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Review Article

Recent progress in 11-β-hydroxysteroid dehydrogenase type 1 (11-β-HSD1) inhibitor development

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CONTENTS

Abstract

Glucocorticoids regulate a plethora of processes via stimulation of nuclear glucocorticoid receptors. Exaggerated effects of glucocorticoids can give rise to multiple clinical features typified by Cushing's disease, a metabolic syndrome comprising hyperglycemia, insulin-resistant diabetes, obesity, dyslipidemia and hypertension. Since the enzyme 11-β-hydroxysteroid dehydrogenase type 1 (11-β-HSD1) locally regenerates active glucocorticoids, tissue-specific inhibitors of 11-β-HSD1 have potential for ameliorating the above conditions. This review describes the recent efforts to identify potent and selective 11-β-HSD1 inhibitors, aimed at correcting abnormalities associated with the metabolic syndrome. The primary focus is on aspects of the metabolic syndrome, but it is recognized that inactivation of 11-β-HSD1 may also be beneficial in other diseases.

Introduction

Glucocorticoid hormones regulate multiple intracellular transcription and expression processes. High circulating levels of the active glucocorticoid cortisol, or hypersensitivity to cortisol, may contribute to metabolic abnormalities in tissues that are central in efforts to find pharmacological intervention points for the treatment of the metabolic syndrome. For instance, hepatic glucose production seems at least partly under the control of glucocorticoids (1, 2), and liver-specific glucocorticoid receptor antagonists effectively suppress glucose production (3). Glucocorticoids impair muscle glucose uptake and glycogen metabolism and increase lipolysis in insulin-sensitive tissues (4, 5). Recently, it was reported that glucocorticoid receptor mRNA levels in muscle highly correlate with insulin resistance and hypertension in men (6, 7). Furthermore, glucocorticoids promote differentiation of pre-adipose tissue (8) and increase triglyceride content in the liver (9). Profound effects have also been demonstrated on pancreatic islets, where the glucocorticoid dexamethasone was shown to inhibit insulin release (10). As a consequence of these additive effects, excessive glucocorticoid activity can result in insulin-resistant diabetes, central obesity, dyslipidemia and hypertension –all established clinical features of Cushing's syndrome (11).

Until recently, it was assumed that the degree of glucocorticoid action was solely determined by the circulating glucocorticoid hormones and the local glucocorticoid receptor density. However, individuals with common forms of obesity or overweight type 2 diabetics do not show abnormal plasma cortisol levels. This discrepancy may involve the prereceptor control of glucocorticoid receptor activation by an enzyme called 11-β-hydroxysteroid dehydrogenase type 1 (11-β-HSD1) (12). At the cellular level, the inactive glucocorticoid cortisone is converted by 11-β-HSD1 to the active component cortisol. Since the total circulating unbound glucocorticoid level consists largely of the precursor cortisone, and to a smaller degree cortisol, 11-β-HSD1 activity could effectively determine the local concentration of cortisol, and hence glucocorticoid receptor modulation (Fig. 1). The activity and/or expression of 11-β-HSD1 is in turn highly regulated by numerous cytokines, growth factors, hormones and steroids, a fact which contributes to a complex picture of the enzyme's role in metabolic regulation. As such, the role of 11-β-HSD1, and consequently its potential as a target in a range of disorders, has been thoroughly reviewed by others (13-15).

The possible involvement of human 11-β-HSD1 in the metabolic syndrome is supported by the high levels of expression in glucocorticoid target tissues (16, 17), including liver (18), certain adipose depots (19) and skeletal muscle (7). Also, the presence of 11-β-HSD1 in human and mouse pancreatic islets has been demon-

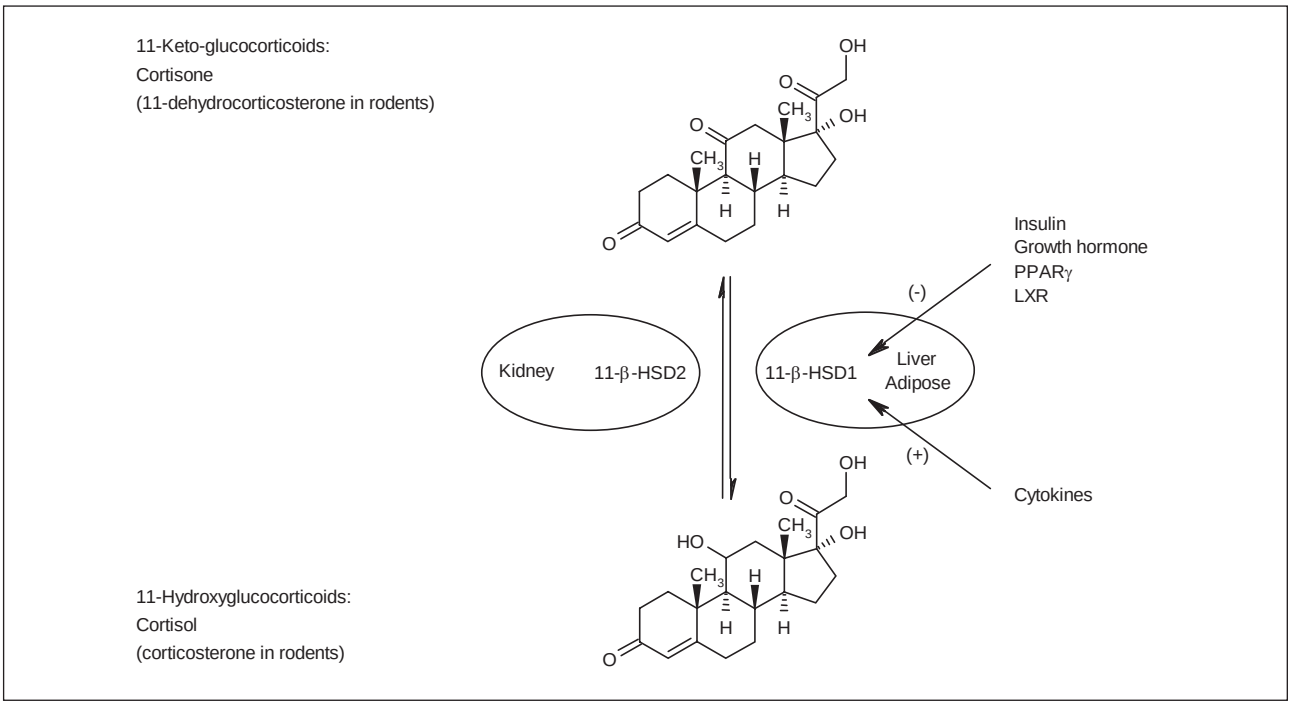

Fig. 1. Schematic representation of the cortisone/cortisol shuttle by 11-β-HSD enzymes.

strated (20). Furthermore, in various species, detectable quantities have been reported in lung, tongue, macrophages, osteoblasts and certain parts of the brain (recently reviewed in 13 and 14).

11-β-HSD1 is a microsomal enzyme and belongs to the family of short-chain dehydrogenases/reductases. The closest relative is 11-β-HSD2, albeit with only 21% sequence homology (21). The activity and direction of the HSD enzymes are determined by the availability of the cofactors NADP(H) for type 1 enzyme and NAD^+ for type 2 enzyme (22). 11-β-HSD1 is in principle bidirectional but appears to act solely as an oxoreductase in intact cells and tissues. 11-β-HSD2 acts exclusively as a high-affinity dehydrogenase, and high levels have been found in the human kidney (23). 11-β-HSD2 protects this organ from inappropriate cortisol-induced mineralocorticoid receptor activation leading to sodium retention, hypokalemia and hypertension (24). Therefore, selectivity of any potential drug candidate relative to this enzyme is important.

Genetically modified mice

A number of highly relevant findings have been reported that strongly support a prominent role for 11-β-HSD1 in obesity and the metabolic syndrome. In 1997, it was demonstrated that mice lacking the 11-β-HSD1 enzyme were developmentally normal, but responded with lower fasting blood glucose levels to stress stimuli or chronic high-fat feeding as compared to wild-type mice (25). Lower activity of the gluconeogenic liver enzymes phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase) in fasted 11-β-HSD1-deficient mice is in agreement with this observation. 11-β-HSD1-null mice fed *ad libitum* displayed lowered plasma triglyceride levels and elevated HDL levels, indicating that inactivation of 11-β-HSD1 could also have beneficial cardioprotective effects (26). Subsequently, the 11-β-HSD1-deficient phenotype was more closely tied to metabolic effects in adipose tissue, where glucocorticoid-inducible transcripts encoding for leptin, resistin and TNF-α were reduced (27). Conversely, higher peroxisome proliferator-activated receptor γ (PPARγ), adiponectin and UCP2 mRNA levels were detected. In addition, 11-β-HSD1$^{-/-}$ mouse adipocytes exhibited higher basal and insulin-stimulated glucose uptake, all suggestive of increased insulin sensitivity, and possibly an increase in energy expenditure. The fact that mice with dietary obesity showed downregulated levels of adipose 11-β-HSD1 is somewhat difficult to comprehend, but may indicate that this enzyme could be part of a regulatory mechanism to counteract the metabolic consequences of increased fat intake (28).

Important information on the relative contribution of 11-β-HSD1 activity in liver and adipose tissue emerged from transgenic mice that selectively overexpressed 11-β-HSD1 in either tissue. Elevated 11-β-HSD1 activity in the liver was associated with mild insulin resistance, and these transgenic mice exhibited fatty liver, dyslipidemia and hypertension (29). Earlier, transgenic mice that selectively overexpressed 11-β-HSD1 in adipose tissue were found to exhibit increased intra-adipose levels of corticosterone and developed visceral obesity, insulin resistance and hyperlipidemia (30). Also, these mice suffered from hypertension (31). Thus, these transgenic phe-

notypes support the notion that intratissue glucocorticoid-dependent pathways play a major role in the biology of metabolic diseases. The beneficial metabolic effects of PPARγ and liver X receptor (LXR) agonists in certain mouse models may be partly derived from their ability to reduce 11-β-HSD1 activity indirectly (32, 33).

Human 11-β-HSD1 in the metabolic syndrome

In keeping with observations in mice, the nonselective 11-β-HSD1 inhibitor carbenoxolone increased hepatic insulin sensitivity in healthy male subjects, possibly due to suppressed hepatic glucose production (34). A later study in nonobese type 2 diabetics confirmed the effects of carbenoxolone administration, with a decrease in glucagon-stimulated glucose production that was attributed to reduced glycogenolysis rather than gluconeogenesis (35). The authors suggest that inhibition of kidney 11-β-HSD2 by carbenoxolone may have increased renal gluconeogenesis due to enhanced local cortisol concentrations, and thus the need for 11-β-HSD1-selective inhibitors is evident. Also, since the observed effects of carbenoxolone appear to be restricted to the liver and kidney, additional benefits are expected with 11-β-HSD1 inhibitors that also target glucocorticoid regeneration in adipose tissue.

Indeed, hepatic 11-β-HSD1 activity appears to be impaired in obese subjects, and enhanced reactivation of cortisol in adipose tissue has been associated with increased weight (36, 37). Several other studies, with a focus on adipose tissue, point in the same direction. For instance, increased levels of 11-β-HSD1 mRNA has been detected in both the abdominal and visceral fat of obese patients (38), and adipose 11-β-HSD1 gene expression was positively correlated with waist circumference and insulin resistance (39). In another study in overweight and lean twins, correlations were found with acquired obesity and insulin resistance and overexpression of 11-β-HSD1 in subcutaneous adipose tissue (40). However, the whole concept remains controversial, since there have also been reports of unaltered depot-specific 11-β-HSD1 activities and expression in human obesity and type 2 diabetes, or even inhibition of 11-β-HSD1 with increasing body mass index (BMI) in simple obesity (41, 42).

Clearly, the exact role of 11-β-HSD1 in certain aspects of the metabolic syndrome in humans remains to be elucidated. Regional differences in adipocyte biology, specifically in processes where 11-β-HSD1 is involved, such as in differentiation and proliferation, require further investigation (43). Also, 11-β-HSD1 activity in skeletal muscle may contribute to the etiology of the metabolic syndrome. 11-β-HSD1 expression in myoblasts was positively correlated with insulin resistance and blood pressure, but only after incubation with physiological concentrations of cortisol (7). In order to fully understand the consequences of pharmacological inhibition of 11-β-HSD1, the discovery of potent and selective inhibitors is needed and a competitive area of pharmaceutical research has developed in the past decade.

Natural products and steroid-based 11-β-HSD1 inhibitors

Early efforts to identify 11-β-HSD1 inhibitors focused on synthetic steroids and natural products. Hult *et al.* expressed recombinant human 11-β-HSD1 in the yeast

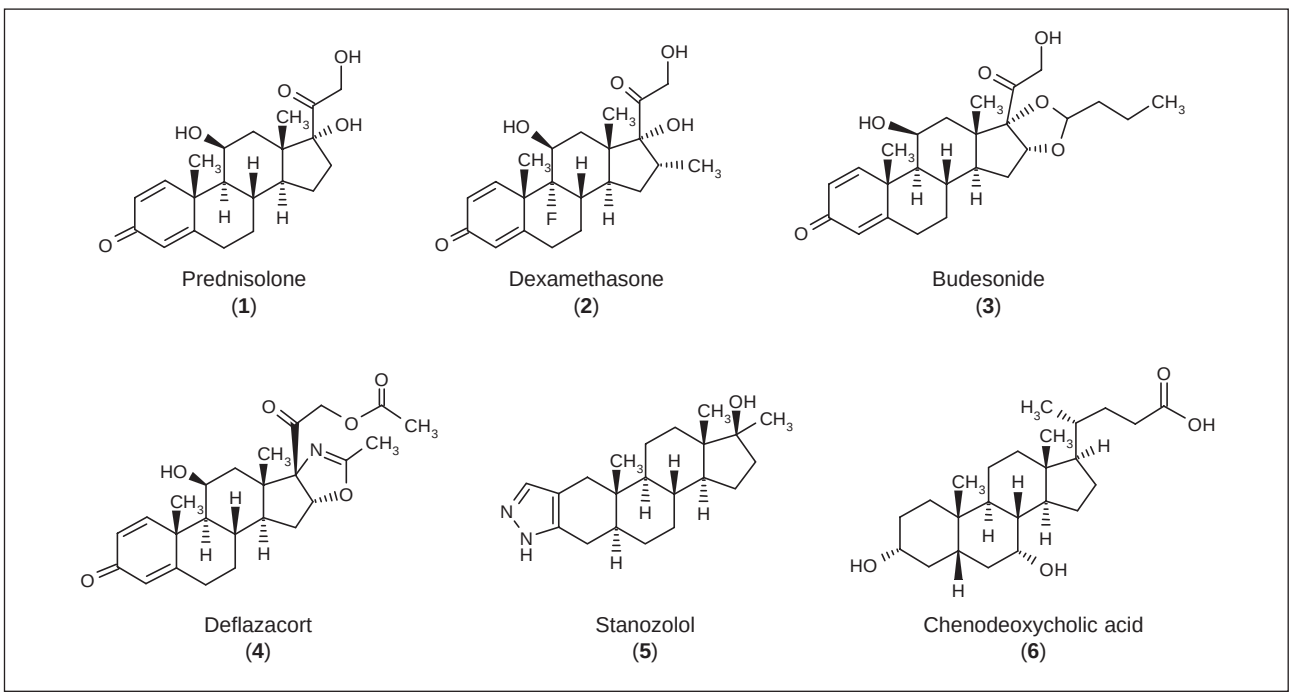

Fig. 2. Chemical structures of steroids.

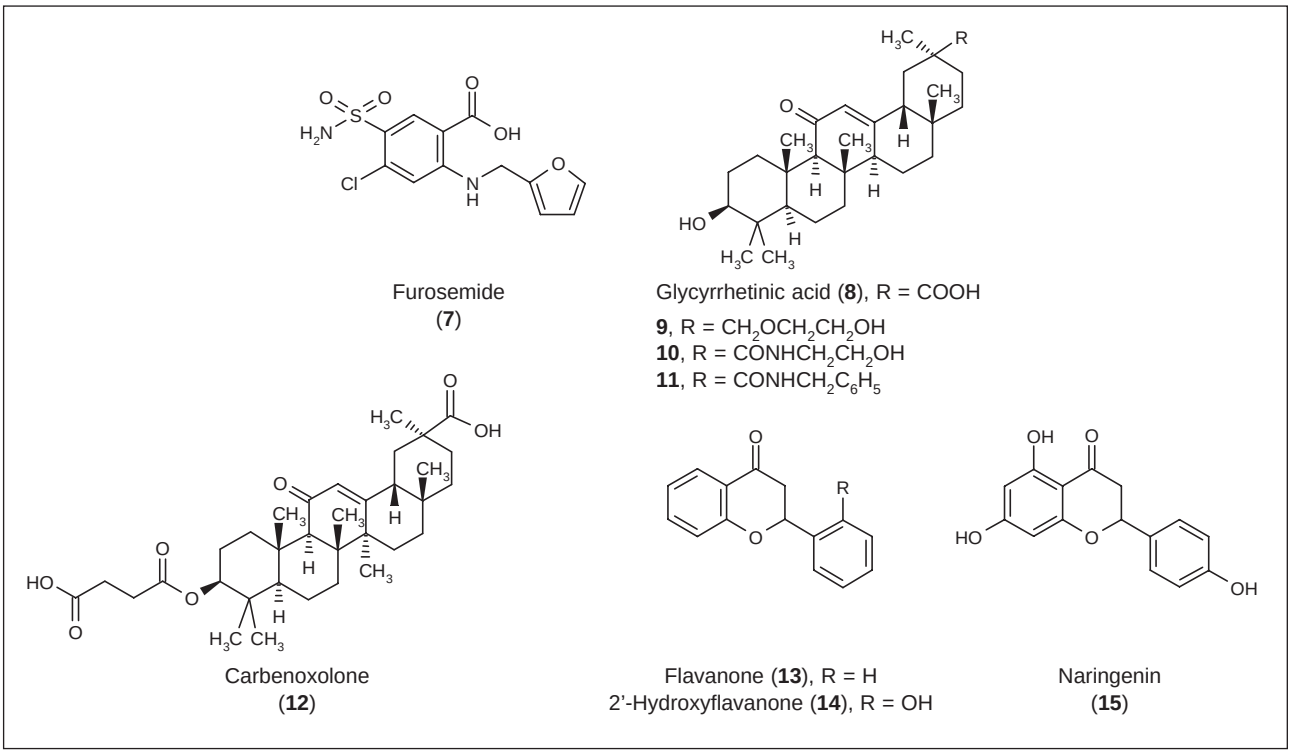

Fig. 3. Chemical structures of natural products.

P. pastoris and found that the reductase reaction dominated the dehydrogenase activity in whole cells (44). This is in line with the enzyme direction reported for intact mammalian cells (45, 46). Substrate specificities and inhibitor constants were determined for synthetic glucocorticoids and a few natural compounds. Prednisone and prednisolone (**1**) were effectively metabolized by 11-β-HSD1, while the antiinflammatory steroids dexamethasone (**2**) and budesonide (**3**) and the oxazoline derivative deflazacort (**4**) were nonsubstrates (Fig. 2). Apparently, a 9α-fluorine substitutent or the introduction of bulk on the D-ring prevents 11-β-dehydrogenation to the 11-keto derivatives. In addition to not being substrates for enzyme activity, the latter compounds were found to inhibit 11-β-HSD1 in both the reduction and oxidation directions with micromolar inhibition constants. In particular, budesonide and deflazacort were approximately 30 times more efficient in blocking the less physiologically relevant dehydrogenase activity, whereas dexamethasone and the androgen stanozolol (**5**) inhibited both directions with similar potencies. The authors also demonstrated that the nonsteroidal molecule furosemide (**7**; Fig. 3) strongly inhibited 11-β-HSD1 with a K_i value of 0.55 μM in the reduction direction. The compounds that are invariably the most potent, albeit nonselective with respect to 11-β-HSD2 are the liquorice ingredient glycyrrhetinic acid (**8**; K_i = 1.87 μM) and its hemisuccinate carbenoxolone (**12**; K_i = 17 nM) (Fig. 3). Diederich *et al.* confirmed the inhibitory potencies in human liver microsomes, and this group reported for the first time that selectivity over the type 2 enzyme could be achieved (47). Chenodeoxycholic acid (**6**; Fig. 2), a bile acid that is clinically useful in gallstone treatment, displayed an IC_{50} value of 2.8 μM using dehydrodexamethasone as the substrate, while 11-β-HSD2 inhibition was absent. Other compounds that selectively inhibit the reductase direction but are devoid of 11-β-HSD2 inhibition are certain flavonoids, such as flavanone (**13**; IC_{50} = 18 μM) and 2'-hydroxyflavanone (**14**; IC_{50} = 10 μM) (Fig. 3) (48). Yet another flavanone derivative, naringenin (**15**), was reportedly less potent (44, 48). Potter and coworkers prepared a number of glycyrrhetinic acid derivatives modified mainly at the C-30 position (**9-11**; Fig. 3), and these compounds were tested on 11-β-HSD1 and 11-β-HSD2 isolated from rat liver and kidney, respectively (49, 50). A few amide analogues such as compounds **9** and **11** displayed a slight preference for rat 11-β-HSD1, but most of the glycyrrhetinic acid analogues inhibited the type 2 enzyme more potently. For instance, compound **10** was reported to have an IC_{50} value of 4 pM against the type 2 isoform.

11-β-HSD1 inhibitors in animal models of obesity and insulin resistance

Lean and obese leptin-resistant Zucker rats administered the nonselective inhibitor glycyrrhetinic acid in the drinking water (280 mg/kg/day) over a 14-week period showed contrasting results on food intake, body weight gain and hepatic 11-β-HSD1 expression levels (51). Treated lean Zucker rats responded with suppressed food intake and weight gain, whereas obese Zucker rats did not. The basal levels of hepatic 11-β-HSD1 mRNA

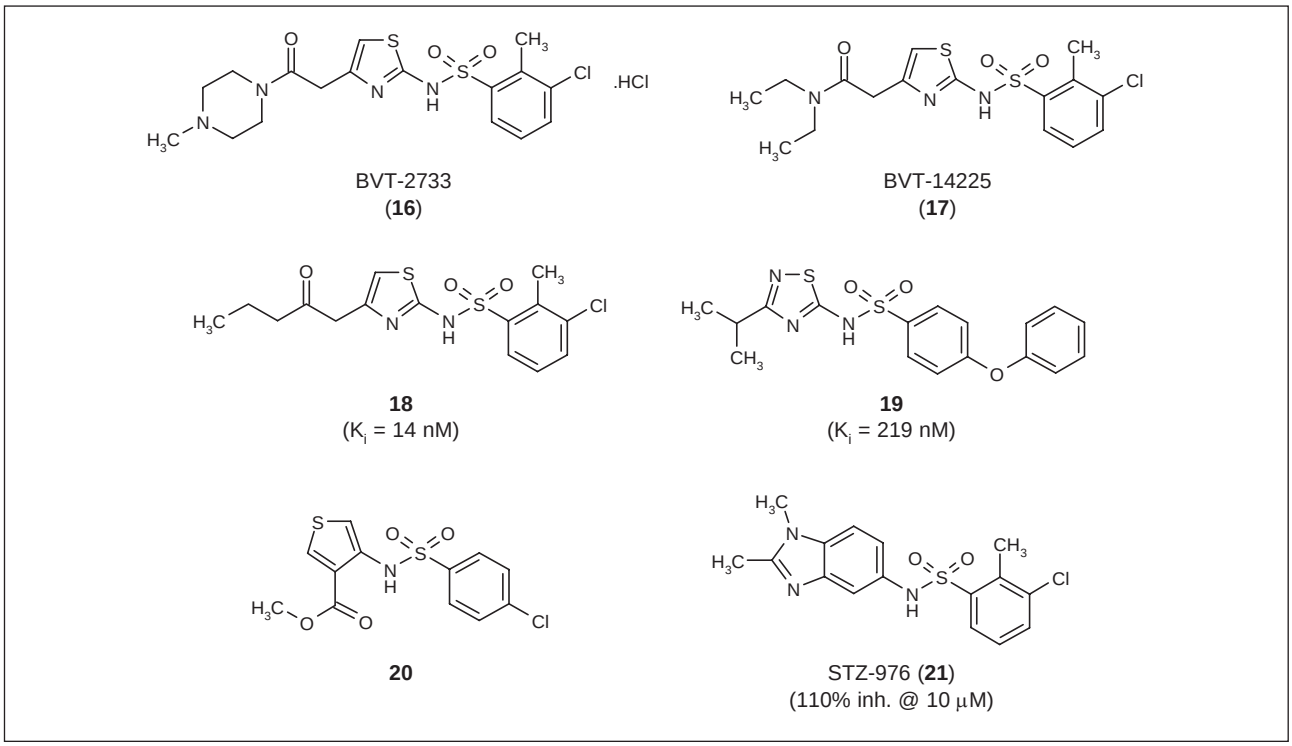

Fig. 4. Structures disclosed by Biovitrum and Sterix.

were lower in obese animals as compared to the lean rats. In contrast, the 11-β-HSD1 mRNA was lower in the latter animals following glycyrrhetinic acid treatment, but unaltered in the former. The authors suggested that drug exposure in obese Zucker rats may have been inadequate or that a weight-lowering effect of the 11-β-HSD inhibitor requires intact leptin receptors. Earlier, Livingstone and coworkers demonstrated that hepatic 11-β-HSD1 activity was attenuated in obese Zucker rats, but that the activity was higher in omental fat, indicative of tissue-specific differences in glucocorticoid metabolism (52). Estradiol administration to gonadectomized rats completely suppressed liver 11-β-HSD1 activity, causing a marked fall in PEPCK mRNA (53). Although estradiol inhibits 11-β-HSD1 via an unknown mechanism, this again shows the involvement of 11-β-HSD1 in the gluconeogenic pathway.

Recently, our group disclosed arylsulfonamidothiazole derivatives as highly selective inhibitors of 11-β-HSD1 (54). Similar to in the 11-β-HSD1-deficient mice, the murine 11-β-HSD1 inhibitor BVT-2733 (**16**; Fig. 4) (IC_{50} = 96 nM) attenuated blood glucose and insulin levels in hyperglycemic KKA^y mice following continuous infusion via osmotic minipumps (167 mg/kg/day) for 7 days (55). The levels of hepatic mRNA encoding for the gluconeogenic enzymes PEPCK and G6Pase were reduced in the same experiment. Oral administration of a single bolus of BVT-2733 (100 mg/kg) resulted in inhibition of hepatic 11-β-HSD1. In an extended study, BVT-2733 (200 mg/kg b.i.d. p.o.) was also shown to reduce circulating glucose and insulin levels in *ob/ob* and *db/db* mice (56). In *ob/ob* and KKA^y mice, BVT-2733 treatment improved whole-body glucose tolerance and increased insulin sensitivity, and in KKA^y mice, cholesterol, triglyceride and FFA levels were reduced following a 4-h fast.

Merck researchers also reported at a recent conference that pharmacological inhibition of 11-β-HSD1 improves the overall metabolic state in mice. The disclosed compound (**22**; Fig. 5) used in the *in vivo* studies has an IC_{50} value of 7.5 nM against human 11-β-HSD1 and of 97 nM against mouse 11-β-HSD1, and is extremely selective over 11-β-HSD2, with respective IC_{50} values of > 3300 nM and > 10,000 nM against the human and mouse orthologs (57).

Oral administration of **22** produced a 7% body weight loss in diet-induced obese (DIO) mice after 10 days of dosing (20 mg/kg b.i.d.) (58). Insulin levels, as well as fasting glucose, were lowered in these animals. In streptozotocin (STZ)-treated mice on a high-fat diet, the same compound (30 mg/kg b.i.d. p.o.) markedly lowered fasting glucose and decreased serum glucose excursions in GTT experiments after 9 days of treatment. A reversal of triglyceride, serum leptin and glucagon levels was demonstrated in these mice. Furthermore, pharmacodynamic analysis upon oral dosing showed a reduction in cortisol levels, indicating successful inhibition of the target enzyme.

A group at Johnson & Johnson (Janssen) demonstrated proof of principle with a potent mouse 11-β-HSD1 inhibitor that most probably belongs to the class of adamantylacetamides (*e.g.*, **28**; Fig. 6). Corticosterone

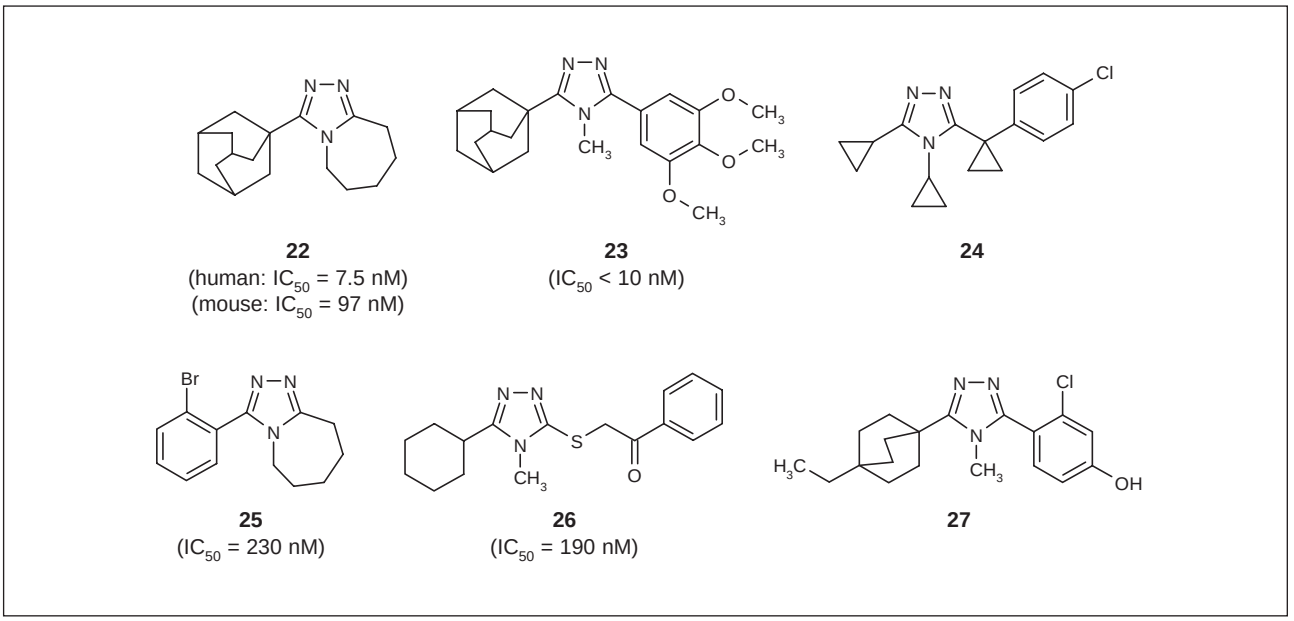

Fig. 5. Structures disclosed by Merck and Novo Nordisk.

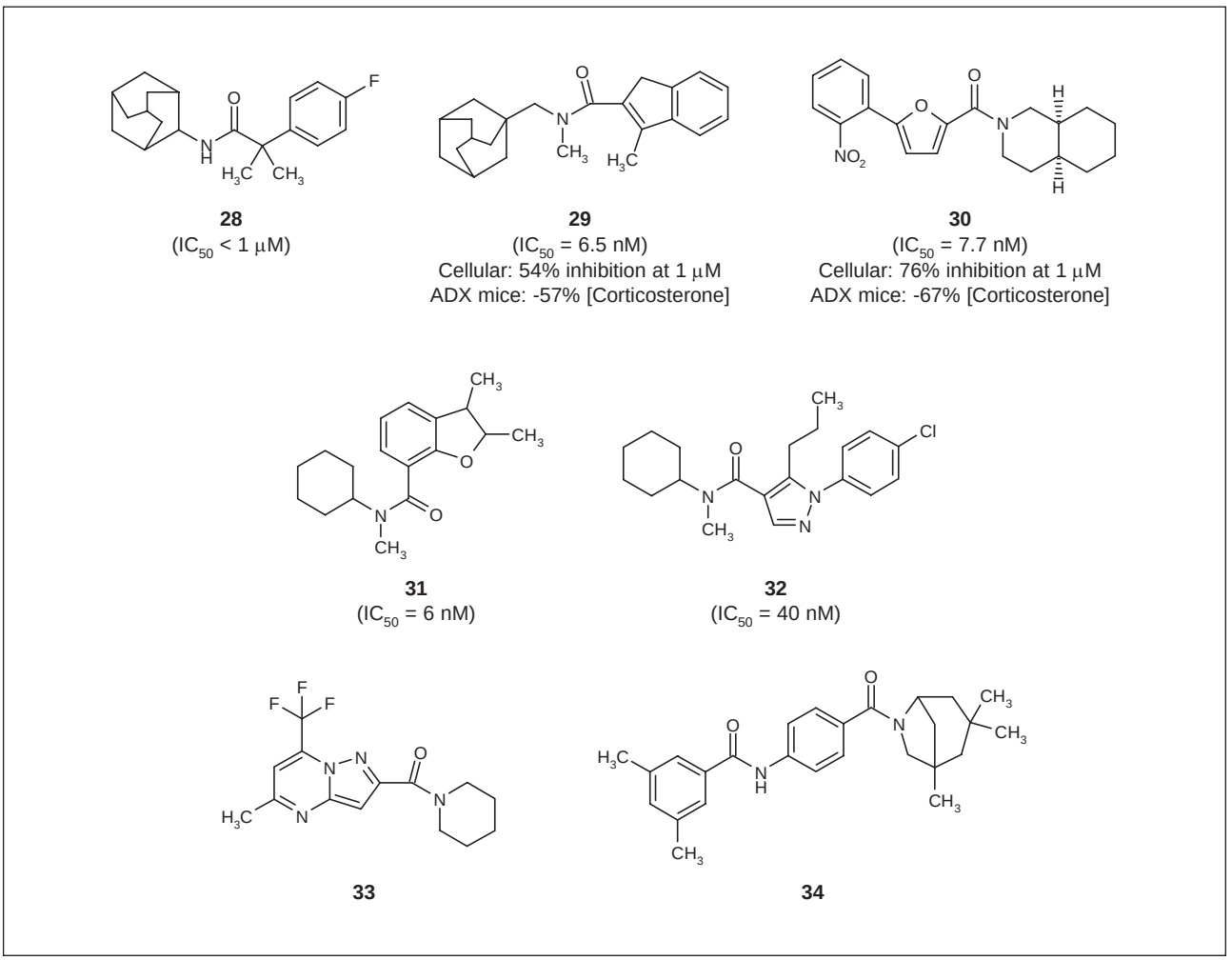

Fig. 6. Structures disclosed by Janssen, Novartis and Novo Nordisk.

formation in the liver and fat was dose-dependently reduced when the compound (pIC_{50} = 8.6) was administered mixed with food (0.075-0.5%; 0.15% equivalent to 125 mg/kg/day) to normal mice (59). Blood glucose levels were attenuated in low-fat diet *db/db* mice and high-fat diet KKA^y mice treated with 0.3% compound in food for 4 weeks, and the latter mice appeared to have reduced fat mass. One month of oral treatment of DIO mice in the food (0.3 and 1% compound) resulted in reduced weight as compared to controls, and this was attributable to lower subcutaneous and epididymal fat pad weights. The fasting blood glucose concentrations were decreased as a consequence of significantly lowered PEPCK and G6Pase gene expression.

The collective results from these research groups show that, independent of chemical class, several of the metabolic abnormalities in mouse models of obesity and diabetes can be reversed by selective 11-β-HSD1 inhibition. Pharmacological inhibition of mouse 11-β-HSD1 has beneficial effects on liver and fat metabolism, and as such can reduce hyperglycemia, insulin resistance, hypertriglyceridemia, body weight and fat mass.

Patent applications

Recent months have seen a flurry of activity with patent applications from a number of companies being made public. This section draws together some of the data and structures presented in these applications. The reader should be aware that direct comparison of results of selected examples evaluated under diverse assay conditions could be misleading.

AstraZeneca

Figure 7 shows the general structure (**35**) around which AstraZeneca's patent application is based, along with some representative examples (**36-38**). A separate application more specifically claims 2-oxoethanesulfonamide analogues such as compound **39**. An additional application covers structures around 1,4-disubstituted piperidines in which the side-chain is linked to the 4-position of the piperidine by a ketone (**40**). The IC_{50} values quoted are derived from a cell-based assay using a β-galactosidase reporter gene as a measure of glucocorticoid receptor activation, and consequently cortisol concentration (60-62).

Biovitrum

The first report of promising biological effects of selective small-molecule inhibitors of 11-β-HSD1 originated from the laboratories of Biovitrum in 2002 (54) following the publication of a series of patent applications. These detail arylsulfonamides of a range of aminoheterocycles, notably thiazoles (**16-18**), thiadiazoles (**19**) and thiophenes (**20**), as potent and selective inhibitors of 11-β-HSD1 (Fig. 4) (63-71).

Hanauske-Abel

Hanauske-Abel has a patent application that concerns the use of small-molecule mimics of corticosteroids as potential inhibitors of 11-β-HSD (Fig. 7). The invention claims the use of an alcohol such as neomenthol (**41**) as a cortisol mimetic to be used to modulate 11-β-HSD1 activity. Indeed, neomenthol has been shown to inhibit 11-β-HSD isolated from rat liver with a K_i value of 35 μM. Conversely, menthone (**42**) is claimed as an inhibitor of the type 2 isoform of 11-β-HSD. Neomenthol was used as a lead to computationally analyze a number of other structures, such as **43**, which incorporate the physicochemical and steric elements required for 11-β-HSD1 inhibition (72).

Janssen

Adamantylacetamides are claimed as selective 11-β-HSD1 inhibitors by Janssen. Compound **28** (Fig. 6) serves as a representative of the examples presented. Enzyme activity was determined *in vitro* in both a noncellular assay, as well as two cell-based environments (differentiated mouse 3T3-L1 cells and rat hepatocytes) (73, 74).

Merck

Merck has applied for a number of patents covering 11-β-HSD1 inhibitors based around a central 1,2,4-triazole ring system exemplified by **22-24** (Fig. 5). Members of this class are claimed to be potent 11-β-HSD1 inhibitors, as well as being selective over the type 2 isoform. Inhibitory activity was assessed by determining the conversion of tritiated cortisone to cortisol. This was carried out by means of a scintillation proximity assay (SPA) *in vitro*, as well as *in vivo* in normal mice after oral dosing of the inhibitor. Certain compounds were further tested for efficacy in *db/db* mice. Merck has also extended its intellectual property to include members of the triazole class in an application for the treatment of obesity using cannabinoid CB_1 antagonists and 11-β-HSD1 inhibitors. Preferred compounds should inhibit the action of both biological targets, but no examples of such dual activity are presented (75-79).

Novartis

A broad range of substituted amides also form the basis of a patent application by Novartis. As we have seen in previously discussed patents, the examples are again characterized by the inclusion of large saturated carbocyles as nitrogen substituents on the central amide group (*e.g.*, **29** and **30**; Fig. 6). A range of pharmacological assays were used to assess the activity of these inhibitors. Determination of the inhibition of cellular 11-β-HSD1 was carried out in primary rat hepatocytes, whilst *in vivo* activity was expressed in terms of changes in

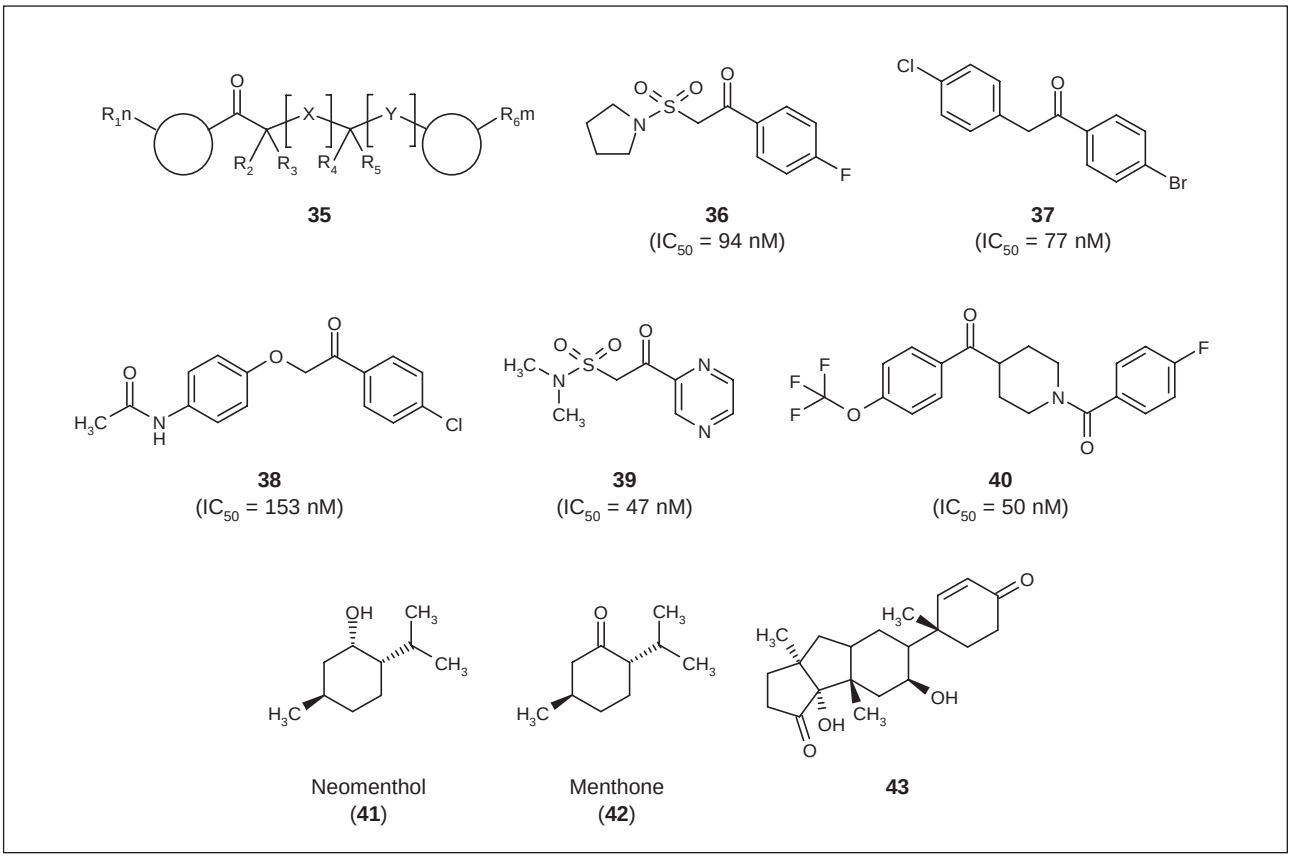

Fig. 7. Structures disclosed by AstraZeneca and Hanauske-Abel.

hepatic corticosterone concentrations in adrenalectomized (ADX) mice after oral administration of inhibitor (80).

Novo Nordisk

Substituted 1,2,4-triazoles also form the basis of two patent applications from Novo Nordisk. In the first, fused triazoles are claimed (Fig. 5). The examples presented uniformly incorporate a saturated or aromatic carbocycle fused to the triazole and a substituted aryl or heteroaryl group, *e.g.,* **25** (IC_{50} values were measured by means of an SPA assay using radiolabeled cortisone/cortisol). The second application covers triazoles, such as **26**, which contain ether or thioether substituents (81-85).

Novo Nordisk has also filed three applications which can be loosely classified as being based around a central arylamide unit, with WO 04089471 (82) more specifically dealing with pyrazolo[1,5-*a*]pyrimidines (*e.g.,* **33** in Figure 6) within this broader structural class. Examples commonly feature tertiary amides in which the nitrogen is substituted with lipophilic saturated alkyl groups, while the carbonyl is generally bonded to an aryl or, more frequently, a nitrogen-containing heteroaryl ring system. Figure 6 also depicts a number of examples from these applications (**31-34**). IC_{50} values were again determined using the previously mentioned SPA assay.

Sterix

Sterix has claimed arylsulfonamides as inhibitors of 11-β-HSD1 that are structurally reminiscent of Biovitrum compounds. They were identified using human liver microsomes. Inhibition was determined using a radioimmunoassay. Alternatively, the formation of [^{3}H]-cortisol was monitored via thin-layer chromatography, followed by radioactivity analysis of the eluted product in a scintillation counter. An exemplary compound is STZ-976 (**21**; Fig. 4), with 110% inhibition at 10 μM against human 11-β-HSD1 (86, 87).

The above summary hopefully illustrates that the community's understanding of 11-β-HSD inhibition is a rapidly evolving area, with nearly all of the patent information having been made public within the past year.

Crystal structures

The family of short-chain dehydrogenases/reductases exhibit a relatively low level of residue homology. Although, the cofactor binding fold is conserved across the family, the topology of the active site differs markedly and this fact is mirrored by the widely varying substrate specificities displayed by members (88-90).

The elucidation of the 3-dimensional structure of 11-β-HSD1 has proved a challenging goal, primarily since it is

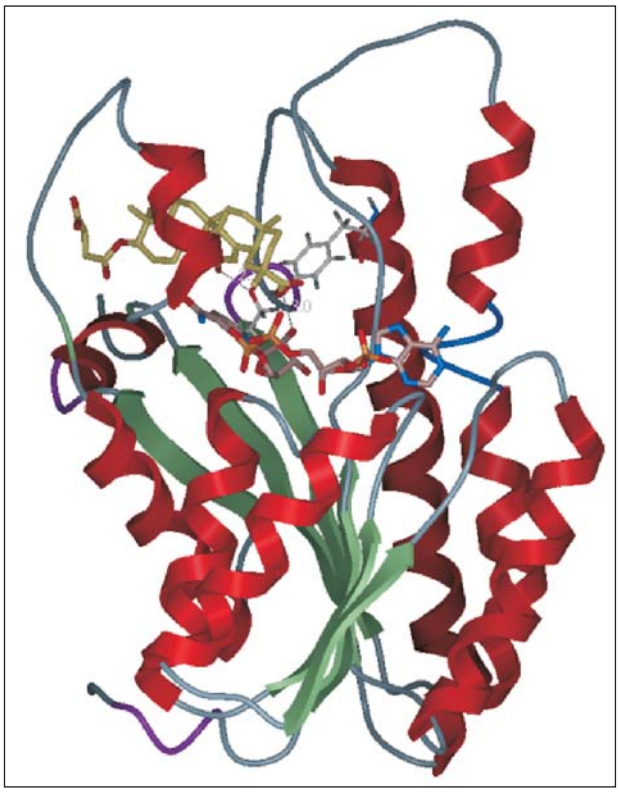

Fig. 8. High-resolution structure of human 11-β-HSD1 in complex with $NADP^+$ and the tight-binding inhibitor carbenoxolone. (Printed with permission from Ref. 94).

a highly hydrophobic glycosylated enzyme which is bound to the membrane of the endoplasmic reticulum. However, progress in this area is now promising to deliver a powerful tool in the search for new inhibitors.

Ogg *et al.* have shown that deletion of the transmembrane domain of guinea pig 11-β-HSD1 resulted in a soluble enzyme which yielded dimeric crystals (91). Interestingly, the dimerization interface extends to the active-site architecture, with the catalytic pocket of each subunit being capped by the *C*-terminus of its dimeric partner. Active-site topography reflects the enzyme's mechanism of action. Reduction proceeds via hydride transfer from the coenzyme. Tyrosine183, with the aid of a proximal lysine187, acts as an acid-base catalyst, while serine170 anchors and stabilizes the substrate carbonyl (92). The enzyme structure's predicted orientation with respect to the membrane surface has led the group to conclude that lipophilic substrates such as cortisol access the catalytic site by way of the lipid membrane.

Hosfield *et al.* recently published a structure of human 11-β-HSD1 bound to the steroid detergent CHAPS, which shows the enzyme adopting a tetrameric complex (93). Oligomerization seems to be modulated by the *C*-terminus of the enzyme, and more specifically through interdimer disulfides formed by cysteine272. The group goes on to speculate that the oxidation state of the 11-β-HSD1 disulfides may play a crucial role in complex formation and consequently enzyme activity.

Structural Genomics has also elucidated the structure of the human enzyme complexed with $NADP^+$ and carbenoxolone (Fig. 8) (94).

These new insights into the structure of 11-β-HSD1 are bound to drive the development of new selective inhibitors as we better understand not only active-site binding, but also the mechanism of substrate capture.

Summary and future directions

Essentially all the tools for the successful development of 11-β-HSD1 inhibitors are available. Published data provide evidence that pharmacological inhibition of intracellular glucocorticoid activation could ameliorate multiple aspects of the metabolic syndrome, including hyperglycemia, insulin resistance, body weight and dyslipidemia. However, is 11-β-HSD1 inhibition the ultimate way to achieve this? A number of hurdles remain and complicate the road to the successful development of 11-β-HSD1 inhibitors. Firstly, cortisol synthesis in the adrenals is regulated by the hypothalamic-pituitary-adrenal (HPA) axis. In addition to endogenous cortisol levels, 11-β-HSD1 expression in the brain regulates the local availability of cortisol, and hence glucocorticoid receptor activation in the hypothalamus and the pituitary gland, which is involved in negative feedback regulation (95). Whole-body enzyme inhibition could therefore result in a compensatory increase in adrenal function and associated pathophysiological consequences (96). For this reason, inhibitors that do not penetrate the blood-brain barrier are probably preferred.

Secondly, two independent laboratories reported recently that, apart from the conversion of glucocorticoids, 11-β-HSD1 also mediates oxysterol metabolism. 7-Ketocholesterol is efficiently transformed to 7β-hydroxycholesterol by human, mouse and rat 11-β-HSD1 *in vitro*, and this process can be blocked by carbenoxolone or the specific human 11-β-HSD1 inhibitor BVT-24829 (97). Liver 11-β-HSD1 was shown to predominantly catalyze the reduction of 7-ketocholesterol, at least in intact rat hepatocytes, hamster liver tissue slices and *in vivo* in rats (98). Since oxysterols have been implicated in the progression of atherosclerotic disease, liver and macrophage 11-β-HSD1 could have a protective role in the detoxification of dietary 7-ketocholesterol. In the various stages of atherosclerotic lesion formation, the reaction direction of the enzyme might be either reductive or oxidative, but it is clear that further studies are required to elucidate the consequences of 11-β-HSD1 inhibition in intact macrophage cell lines. Collectively, these preliminary data suggest that inhibitors of 11-β-HSD1 should be targeted at adipose tissue.

Lastly, a general problem that every research group has to overcome is the level of sequence homology between species. Although relatively high, a few amino acid differences in the catalytic site have rendered inhibitors that can be active for human 11-β-HSD1 but completely inactive at rodent 11-β-HSD1, or *vice versa*. This automatically narrows down the number of useful

pharmacological tools at hand, and hampers the extrapolation to potential clinical candidates. In spite of this, the efforts in this competitive field have nearly come to fruition, and the first 11-β-HSD1 inhibitors have already entered clinical trials.

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