Disruption of Glucocorticoid and Mineralocorticoid Receptor-Mediated Responses by Environmental Chemicals

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Abstract: Glucocorticoids and mineralocorticoids are key endocrine hormones modulating essential physiological processes such as energy metabolism, cell growth and differentiation, maintenance of blood pressure and immune responses. Despite their importance and the fact that their impaired function has been associated with various diseases, there are only few studies on the potential disruption of glucocorticoid and mineralocorticoid action by xenobiotics. To facilitate the identification and characterization of such chemicals, we established cell-based assays to determine the impact of xenobiotics on different steps of corticosteroid hormone action. Screening of a small library of chemicals led to the identification of several compounds inhibiting the 11β-hydroxysteroid dehydrogenase (11β-HSD) prereceptor enzymes 11β-HSD1 and/or 11β-HSD2 and of chemicals blocking the function of glucocorticoid receptors (GR) and mineralocorticoid receptors (MR). These findings build a basis to extend the search for chemicals acting on additional targets of the corticosteroid hormone pathway and to apply in silico prediction tools in combination with biological testing to screen large numbers of chemicals. The identification of chemicals interfering with corticosteroid action and the elucidation of the underlying molecular mechanisms are relevant with respect to the potential contribution to common diseases such as metabolic syndrome, immune diseases, brain disorders and cancer.

Keywords: Cortisol · Endocrine disruptor · Glucocorticoid receptor · Glucocorticoid · 11β-Hydroxysteroid dehydrogenase · Mineralocorticoid receptor · Xenobiotics

1. Physiological Role of Glucocorticoids and Mineralocorticoids

Corticosteroid hormones are divided into glucocorticoids (cortisol in humans and corticosterone in rodents) and mineralocorticoids (aldosterone). They are involved in the regulation of many physiological processes, including energy metabolism, electrolyte and blood pressure control, bone metabolism, regulation of brain function, cell cycle control, and modulation of stress and inflammatory responses (Fig. 1).[1] An impaired regulation of glucocorticoid action has been associated with various complications including metabolic and cardiovascular diseases, osteoporosis, cataracts, immune diseases, mood and cognitive disorders and cancer.[2-5] Disturbances of mineralocorticoid action have been linked to the occurrence of hypertension and cardiovascular diseases.[6,7] The incidence of these complex diseases increases with increasing age, and in addition to genetic predisposition, factors including life style and the exposure to xenobiotics are likely to play a role in these pathological processes. In developed countries, the exposure to chemicals from the environment may contribute to the high incidence of allergic diseases, cancer and metabolic disturbances.[8-10]

2. Major Targets of the Corticosteroid Hormone Pathway

Exogenous chemicals can cause disturbances of corticosteroid hormone action at several steps (Fig. 2). Corticosteroids are produced in the adrenal cortex involving several steroidogenic enzymes, and the synthesis of these hormones is tightly regulated by corticotrophin releasing factor (CRF) and adrenocorticotropic hormone (ACTH) via the hypothalamus-pituitary-adrenal (HPA) axis. Glucocorticoids control their own synthesis by a negative feedback response mediated by inhibition of CRF and ACTH. Upon release into the bloodstream, corticosteroid hormones are mainly bound to carrier proteins (transcor tin, albumin) and reach cells in peripheral tissues.

At the cellular level, cortisol or corticosterone exert their action through GR and aldosterone acts by activating MR. Importantly, the local activation of the receptors is controlled by two distinct 11β-HSD prereceptor enzymes.[11] 11β-HSD1 is expressed ubiquitously and catalyzes predominantly the reduction of inactive 11-ketoglucocorticoids (cortisone, 11-dehydrocortisone) into active 11β-hydroxyglucocorticoids (cortisol, corticosterone).[12] This enzyme has a crucial role in potentiating local GR activation in metabolic processes and in the immune system.
The second enzyme, 11β-HSD2, is expressed in cortical collecting ducts and distal tubules in the kidney and in distal colon, where it protects MR from glucocorticoids by converting 11β-hydroxyglucocorticoids into 11-keto-glucocorticoids. The MR has similar affinities to bind aldosterone and cortisol and circulating concentrations of the latter are 100–1000 times higher, thus, the close proximity of 11β-HSD2 to the receptor allows aldosterone to bind. 11β-HSD2 is also expressed in the syncytiotrophoblast layer of the human placenta, where it protects the fetus from high maternal glucocorticoid concentrations.

The tissue-dependent responses upon activation of GR and MR strongly depend on the presence of coactivator and corepressor proteins that interact with the receptor complex, as well as on post-translational modifications of both receptor and associated proteins. Finally, enzymes responsible for the degradation and excretion of the steroid hormones are important to terminate hormone action.

The proteins described above all recognize corticosteroid molecules despite the fact that they belong to different classes of proteins and share very low sequence similarity. This suggests that the binding pockets of these proteins share structural similarity and that the chemicals mimicking corticosteroid molecules might interact with more than one of these proteins. Thus, to assess the potential disruption of corticosteroid hormone action by exogenous chemicals, suitable bioassays are required to measure the activities of the different proteins, with roles in regulation, biosynthesis, transport, intracellular metabolism, receptor and degradation.

3. Bioassays

Compared to the extensively studied field of estrogen- and androgen-like actions by environmentally relevant chemicals, there are only few studies focusing on corticosteroid hormones. As a starting point to identify chemicals that act on different steps of glucocorticoid and mineralocorticoid regulation, we established assays to measure the function of human corticosteroid hormone receptors (GR and MR) and glucocorticoid metabolizing enzymes (11β-HSD1 and 11β-HSD2). To distinguish between different steps of receptor activation, the HEK-293 cell line was selected that is devoid of endogenous expression of corticosteroid receptors and hormone metabolizing enzymes. Other cells that are suitable alternatives include COS-1 and CV-1 cells, but they are not of human origin.

Receptor activity was measured in intact cells transiently expressing recombinant receptor or a green-fluorescence (GFP)-chimeric receptor. This allowed the assessment of the effect of a given chemical on ligand binding to the receptor, subsequent translocation of the receptor into the nucleus and receptor-mediated transcriptional activation of a reporter gene. It is important to distinguish the different steps of receptor activation to understand the inhibitory mechanism of a chemical. Spironolactone, as an example, efficiently binds to MR and induces translocation of the receptor into the nucleus. It acts as a partial agonist and therefore antagonizes the potent agonist effect of aldosterone. The bile acid chenodeoxycholic acid inhibits 11β-HSD2 and leads to glucocorticoid-induced activation of MR. Although it also induces nuclear translocation of MR in the absence of 11β-HSD2, chenodeoxycholic acid neither activates nor antagonizes MR activation by cortisol or aldosterone.

To assess the effect of chemicals on 11β-HSD prereceptor enzyme function, the conversion of cortisone to cortisol or the reverse reaction was measured in lysates or intact HEK-293 cells stably expressing C-
and it is more likely that other, as yet un-
identified, compounds are responsible for
inhibition of 11β-HSD2. Additional com-
ponents of the triterpenoid and flavonoid
class of chemicals that inhibited 11β-HSD2
include abietic acid, gossypol, magnolol and
tea polyphenols. However, their rela-
tively weak activities observed in intact
cell assays suggest that these compounds
are unlikely to cause physiological conse-
quences by inhibiting 11β-HSD2.\[33\]

We then tested about 100 environmen-
tally relevant chemicals that were selected
based on evidence in the literature for po-
tential interference with corticosteroid ac-
tion, for their effect on 11β-HSD2 activity.
In addition to the compounds mentioned
above, abietic acid, fusicidic acid, zearela-
none, 4-tert-octylphenol, 4-nonylphenol,
bispHENol A, endosulfan and several di-
thiocarbamates and organotins inhibited
11β-HSD2.\[34\] Among these, abietic acid,
bispHENol A, dithiocarbamates and organo-
tins inhibited 11β-HSD2 at subcytotoxic
concentrations. Dithiocarbamates and or-
ganotins were studied in more detail.

Dithiocarbamate chemicals are widely
used in agriculture as pesticides or fungi-
cides and as vulcanization accelerators in
latex production (mainly in gloves). Dithio-
carbamates are considered to be responsi-
ble for the allergic reactions against rubber
products.\[34\] Interestingly, disulfiram, known
as Antabus to treat alcoholic patients,
was also active. Among the dithiocarbam-
ates analyzed, disulfiram and thiram were
most potent with an IC\(_{50}\) value of 130 nM,
followed by maneb, diethylthiocarbamate
and zineb.\[35\] The inhibitory potential of
disulfiram and thiram to inhibit 11β-HSD2
was comparable with that for aldehyde de-
hydrogenase. These chemicals irreversibly
inhibited 11β-HSD2, probably by covalent
carbamoylation of catalytically important
cysteine residues. Glutathione protected
11β-HSD2 from inhibition by dithiocarba-
mates, suggesting that these chemicals
are most critical in situations of oxidative
stress, when intracellular glutathione con-
centrations are low. The exposure to the
dithiocarbamates maneb and zineb has been
associated with acute renal failure and
nephrotic syndrome in agricultural work-
ers, as well as kidney damage and reduced
body weights in the offspring from exposed
pregnant rats. The inhibition of 11β-HSD2
may contribute to some of the observed
toxic effects of these chemicals in kidney
and on blood pressure, as well as in pla-
centa and on fetal development.

We identified several organotins includ-
ing trialkyls and dialkyls that potently
inhibited 11β-HSD2 but not 11β-HSD1,
17β-HSD1 or 17β-HSD2.\[36\] They re-
versibly inhibited the enzyme with comparable
potencies in assays with cell lysates or in-
tact cells. Analysis of the inhibitory mech-
anism suggested that organotins interfere
with 11β-HSD2 function by modification of
cysteine residues. Dithiothreitol, but not
glutathione, protected from organotin-
dependent inhibition. Enhanced glucocor-
ticoid concentrations, due to disruption
of 11β-HSD2 function, may contribute to the
observed organotin-dependent toxicity in
some glucocorticoid-sensitive tissues such
as thymus and placenta. Reduced birth
weight and thymus involution, observed
upon exposure to organotins, can also be
carried by excessive glucocorticoid levels.

5. Chemicals Disrupting MR
Activation

The same set of about 100 chemicals
was screened using the MR nuclear trans-
location assay to test them for agonist or an-
tagonist properties. Aldosterone-mediated
nuclear translocation of MR was inhibited
by various chemicals with different prop-
erties, including bisphenol A, endosulfan,
4-nonylphenol, vinclozolin, zearelanone,
and some phthalate derivatives. All of these
chemicals inhibited nuclear translocation of
MR at high concentrations of 20–50 \(\mu\)M
that are unlikely to be physiologically rel-
vant. The screening of this small number of
chemicals showed that chemicals interfer-
ing with MR activation exist, although
none of the identified compounds was of
high potency. It cannot be excluded at this
point that some of the identified chemicals
have more pronounced effects under certain
conditions such as oxidative stress or upon
preincubation for a prolonged time. Clearly,
a more extensive screening will be neces-
sary for the identification of chemicals with
physiologically relevant effects.

The contribution of dithiocarbamate-
dependent 11β-HSD2 inhibition to cor-
tisol-induced activation of corticosteroid
receptors is unclear, since we found that
high concentrations of these chemicals also
could block MR and GR. This is in line with
a recent study by Garbrecht et al., who de-
derived thiram-dependent inhibition of GR
activation.\[37\] An inhibition of 11β-HSD2
and/or MR and GR is only likely to occur
under conditions of glutathione depletion,
and further studies are required to see which
protein is inhibited at the lowest concen-
trations.

6. Compounds Inhibiting 11β-HSD1

Inhibition of 11β-HSD1-dependent
glucocorticoid activation is currently con-
sidered as a promising strategy to treat pa-
tients with metabolic syndrome\[5\] and
many medium and large pharmaceutical com-
panies have inhibitor development programs.
While preventing excess production of ac-
tive glucocorticoids is important to avoid
adverse metabolic effects and inhibition of 11β-HSD1 is beneficial in such situations, glucocorticoid deficiency has been associated with impaired immune responses in inflammatory reactions and a reduced 11β-HSD1 expression has been found in some forms of cancer. Thus, depending on the tissue and on the metabolic state, inhibition of 11β-HSD1 may have different consequences.

Several compounds inhibiting 11β-HSD1 were identified upon screening our small library. Zearalenone, 4-tert-octylphenol, 4-nonylphenol, methyljasmonate, dibenzoylmethane and 2,2'-dihydroxybiphenyl were weak inhibitors, unlikely to be of physiological relevance. Abietic acid and flavanone as well as some monohydroxylated flavanone derivatives were more potent. While abietic acid displayed a similar activity as the one observed with glycyrrhetinic acid and carbenoxolone and inhibited both 11β-HSD1 and 11β-HSD2, flavanone and its derivatives selectively inhibited 11β-HSD1 by competing with substrate binding. Analysis of the structural requirements for the inhibitory effect of flavanones revealed that hydroxylation at position 6 leads to reduced potency. Multiple hydroxylated flavanones such as the trihydroxylated naringenin also showed weak inhibitory activities on 11β-HSD enzymes. In addition, the inhibitory effect was lost in flavones, which have a double bond between atoms C2 and C3. In a recent study, we provided evidence that coffee contains compounds inhibiting 11β-HSD1, which might explain in part the anti-diabetic effect associated with regular coffee consumption. Thus, some natural compounds may contribute to the positive health effects of fruits and vegetables by inhibiting 11β-HSD1 and they might be used as food supplements.

11β-HSD1 is a relatively unspecific enzyme and accepts several substrates. Several reports suggested a role for 11β-HSD1 in phase I detoxification of carboxyl group containing xenobiotics. In intact cells, 11β-HSD1 catalyzed exclusively the conversion of 7-ketocholesterol to 7β-hydroxycholesterol. 7-Ketcholesterol is a major oxidized cholesterol metabolite and is formed mainly upon processing cholesterol-rich food. It is found at micromolar concentrations in cataract lenses and in atherosclerotic plaques. The role of 11β-HSD1 in these pathological conditions and the impact of its inhibition remains to be investigated. Nevertheless, an accumulation of orally administered 7-ketocholesterol was observed in rats treated with the inhibitor carbenoxolone.

More recently, we found that 11β-HSD1 metabolizes 7-ketodehydroepiandrosterone and 7-ketopregnenolone, suggesting a role not only in the detoxification of oxidized cholesterol from food but also of oxidized steroid hormone metabolites.

7. Interference of Chemicals with GR Function

The same set of about 100 chemicals was screened using our nuclear GR translocation assay. Bisphenol A, zearalenone and some phthalate derivatives inhibited cortisol-induced nuclear translocation of GR. These chemicals also inhibited nuclear MR translocation. Bisphenol A and zearalenone inhibited the function of all four proteins tested, suggesting either an unspecific mechanism or that these chemicals mimic the steroid hormone cortisol and are therefore recognized by all four proteins. In addition, dibenzoylmethane and abietic acid at concentrations of 20–50 μM were shown to inhibit GR translocation. Due to the high concentrations required for inhibition, a direct effect on GR was considered not physiologically relevant. In these experiments, effects on transcriptional expression or on protein stability after prolonged incubation with the chemical of interest have not been assessed.

In a recent study, we found that the organotin dibutyltin inhibits the activation of GR at submicromolar concentrations (unpublished observations). An inhibition of GR function by dibutyltin might explain some of the immunotoxic effects and effects on energy metabolism of this chemical, with relevance to chronic inflammatory disorders as well as metabolic diseases.

8. Outlook

Screening of a very small library of environmentally relevant chemicals with cell-based assays for GR, MR, 11β-HSD1 and 11β-HSD2 led to the identification of several compounds that interfere with the function of these proteins, emphasizing the importance to consider potential interference of glucocorticoid and mineralocorticoid hormone action for the safety assessment of chemicals. Disruption of corticosteroid hormone action by xenobiotics might contribute to metabolic and cardiovascular diseases, impaired brain function, developmental disorders, immune diseases and cancer. To assess the safety of chemicals and avoid interferences with corticosteroid hormone action, the development of additional biological in vitro and in vivo tests is required for the detection of disturbances at various levels of hormone action, including HPA axis regulation, steroiogenesis, transport protein activity, activities of metabolizing enzymes and receptor function. The development of suitable in silico prediction tools and systemic approaches, including analyses of effects of xenobiotics on the transcriptome and proteome, is necessary for the identification of chemicals disrupting corticosteroid hormone action and to understand their mechanisms of action.

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