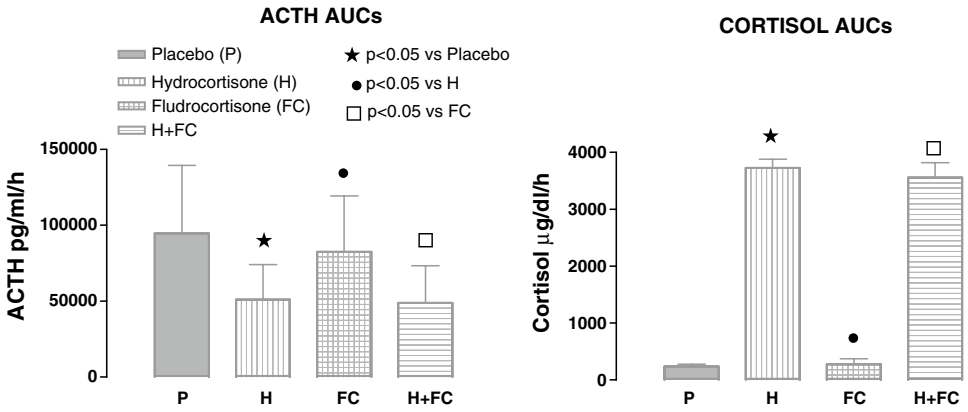

Fig. 2 ACTH and cortisol $AUCs_{0–300}$ during placebo, hydrocortisone (H), fludrocortisone (FC) and H + FC administration

i.v. session starting from the time point 80 min for ACTH and from 40 min for cortisol ($p < 0.01$, $p < 0.01$; Fig. 1) as well for the $AUCs_{0–300}$ ($p < 0.01$, $p < 0.01$; Fig. 2).

Side effects

No side effects were reported by the patients during the sessions.

Discussion

In the present study, we evaluated in a patient population with Addison's disease the effect on corticotrope secretion of fludrocortisone, a MRs agonist commonly used in the replacement therapy for primary adrenal failure. The study demonstrated, to our knowledge for the first time, that fludrocortisone does not exert, differently from that observed in normal subjects, an inhibitory effect on corticotrope activity in patients naturally deprived of glucocorticoids. In fact, our previous study provided evidence that the acute administration of fludrocortisone is able to clearly inhibit the HPA response to the strong hypophysial stimulus hCRH in physiological conditions [28].

Animal studies have demonstrated that MRs in CNS, besides their expression at the hippocampal level, are also expressed in the pituitary in different species [29, 30], whereas we are unaware of any data concerning pituitary MRs in humans. Therefore, a possible inhibitory effect of fludrocortisone in humans on the HPA axis through MRs situated at the hypophysial level cannot be excluded and further research is mandatory to confirm this hypothesis. Alternatively, the given data in vitro studies that fludrocortisone is a GR agonist with a GR potency higher than that of cortisol [31] suggesting its possible GR-mediated effect directly at the pituitary level, could allow to assume that fludrocortisone may be a more potent GR agonist than commonly thought. This hypothesis led us to postulate a possible concern about the therapeutic approach of Addison's disease, assuming an additive glucocorticoid activity to that naturally offered by the glucocorticoids usually administered to these patients.

Surprisingly, unlike that observed in normal subjects, fludrocortisone, at the same dose used in normal volunteers, did not show any inhibitory effect on ACTH secretion in patients with corticotrope hyperactivation induced by cortisol deprivation, such as Addison's disease.

Our study also demonstrated that the co-administration of hydrocortisone and fludrocortisone has an effect on ACTH release overlapping with that after hydrocortisone alone, thus suggesting that, actually, fludrocortisone does not interfere with the GC-mediated action of hydrocortisone on corticotrope function in this clinical condition.

Based on these results, we can assume a different neuroregulation of the HPA axis between normal subjects, in whom fludrocortisone inhibits both basal and hCRH-stimulated corticotrope activity, and Addison patients, in whom the disease *per se* may have altered the normal control of the MR- and GR-mediated feedback mechanisms. This hypothesis can be related to the reduced sensitivity to the negative feed-back activity exerted by fludrocortisone on ACTH release, regardless of the receptor mechanism by which it can be mediated. In agreement with this hypothesis, we cannot exclude a dose-dependent effect of FC on HPA activity in Addison patients when administered at higher doses than those commonly used in the hormonal replacement therapy.

A possible explanation for the reduced sensitivity to the negative feed-back activity exerted by fludrocortisone on ACTH release in Addison patients could be sought in the long duration of the disease from which these patients are affected. It is possible that patients suffering from Addison's disease for a long time may change the mechanisms of the neuroendocrine control of corticotrope release, with the occurrence of a somewhat corticotrope desensitization [32]. Unfortunately, we could not prove this hypothesis, as the cohort enrolled in our study did not include patients with newly diagnosed disease, in whom a still preserved neural control of corticotrope function

could be conceivable. Moreover, the study lacks an important control population, e.g., patients with post-TBC Addison's disease, in whom mineralocorticoid production is often preserved and a selective deficit of cortisol is observed. It would be very interesting to compare the response of the HPA axis in patients affected by post-TBC or autoimmune Addison. It is possible to assume that in the post-TBC Addison, an FC-mediated effect on the HPA axis could be similar to that observed in healthy subjects, reflecting a possible more integrity of the MR receptor systems with respect to the autoimmune Addison's disease. Unfortunately, our cohort of patients with Addison's disease is rather small in tuberculosis forms; thus, a comparison with autoimmune Addison's disease was not possible.

From a clinical point of view, regarding the therapeutic management of patients with Addison's disease, the evidence provided by our data seems to exclude the possibility that fludrocortisone can act as a glucocorticoid agonist, avoiding the fear of a possible excess of glucocorticoid replacement during the combined treatment with fludrocortisone and exogenous glucocorticoids.

In conclusion, our study showed a lack of fludrocortisone effect on corticotrope secretion in patients affected by Addison's disease, differently from that observed in physiological conditions. As a glucocorticoid receptor-mediated inhibiting effect exerted by fludrocortisone on ACTH release has been postulated, our present results would allow us to consider the use of this compound without risks of glucocorticoid excess in primary adrenal insufficiency.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All the procedures involved in the study were in accordance with the ethical standards of the ethical institutional committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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