Modulation of 11β-hydroxysteroid dehydrogenase functions by the cloud of endogenous metabolites in a local microenvironment: The glycyrrhetinic acid-like factor (GALF) hypothesis

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A R T I C L E I N F O
Keywords:
11β-Hydroxysteroid dehydrogenase Inhibitor Glycyrrhetinic acid GALF Essential hypertension Mineralocorticoid Glucocorticoid Oxysterol Bile acid Gut microbiota

A B S T R A C T
11β-Hydroxysteroid dehydrogenase (11β-HSD)-dependent conversion of cortisol to cortisone and corticosterone to 11-dehydrocorticosterone are essential in regulating transcriptional activities of mineralocorticoid receptors (MR) and glucocorticoid receptors (GR). Inhibition of 11β-HSD by glycyrrhetinic acid metabolites, bioactive components of licorice, causes sodium retention and potassium loss, with hypertension characterized by low renin and aldosterone. Essential hypertension is a major disease, mostly with unknown underlying mechanisms. Here, we discuss a putative mechanism for essential hypertension, the concept that endogenous steroidal compounds acting as glycyrrhetinic acid-like factors (GALFs) inhibit 11β-HSD dehydrogenase, and allow for glucocorticoid-induced MR and GR activation with resulting hypertension. Initially, several metabolites of adrenally produced glucocorticoids and mineralocorticoids were shown to be potent 11β-HSD inhibitors. Such GALFs include modifications in the A-ring and/or at positions 3, 7 and 21 of the steroid backbone. These metabolites may be formed in peripheral tissues or by gut microbiota. More recently, metabolites of 11β-hydroxyΔ4-androstene-3,17-dione and 7-oxygenated oxysterols have been identified as potent 11β-HSD inhibitors. In a living system, 11β-HSD isoforms are not exposed to a single substrate but to several substrates, cofactors, and various inhibitors simultaneously, all at different concentrations depending on physical state, tissue and cell type. We propose that this “cloud” of steroids and steroid-like substances in the microenvironment determines the 11β-HSD-dependent control of MR and GR activity. A dysregulated composition of this cloud of metabolites in the respective microenvironment needs to be taken into account when investigating disease mechanisms, for forms of low renin, low aldosterone hypertension.

1. Introduction

Licorice, isolated from the root succus liquiritiae or from the licorice plant glycyrrhiza glabra, was initially used to treat gastric ulcers, and it is still used in traditional Japanese Kampo medicine, in traditional Chinese medicine, in tobacco products and as a confection [1,2]. It is well-known that licorice when taken in excess can cause mineralocorticoid side effects including edema and increased blood pressure [3]. Later studies revealed the underlying mechanism of this low renin, low aldosterone form of hypertension by demonstrating that the major licorice constituent 18β-glycyrrhetinic acid (GA) is a potent inhibitor of 11β-hydroxysteroid dehydrogenase (11β-HSD) activity, resulting in cortisol-induced activation of mineralocorticoid receptors (MR) in renal cortical collecting ducts and distal tubules [4–6]. In fact, GA was found to be about ten times more potent towards 11β-HSD1 dehydrogenase than 11β-HSD1 oxoreductase [7]. More recent studies suggested 18β-glycyrrhetinyl-3-O-sulfate, formed by SULT2A1 from GA and exhibiting more potent inhibitory activity against 11β-HSD2 and reaching higher serum concentrations than GA, to be the causative agent of the licorice-induced pseudohyperaldosteronism in human [8–10]. Although

Abbreviations: 11β-HSD, 11β-hydroxysteroid dehydrogenase; AME, apparent mineralocorticoid excess; CYP, cytochrome P-450; DOC, 11-deoxycorticosterone; GALF, glycyrrhetinic acid-like factor; GR, glucocorticoid receptor; MR, mineralocorticoid receptor.

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https://doi.org/10.1016/j.jsbmb.2021.105988
Received 14 June 2021; Received in revised form 8 August 2021; Accepted 25 August 2021
Available online 28 August 2021
essential hypertension affects about 25% of the adult population, the underlying cause remains unknown in the majority of cases, but impaired 11β-HSD dehydrogenase activity may be involved in a part of this population [11].

The concept that endogenous substances exist that exert potent glycyrrhetinic acid-like factor (GALF) inhibitory activity against both 11β-HSD2 and 11β-HSD1 dehydrogenase has evolved over the decades [12–15] (Fig. 1). Initial work testing partially purified Sep-Pak extracts of urine samples from normotensive men and from non-pregnant and pregnant women demonstrated inhibition of 11β-HSD dehydrogenase and 5β-reductase activities [16]. The inhibitory effects of such extracts increased throughout pregnancy and the bioactive substances seemed to be heat stable. The majority of the substances were not extractable with ethyl acetate, likely representing glucuronidated and sulfated compounds. Hydrolysis with β-glucuronidase of these Sep-Pak extracts released ethyl acetate extractable GALFs, which possessed two to ten times higher inhibitory activity towards both sheep kidney 11β-HSD dehydrogenase and rat liver 11β-HSD1 dehydrogenase [17]. As extracts contain a mixture of compounds, later work focused on investigating inhibitory activities of individual steroidal derivatives that were found in urine or plasma samples. Some GALF compounds can also inhibit 11β-HSD1 oxidoreductase activity [12], thereby likely affecting the regulation of cardio-metabolic processes, neurological activity, behavior and the immune system [18,19]. This review will focus on endogenous steroidal metabolites as potential GALFs, although other endogenous non-steroidal compounds inhibiting 11β-HSD dehydrogenase likely also exist. We fully recognize that other types of substances exist in nature which when included in the dietary intake or taken up by other routes such as skin and lung would inhibit these enzymes [20–22].

Several potent active GALFs are derived from the adrenal steroids cortisol, corticosterone, 11β-hydroxy-18-deoxycorticosterone and aldosterone [23,24]. Some of these GALFs circulate freely in the blood stream, reaching their target tissues. Other GALFs require further modification by specific enzymes in the gut microbiota and reach their target tissues via the enterohepatic recirculation [12,23–25]. Complicating matters further, some GALFs may be directly synthesized locally from androgens and progestogens in the very target tissues where they function (see below) [26–28]. Besides, some 7-oxygenated steroids, bile acids and oxysterols have been found to inhibit 11β-HSD enzymes, acting potentially as GALFs [29–34]. GALFs may act as competitive substrates (including some 11β- and 7β-hydroxy-derivatives competing with cortisol and corticosterone dehydrogenation and the corresponding 11- and 7-oxo-derivatives with cortisone and 11-dehydrocorticosterone oxoreduction), whilst others exclusively act as inhibitors.

Stewart and Edwards discovered that the licorice constituent GA inhibited both the oxidation of cortisol to cortisone and the hepatic glucocorticoid reduction by 5β-reductase [6]. Later, those observations were expanded to the kidney where isomers of 11β-HSD oxidize cortisol to cortisone and corticosterone to 11-dehydrocorticosterone [5]. When the 11β-HSD dehydrogenase is inhibited, cortisol becomes able to bind to and activate MR, causing renal sodium retention, potassium loss and hypertension [38–40]. This landmark finding also accounted for the hypertension observed in children with apparent mineralocorticoid excess (AME), carrying genetic mutations in the gene encoding 11β-HSD2 [41–44].

As mentioned above, early studies by the Morris group, at a time where LC–MS/MS methods were not established, reported the presence of endogenously produced substances, termed GALFs, in extracts of human urine [16]. These GALFs acted like licorice, inhibiting the dehydrogenase activity of both 11β-HSD2 and 11β-HSD1. This led to the hypothesis that endogenous steroidal compounds might function as potential competitive enzyme inhibitors and, based on this, more than 100 steroids were screened for their GALF-like inhibitory properties. The more potent GALF substances derive from two major families of adrenal steroids, corticosterone and cortisol [12,13,23]. They include 3α,5α-tetrahydrocorticosterone (5αTHB), 11β-hydroxyprogesterone (11βOH-pr og), 3α,5α-tetrahydro-11β-hydroxyprogesterone (5αTH-11βOH-prog) pruced by 21-deoxxygenation of corticosterone and its 5α-pathway products by gut microbiota and 3α,5α-tetrahydro-11α-hydroxytestosterone (5αTH-11αOH, C19-steroïd, a derivative produced following side-chain cleavage of cortisol or following hydroxylation of androgens). All are potent inhibitors of 11β-HSD2 and some of 11β-HSD1 dehydrogenase [12,13,23]. Interestingly, the 3α,5β-tetrahydro (TH) derivatives of these 11-oxygenated adrenocorticoïds possessed no inhibitory activity at all [13]. The 11-oxo metabolites 3α,5α-tetrahydro-11-dehydrocorticosterone (5αTHA), 3β,5α-tetrahydro-11-ketotestosterone, and 3α,5α-tetrahydrocortisone (5αTHC), are all potent inhibitors of 11β-HSD1 oxidoreductase [12].

The microbial metabolite, 21-desoxycorticisol, derived from 21-dehydroxylaton of cortisol by intestinal bacteria, also potently inhibited 11β-HSD2 (but not 11β-HSD1) with an IC50 of 0.35 μM [12,13] (Table 1). Plasma levels of 21-desoxycortisol are about two times elevated in humans with hypertension when compared to normotensive controls [45]; however, plasma concentrations were found to be in the sub-nanomolar range. Nevertheless, conjugated metabolites should be considered, as demonstrated for GA and its sulfated metabolite 18β-glycyrrhetin-3-O-sulfate, and tissue levels of potential GALFs need to be determined as they can substantially be different from plasma levels.

Humans have evolved and synthesize two glucocorticoids, cortisol and corticosterone, which display their own individual characteristics [46]. That specific intestinal bacteria have likewise adapted to metabolize and utilize both glucocorticoids may not be surprising. These gut bacteria further metabolize endogenous glucocorticoids and provide additional glucocorticoid derivatives that, following reabsorption into the blood stream, may influence sodium and potassium homeostasis, blood pressure as well as pro- or anti-inflammatory pathways.

Fig. 1. Schematic overview of glucocorticoid metabolism by 11β-HSD enzymes and inhibition by GALFs. 11β-HSD1, especially in the presence of H6PDH, converts inactive cortisone and 11-dehydrocorticosterone to the potent 11β-hydroxylglucocorticoids cortisol and corticosterone (blue arrow). The reverse reaction is catalyzed by 11β-HSD2 and 11β-HSD1 dehydrogenase (red arrows). Inhibition of 11β-HSD dehydrogenase activity by GALF substances result in elevated cortisol and corticosterone concentrations, ultimately leading to adverse health effects caused by the excessive activation of MR and GR (highly simplified schematic).
Currently known steroidal GALFs and inhibition of 11β-HSD enzymes.

| Chemical name (abbreviation) | 11β-HSD2 dehydrogenase | 11β-HSD1 dehydrogenase | 11β-HSD1 oxoreductase |
|-----------------------------|-------------------------|------------------------|-----------------------|
| **11-Oxygenated GALFs**     |                         |                        |                       |
| 5α-Dihydrocortisol (5αDHF)  | 3.1 d                   | 40 e                   | n.a.                  |
| 3α,5α-Tetrahydrocortisol (5αTHF) | 8.0 d | 14 b | 180 a | n.i. c |
| 3α,5α-Tetrahydrocortisone (5αTHE) | 5.5 d | 240 a | 4.3 a |
| 21-Desoxycorticisol | 0.35 d | 100 a | n.a. |
| 3α,5α-Tetrahydroaldosterone (5αTH-Aldo) | 0.5 d | 25 b | n.i. c |
| 5α-Dihydrocorticosterone (5αDHB) | 0.15 d | 2.1 b | 7.5 a | 6.3 c |
| 3α,5α-Tetrahydrocorticosterone (5αTHB) | 0.26 d | 1.3 b | 50 e |
| 11-Dehydrocorticosterone | 0.47 d | n.a. | n.a. |
| 3α,5α-Tetrahydro-11-dehydrocorticosterone (5αTH-11OHT) | 0.80 d | 8.0 b | 0.7 c |
| 11β-Hydroxy pregnenolone (11βOH-preg) | 0.1 d | 9.5 e | n.a. |
| 11β-Hydroxyprogesterone (11βOH-preg) | 0.05 d | 5.6 b | 11 e |
| 3α,5α-Tetrahydro-11β-hydroxyprogesterone (5αTH-11βOH-preg) | 0.12 d | 3.0 b | 16 a | n.i. c |
| 11-Ketopregesterone (11k-preg) | 0.40 a | 190 p | 9.5 a |
| 3α,5α-Tetrahydro-11-ketopregesterone (5αTH-11k-preg) | 1.50 a | n.i. b | 0.8 c |
| 3α,5α-Tetrahydro-11-ketopregesterone (5αTH-11k-preg) | n.i. c | 65 a |
| 11β-Hydroxytestosterone (11βOH-T) | 0.35 a | 9.0 b | n.i. c |
| 3α,5α-Tetrahydro-11β-hydroxytestosterone (5αTH-11βOH-T) | 4.50 a | 5.0 b | 11.5 c |
| 11β-Ketotestosterone | 1.35 a | n.i. b | 18 c |
| 11β-Hydroxy-Δ4-androstene-3,17-dione (11βOH-AD) | 7.80 a | n.i. b | n.i. c |
| 3β-Tetrahydro-11β-ketotestosterone | 8.0 a | 50 b | 0.65 c |
| **11-Deoxy steroidal GALFs** |                         |                        |                       |
| 3α,5α-Tetrahydro-11-β-deoxycorticosterone (5αTH-DOC) | 2.4 d | 31 e | n.a. |
| 3α,5α-Tetrahydro-11-β-deoxycorticosterone (TH-DOC) | > 10 | 3.0 a | n.a. |
| **7-Oxygenated steroidal GALFs** |                         |                        |                       |
| 7α-hydroxydehydroepiandrosterone (7αOH-DHEA) | n.a. | 1.85 r | n.a. |
| 7β-hydroxydehydroepiandrosterone (7βOH-DHEA) | n.a. | 0.255 r | n.a. |
| 7α-oxodehydroepiandrosterone (7αKHDEA) | n.a. | n.a. | 1.13 r |
| 7α-hydroxyepiandrosterone (7αOH-EpiA) | n.a. | 13.6 q | n.a. |
| 7β-hydroxyepiandrosterone (7βOH-EpiA) | n.a. | 1.0 s | n.a. |
| 7α-oxoepiandrosterone (7αEpiA) | n.a. | 1.6 q | 2.2 t |
| 7,20α-Hydroxycholesterol (7β20αHC) | 0.091 f | n.a. | 3500 m |
| 7α,25α-Hydroxycholesterol (7α25αHC) | > 3 f | 5.9 m |
| 7α,20α-Hydroxycholesterol (7β20αHC) | 0.011 h | n.a. | 0.405 f |
| 7α,25α-Hydroxycholesterol (7α25αHC) | 0.015 h | n.a. | > 3 f |

Details on reaction conditions (biological material, substrate, cofactor, reference):

a,d sheep kidney microsomes, 50 nM corticosterone, 200 μM NAD+/α [12], d [13].

b rat Leydig cell homogenates, 600 nM corticosterone, 3 mM NADP+/β [12].

i rat Leydig cell homogenates, 600 nM 11-dehydrocorticosterone, 3 mM NADPH [12].

i rat liver tissue homogenates, 5 μM corticosterone, 3.4 mM NADP+/i [13].

i, h, k HEK-293 lysates expressing human enzyme, 50 nM cortisol, 500 μM NAD+/i [34], h [33], k [29].

e, f HEK-293 lysates expressing human enzyme, 200 nM cortisol 500 μM NADP+/e [34], f [33].

i rat kidney cortex microsomes, 100 nM corticosterone, 1 mM NADP+ [35].

m rat kidney cortex microsomes, 100 nM 11-dehydrocorticosterone, 1 mM NADPH [35].

n human liver microsomes, 100 nM corticosterone, 1 mM NADPH [36].

o human liver microsomes, 100 nM 11-dehydrocorticosterone, 1 mM NADPH [36].

p rat kidney microsomes, 5 μM corticosterone, 3.4 mM NADP+/p [37].

q, t K, cortisol for oxidation and cortisone for oxoreduction, NADPH regenerating system q [31], t [30].

Data on human enzymes are indicated in bold.
2. Measurement of steroidal GALFs in human hypertension

2.1. AME (apparent mineralocorticoid excess) syndrome

When Stewart and Edwards initially described the inhibition of 11β-HSD as a cause for the hypertension in patients with AME [6,38], the exact mechanism remained unknown. Due to the similar phenotype of licorice induced hypertension, the existence of endogenous inhibitors was investigated by Morris and coworkers. They analyzed urine samples obtained from several children with AME, off all medications (provided by M. New and S. Ulick), for their GALF inhibitory activity against both sheep kidney isoforms of 11β-HSD (likely 11β-HSD2 and 11β-HSD1 dehydrogenase) and rat liver 11β-HSD1 dehydrogenase activity; however, no GALF activity was measurable (other than in 1 case) [24,46]. This was later explained by the discovery of genetic mutations leading to a loss of 11β-HSD2 activity in AME [42,47]. Defects in the gene encoding 11β-HSD2 are very rare, thus, from a teleological perspective, having found no GALFs in children with AME suggests that GALFs may play a role in a sub-set of patients with essential hypertension.

2.2. 17α-Hydroxylase deficiency

A 24-h urine sample was collected from one of the sisters with 17α-hydroxylase deficiency (diagnosed by Mallin) [48] and analyzed for GALF activity. The urine sample from this patient was obtained after all glucocorticoid medication was discontinued for 96 h. Markedly elevated GALF activity (i.e. 11β-HSD dehydrogenase inhibition) was seen using sheep kidney microsomal preparations, with liver preparations, and with vascular smooth muscle cell preparations (approximately 300 times greater, and >400 times greater, respectively) compared to urine samples from normotensive female controls [49]. Two earlier studies described females diagnosed with 17α-hydroxylase deficiency [50,51] who had increased urinary excretion of corticosterone metabolites and their 21-dehydroxylated derivatives, including high concentrations of 5αTHB and its derivatives 5αTH-11βOH-prog and 3α,5α-tetrahydro-11-ketoprogesterone (5αTH-11β-ket-prog) (all metabolites were identified using GC–MS). Although currently neither plasma nor tissue concentrations of the above mentioned metabolites are available, an increased presence of such metabolites in the urine may indicate a greater production and/or formation by local metabolism, providing an explanation for the observed hypertension and hypokalemia. Future studies will need to determine plasma as well as tissue concentrations of various GALF substances in hypertensive animal models and, if possible, in patients.

In 17α-hydroxylase deficiency (mutations in the gene encoding CYP17A1) the adrenal production of mineralocorticoids is greatly increased as a result of the activated hypothalamus-pituitary-adrenal axis in an attempt to restore glucocorticoid levels. The elevated plasma corticosterone (aldosterone was found only slightly elevated or not altered) and 11-deoxycorticosterone (DOC, in some but not all patients with the genetic defects or upon pharmacologic inhibition of CYP17A1) [52,53], together with GALF metabolites of these two corticosteroids, are likely responsible for the observed MR activation and resulting sodium retention, potassium loss and increased blood pressure. In addition to the elevated concentrations of the above mentioned GALFs other as yet unidentified steroidal GALFs may contribute to the observed clinical mineralocorticoid-like effects and deserves further investigation.

2.3. Human essential hypertension

Patients with essential hypertension have marked increases in the ratio of 5α/5β-reduced steroid metabolites of cortisol and corticosterone excreted in the urine [54,55]. Also, the ratio of 11β-hydroxylated metabolites to 11-dehydro metabolites, (THF + 5αTHF)/THE, was significantly higher than in healthy controls, a pattern similar to individuals following licorice ingestion [6]. These changes in the routes of steroid metabolism were thought to account for the prolonged half-life (t1/2) of 11α-13C2-cortisol observed in a large proportion of patients with essential hypertension [56]. Thus, a sub-set of patients with essential hypertension can exhibit endogenous GALF-like inhibitors, which might include endogenous (allo-) 3α,5α-reduced pathway steroidal products. The relative contribution of adrenal corticosteroids and their metabolites acting as GALFs and influencing electrolyte balance and blood pressure increase remains unclear and requires further investigation. Since plasma concentrations may differ significantly from the intracellular GALF concentrations in the affected tissues (kidney, vascular cells), we need to study their concentrations in both environments.

Urinary 11β-HSD2 inhibitory GALFs were found to be significantly higher in a sub-set of high/normal-renin hypertensive subjects when challenged with a low Na+ diet [17]. These elevated 11β-HSD2 inhibitory GALFs (measured using sheep kidney microsomal preparations) strongly correlated with both increased urinary free cortisol and plasma renin activity in these patients [57].

The GALFs present in the human urine samples may also contain 11β- or other hydroxylated steroidal substances, which themselves may be substrates for inactivation by renal 11β-HSD2 (or 11β-HSD1 dehydrogenase) and/or other conjugating enzymes as well as C20-reductases [17,57]. Furthermore, so far mainly unconjugated steroid metabolites have been considered in testing for inhibitory activities and conjugated metabolites need to be included, in analogy to the potent inhibitory effect of the sulfated GA metabolite. The 11-keto derivatives and other further metabolic products may be far less active than the “parent” circulating GALFs; hence underestimating the true circulating or tissue concentrations of the active inhibitory compounds. This may be relevant since studies undertaken so far to measure GALF substances in essential hypertensive individuals did not consider this possibility.

3. Transformation of adrenal steroids into GALFs by gut microbiota

3.1. 21-Dehydroxylation of cortisol and corticosterone by intestinal flora

Ericksson et al. using germ-free rats were the first to demonstrate the 21-dehydroxylolation of corticosterone to 11βOH-prog by intestinal bacteria [58]. Similarly, 11-deoxycorticosterone (DOC) was then shown to be excreted in urine of humans mainly as pregnanediol metabolites following anaerobic 21-dehydroxylation by intestinal flora [59]. Soon after, cortisol, aldosterone, and corticosterone, and their (allo) 3α, 5α-tetrahydro (5αTH) metabolites (but not their 3α,5β-tetrahydro (TH) metabolites) were also found to be 21-dehydroxylated in human feces by Eggerthella lenta [60,61]. The products, 21-deoxycortisol (11β-hydroxylated 17αOH-prog), 11β(OH) prog and 5αTH-11β-hydroxypregnanolone all possess potent GALF inhibitory activity against 11β-HSD2, and 11βOH-prog and 5αTH-11β-OH-pregnanolones against the 11β-HSD1 dehydrogenase [12,13,23].

All these compounds, as well as 5αTHB, can induce hypertension when infused into adrenally intact male Sprague Dawley rats [46,49,62], 11βOH-prog, 11β-hydroxysteroid (11βOHT) and 5αTH-11βOHT all can allow endogenous glucocorticoids to activate MR and enhance Na+ retention, potassium loss, and hypertension [46,62]. Moreover, when aortic rings were simultaneously preincubated with 11βOH-prog and corticosterone, Brem and colleagues [63] demonstrated enhanced vasoconstriction with catecholamines, and in separate experiments later with angiotensin II [64], when compared with controls. These findings were consistent with 11βOH-prog acting as an inhibitor of vascular 11β-HSD dehydrogenase activity. The generation of GALFs derived from corticosterone likely induced the experimental form of hypertension observed in rats by Honour [65], since the hypertension was reversed when the gut microbiota was eliminated by oral antibiotic treatment, i.e. neomycin [65,66]. This was the first report to suggest that neomycin can prevent the hypertension by
inhibiting the formation of the 21-dehydroxylated steroid metabolites of corticosterone produced by intestinal flora [65]. Murphy had earlier identified a steroidal GALF substance which inhibited 11β-HSD dehydrogenase activity in placental tissue [15,67]. This GALF, with a putative chemical structure of 11βOH-20-di-hydro-prog, was suggested to regulate placental cortisol bathing the fetus, and affecting fetal size, and parturition date [15]; parameters subsequently related to cardiovascular disease, including hypertension, later in life [68].

Gut dysbiosis has been associated with hypertension in other studies [69–71]. Galla, Joe and coworkers studied microbial effects following treatment with three different families of antibiotics, i.e. neomycin, minocycline, and vancomycin, on two genetic models of hypertension, the Dahl S rat and the spontaneously hypertensive SHR rat [69,70]. The disparate effects on blood pressure were accompanied by significant alterations in gut microbiota, suggesting role(s) they may play in different animal models of hypertension and possibly subsets of patients with varying forms of hypertension [69]. Other studies showed that decreases in bacterial strains known to produce the short-chain fatty acid butyrate accompanied the increase in blood pressure following transplanting cecal contents from hypertensive rats with obstructive sleep apnea (OSA) (on high-fat diet) into OSA recipient rats (on normal chow diet) [72]. Additionally, two olfactory sensory receptors, Olfr78 and G protein-coupled receptor 41 (Gpr41), have been implicated as novel regulators of blood pressure [72]. These receptors found in the vascular bed, serve as receptors for short-chain fatty acids, specifically acetate and propionate, synthesized by gut microbes and when activated can modify blood pressure [74].

Questions remain about the importance of gut microbial metabolism in the generation of potential steroidal GALFs, particularly whether the concentrations of the metabolites at the site of the corresponding enzyme and receptor are sufficient to affect blood pressure through inhibition of renal or vascular 11β-HSD2 or by direct activation of MR and/or GR. Individuals with polymorphisms in HSD11B2 that reduce functional activity may be especially susceptible to effects from endogenous steroidal GALFs.

3.2. Side-chain cleavage of cortisol by intestinal flora

Cerone-McLernon et al. [75] established that the gut microbiota is capable of catalyzing the side-chain cleavage of cortisol; C-19 17α-hydroxy metabolites of cortisol were observed after incubation with human fecal microbiota [75–77]. Ridlon and colleagues have confirmed that human feces gut microbial isolates, such as Clostridium scindens ATCC 35704, can readily metabolize cortisol to 11β-hydroxy-α,4-androstene-3,17-dione (11βOH-AD), and that 5αTHF, (but importantly not THF), is similarly transformed to 3α,5α-tetrahydro-11βOH-AD [24,76,77]. Several bacteria in humans also possess 17β-HSD activity. The bacterial genes responsible for the side-chain cleavage desmolase activity and other adrenal steroid metabolizing bacterial enzymes have recently been reviewed in detail [25].

As mentioned earlier, all these cortisol-derived products also possess potent GALF activity [12,13,24]. The clinical relevance of these bacterially generated compounds becomes evident when they are reabsorbed from the intestine into the circulation. The desmolase activity of Clostridium scindens and other gut bacteria which contain 17β-HSD activity are able to metabolize cortisol into androgens [25,76]. 11βOHT, 11-ketotestosterone, 5αTH-11βOHT, all potently inhibit 11β-HSD2. Additionally, 11βOHT and 5αTH-11βOHT inhibit 11βHSD1 dehydrogenase [12]. 11βOH-AD has moderate GALF activity [12,14,15]. The recent discovery that the androgen 11βOH-AD is adenally synthesized and leads to peripheral metabolism to other 11β- and 11-keto androgens [25,78] raises the question as to what physiological functions do they serve by affecting 11β-HSD2 as well as the functional equilibrium of bi-directional 11β-HSD1. As mentioned above, plasma and tissue concentrations of these 11-oxo-steroids need to be determined and possible direct modulatory effects towards MR and GR have to be assessed.

Furthermore, Odermatt and coworkers have drawn attention to the fact that the inhibitory activity and relative IC50 values can be very different when measured against the human, rat and mouse 11β-HSD isoforms [7,33,79]. Given the apparent species differences, we need to establish the relative potencies of GALF substances against human 11β-HSD2 and 11β-HSD1 dehydrogenase and reductase in order to determine physiological relevance. Results from studies with experimental animals cannot be directly extrapolated to human.

Thus, specific bacteria in the gut have adapted to metabolize endogenous glucocorticoids, thereby forming GALFs that may directly or indirectly influence a variety of MR- and GR-mediated processes, including sodium reabsorption, potassium excretion and blood pressure, as well as oxidative stress and inflammatory responses. The symbiotic relationship between the gut microbiota and the human host is clearly intriguing especially when considering mechanisms of hypertension and possibly other diseases.

4. 7-Oxygenated steroidal GALFs

4.1. 7-Oxygenated androgens as substrates and inhibitors of 11β-HSDs

Another group of endogenous modulators of 11β-HSD enzyme activities comprises 7-oxygenated androgens, which may act as substrates and/or inhibitors. Due to rotational symmetry of the steroid backbone, the C7 and C11 substituents can adopt similar positions in the substrate binding pocket of the enzymes [80,81] (Fig. 2).

Several studies by Morfin and coworkers using microsomal preparations from yeast expressing recombinant enzyme showed that human 11β-HSD1 accepts 7-oxygenated metabolites of dehydroepiandrosterone (DHEA), epipandrosterone and 5α-androstane-3β,17β-diol. Therefore, these metabolites may inhibit 11β-HSD1 oxoreductase-mediated regeneration of active glucocorticoids, which has been introduced as the anti-glucocorticoid paradigm [30–32,82,83]. A comparison of kinetic parameters revealed a preference for the oxoreductase over the dehydrogenase activity for 7-oxygenated metabolites of these steroids, and a preference for the formation of the 7β-epimer over the 7α-epimer was observed [84]. Supporting these observations, Nashev et al. used intact transfected HEK-293 cells to show that 11β-HSD1 catalyzes the interconversion of 7-oxo and 7-hydroxy metabolites of DHEA, pregnenolone and 5α-androstane-3β,17β-diol [85]. As expected the respective 7-oxo metabolites were potent inhibitors of 11β-HSD1 oxoreductase and the 7β-hydroxy metabolites of the dehydrogenase, whilst the 7α-epimers had weak effects. Importantly, an epimerization of 7α-hydroxy to 7β-hydroxy metabolites could be observed, in line with results from Morfin and coworkers [84]. Thus, the 7β-hydroxy metabolites are potent inhibitors of 11β-HSD1 dehydrogenase, while their 7-oxo forms potentely inhibit the reductase.

To our knowledge, a detailed analysis of inhibitory effects of these steroids against 11β-HSD2, with a possible role in regulating MR and GR activation by glucocorticoids in inflammation or in MR-dependent sodium retention and hypertension, remains to be conducted. Although plasma concentrations of these 7-oxygenated steroids are expected to be low, they may exert modulatory effects on 11β-HSD-mediated glucocorticoid metabolism in specific tissues and cells including inflamed tissues.

4.2. Bile acids potentially acting as GALFs

Evidence for an inhibition of 11β-HSD1 and 11β-HSD2 dehydrogenase by 7-hydroxylated bile acids and contribution to renal sodium retention and potassium loss in cholestatic liver cirrhosis has been reported over 25 years ago (reviewed in [86]). The infusion in adrenalectomized rats of chenochoexylic acid (CDCA), but not cholic acid (CA) or deoxycholic acid (DCA), enhanced sodium retention and potassium excretion, an effect that was prevented by co-treatment with the MR antagonist RU-28318 [37]. Furthermore, infusion of CDCA
enhanced blood pressure [87]. The mechanisms underlying these observations seem to be complex and are still not fully understood.

Mechanistic studies using rat kidney and liver microsomal preparations with NADP⁺ or NADPH to assess 11β-HSD1 oxidation and oxoreduction of corticosterone and 11-dehydrocorticosterone, respectively, revealed lithocholic acid (LCA) and CDCA as most potent dehydrogenase but weak oxoreductase inhibitors [35–37]. These observations were supported using recombinant human 11β-HSD1 enzyme [88]. The highly toxic LCA, present at only low levels in human plasma, was the most potent inhibitor with a slight preference for the dehydrogenase over the oxoreductase activity. The major primary bile acid CDCA (bearing a 7α-hydroxyl) was equally potent towards the dehydrogenase as LCA (lacking a 7-oxygen) but more than tenfold less potent against the oxoreductase. Also taurochenodeoxycholic acid (TCDC) and glycochenodeoxycholic acid (GCDC) showed a 20-fold preference to inhibit 11β-HSD1 dehydrogenase over oxoreductase. Ursodeoxycholic acid (UDCA, bearing a 7β-hydroxyl) and DCA (lacking a 7-oxygen) were found to be weak inhibitors and CA did not inhibit. The 11β-HSD1 oxoreductase was found to be potently inhibited by 7α-amino-lithocholic acid (7α-oxo-LCA), which was identified as substrate to be reduced preferentially to CDCA and at a lower extent to UDCA by the human enzyme [88–90].

An inhibition of 11β-HSD2 has been proposed to be responsible at least in part for the sodium retention and potassium loss in cholestatic cirrhosis [86]. Early experiments with human, rat and sheep microsomal preparations showed clearly less potent inhibition of 11β-HSD2 compared to 11β-HSD1 dehydrogenase activity for both LCA and CDCA [36,87,91]. Measurements using lysates of HEK-293 cells expressing human 11β-HSD2 revealed that LCA, CDCA and DCA moderately inhibited the enzyme with micromolar IC₅₀ values [29] that were at least an order of magnitude higher than the values against 11β-HSD1 dehydrogenase [88]. In contrast to CDCA, UDCA did not inhibit 11β-HSD2.

Nevertheless, serum bile acid concentrations in patients with cholestasis due to biliary obstruction caused by gallstones reached levels for CDCA and DCA that exceeded the IC₅₀ to inhibit 11β-HSD2 in a majority of patients [29,92]. Thus, inhibition of 11β-HSD2 likely contributes to glucocorticoid-induced MR activation and subsequent sodium retention and potassium excretion in these patients. In line with this assumption, the (5αTHF + THF)/THE ratio (a urinary marker of the sum of renal proximal and distal 11β-HSD dehydrogenase activity) was significantly increased in the cholestatic patients, and reversed to normal values together with a normalization of the serum bile acid concentrations following surgical removal of the biliary obstruction [29,92].

Several mechanisms are likely to contribute to MR-mediated sodium retention and potassium loss in cholestatic liver disease. Elevated inflammatory cytokines can result in increased hepatic 11β-HSD1 and decreased renal 11β-HSD2 [93,94], thus shifting the balance from inactive to active glucocorticoids (Fig. 3). Regarding direct inhibition, CDCA seems to be the most relevant metabolite, reaching concentrations in cholestatic patients that can inhibit 11β-HSD2 and allow for glucocorticoid-mediated MR activation. Additionally, the about tenfold more effective inhibition of 11β-HSD1 dehydrogenase in renal proximal tubules is expected to contribute to elevated concentrations of active glucocorticoids in the kidney. The combined effect of gene expression and direct inhibition of 11β-HSD2 dehydrogenase determines the glucocorticoid-induced MR activation, resulting in sodium retention and potassium excretion. In addition to the effects on renal volume regulation, inhibition of 11β-HSD1 dehydrogenase activity in vascular cells likely enhances vasoconstriction via glucocorticoid-induced activation of MR and/or GR [95], thereby contributing to increased blood pressure.

4.3. 7-Oxygenated oxysterols modulating 11β-HSD activities

7-Oxygenated cholesterol metabolites also need to be added to the list of GALFs. 11β-HSDs accept some 6- and 7-oxygenated oxysterols as substrates [33,34,96–98], and their elevated levels might impact 11β-HSD-dependent glucocorticoid metabolism (Fig. 3). 7-oxocholesterol (or 7-ketocholester, 7kC) and 7β-hydroxycholesterol (7β-OHC) represent major oxysterols taken up through oxidized cholesterol-rich enhanced bile.

Fig. 2. Symmetry of steroid molecules. A) the three axes of the sterol molecule are depicted, including numbers of the carbon atoms. B) following rotation around the x-axis, an 11β-hydroxyl can adopt a similar orientation than a hydroxyl at 7α position. Alternatively, if rotating 180° around the z-axis the 11β-hydroxyl can adopt a similar pose than a 7β-hydroxyl.

Fig. 3. Simplified scheme of impact of inflammation and CDCA on 11β-HSD-dependent glucocorticoid metabolism. Inflammation enhances the expression of 11β-HSD1 and decreases 11β-HSD2. CDCA inhibits the dehydrogenase activity of 11β-HSD1 and 11β-HSD2. The combined effect of enzyme expression and inhibition results in elevated cortisol (and similarly corticosterone) concentrations.
food [99] or formed by autoxidation of cholesterol in cellular membranes or cholesterol bound to low-density lipoprotein (LDL) during oxidative stress [100–102]. 7kC and 7βOHC concentrations are in the lower nanomolar range under normal conditions in healthy individuals but are strongly increased in patients with genetic diseases such as Cerebrotendinous xanthomatosis and Smith-Lemli-Opitz syndrome as well as in diseased tissues including atherosclerotic plaques and in cataract lenses [103–107]. 7kC and 7βOHC were first described as substrates of 11β-HSD1 using liver microsomal preparations or lysates of cells expressing recombinant enzyme from different species [7,96,97,108].

In intact cells expressing H6PD, 11β-HSD1 predominantly catalyzes the oxoreduction of 7kC. Interestingly, in intact cells as well as in lysates of HEK-293 cells expressing recombinant human 11β-HSD1 7kC seemed not to be able to inhibit 11β-HSD1-dependent cortisone reduction; however, it did so moderately in human THF-1 macrophage expressing endogenous levels of the enzyme [109]. Similarly, 7kC weakly inhibited the oxoreduction of 11-dehydrocorticosterone by murine 11β-HSD1 upon over expression in HEK-293 cells [110] but readily inhibited the enzyme in mouse serum. In these mouse adipocyte cell lines incubation with 7kC diminished the differentiation of preadipocytes to mature adipocytes and abolished lipid loading. This was explained by an inhibited generation of active from inactive glucocorticoids by 7kC and its ability to repress GR transcriptional activity. In contrast, incubation of the cells with 7βOHC was found to stimulate GR activity, due to its inhibition of the oxidation/inactivation of glucocorticoids. However, since these experiments were performed in intact cells, the formation of metabolites of 7kC and 7βOHC causing the observed effects cannot be excluded. Furthermore, a study in healthy volunteers administered i.v. deuterium labeled 7kC or 7βOHC suggested a rather short half-life of 1.5 h and 1.9 h, respectively [112].

Based on the above observations it seems rather unlikely that 7kC and 7βOHC by themselves alter 11β-HSD-dependent glucocorticoid metabolism. In this respect, both oxysterols are efficiently metabolized by CYP27A1 in the liver to their 27-hydroxylated metabolites and 7kC and 7βOHC are potent inhibitors of 11β-HSD1 oxoreductase and dehydrogenase, respectively, and that these compounds also belong to the most potent inhibitors of human 11β-HSD2 described so far [33,34] (Fig. 4). Cholesterol-25-hydroxylase (CH25H), responsible for the 25-hydroxylatation of 7kC and 7βOHC, and CYP27A1 are both expressed in activated M1 phenotypic macrophage [114]. The generated 7,25- and 7, 27-dioxycorticoids may be more relevant in the vasculature of elevated oxidative stress such as diabetic nephropathy. Whether sufficient high plasma or intra-tissue concentrations of the dioxygenated metabolites are generated to inhibit 11β-HSD enzymes, remains to be investigated.

5. The cloud concept

The purpose of this review is to re-examine the GALF hypothesis in light of these new exciting findings and think more broadly about what this may mean in the context of key regulatory mechanisms related to homeostasis and regarding the consequences of dysregulation in disease processes.

Mechanistic information on enzyme activity and effects of inhibitors is traditionally provided by assays using purified proteins and preparations where the enzyme is accessible to compounds such as cellular fractions, cell lysates or tissue homogenates. In case of short-chain dehydrogenase/reductase enzymes (such as 11β-HSDs), assays are usually performed in the presence of saturating concentrations of co-factor and a defined substrate and inhibitor by incubations for a relatively short time period. Further information on inhibitory efficiency can be obtained from measurements using intact cells and adding defined concentrations of substrate and inhibitor. This experimental setting, although essential to discover new functions and assess biochemical properties of enzymes, may limit the understanding of (patho)physiological situations in living cells that are highly dynamic. In living systems several substrates and inhibitors may be present simultaneously and the co-factor concentrations for oxidation and oxoreduction are both present and may vary depending on the physiological conditions and on the cell type (Fig. 5). Moreover, unconjugated and conjugated (by amidation, esterification, glucuronidation, sulfation) GALF substances may possess markedly different enzyme inhibitory properties and cause different effects at the hormonal receptor level, as exemplified for 25-hydroxycholesterol and its sulfated form [115]; and they may be present at different concentrations and composition in different cell types and tissues in healthy and disease situations.

6. The local microenvironment concept

We now wish to extend the GALF concept by the idea that these steroidal compounds inter-relate with each other in the local microenvironment at the active site of 11β-HSD enzymes, the MR and GR, and with other proteins involved in steroid metabolism, conjugation, transport or signaling. Many adrenal and locally generated steroid metabolites, as well as bile acids and oxysterols, may have additional as yet undiscovered local activities in target tissues that have been overlooked.

Circulating endogenous steroid hormones and potential GALFs enter tissues and from there their target cells. The rate of entry, local metabolism and efflux all determine their concentrations in the cytosol and in organelles, which can markedly differ among tissues and cell types as well as in different cellular compartments. For a given GALF to be active, it has to be present in adequate amounts locally with endogenous levels of the enzyme [104,113]. The relevance of CYP27A1 for 7kC metabolism is seen in Cerebrotendinous xanthomatosis patients who show accumulation of this oxysterol [103,106]. Importantly, two recent studies showed that the 25-hydroxylated as well as 27-hydroxylated metabolites of 7kC and 7βOHC are potent inhibitors of 11β-HSD1 oxoreductase and dehydrogenase, respectively, and that these compounds also belong to the most potent inhibitors of human 11β-HSD2 described so far [33,34] (Fig. 4).

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Circulating endogenous steroid hormones and potential GALFs enter tissues and from there their target cells. The rate of entry, local metabolism and efflux all determine their concentrations in the cytosol and in organelles, which can markedly differ among tissues and cell types as well as in different cellular compartments. For a given GALF to be active, it has to be present in adequate amounts locally with endogenous glucocorticoid adjacent to the target (11β-HSD) enzyme. Complicating matters, steroid substrates, their metabolites and GALFs comprise a plume or a “cloud” of biologically active compounds that all potentially interact with each other to influence the catalytic efficiency of each of the 11β-HSD isoforms. Moreover, local co-factor concentrations and the redox state within the corresponding cellular compartment also influence the catalytic efficiency of each 11β-HSD isoform. This cloud of substances will constantly change in its size, dimension and

![Fig. 4. Schematic overview of inhibition of 11β-HSD enzymes by 7-oxygenated oxysterols. A) 7k27OHC and 7k25OHC are reduced by 11β-HSD1 to the corresponding 7β-hydroxylated oxysterol, thereby competing with cortisone reduction and decreasing the regeneration of cortisol. B) 7β27OHC and 7β25OHC are substrates of 11β-HSD2 (and possibly 11β-HSD1) dehydrogenase, resulting in diminished metabolism of cortisol.](image-url)
composition, possessing a 3-dimensional character. Thus, reactions conducted in a test tube with purified enzymes, homogenates or whole cell suspensions (expressing endogenous or recombinant proteins) or in modified animal models may optimize the observations but may not be the true indicator of biological activity. The overall directionality and functional equilibrium of 11β-HSD isoenzymes is not constant but variable and complex, and dependent on the overall local microenvironment. As described above, several 7β-hydroxy-oxysterols are substrates and potent inhibitors of human 11β-HSD2 (and likely also of 11β-HSD1) dehydrogenase (with very low IC₅₀ values) [33,34]. Similarly, the corresponding 7-α-oxy-oxysterols serve as competitive inhibitors against 11β-HSD1 oxidoreductase. Besides, several 11β-hydroxy progestogens and androgens as well as 7β-hydroxy androgens may inhibit 11β-HSD2 (and 11β-HSD1) dehydrogenase and their 7-α-oxygen forms 11β-HSD1 oxidoreductase. The inhibitory effects of glucocorticoid metabolism by these non-glucocorticoid substrates, along with those of individual GALFs (also with low IC₅₀ values), are cumulative and the sum of their individual substrate concentrations in the local microenvironment determines the extent of inhibition of 11β-HSD2 and 11β-HSD1 dehydrogenase and also that of the 11β-HSD1 oxidoreductase activity towards glucocorticoids, thereby regulating MR and GR transcriptional activities. Thus, the functional equilibrium and biological activity of 11β-HSD2 and 11β-HSD1 dehydrogenase/reductase highly depends on the local presence of the various substrates and endogenous inhibitors.

Future studies should exploit the recent advances in analytical methods. Steroidomic and metabolomics quantitative analyses of the presence of GALFs and above mentioned 7-oxygenated oxyosterol metabolites in plasma as well as in tissues of interest should provide important information on the 11β-HSD-mediated regulation of MR and GR in health and disease situations.

6.1. Local synthesis of steroid hormones

The microenvironment can also be influenced by the local synthesis of several steroid hormones that are known to be produced in the adrenals. Evidence has been presented that cortisol, corticosterone, DOC and aldosterone can be generated in target tissues such as the brain, the vascular system, the intestine and skin [116-124]. The discovery of CYP11B1 and CYP11B2 in variety of tissues indicates that potent glucocorticoids and mineralocorticoids can be produced locally, with far more potent effects at the cellular level than their circulating concentrations might suggest. Locally produced steroid metabolites, including neurosteroids such as 7-oxygenated androgens as well as some 5α-reduced steroids, may exert highly tissue- and cell-specific functions. The receptors and mechanisms-of-action of several such steroid metabolites remain yet to be uncovered.

6.2. Local generation of GALF substances

Local enzymes including 11β-HSD isoforms, Ring-A 5α-reductases, 3α-HSDs and 20α/β-HSDs are present in many glucocorticoid and mineralocorticoid target tissues. GALFs derived from the circulation can be further modified in the local microenvironment. For example, 5α-dihydroaldosterone and 3α,5α-tetrahydroaldosterone (5αTH-Aldo) are synthesized from aldosterone in rat kidney subcellular fractions, an effect blunted by the mineralocorticoid antagonist spironolactone [125]. 5αTH-Aldo displays potent 11β-HSD2 GALF as well as moderate 11β-HSD1 GALF dehydrogenase inhibitory activity (even though aldosterone and 3α,5α-tetrahydroaldosterone are totally inactive) [12,23]. 5αTH-Aldo functions as a mineralocorticoid [126] and hence its synthesis locally in a specifically relevant microenvironment demands further investigation. Many target tissues of mineralocorticoids and glucocorticoids including brain, vasculature, intestine and eye, all express considerable 5α-reductase and 3α-HSD and 3β-HSD enzymatic activity [127-129]. Similar local enzymatic modifications may generate GALFs from other parental compounds including oxysterols and their sulfated derivatives [115], as well as other steroid metabolites and their conjugates.

The complexity of the different isoforms of 11β-HSD and their properties has recently been reviewed in detail [27]. Many currently held beliefs have been discussed and challenged. The protective and specificity conferring role(s) of the 11β-HSD isoforms and their relationship to MR and GR has evolved and seemingly ever more complex [130-134]. The concept of a cloud of interrelated cumulative adrenal steroid metabolites, alternative steroid and steroid-like substrates and GALF substances, all influencing enzyme activities in the microenvironment, is undoubtedly complicated, challenging, and remains worthy of many prospective investigations.

Author statement

David J Morris, Andrew S Brem and Alex Odermatt all contributed to literature search, interpretation of content and all wrote and revised the paper.

Declaration of Competing Interest

The authors report no declarations of interest.

Acknowledgement

This work was supported by the Swiss National Science Foundation grant 31003A-179400.
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