Part One Steroid Hormones

1
Glucocorticoids and Mineralocorticoids

Eilidh Craigie, John J. Mullins, and Matthew A. Bailey

Glucocorticoids and mineralocorticoids are members of the corticosteroid hormone family, synthesized in the adrenal gland from the precursor sterol cholesterol via the intermediate pregnenolone (Figure 1.1). The principal glucocorticoid in humans is cortisol (in rodents corticosterone) and the principal mineralocorticoid is aldosterone. Sharing a common synthesis pathway, cortisol and aldosterone are structurally similar (Figure 1.1), and exhibit a degree of cross-receptor affinity and function. Nevertheless, small differences in structure permit important differences in physiological function. Aldosterone classically acts via the mineralocorticoid receptor (MR) to promote sodium transport in the kidney and gut, thereby regulating long-term electrolyte homeostasis and blood pressure control. Cortisol, by comparison, exhibits a wide range of metabolic and stress-related response effects.

1.1
Synthesis of the Corticosteroids

Steroid synthesis occurs principally in the adrenal gland but also occurs in the steroidogenic cells of the testes, ovary, placenta and brain. The intramitochondrial delivery of cholesterol is the rate-limiting step for steroid synthesis and is mediated by steroidogenic acute regulatory protein (StAR) [1]). Defects in cholesterol transport associated with mutations in StAR [2] cause the autosomal recessive disorder of lipoid congenital adrenal hyperplasia (CAH; Online Mendelian Inheritance in Man (OMIM) #201710). This rare condition presents with large adrenal glands containing high levels of cholesterol. Lipoid CAH is lethal within a few days without hormone replacement therapy. Over 30 mutations in StAR have been reported to cause lipoid CAH, all of which result in varying degrees of defective cholesterol transport (for review, see [3]). Mice null for StAR, generated by homologous recombination, emphasize the key role of this protein. Homozygous null pups fail to thrive and die within a week of birth: corticosterone and aldosterone levels are very low despite elevated ACTH (adrenal corticotropic hormone) and CRH (corticotropin-releasing hormone) [4]. Lipoid CAH can also arise from

Cardiovascular Hormone Systems. Edited by Michael Bader

ISBN: 978-3-527-31920-6

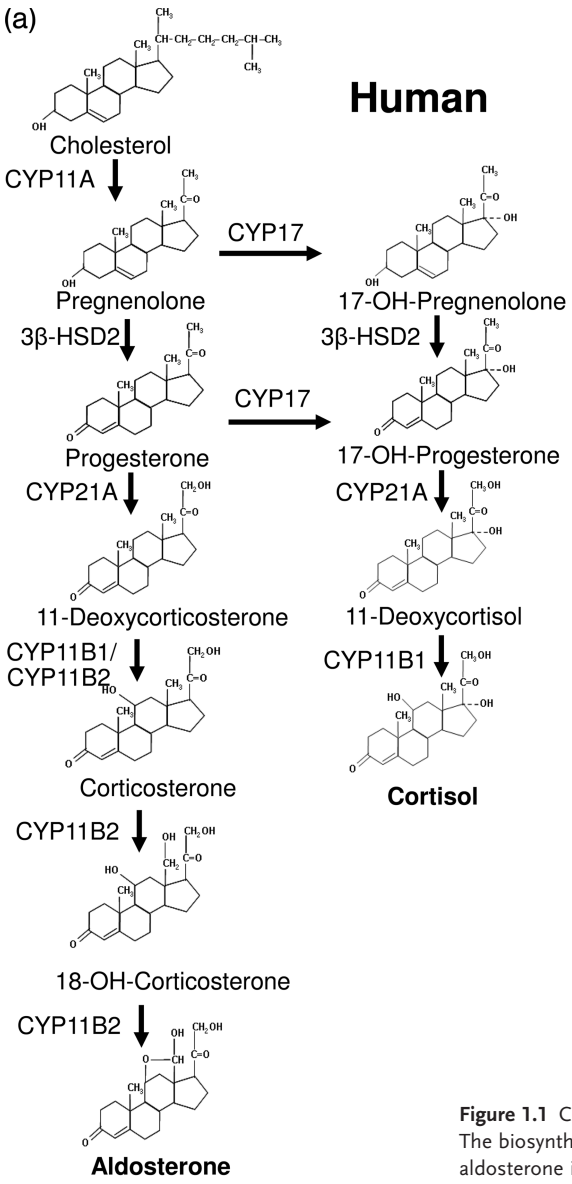

Figure 1.1 Corticosteroid biosynthesis. The biosynthesis pathways of cortisol and aldosterone in (a) humans and (b) rodents.

mutations in P450scc [5], an enzyme that cleaves cholesterol to produce pregnenolone – the common precursor for both cortisol and aldosterone synthesis (Figure 1.1). Indeed the biosysthetic pathways of both share a number of intermediates and enzymes (Figure 1.1), becoming fully exclusive only at 11-deoxycortisol (DOC; cortisol pathway) and 11-deoxycorticoisteroid (aldosterone pathway). In rodents, exclusivity occurs at 11-deoxycorticoisteroid (Figure 1.1b).

The final step in cortisol synthesis, the conversion of DOC to cortisol, is catalyzed by 11β-hydroxylase (CYP11B1 gene), while the final three stages of aldoste-

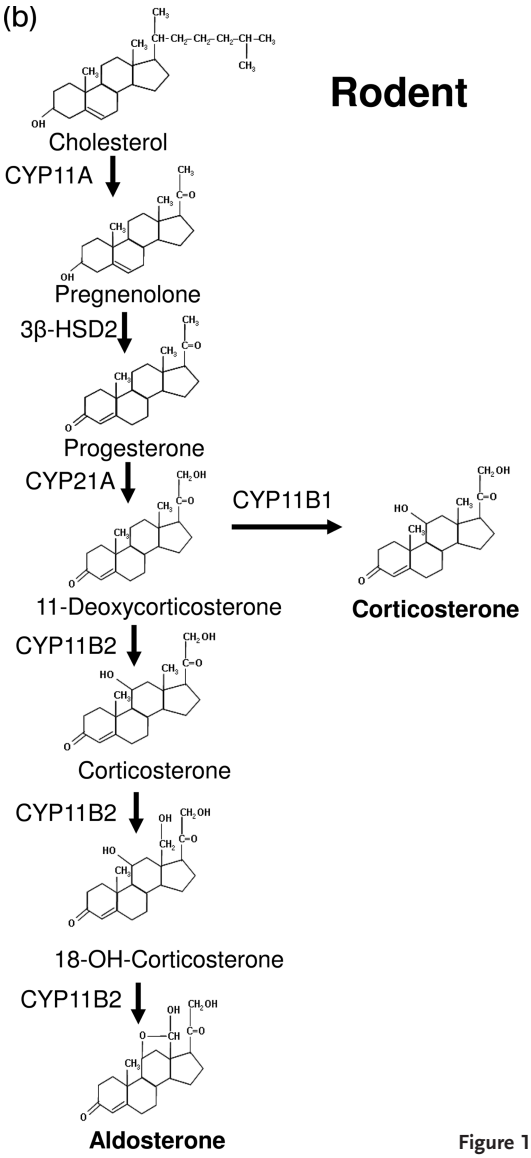

Figure 1.1 *Continued*

rone synthesis require aldosterone synthase (CYP11B2 gene). There is a differential spatial expression of these two enzymes in the cortex of the adrenal gland which is divided into three distinct zones: zona glomerulosa, zona fasciculata and zona reticularis. Cortisol is synthesized primarily in the zona fasciculata, with a small amount being produced by neighboring cells in the zona reticularis. 11β-Hydroxylase is present in both these zones. Aldosterone is produced in the zona glomerulosa, where aldosterone synthase expression is exclusively expressed.

Glucocorticoid remedial hypertension (GRA; OMIM #103900) is an autosomal dominant disorder that occurs when there is unequal crossing over between

CYP11B1 and CYP11B2, which are highly homologous and located in tandem at chromosome 8q24.3, approximately 45 kb apart [6]. In this situation, a chimeric gene is created in which the 5' regulatory regions of the 11β-hydroxylase gene are fused to the coding sequence of the aldosterone synthase gene. There is ectopic expression of the aldosterone synthase in the zona fasciculata, which is now strongly controlled by ACTH. GRA presents with constitutive release of aldosterone and hypertension associated with sodium retention and potassium wasting. Administration of exogenous glucocorticoids suppresses the HPA axis and alleviates the symptoms (see Section 1.2.2 and Figure 1.3).

CAH (OMIM +201910) is an autosomal recessive disorder of cortisol synthesis in which patients have low levels of cortisol and accumulation of DOC and 11-deoxycorticosterone. Approximately 11% of CAH arises from mutations in CYP11B1, the majority being caused by loss of 21-hydroxylase function. Regardless of genetic causality, CAH is associated with neonatal lethality, perhaps leading to an underestimation of the prevalence of the syndrome. Hypertension (DOC is a potent mineralocorticoid, see below) and symptoms of androgen excess, such as precocious puberty and the development of intersexual genitalia, are also features. The only mouse model of CAH available is the H-2(aw) strain which carries a variety of loss-of-function mutations in 21-hydroxylase [7, 8]. Homozygosity for the mutation causes neonatal death; mice heterozygous for mutations have compromised steroidogenesis and faithfully reproduce CAH.

Mutations in CYP11B2 cause the autosomal disorder of corticosterone methyoxidase deficiency (CMO), types 1 and 2 [9]. In CMO1 (OMIM #203400), there is no enzyme activity and aldosterone is undetectable. Patients have marked growth retardation and fail to thrive. Altered renal electrolyte balance leads to hyponatremia and hyperkalemia, and hypotension is evident. This is presumably secondary to volume depletion; however, since activation of MR in vascular smooth muscle potentiates the action of vasoconstrictors (see below), a contribution of vasodilation to the hypotension cannot be discounted. CMO2 (OMIM #610600) is a milder form of the disease: mutations impair but do not ablate aldosterone synthase activity. Lee *et al*. have modeled CMO1 in the mouse, replacing the first two of nine exons with enhanced green fluorescent protein (EGFP), thereby creating a gene expression reporter while concomitantly abolishing enzyme activity [10]. *cyp11b2* null mice were born in normal Mendelian ratios, but a third of the homozygous null animals died prior to weaning, with the rest showing marked retardation of growth, hyperkalemia and altered renal electrolyte handling [10, 11]. Plasma renin activity was elevated (45-fold) in *cyp11b2* null mice, and renin expression was induced in both the zona glomerulosa and fasciculata of the adrenal gland. That these changes failed to maintain blood pressure (null mice were mildly hypotensive), despite the high levels of angiotensin (Ang) II, underscores the essential role for aldosterone in blood pressure homeostasis. Salt supplementation rescued the electrolyte disturbances but did not correct blood pressure. In experimental animals, adrenalectomy or genetic ablation of MR will cause death unless salt therapy is administered. Aldosterone synthase null mice do not require salt supplements to survive with only modestly compromised blood pressure regulation, the

implication being that a degree of MR activation persists. The *cyp11b2* null mouse has provided important data concerning the role and regulation of aldosterone synthesis. Induction of the EGFP construct was used to indicate gene activation. Surprisingly the strongest signals were present in the transition zone between cortex and medulla. It was demonstrated that this zone was rich in cells undergoing apoptotic cell death, suggesting that abnormal aldosterone synthesis has an extensive effect on adrenal gland structure and function. In addition to the expected expansion of the zona glomerulosa, cortical architecture becomes disorganized and there is significant accumulation of lipid in steroidogenic cells.

1.2
Regulation of Corticosteroid Synthesis

1.2.1
Aldosterone

The production of aldosterone from the zona glomerulosa is controlled by the renin–angiotensin system (RAS) (Figure 1.2) and plasma potassium. ACTH can also stimulate aldosterone secretion, particularly in rodents; however, since hypophysectomy or suppression of ACTH by dexamethasone administration does not alter basal aldosterone secretion (or, indeed, the response to salt deprivation), ACTH is not considered to be a key regulator. Other factors, such as plasma sodium, catecholamines, β-endorphins and serotonin, may also play a role, but compared to the RAS and potassium these are minor. Indeed, of all these agents, only Ang II and potassium exert a trophic effect on adrenal gland structure, promoting both hypertrophy of the zona glomerulosa and an increased sensitivity of secretion to their action [12]. Net secretion of aldosterone normally results from the integration of several signals.

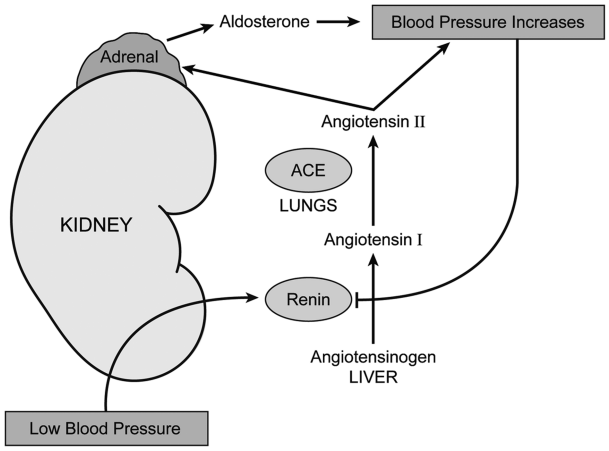

Figure 1.2 The RAS and the control of blood pressure.

Angiotensinogen, primarily synthesized in the liver, is cleaved by the aspartyl protease renin to produce Ang I. This is further cleaved by angiotensin-converting enzyme (ACE) to yield the octapeptide, Ang II. Ang II, acting via AT_1 and AT_2 receptors, will increase blood pressure due to effects on renal sodium reabsorption and vascular resistance. Furthermore, the RAS is a biologically significant regulator of angiogenesis [13]. Therefore, Ang II is an important cardiovascular hormone in its own right, quite separately from its effects on aldosterone synthesis, as covered in Section 3.1.

Ang II, and its metabolite Ang III, rapidly stimulate aldosterone production by activation of both early and late stages of steroid biosynthesis [14]. Both angiotensins are equally efficacious, but Ang II is present in the circulation at much higher concentrations and is, therefore, more important.

Classically, the RAS operates at a systemic level. Recent evidence, however, demonstrates that the RAS can operate independently at the level of the tissue and exert powerful cardiovascular effects quite independently of the systemic system [15]. Although there are no strong quantitative trait locus associations between the RAS and primary hypertension, the involvement of this system in the misregulation of blood pressure is undisputed: the beneficial effects of ACE inhibitors and AT_1 receptor blockers in patients with cardiovascular disease has been demonstrated many times in large-scale clinical trials [16]. More recently, a second form of ACE (ACEII) has been implicated in cardiovascular disease [17] and is a novel therapeutic target. Similarly, the new renin inhibitor, aliskiren, is effective in the treatment of moderate hypertension, although long-term outcome data are not yet available [18]. As for other systems, the use of transgenic animals has clearly demonstrated the major role of the RAS in cardiovascular homeostasis (for a detailed review of this subject, see [19]). Nevertheless, in the majority of these models, the primary abnormality in blood pressure relates to alterations in circulating Ang II, rather than aldosterone. That aldosterone synthesis occurs despite compromised RAS indicates the important regulatory role of plasma potassium levels. This is further supported by the observation that the circadian rhythm of aldosterone secretion does not coincide with that for renin, but for potassium.

An increase in plasma potassium concentration increases the synthesis of aldosterone and, conversely, potassium depletion reduces aldosterone synthesis. The regulation of aldosterone synthesis by potassium is very sensitive: changes of ±0.1 mM can alter the rate of production independently of either Ang II or plasma sodium [12]. There is, moreover, reciprocal regulation: if plasma potassium rises, the rapid increase in aldosterone synthesis promotes kaliuresis and a redistribution of potassium from the extracellular fluid into the cytosol, thereby returning plasma potassium levels to normal [20]. This feedback loop is so persuasive that it can, under conditions of sodium depletion for example, uncouple the secretion of aldosterone from control by Ang II [21]. Thus, the sodium-retaining (and pressor) effects of Ang II may be more important for blood pressure homeostasis than the effects of aldosterone on either the kidney or the vasculature, with the latter acting principally as a regulator of potassium [20].

1.2.2 Glucocorticoids

Glucocorticoid synthesis is regulated by the HPA axis via ACTH (Figure 1.3). ACTH is synthesized by the posterior pituitary mainly in response to two synergistic factors – CRH and antidiuertic hormone (ADH or vasopressin), both of which produced in the paraventricular nucleus of the hypothalamus. These peptides travel through the neurohypophyseal stalk to the median eminence from where they enter the portal circulation and stimulate release of ACTH via binding to the CRF type 1 receptor or V1b receptor, respectively. ACTH stimulates the synthesis of cortisol in the adrenal. Cortisol itself exerts negative feedback on the HPA axis by inhibiting both the release and actions of CRH. ACTH also exerts a short-loop negative feedback by inhibiting its own secretion.

Of the two peptides, CRH is the more important. Mice in which this is deleted have impaired HPA axis, ablated stress response and a loss of the normal circadian rhythm for glucocorticoid production [22]. CRH acts principally at the type 1 receptor with CRF-R1 null mice having a marked impairment of the HPA axis [23]. Unstressed ACTH levels in these animals are, however, normal and they are still able to mount a stress response. This is mediated in part through a second receptor for CRH, as shown by a double knockout strategy [24]. Nevertheless, injection of antisera to ADH was shown to reduce ACTH levels by 60%, indicating a key role for ADH [23] in the compensatory response. ADH acts synergistically to CRF but is not an absolute requirement for ACTH release: Brattleboro rats, which are congenitally devoid of ADH, have a normal HPA axis [25], and mice lacking the

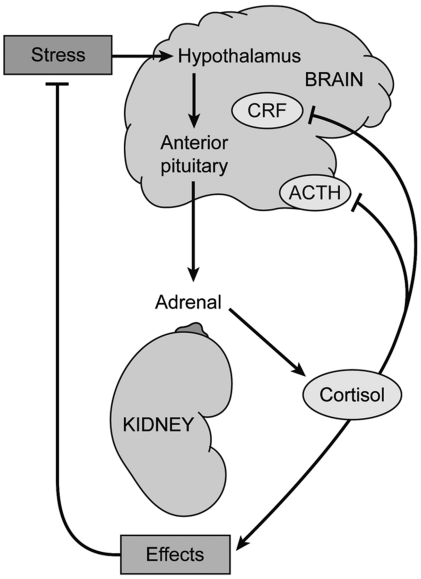

Figure 1.3 The HPA axis.

V1b receptor [26] have normal ACTH and corticosterone levels. Such studies do, however, demonstrate a key role for ADH in sustaining the ACTH response to stress.

That the HPA axis is not abolished by combined administration of antisera to CRF and AVP – or indeed by double knockout of the receptors – indicates other regulatory factors about which less is known. A number of neuroactive compounds, such as Ang II [27], catecholamines and glutamate [28], have been implicated.

ACTH circulates unbound to plasma with a half-life of approximately 15 minutes and exerts its effects via G-protein-coupled receptors belonging to the melanocortin receptor subfamily known as ACTH-R. ACTH-R is mainly expressed in the adrenal cell plasma membrane, with low expression levels being reported in skin and adipose tissue [29]. Although ACTH-R is specific for its ligand, ACTH itself is also recognized by the other four melanocortin receptors. The receptors are coupled to adenylyl cyclase: the cAMP–protein kinase A cascade causes the hydrolysis of cholesterol esters stored in the zona fasciculata and the synthesis of cortisol. The human inheritable condition of familial glucocorticoid deficiency [FGD; OMIM (202200)] has been attributed to mutations within the ACTH-R gene and several different FGD mutations within the gene have been so far identified [30]. FGD is characterized by glucocorticoid deficiency with high plasma ACTH levels and a normal RAS.

Administration of intravenous ACTH in humans is followed by a rapid (within minutes) increase in cortisol plasma levels [31], primarily due to *de novo* synthesis. Although the concentration of steroids is 2- to 3-fold higher in the adrenal gland than in the plasma, this does not act as a reservoir. A sustained increase in ACTH levels results in hypertrophy of the adrenal gland due to an increase in cell size, not number, thereby permitting increased storage of cholesterol. Conversely, adrenal atrophy occurs if ACTH levels remain chronically low.

Plasma cortisol levels fluctuate throughout the day as release occurs in an episodic, rather than constitutive, manner. Nevertheless, the episodes of release are more frequent in the late evening and early morning, and there is a true circadian rhythm (although light does have some effect on the cycle): most secretion occurs from the third hour of sleep to the early hours of wakefulness and plasma cortisol can be undetectable during the rest of the day. The rhythm synchronizes, to an extent, with plasma ACTH concentration and there is a peak in hypothalamic CRF preceding that of cortisol by 4–5 h. However, the circadian rhythm of glucocorticoid production persists even when CRF/ACTH levels are clamped [32] suggesting that the periodicity of release is entrained by other factors. Several agents have been suggested, although neither catecholamines nor serotonin appear to be involved. The rhythm is disrupted, however, by adrenal denervation, spinal chord transection or lesions in the ventromedial nucleus of the hypothalamus [33]. The circadian fluctuations are of major importance to the normal regulation of the HPA axis. Furthermore, glucocorticoids demonstrably influence the phase of peripheral oscillators in the kidney, heart and liver, although not in the central "clock" of the supra chiasmatic nuclei [34]. Studies in genetically modified mice

suggest that disturbances in HPA signaling are connected with vulnerability to behavioral abnormalities [35]. Moreover, there is a well-established circadian variation in cardiovascular risk events, with an increase in events in the morning compared to other times of day [36], coinciding with elevated glucocorticoid and aldosterone levels. Indeed, there is growing literature showing cortisol secretion/metabolism to be directly associated with cardiovascular risk. In contrast, an association study found no link between a glucocorticoid receptor (GR) polymorphism (with a trend toward elevated cortisol) and adverse cardiovascular events [37].

1.3 Corticosteroid Receptors and Control of Ligand Access

1.3.1 Steroid Receptors

GR and MR are intracellular receptors responsible for binding and mediating the "classic" effects of cortisol and aldosterone, respectively. They belong to subfamily 3C of a large and diverse family of transcription factors known as the nuclear receptor family. Other members of subfamily 3C include the progesterone receptor (PR) and the androgen receptor (AR). GR and MR share a high degree of structural homology, reflecting the structural similarities between their corticosteroid ligands. The structural homology is highest at the DNA-binding domains (DBDs) (94%) and 56% between the ligand-binding domains (LBDs) [38]. This high degree of homology suggests that the two receptors are closely associated in evolutionary terms and are most likely descended from a common ancestral receptor. A polar surface within the ligand-binding pocket of MR, lacking in GR and other receptors of the family, permits preferential binding of aldosterone. Nevertheless, the cloning and expression of MR [39] revealed considerable ligand promiscuity with receptor specificity being governed by ligand access.

Unactivated, GR and MR are sequestered in the cytoplasm by complexing with heat-shock protein (HSP). The HSP acts to stop the receptors entering the nucleus in the absence of an appropriate activation signal. Cortisol and aldosterone circulate in plasma bound to plasma proteins, and can easily diffuse through the cell membrane into the cytoplasm. Here they will act to displace the HSP from their receptor to allow the formation of a hormone–receptor complex. This changes the conformation of the receptor, allowing it to form a homodimer, which can now readily enter the nucleus where it will recognize specific hormone response elements (HREs) associated with target genes, acting as a ligand-dependent transcription factor (Figure 1.4). Interestingly, a recent study has shown that GR and MR can form heterodimers [40], which can translocate to the nucleus: the downstream effects on DNA transcription of this complex are unknown. HREs are typically located within a gene enhancer, which can be several kilobases away from the gene promoter. GR and MR, along with PR and AR, recognize response elements whose

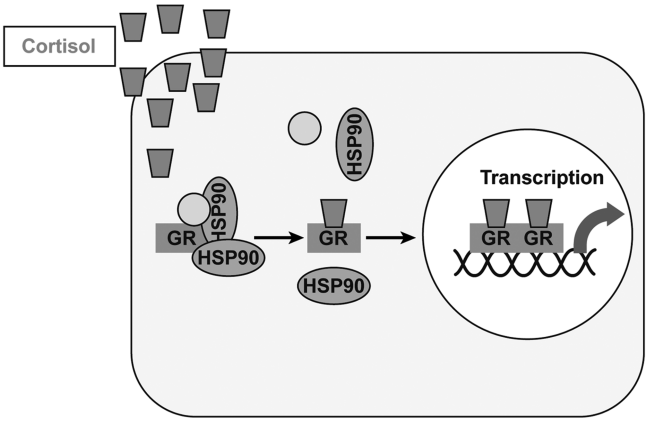

Figure 1.4 Nuclear translocation of GR on ligand binding. Schematic representation of the translocation of GR homodimer to the nucleus for transcriptional effects after ligand binding (cortisol) releases GR from its associated HSP inhibitory complex. MR activation behaves in a similar manner (see Figure 1.6).

HRE sequence consists of two hexameric half-sites (TGTTCT). Glucocorticoids can also affect transcription independent of direct DNA binding by interacting with protein transcription factors [41]. This allows the transcription of genes that mediate GR- and MR-induced responses to be tightly regulated by appropriate ligand binding and hormone–receptor complex conformation.

1.3.2 Control of Ligand Access

The two 11β-hydroxysteroid dehydrogenase (11βHSD) enzymes, types 1 and 2, are key determinants of ligand access to GR and MR, respectively. The enzymes interconvert cortisol (active) and cortisone (inactive), thereby controlling the local concentrations of glucocorticoids (Figure 1.5).

11βHSD1 is the product of HSD11B1 found on chromosome 1 in both mice and humans. The enzyme has a wide distribution but its major areas of action, in terms of both transcript expression and activity, are the liver, adipose and brain [42]. HSD11B2, found on chromosome 16 in humans and on chromosome 8 in mice, encodes the second isozyme. 11βHSD2 has a more limited distribution than 11βHSD1, being expressed predominantly in aldosterone target tissues such as the distal nephron and colon [43]. It is also found in the placenta [44, 45] and in the vascular endothelium [46]. 11βHSD1 was cloned from the liver [47] and 11BHSD2 from the kidney [48]. Although both enzymes belong to the same superfamily of short-chain alcohol dehydrogenase reductases [49], sequence comparison reveals little identity with the exception of the regions encompassing cofactor binding (NAD or NADP[H]) and the active site [50]. In cell culture systems, both

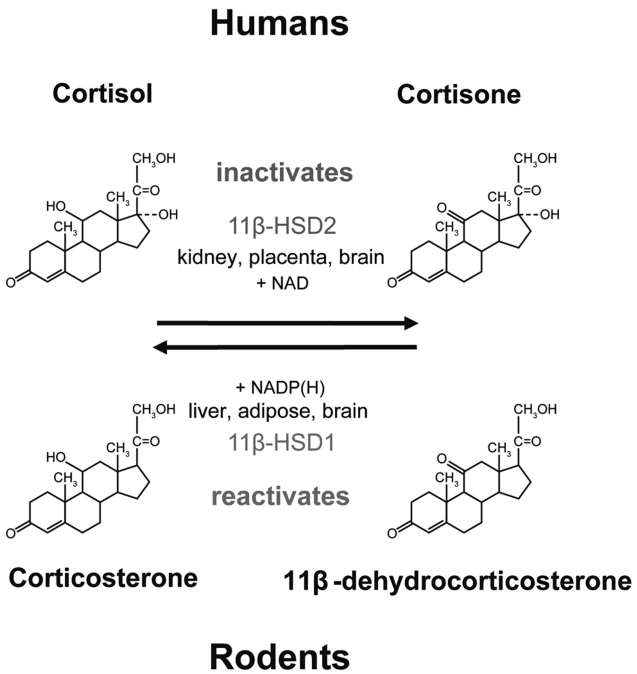

Figure 1.5 Glucocorticoid interconversion. Diagrammatic demonstration of the interconversion between active and inactive glucocorticoids by 11βHSD1 and11βHSD2 in humans and rodents.

enzymes are single-chain polypeptides localized to the membrane of the endoplasmic reticulum, with opposing orientations of their catalytic sites [51, 52]. *In vivo*, however, homodimerization may provide additional regulation of enzyme activity. Dimerization supports full activity of 11βHSD1 [53], but inactivates 11βHSD2 [54].

1.3.2.1 **11βHSD1**

Although there appears to be no physical association of 11βHSD1 with GR, the enzyme governs *in vivo* the extent to which the receptor is activated by glucocorticoids. This occurs by converting inactive cortisone to active cortisol (see Figure 1.5) thereby maintaining glucocorticoid signaling at a local level [50]. Due to its widespread distribution and the lack of specific inhibitors, functional dissection of the role of the enzyme in specific tissues is difficult. However, significant advances in our understanding have come from the generation of genetically modified mice. For example, the 11βHSD1 null mouse has elucidated major functions for the enzyme in the response to stress and in regulation of the HPA [50]. For the latter, regeneration of glucocorticoids by the liver appears to be particularly important [55]. In addition, 11βHSD1 null mice are resistant to age-related

cognitive impairment [56], indicating roles in the brain. This literature has recently been reviewed [50] and the role of 11βHSD1 in the regulation of metabolism and cardiovascular function is discussed later in the chapter. These studies have important implications for human disease, suggesting that 11βHSD1 is an exciting therapeutic target. This area will no doubt be advanced by the recent crystallization of the enzyme [57].

11βHSD1 null mice have improved glucose tolerance, a favorable lipoprotein profile, and increased sensitivity of the liver and fat to insulin [175]. Moreover, on the obese-prone C57BL/6J background, mice carrying the null mutation were resistant to the weight gain induced by high-fat feeding [176]. That loss of 11βHSD1 confers a cardioprotective metabolic profile is intriguing and most probably results from a lack of glucocorticoid regeneration in adipocytes. However, these mice also had an increase calorific intake, suggesting that energy expenditure was also stimulated (Morton *et al.*, 2004).

The generation of a mouse that overexpresses 11βHSD1 under the control of an adipocyte-specific promotor [58] further highlights the role of the enzyme in metabolic function. The amplification of glucocorticoids was relatively modest and confined to the adipocyte, circulating corticosterone being normal. Nevertheless, these transgenic mice developed central obesity, insulin resistance and glucose intolerance. In addition, these animals were hypertensive due to a chronic activation of the RAS [59]. This clustering of metabolic and cardiovascular phenotypes is characteristic of the metabolic syndrome, which is discussed later in this chapter. The level of enzyme function in the adipocyte appears therefore, to play a critical role in setting of metabolic profile.

1.3.2.2 **11βHSD2**

In vitro, MR can be activated with equal potency both by aldosterone and cortisol [39]. *In vivo*, ligand access to MR is determined by colocalization with 11βHSD2 (Figure 1.6). By catalyzing the rapid conversion of cortisol into cortisone (Figure 1.5), which does not activate MR, 11βHSD2 confers upon MR the specificity to aldosterone that it inherently lacks [60, 61]. MR and 11βHSD2 have overlapping distributions in those tissues classically held to be aldosterone selective [43]. In addition to protecting MR from activation by glucocorticoids, there is evidence of a direct association of the proteins [62] and 11βHSD2 may directly regulate MR activation by aldosterone.

Inactivating mutations in the gene *HSD11B2* cause the Syndrome of Apparent Mineralocorticoid Excess (SAME; OMIM #218030). In this setting, cortisol activates MR [63–67], resulting in severe hypertension thought to arise from volume expansion secondary to renal sodium retention [63, 64, 66]. Dexamethasone can be therapeutically effective [67] as it will suppress endogenous glucocorticoids, but does not activate MR. In addition, dexamethasone may act as a chaperone and stabilize mutant enzyme [68]. Nevertheless, neither synthetic glucocorticoid nor MR blockade has a consistent antihypertensive effect [69].

SAME has been modeled by targeted disruption of the 11βHSD2 locus, producing a mouse in which the cardinal features of the disorder were preserved [70].

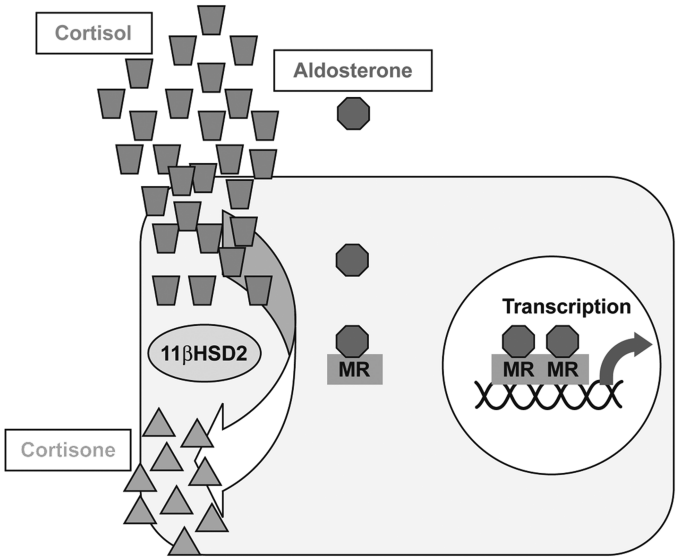

Figure 1.6 MR aldosterone specificity. Schematic representation of 11βHSD2-conferred MR aldosterone specificity.

Although animals were born in normal Mendelian ratios, there was high neonatal mortality in the homozygote null animals, and the remainder were hypertensive and severely hypokalemic. The RAS was suppressed and plasma aldosterone was also low [71, 70]. In one patient with SAME, the disorder was fully corrected by kidney transplant [72], indicating the disease is of renal origin. In support of this, 11βHSD2 null mice have excess renal sodium reabsorption due to activation of the epithelial sodium channel [71]. Nevertheless, sodium retention was found to be transient, and the hypertension moves from a renal to a central and ultimately vascular disorder through activation of the sympathetic nervous system [71]. It is possible that the sympathetic nervous system is activated by the hypernatremia that is sustained beyond the period of sodium retention. However, 11βHSD2 is also expressed in the nucleus of the solitary tract and amygdala of the mouse brain [73, 74], regions important to the central of blood pressure. Thus, SAME may reflect overactivation of MR in regions other than the kidney. This is supported by experiments showing that central administration of either aldosterone [75] or 11βHSD2 inhibitors [76] have a sustained hypertensive effect. In addition, inhibition (pharmacological or antisense) of 11βHSD2 sensitizes the vasculature to both Ang II [77] and catecholamines [78]. Vascular reactivity to noradrenalin is enhanced in a patient with SAME [79]. The 11βHSD2 null mice have endothelial dysfunction, with enhanced sensitivity to vasoactive agents being underpinned by a reduction in nitric oxide (NO) production [46, 80]. However, the extent of the endothelial dysfunction following targeted disruption of 11βHSD2 is dependent on the underlying background strain of the mouse [71] and may not, therefore, contribute in a major way to altered blood pressure homeostasis.

The enzyme is also expressed in the placenta where it serves to prevent maternal–fetal transfer of high levels of glucocorticoid. The deleterious effects on fetal development of *in utero* exposure to high levels of glucocorticoid are well documented, and such programming is associated with low birth weight and adverse cardiovascular risk. This subject has recently been reviewed [81].

Although SAME is an extreme phenotype and very rare, it illustrates the role that 11βHSD2 in the kidney, brain and vasculature has in the regulation of cardiovascular homeostasis. Indeed, it is possible that mild mutations are prevalent in the essential hypertensive population [82], particularly in those individuals with low-renin or salt-sensitive hypertension. Human molecular genetic studies in hypertensive populations have sought associations between blood pressure and loss-of-function polymorphisms in HSD11B2, with conflicting results (e.g. [83–85]). A more direct relationship between 11βHSD2 and blood pressure was obtained in nonhypertensive individuals subject to salt loading. For those individuals with salt-sensitive blood pressure, the extent of the rise in blood pressure following salt load was indirectly related to 11βHSD2 activity. That is, the lower the activity, the more exaggerated the response to salt. Our preliminary observations support this relationship finding that mice heterozygote null for *hsd11b2* have salt-sensitive blood pressure and an impaired ability to excrete sodium. These observations are of particular relevance to Western populations in which hypertension and excessive salt intake are common.

1.4 Cardiovascular Effects of Aldosterone

The well-documented effects of aldosterone on electrolyte transport in the epithelia of the distal nephron and colon [86, 87] can affect blood pressure and abnormal regulation of the RAS is implicated in hypertension [19, 88]. Mineralocorticoids also have actions in the heart [89], vasculature and brain [90] that can influence blood pressure homeostasis and cardiovascular control. Aldosterone is required for adaptation of blood pressure to postural changes and the clinical treatment of postural hypotension is fludrocortisone. These rapid changes in blood pressure occur long before any alteration in plasma volume and therefore lie outwith the control of renal MR. Furthermore, the antihypertensive effects of MR blockade do not correlate with any effects on renal salt balance [91]. The actions in nonepithelial tissue are informative in that they provide insights into "nonclassical" activation of MR and challenge the conventional view of receptor–ligand relationships.

1.4.1 Aldosterone and the Heart

Although aldosterone has both genomic and nongenomic effects on the biophysical properties of the cardiomyocyte (e.g. [92]), a physiological role has been dis-

counted [93] on the basis that MR are antagonized by glucocorticoids under normal circumstances (see below). In contrast a pathological role for MR activation, particularly in the setting of mineralocorticoid excess or salt loading, has been demonstrated since the 1940s.

In the early 1990s a study by Brilla and Weber investigated the effects of mineralocorticoid excess with relation to cardiovascular function, observing that rats exposed to high levels of both aldosterone and salt developed hypertension and cardiac fibrosis [94]. This triggered resurgence in clinical interest with data suggesting that actual mineralocorticoid excess was associated with cardiac abnormalities [95]. Treatment of cardiac abnormalities through MR blockade was recommended following positive outcomes of two clinical trials: RALES and EPHESUS [96, 97]. In the RALES study, patients with severe heart failure were administered the MR antagonist spironolactone, alongside their continuing conventional medication. This produced a 30% reduction in mortality and a 35% lower frequency of hospitalization versus placebo-treated patients. Further verification of the beneficial effects of MR blockade and aldosterone antagonism was provided by the EPHESUS study in which eplerenone, an antagonist at MR more selective than spironolactone, was administered to patients who had suffered an acute myocardial infarction. Again, the results of MR blockade were particularly positive in terms of patient morbidity and mortality.

These trials show MR blockade to be beneficial in the treatment of heart disease, but the underlying mechanisms of action were not clear. The most straightforward explanation was that MR blockade inhibited the effects of aldosterone in the heart and was therefore cardioprotective. Indeed, it has been shown experimentally that increased aldosterone levels coupled with increased salt levels instigates deleterious cardiac and vascular pathologic responses [98] and, circumstantially, aldosterone levels are often raised in congestive heart failure [99]. However, in neither RALES nor EPHESUS were plasma aldosterone levels elevated [96, 97]. Similarly, in Dahl-salt-sensitive rats fed a high salt diet, MR blockade prevented the development of cardiac hypertrophy and the onset of chronic heart failure, despite the fact that aldosterone was lower in this group than controls [100]. In this case, the cardioprotective effects of eplerenone were independent of the antihypertensive effect of MR blockade, as has been reported elsewhere [91]. Together these data indicate that MR activation *per se*, rather than excess of agonist is critical to the developing pathology. Experiments using transgenic approaches are not so clear: mice overexpressing human MR display only mild cardiomyopathy [101] and mice in which MR has been knocked down via an inducible antisense transgene have severe heart failure (Figure 1.7) [103].

In contrast to classical aldosterone target tissues, occupancy of cardiac MR by glucocorticoids is the physiological norm [104]: 11βHSD2 is not normally expressed in cardiomyocytes at physiologically relevant levels. This would indicate that the benefits of MR blockade could be ascribed to relief from stimulation by glucocorticoids. However, the mode of glucocorticoid action at cardiac MR is not clear. Experiments designed to test this hypothesis followed the generation of a mouse

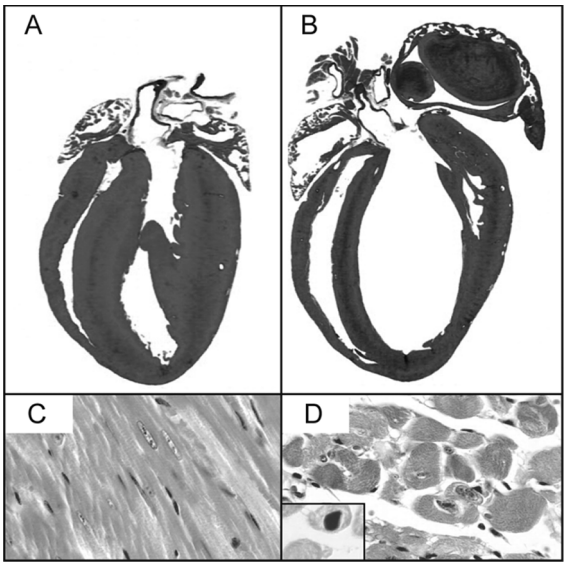

Figure 1.7 Cardiac damage in transgenic models of altered MR activation. Cardiac dilated hypertrophy in transgenic mice overexpressing 11βHSD2 in cardiomyocytes (B). (A) Heart of a nontransgenic mouse [102]. Histological analysis of cardiac remodeling induced by MR antisense mRNA expression in cardiomyocytes (D), showing large hyperchromatic nuclei, myocardial fiber disarray and myocyte hypertrophy, all of which are absent from the control mouse heart displayed in (C). (Adapted from [103]).

expressing 11βHSD2 selectively in cardiomyoctes [102]. Surprisingly, these mice developed severe cardiac hypertrophy and fibrosis, and died from accelerated heart failure (Figure 1.7). Moreover, an MR antagonist rescued the cardiopathology, whereas a GR antagonist did not. These data indicate that (i) glucocorticoids normally occupy cardiac MR, but act as an antagonist rather than agonist, and (ii) that aldosterone activation of MR – only observed when 11βHSD2 prevents glucocorticoid occupancy – is detrimental to heart function.

The data above are confusing and often conflicting, suggesting that MR blockade is both beneficial and damaging and that glucocorticoids can both activate and antagonize the MR. Recent data may reconcile these observations [105]. In isolated cardiomyocytes, aldosterone will activate the Na^+–K–$2Cl^-$ cotransporter, whereas cortisol will not [106]: coadministration of cortisol with aldosterone blocks the activation of the cotransporter. Moreover, if the redox state is altered to mimic production of reactive oxygen species, cortisol no longer antagonizes the actions of aldosterone and even acts as a mineralocorticoid. Thus, the question of what prompts the glucocorticoids to turn from tonic antagonists into pathological agonists may well rest with the generation of reactive oxygen species that can occur following cardiac trauma [89, 105].

1.4.2 Vasculature

MR have been located in freshly isolated vascular tissue and in both cultured vascular smooth muscle cells (VSMCs) and the vascular endothelium [107]. 11βHSD2 is also present in human VSMC, the adventitial fibroblasts and endothelial cells (ECs) [77, 108, 109]. In the mouse thoracic aorta, however, mRNA for 11βHSD2 is confined to the endothelium [46], as it is in cultured rat aortic cells [110]. Whether this is a species difference or reflects the sensitivity of enzyme expression to conditions of culture remains uncertain and resolution awaits the development of reliable antibodies.

Physiologically adrenal steroids – both aldosterone and glucocorticoids – potentiate the action of vasoconstrictors. This effect was first described in the 1950s for catecholamines but is also true for other vasoactive agents, notably Ang II [111]. There is some evidence to suggest that the potentiating effect of corticosteroids differs throughout the vasculature. For example, in the deoxycorticosterone acetate (DOCA)-salt-hypertensive rat model, the pressor effects of Ang II are exacerbated, indicating an increased sensitivity of the resistance vasculature to vasoconstrictors [112]. The conduit vasculature, in contrast, was not sensitized. However, the opposite has been reported for catecholamines: the conduit vasculature being sensitized and the resistance vessels desensitized to phenylephrine [113].

The mechanisms of potentiation have focused on increased receptor density, in part due to the actions of corticosteroids as transcription factors, but also because the effects are seen *ex vivo* and are therefore a property intrinsic to the vessel. For Ang II this appears to hold true, since both aldosterone and glucocorticoids greatly enhance receptor density [111]. Moreover, the increase in receptor number is transduced to a downstream effect, there being a more robust activation by Ang II of intracellular signaling cascades following exposure to mineralocorticoids [114]. These effects are exclusive to the AT_1 receptor [115], consistent with the fact that the promotor region for the gene contains several steroid response elements [116]. In addition to the effects on receptor density, mineralocorticoids also lead to activation of a localized RAS, with increased angiotensinogen formation [117] and ACE activity being found in both ECs and VSMCs [118].

Mineralocorticoid increases the expression of α-adrenergic receptors [119], whereas adrenalectomy reduces receptor density [120]. However, binding studies indicate that receptor affinity moves in the opposite direction to number, thereby offsetting greatly the theoretical "stimulatory" effects of mineralocorticoid. There are several conflicting reports in the literature, but overall convincing data to suggest a receptor number-based response to corticosteroids is lacking [111]. Neither does altered release or uptake of catecholamines at nerve terminals contribute to potentiation by aldosterone [121]. A clue to the underlying mechanism came from the observation that endothelium-dependent vasodilation was impaired in DOCA-salt rats [122]. This was initially attributed to damage secondary to chronic hypertension but other studies demonstrated that the heightened pressor

responses to noradrenalin were, in fact, due to a reduced synthesis of the vasodilator prostaglandin E2 from the endothelium [123]. It is now clear that attenuation of endothelium-derived vasodilation contributes to the potentiation by corticosteroids of the response to catecholamines. This is NO-dependent in conduit vasculature, but not in resistance vessels. More recently, experiments have described direct effects of aldosterone on VSMCs. By improving the coupling of α_1-adrenoceptors to downstream signaling pathways, mineralocorticoids improve vascular tone [124].

The molecular mechanism of aldosterone's action in the vasculature involves genomic effects but these are observed more than 2 h after exposure. That vasoactive responses can be observed almost immediately after infusion of aldosterone (i.e. prior to the onset of *de novo* protein synthesis) suggests nongenomic action in the vasculature [104]. Although *in vivo* systemic infusions of aldosterone almost always promote an immediate pressor response [125], vasoconstriction is not a universal finding *ex vivo*. Indeed, local infusion of aldosterone into the forearm increases local blood flow [125]. This vasodilation results from stimulation of NO production by the endothelium [125]. There is, however, also an effect on the VSMCs to promote vasoconstriction and the net effect on vascular function depends on the balance between the two opposing forces. Thus, inhibition of endothelial NO synthase with *N*-monomethyl-L-arginine leads to a powerful and sustained vasoconstriction [126]. These observations explain why the effect of aldosterone on vascular tone may vary in different vascular beds. Moreover, local vasodilation may be offset by blunting of the baroreceptor reflex [127] and indirect activation of the sympathetic nervous system.

In addition to these well-documented responses, both mineralocorticoids and glucocorticoids will stimulate the local release of endothelin from the vasculature [128]. This is a potent vasoconstrictor (covered in Chapter 6) and could mediate aldosterone-stimulated increases in total peripheral resistance.

Aldosterone also exerts profound pathological effects in the vasculature. The accumulative and slowly developing disease atherosclerosis is a major cause of heart disease. Disruption of vascular homeostasis predisposes the endothelial vessel wall to vasoconstriction, inflammation and atherosclerosis, all of which can be contributors towards cardiac disease onset. The development and progression of atherosclerosis is largely associated with endothelial dysfunction (for review, see [129]), and it has been suggested that the positive clinical outcomes of the RALES and EPHESUS studies may be in part due alleviation of vascular endothelial aldosterone and/or MR antagonism. Using cultured human umbilical vein ECs, Oberleithner *et al.* demonstrated that aldosterone promotes remodeling of the endothelium *in vitro* [130]. They observed that aldosterone administration caused the cells to increase in both size and stiffness, which would *in vivo* lead to endothelial dysfunction and associated pathogenesis. Endothelial dysfunction can be rescued in the stroke-prone spontaneously hypertensive rat by administration of eplerenone [177]. Aldosterone also plays a major role in Ang II-induced vascular inflammation in the setting of high salt intake [98].

It is not easy to present a unifying view of the effect of aldosterone on vascular function since the literature is often divergent. It would appear, however, that the net effect of systemic aldosterone is to increase blood pressure by potentiating the action of vasoconstrictors, "activating" VSMCs and increasing sympathetic drive (either directly or indirectly). The action on the endothelium is less clear. Physiologically and in healthy individuals, it would appear that aldosterone promotes vasodilation, both acutely and chronically [131]. In the setting of hypertension or mineralocorticoid excess coupled with high salt, aldosterone might promote endothelial dysfunction [107]. Although controversial, some evidence suggests that the vasculature can synthesize aldosterone locally [132], adding a further level of complexity to the field. Indeed, locally activated RAS is proinflammatory and promotes detrimental remodeling of the vasculature in hypertension [133]. These findings, together with the positive outcomes of clinical trials, would advocate the use of MR antagonists in hypertension and cardiovascular disease. Indeed, the Framingham Heart Study reports a complex but positive correlation between serum aldosterone in the physiological range and cardiovascular risk [134].

The use of MR antagonists to treat cardiovascular disease is limited, as they tend to promote hyperkalemia due to actions in the kidney. Furthermore, targeted disruption in mice of the gene encoding MR has not been particularly informative in terms of the role of aldosterone in cardiovascular function. MR null mice die within 8 days of birth due to uncorrected salt wasting [135]. Despite significant activation of the RAS, MR null mice were unable to activate the epithelial sodium channel, modeling well the autosomal dominant form of pseudohypoaldosteronism type 1 (OMIM #177735), in which inactivating mutations in MR are reported. These experiments not only indicate the critical role for renal MR in the long-term regulation of blood pressure, but also demonstrate that activation of the GR does not compensate for loss of MR. In order to circumvent the problem of early postnatal death associated with global MR deficiency, the gene has been "floxed" allowing targeted deletion through use of the Cre–loxP system [136]. Surprisingly, when MR was deleted in the distal nephron, mice were able to thrive, albeit with much-elevated aldosterone [137]. This would suggest that MR in other systems could compensate for the loss of renal MR and this was indeed found in the colon. Nevertheless, the principal cell mutant mice were able to maintain near perfect salt balance, even on a low-sodium diet, via upregulation of the epithelial sodium channel and it was found that in a small percentage of principal cells in the early connecting tubule MR had not been deleted. Although this highlights a pitfall of the Cre–loxP system, it is expected that future experiments using the "floxed" MR will be informative.

1.5
Cardiovascular Effects of Glucocorticoids

Glucocorticoids are responsible for a wide range of physiological effects (Figure 1.8), the majority of which are united under the common subheading of stress

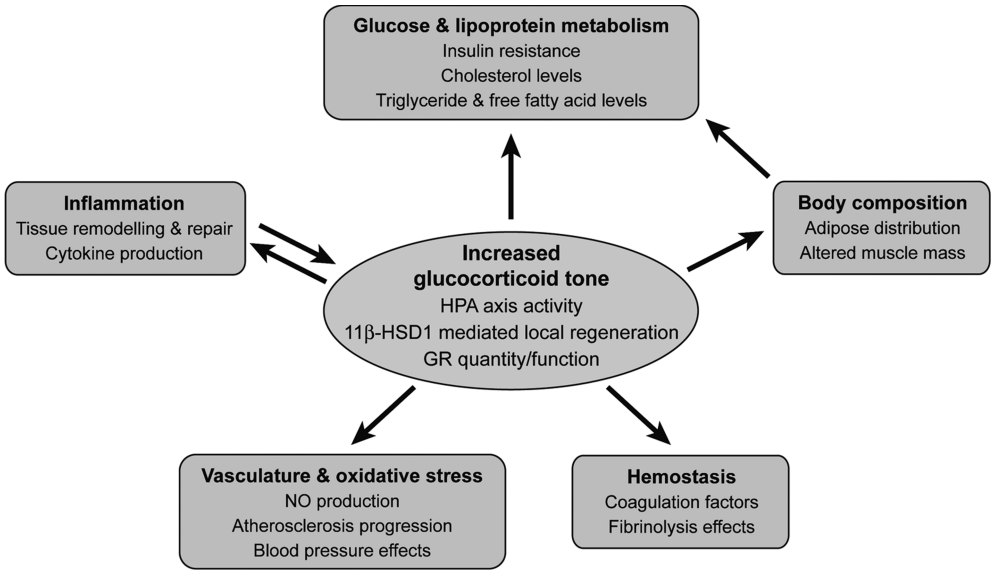

Figure 1.8 Glucocorticoid cardiovascular effects.
Glucocorticoid effects upon cardiovascular risk factors.

responses. The release of glucocorticoids following stress-induced stimulation of the HPA axis promotes coordination of endocrine, immune and nervous system responses to the initial stimuli. Examples of this include inducing the mobilization of energy resources in response to physical stresses such as starvation and the "fight or flight" response by stimulating gluconeogenesis and lipolysis, and inhibiting glucose uptake by peripheral tissues. Glucocorticoids also act to suppress inflammatory responses, cellular proliferation and tissue repair, suggesting a regulatory role to prevent these responses becoming undisciplined and destructive.

Several clinical disorders associated with cortisol deregulation – whether a consequence of synthesis, HPA axis or GR-mediated effects – have been associated with an increased rate of morbidity and mortality, which in turn is possibly corollary to an increased risk of cardiovascular events (for review, see [138]).

It is difficult to separate direct primary effects of glucocorticoids on the heart and vasculature from secondary changes arising from activation of GR in other systems (Figure 1.8). However, evidence from human patients and transgenic mice have helped to establish the nature of these primary responses. It appears that glucocorticoids, at physiological concentrations, may be beneficial to cardiac function, potentially by antagonizing the MR as described above. Furthermore, glucocorticoids potentiate the action of vasoactive substances and so can clearly influence vascular tone. That glucocorticoids are important cardiovascular hormones is illustrated through the extremes of altered glucocorticoid secretion:

Addison's disease presents with life-threatening hypotension and vascular collapse while high blood pressure is a common feature of Cushing's syndrome [139]

1.5.1 Transgenic Models

That no complete loss-of-function mutations in the GR are present in the human population indicates that glucocorticoid activity is essential for life. Transgenic mice with mutations in the GR gene, leading to partial or complete ablation of GR function, support this hypothesis (for review, see [140]) and have not been particularly informative due to high levels of mortality: GR null mice die a few hours after birth due to respiratory failure (glucocorticoids are critical in fetal lung maturation) [141].

Other models in which GR activity is only partially affected are more useful. For example, transgenic mice were generated with a point mutation in the GR gene that abolished DNA-binding capability without affecting the other actions of GR [142]. Approximately one-fifth of these mice survived until adulthood, suggesting that the DNA-binding capability of GR is not essential for survival. Surviving mice had impaired induction of gluconeogenic enzymes, increased ACTH levels as well as adrenal hypertrophy, hyperplasia and an overproduction of steroidogenic enzymes. Of interest, there were no obvious phenotypes usually associated in humans with glucocorticoid excess, such as altered fat disposition. This provides critical information regarding those pathways that require binding of GR to DNA. A second point mutation, introduced into exon 4 of mouse GR [143], impairs GR dimerization. The resulting mice are viable, again indicating that GR DNA binding is not essential for survival. Serum levels of corticosteroid and expression of key steroidogenic enzymes were upregulated, but no change was found in ACTH serum levels (although it was 2.2-fold increased in the anterior pituitary) or adrenal morphology. The data from this model indicate that certain downstream effects of GR activation do not require receptor dimerization in the classical mode.

GR tissue-specific null mice have also been developed using the Cre–loxP system. These mutations, so far used to target gene deletion to the either the liver or nervous system, circumvent the lethality of somatic GR ablation. Both have altered HPA axis regulation. In the liver-specific null, growth retardation was observed, even though serum glucocorticoids and growth hormone levels were normal [144]. Following deletion of GR in the nervous system, behavioral abnormalities were observed [145]. Utilizing this technology to develop a heart and/or vasculature GR-specific null model could reveal valuable insights into how glucocorticoids specifically affect the cardiovascular system.

Taking the opposite approach, a recent study has over-expressed GR exclusively in cardiomyocytes under a "tet-on, tet-off" system [146]. The mice displayed electrocardiogram abnormalities, which were completely reversible with GR overexpression shutoff. Isolated ventricular cardiomyocytes displayed major ion channel

remodeling, as well as changes in cell calcium homeostasis. The electrophysical phenotyping of this model indicates that the cardiac GR overexpression produces defects in conduction with a high degree of atrio-ventricular block. This may reflect, in a magnified and acute setting, the physiological effects that excessive glucocorticoids levels have upon the heart.

1.5.2 Lessons from Human Disease

1.5.2.1 Cushing's Syndrome

Cushing's syndrome is the result of prolonged exposure to excessive cortisol, either as a consequence of hypersecretion of endogenous cortisol outwith the normal physiologic feedback of the HPA axis, or as a result of intensive exogenous exposure in the form of steroid treatment. The most common cause of endogenous Cushing's syndrome is pituitary adenoma, otherwise known as Cushing's disease (OMIM #219090), in which excess ACTH is secreted. Other causes involve ectopic ACTH secretion due to a carcinoid tumor and excess cortisol secretion from adrenal adenomas or carcinomas. Clinical symptoms of Cushing's syndrome include central obesity, hypertension, glucose intolerance, insulin resistance and dyslipidemia [147]. Cardiovascular disease is the main cause of death and disease in Cushing's syndrome patients, and an elevated risk remains even after successful treatment of other symptoms [148]. Epidemiological studies of Cushing's syndrome show patients have a mortality rate 4 times higher than average (age- and sex-matched controls) due to cardiovascular complications [149].

Hypertension is one of the most important cardiovascular risk factors associated with Cushing's syndrome, being present in around 80% of adult patients [150]. The hypertensive phenotype is an effect of interactions between several of the associated pathophysiological mechanisms underlying Cushing's syndrome including effects upon plasma volume, peripheral vascular resistance and cardiac output, all of which have a tendency to be increased [151]. Cushing's associated blood pressure abnormalities present initially with a deregulation of blood pressure circadian rhythm, characterized by a loss of the typical nocturnal fall. This transition from a "dipper" to a "nondipper" phenotype (nocturnal hypertension) is recognized as a cardiovascular risk in its own right [152].

The key underlying mechanism of hypertension is glucocorticoid excess. Part of this can be attributed to illicit MR occupation, but this is not the only cause as MR antagonists do not fully alleviate hypertension [148]. Other blood pressure effects are mediated by excessive activation of the RAS, potentiation of vasoactive substances (see above), and/or suppression of vasodilators (for further details, see [151]). Hypertension is generally resolved in patients after successful treatment, but in a few cases the hypertension persists, suggesting permanent damage to, and remodeling of, the renal and cardiovascular systems. The gain in body weight and partitioning of adipose tissue towards visceral obesity also plays a role in the cardiovascular risk associated with Cushing's syndrome.

1.5.2.2 The Metabolic Syndrome and Tissue-Specific Regulation of Glucocorticoids

The metabolic syndrome (or Syndrome X; OMIM &605552) is defined as the clustering of a plethora of metabolic and cardiovascular phenotypes, including hypertension, hyperglycemia (type 2 diabetes), dyslipidemia and abdominal obesity [153]. The metabolic syndrome is a major cardiovascular risk factor, the prevalence in society of which may be related to relatively recent lifestyle changes, such as dramatic increases in calorific/salt intake and sedentary life habits [154]. However, the metabolic interactions leading to the clustering if the metabolic syndrome phenotypes are not completely understood [155]. The phenotypic similarities between the metabolic syndrome and Cushing's disease cannot help but be noticed and glucocorticoid excess/resistance is certainly a viable as a candidate for an underlying cause of metabolic syndrome [156]. As GR are ubiquitously expressed throughout all tissues, the effects of a global increase in plasma glucocorticoids levels can have many varied consequences that may detract the needed effects away from the factor causing the initial stress. A mechanism to control this is to regulate the concentration of available glucocorticoids at a more local, tissue-specific level. As mentioned previously, 11βHSD1 is globally expressed and is responsible for the conversion of the inactive cortisone to the active cortisol. Studies have shown that 11βHSD1 acts as a regulator of locally available cortisol, as an upregulation in 11βHSD1 would result in increased generation of cortisone to cortisol at a local level. These phenotypes observed in 11βHSD1 transgenic mice are all characteristic of the metabolic syndrome as described above. The level of enzyme function in the adipocyte appears to play a critical role in setting of metabolic profile. Further support for 11βHSD1 involvement in local glucocorticoid regulation in relation to metabolic syndrome phenotypes can be found in other rodent models of obesity, such as the obese Zucker rat model. Corticosteroid metabolism was measured in these obese rats, and 11βHSD1 was enhanced in omental adipose tissue, an indicator that local GR activation may be underling the local promotion of obesity [157, 158]. Evidence of 11βHSD1 upregulation in human obesity has also been shown specifically in adipose tissue in both men [159] and women [160]. Observations such as these raise the possibility of 11βHSD1-specific inhibition as a potential novel treatment for the phenotypes of the metabolic syndrome.

1.5.2.3 Glucocorticoid Resistance Syndrome

Glucocorticoid resistance syndrome (GRS; OMIM +138040) is a rare syndrome characterized by diminished cortisol action mediated by altered GR [161]. This results in a compensatory increase in adrenal ACTH secretion through hyperactivation of the HPA axis, leading to increased levels of circulating glucocorticoids, mineralocorticoids and androgens. A number of mutations in the human GR (hGR) gene LBD or DBD have been identified as underlying molecular mechanisms of familial GRS [162]. Although the different locations of these GRS mutations within the hGR gene result in variable clinical phenotypes, some phenotypes are more persistently expressed than others. These vary from hypertension with

or without hypokalemia to hirsutism and infertility [163]. Cardiovascular morbidity and mortality is increased if the disease is not successfully treated.

1.5.2.4 GR Polymorphisms

Glucocorticoid variation also exists in the general population, as has been experimentally shown by varied responses to dexamethasone (asynthetic glucocorticoid that has no affinity for the MR) by an elderly experimental cohort [164]. Several GR polymorphisms have been identified that seem to be associated with altered glucocorticoids sensitivity and metabolic parameters (for review, see [165]). The N363S polymorphism has been shown to increase glucose sensitivity and the insulin response to dexamethasone, as well as an increased body mass index (BMI). In certain populations there was found to be an increased cardiovascular risk due to high cholesterol and triglyceride concentrations [164]. The N363S variant is associated with obesity, angina and coronary artery disease [166], suggesting a role for altered GR variation in these conditions. The Bcl-1 restriction fragment length polymorphism increases glucocorticoid sensitivity and abdominal obesity in middle age, but then conversely towards old age BMI tends to be lower, perhaps due to accelerated muscle atrophy [167]. Both of these mutations could be described as glucocorticoid hypersensitivity.

Conversely, the ER22/23EK polymorphism [168] is associated with glucocorticoid-resistant phenotypes. Carriers display lower total cholesterol and improved insulin sensitivity, and elderly male carriers of the ER22/23EK polymorphism tend to be protected from cardiovascular damage and therefore have improved survival [169]. In young males and females, the ER22/23EK polymorphism is associated with "improved" body shape and lower body weight, compared to age- and sex-matched controls [170]. This evidence implies that the ER22/23EK polymorphism may predispose towards a healthier metabolic profile, which is regarded as an important aspect of reducing an individual's cardiovascular risk. A recent study identified another novel mutation in the LBD of the hGR which results in generalized glucocorticoid resistance [162], underlining the importance of the role that hGR polymorphisms may play in the general population for contributing towards an individuals cardiovascular risk profile.

1.5.3 Endothelial Dysfunction, Vascular Tone and Atherosclerosis

Atherosclerosis is a major cause of morbidity and mortality in cardiovascular disease, and occurs as a result of a chronic inflammatory response to the deposition of lipoproteins in arterial walls [171]. This leads to the formation of an atheromatous plaque in the arterial wall, which can protrude into the arterial lumen and alter blood flow through the artery. Narrowing of the arterial lumen causes an increase in blood pressure and the sheering stress caused by this can cause the atheromatous plaque to rupture, leading to an occlusion of blood flow and myocardial infarction. There is epidemiological evidence that long-term exposure to glucocorticoid excess (such as in Cushing's syndrome) is associated with accel-

erated atherosclerosis [172]. Administration of 11βHSD1-specific inhibitors in a mouse model of atherosclerosis resulted in a reduced progression of an atheromatous plaque, suggesting that intracellular inactivation of glucocorticoids can have a direct positive effect upon the progression atherosclerosis [173]. Moreover, chronic administration of glucocorticoids to patients with rheumatoid arthritis increases the incidence of atherosclerosis [174].

References

1 Clark, B.J., Wells, J., King, S.R. and Stocco, D.M. (1994) The purification, cloning, and expression of a novel luteinizing hormone-induced mitochondrial protein in MA-10 mouse Leydig tumor cells. Characterization of the steroidogenic acute regulatory protein (stAR). *Journal of Biological Chemistry*, **269**, 28314–22.

2 Lin, D., Sugawara, T., Strauss, J.F., 3rd, Clark, B.J., Stocco, D.M., Saenger, P., Rogol, A. and Miller, W.L. (1995) Role of steroidogenic acute regulatory protein in adrenal and gonadal steroidogenesis. *Science*, **267**, 1828–31.

3 Stocco, D.M. (2002) Clinical disorders associated with abnormal cholesterol transport: mutations in the steroidogenic acute regulatory protein. *Molecular and Cellular Endocrinology*, **191**, 19–25.

4 Caron, K.M., Soo, S.C., Wetsel, W.C., Stocco, D.M., Clark, B.J. and Parker, K.L. (1997) Targeted disruption of the mouse gene encoding steroidogenic acute regulatory protein provides insights into congenital lipoid adrenal hyperplasia. *Proceedings of the National Academy of Sciences of the United States of America*, **94**, 11540–5.

5 Bhangoo, A., Anhalt, H., Ten, S. and King, S.R. (2006) Phenotypic variations in lipoid congenital adrenal hyperplasia. *Pediatric Endocrinology Reviews*, **3**, 258–71.

6 Lifton, R.P., Dluhy, R.G., Powers, M., Rich, G.M., Cook, S., Ulick, S. and Lalouel, J.M. (1992) A chimaeric 11 beta-hydroxylase/aldosterone synthase gene causes glucocorticoid-remediable aldosteronism and human hypertension. *Nature*, **355**, 262–5.

7 Gotoh, H., Sagai, T., Hata, J., Shiroishi, T. and Moriwaki, K. (1988) Steroid 21-hydroxylase deficiency in mice. *Endocrinology*, **123**, 1923–7.

8 Riepe, F.G., Tatzel, S., Sippell, W.G., Pleiss, J. and Krone, N. (2005) Congenital adrenal hyperplasia: the molecular basis of 21-hydroxylase deficiency in H-2(aw18) mice. *Endocrinology*, **146**, 2563–74.

9 White, P.C., Curnow, K.M. and Pascoe, L. (1994) Disorders of steroid 11 beta-hydroxylase isozymes. *Endocrine Reviews*, **15**, 421–38.

10 Lee, G., Makhanova, N., Caron, K., Lopez, M.L., Gomez, R.A., Smithies, O. and Kim, H.S. (2005) Homeostatic responses in the adrenal cortex to the absence of aldosterone in mice. *Endocrinology*, **146**, 2650–6.

11 Makhanova, N., Lee, G., Takahashi, N., Sequeira Lopez, M.L., Gomez, R.A., Kim, H.S. and Smithies, O. (2006) Kidney function in mice lacking aldosterone. *American Journal of Physiology – Renal Physiology*, **290**, F61–69.

12 Quinn, S.J. and Williams, G.H. (1988) Regulation of aldosterone secretion. *Annual Review of Physiology*, **50**, 409–26.

13 Heffelfinger, S.C. (2007) The renin angiotensin system in the regulation of angiogenesis. *Current Pharmaceutical Design*, **13**, 1215–29.

14 Kramer, R.E., Gallant, S. and Brownie, A.C. (1980) Actions of angiotensin II on aldosterone biosynthesis in the rat adrenal cortex. Effects on cytochrome P-450 enzymes of the early and late pathway. *Journal of Biological Chemistry*, **255**, 3442–7.

15 Lazartigues, E., Feng, Y. and Lavoie, J.L. (2007) The two fACEs of the tissue renin–angiotensin systems: implication

in cardiovascular diseases. *Current Pharmaceutical Design*, **13**, 1231–45.
16 Stojiljkovic, L. and Behnia, R. (2007) Role of renin angiotensin system inhibitors in cardiovascular and renal protection: a lesson from clinical trials. *Current Pharmaceutical Design*, **13**, 1335–45.
17 Hamming, I., Cooper, M.E., Haagmans, B.L., Hooper, N.M., Korstanje, R., Osterhaus, A.D., Timens, W., Turner, A.J. and Navis G., and van Goor, H. (2007) The emerging role of ACE2 in physiology and disease. *Journal of Pathology*, **212**, 1–11.
18 Van Tassell, B.W. and Munger, M.A. (2007) Aliskiren for renin inhibition: a new class of antihypertensives. *Annals of Pharmacotherapy*, **41**, 456–64.
19 Mullins, L.J., Bailey, M.A. and Mullins, J.J. (2006) Hypertension, kidney, and transgenics: a fresh perspective. *Physiological Reviews*, **86**, 709–46.
20 Young, D.B. (1985) Analysis of long-term potassium regulation. *Endocrine Reviews*, **6**, 24–44.
21 Cowley, A.W. and McCaa, R.E. (1976) Acute and chronic dose–response relationships for angiotensin, aldosterone, and arterial pressure at varying levels of sodium intake. *Circulation Research*, **39**, 788–97.
22 Muglia, L.J., Bethin, K.E., Jacobson, L., Vogt, S.K. and Majzoub, J.A. (2000) Pituitary–adrenal axis regulation in CRH-deficient mice. *Endocrine Research*, **26**, 1057–66.
23 Turnbull, A.V., Smith, G.W., Lee, S., Vale, W.W., Lee, K.F. and Rivier, C. (1999) CRF type I receptor-deficient mice exhibit a pronounced pituitary–adrenal response to local inflammation. *Endocrinology*, **140**, 1013–7.
24 Bale, T.L., Picetti, R., Contarino, A., Koob, G.F., Vale, W.W. and Lee, K.F. (2002) Mice deficient for both corticotropin-releasing factor receptor 1 (CRFR1) and CRFR2 have an impaired stress response and display sexually dichotomous anxiety-like behavior. *Journal of Neuroscience*, **22**, 193–9.
25 Makara, G.B., Mergl, Z. and Zelena, D. (2004) The role of vasopressin in hypothalamo-pituitary–adrenal axis activation during stress: an assessment of the evidence. *Annals of the New York Academy of Sciences*, **1018**, 151–61.
26 Lolait, S.J., Stewart, L.Q., Jessop, D.S., Young, W.S., 3rd and O'Carroll, A.M. (2007) The hypothalamic–pituitary–adrenal axis response to stress in mice lacking functional vasopressin V1b receptors. *Endocrinology*, **148**, 849–56.
27 Phillips, M.I. (1987) Functions of angiotensin in the central nervous system. *Annual Review of Physiology*, **49**, 413–35.
28 Jezova, D., Makatsori, A., Smriga, M., Morinaga, Y. and Duncko, R. (2005) Subchronic treatment with amino acid mixture of L-lysine and L-arginine modifies neuroendocrine activation during psychosocial stress in subjects with high trait anxiety. *Nutritional Neuroscience*, **8**, 155–60.
29 Elias, L.L. and Clark, A.J. (2000) The expression of the ACTH receptor. *Brazilian Journal of Medical and Biological Research*, **33**, 1245–8.
30 Wu, S.M., Stratakis, C.A., Chan, C.H., Hallermeier, K.M., Bourdony, C.J., Rennert, O.M. and Chan, W.Y. (1998) Genetic heterogeneity of adrenocorticotropin (ACTH) resistance syndromes: identification of a novel mutation of the ACTH receptor gene in hereditary glucocorticoid deficiency. *Molecular Genetics and Metabolism*, **64**, 256–65.
31 Lachelin, G.C., Barnett, M., Hopper, B.R., Brink, G. and Yen, S.S. (1979) Adrenal function in normal women and women with the polycystic ovary syndrome. *Journal of Clinical Endocrinology and Metabolism*, **49**, 892–8.
32 Schulte, H.M., Chrousos, G.P., Gold, P.W., Booth, J.D., Oldfield, E.H., Cutler, G.B., Jr and Loriaux, D.L. (1985) Continuous administration of synthetic ovine corticotropin-releasing factor in man. Physiological and pathophysiological implications. *Journal of Clinical Investigation*, **75**, 1781–5.
33 Sherman, B., Wysham, C. and Pfohl, B. (1985) Age-related changes in the circadian rhythm of plasma cortisol in man. *Journal of Clinical Endocrinology and Metabolism*, **61**, 439–43.

34 Balsalobre, A., Brown, S.A., Marcacci, L., Tronche, F., Kellendonk, C., Reichardt, H.M., Schutz, G. and Schibler, U. (2000) Resetting of circadian time in peripheral tissues by glucocorticoid signaling. *Science*, **289**, 2344–7.

35 Howell, M.P. and Muglia, L.J. (2006) Effects of genetically altered brain glucocorticoid receptor action on behavior and adrenal axis regulation in mice. *Frontiers in Neuroendocrinology*, **27**, 275–84.

36 Giles, T.D. (2006) Circadian rhythm of blood pressure and the relation to cardiovascular events. *Journal of Hypertension. Supplement*, **24**, S11–16.

37 Rosmond, R., Chagnon, Y.C., Chagnon, M., Perusse, L., Bouchard, C. and Bjorntorp, P. (2000) A polymorphism of the 5′-flanking region of the glucocorticoid receptor gene locus is associated with basal cortisol secretion in men. *Metabolism*, **49**, 1197–9.

38 Lu, N.Z., Wardell, S.E., Burnstein, K.L., Defranco, D., Fuller, P.J., Giguere, V., Hochberg, R.B., McKay, L., Renoir, J.M., Weigel, N.L. *et al.* (2006) International Union of Pharmacology. LXV. The pharmacology and classification of the nuclear receptor superfamily: glucocorticoid, mineralocorticoid, progesterone, and androgen receptors. *Pharmacological Reviews*, **58**, 782–97.

39 Arriza, J.L., Weinberger, C., Cerelli, G., Glaser, T.M., Handelin, B.L., Housman, D.E. and Evans, R.M. (1987) Cloning of human mineralocorticoid receptor complementary DNA: structural and functional kinship with the glucocorticoid receptor. *Science*, **237**, 268–75.

40 Nishi, M. and Kawata, M. (2007) Dynamics of glucocorticoid receptor and mineralocorticoid receptor: implications from live cell imaging studies. *Neuroendocrinology*, **85**, 186–92.

41 Bamberger, C.M., Schulte, H.M. and Chrousos, G.P. (1996) Molecular determinants of glucocorticoid receptor function and tissue sensitivity to glucocorticoids. *Endocrine Reviews*, **17**, 245–61.

42 Seckl, J.R. and Walker, B.R. (2004) 11Beta-hydroxysteroid dehydrogenase type 1 as a modulator of glucocorticoid action: from metabolism to memory. *Trends in Endocrinology and Metabolism*, **15**, 418–24.

43 Brown, R.W., Diaz, R., Robson, A.C., Kotelevtsev, Y.V., Mullins, J.J., Kaufman, M.H. and Seckl, J.R. (1996) The ontogeny of 11 beta-hydroxysteroid dehydrogenase type 2 and mineralocorticoid receptor gene expression reveal intricate control of glucocorticoid action in development. *Endocrinology*, **137**, 794–7.

44 Thompson, A., Han, V.K. and Yang, K. (2002) Spatial and temporal patterns of expression of 11beta-hydroxysteroid dehydrogenase types 1 and 2 messenger RNA and glucocorticoid receptor protein in the murine placenta and uterus during late pregnancy. *Biology of Reproduction*, **67**, 1708–18.

45 Thompson, A., Han, V.K. and Yang, K. (2004) Differential expression of 11beta-hydroxysteroid dehydrogenase types 1 and 2 mRNA and glucocorticoid receptor protein during mouse embryonic development. *Journal of Steroid Biochemistry and Molecular Biology*, **88**, 367–75.

46 Christy, C., Hadoke, P.W., Paterson, J.M., Mullins, J.J., Seckl, J.R. and Walker, B.R. (2003) 11Beta-hydroxysteroid dehydrogenase type 2 in mouse aorta: localization and influence on response to glucocorticoids. *Hypertension*, **42**, 580–7.

47 Agarwal, A.K., Monder, C., Eckstein, B. and White, P.C. (1989) Cloning and expression of rat cDNA encoding corticosteroid 11 beta-dehydrogenase. *Journal of Biological Chemistry*, **264**, 18939–43.

48 Agarwal, A.K., Rogerson, F.M., Mune, T. and White, P.C. (1995) Analysis of the human gene encoding the kidney isozyme of 11 beta-hydroxysteroid dehydrogenase. *Journal of Steroid Biochemistry and Molecular Biology*, **55**, 473–9.

49 Grundy, W.N., Bailey, T.L., Elkan, C.P. and Baker, M.E. (1997) Hidden Markov model analysis of motifs in steroid dehydrogenases and their homologs.

Biochemical and Biophysical Research Communications, **231**, 760–6.

50 Paterson, J.M., Seckl, J.R. and Mullins, J.J. (2005) Genetic manipulation of 11beta-hydroxysteroid dehydrogenases in mice. *American Journal of Physiology – Regulatory Integrative and Comparative Physiology*, **289**, R642–52.

51 Naray-Fejes-Toth, A. and Fejes-Toth, G. (1996) Subcellular localization of the type 2 11beta-hydroxysteroid dehydrogenase. A green fluorescent protein study. *Journal of Biological Chemistry*, **271**, 15436–42.

52 Odermatt, A., Arnold, P., Stauffer, A., Frey, B.M. and Frey, F.J. (1999) The N-terminal anchor sequences of 11beta-hydroxysteroid dehydrogenases determine their orientation in the endoplasmic reticulum membrane. *Journal of Biological Chemistry*, **274**, 28762–70.

53 Maser, E., Volker, B. and Friebertshauser, J. (2002) 11Beta-hydroxysteroid dehydrogenase type 1 from human liver: dimerization and enzyme cooperativity support its postulated role as glucocorticoid reductase. *Biochemistry*, **41**, 2459–65.

54 Gomez-Sanchez, E.P., Ganjam, V., Chen, Y.J., Liu, Y., Clark, S.A. and Gomez-Sanchez, C.E. (2001) The 11beta hydroxysteroid dehydrogenase 2 exists as an inactive dimer. *Steroids*, **66**, 845–8.

55 Paterson, J.M., Holmes, M.C., Kenyon, C.J., Carter, R., Mullins, J.J. and Seckl, J.R. (2007) Liver-selective transgene rescue of hypothalamic–pituitary–adrenal axis dysfunction in 11beta-hydroxysteroid dehydrogenase type 1-deficient mice. *Endocrinology*, **148**, 961–6.

56 Yau, J.L., Noble, J., Kenyon, C.J., Hibberd, C., Kotelevtsev, Y., Mullins, J.J. and Seckl, J.R. (2001) Lack of tissue glucocorticoid reactivation in 11beta-hydroxysteroid dehydrogenase type 1 knockout mice ameliorates age-related learning impairments. *Proceedings of the National Academy of Sciences of the United States of America*, **98**, 4716–21.

57 Zhang, J., Osslund, T.D., Plant, M.H., Clogston, C.L., Nybo, R.E., Xiong, F., Delaney, J.M. and Jordan, S.R. (2005) Crystal structure of murine 11 beta-hydroxysteroid dehydrogenase 1: an important therapeutic target for diabetes. *Biochemistry*, **44**, 6948–57.

58 Masuzaki, H., Paterson, J., Shinyama, H., Morton, N.M., Mullins, J.J., Seckl, J.R. and Flier, J.S. (2001) A transgenic model of visceral obesity and the metabolic syndrome. *Science*, **294**, 2166–70.

59 Masuzaki, H., Yamamoto, H., Kenyon, C.J., Elmquist, J.K., Morton, N.M., Paterson, J.M., Shinyama, H., Sharp, M.G., Fleming, S., Mullins, J.J. *et al.* (2003) Transgenic amplification of glucocorticoid action in adipose tissue causes high blood pressure in mice. *Journal of Clinical Investigation*, **112**, 83–90.

60 Funder, J.W., Pearce, P.T., Smith, R. and Smith, A.I. (1988) Mineralocorticoid action: target tissue specificity is enzyme, not receptor, mediated. *Science*, **242**, 583–5.

61 Stewart, P.M., Wallace, A.M., Valentino, R., Burt, D., Shackleton, C.H. and Edwards, C.R. (1987) Mineralocorticoid activity of liquorice: 11-beta-hydroxy-steroid dehydrogenase deficiency comes of age. *Lancet*, **2**, 821–4.

62 Odermatt, A., Arnold, P. and Frey, F.J. (2001) The intracellular localization of the mineralocorticoid receptor is regulated by 11beta-hydroxysteroid dehydrogenase type 2. *Journal of Biological Chemistry*, **276**, 28484–92.

63 Dave-Sharma, S., Wilson, R.C., Harbison, M.D., Newfield, R., Azar, M.R., Krozowski, Z.S., Funder, J.W., Shackleton, C.H., Bradlow, H.L., Wei, J.Q. *et al.* (1998) Examination of genotype and phenotype relationships in 14 patients with apparent mineralocorticoid excess. *Journal of Clinical Endocrinology and Metabolism*, **83**, 2244–54.

64 Monder, C., Shackleton, C.H., Bradlow, H.L., New, M.I., Stoner, E., Iohan, F. and Lakshmi, V. (1986) The syndrome of apparent mineralocorticoid excess: its association with 11 beta-dehydrogenase and 5 beta-reductase deficiency and some consequences for corticosteroid metabolism. *Journal of Clinical Endocrinology and Metabolism*, **63**, 550–7.

65 Morineau, G., Sulmont, V., Salomon, R., Fiquet-Kempf, B., Jeunemaitre, X., Nicod, J. and Ferrari, P. (2006) Apparent mineralocorticoid excess: report of six new cases and extensive personal experience. *Journal of the American Society of Nephrology*, **17**, 3176–84.

66 New, M.I. and Wilson, R.C. (1999) Steroid disorders in children: congenital adrenal hyperplasia and apparent mineralocorticoid excess. *Proceedings of the National Academy of Sciences of the United States of America*, **96**, 12790–7.

67 Stewart, P.M., Corrie, J.E., Shackleton, C.H. and Edwards, C.R. (1988) Syndrome of apparent mineralocorticoid excess. A defect in the cortisol-cortisone shuttle. *Journal of Clinical Investigation*, **82**, 340–9.

68 Atanasov, A.G., Ignatova, I.D., Nashev, L.G., Dick, B., Ferrari, P., Frey, F.J. and Odermatt, A. (2007) Impaired protein stability of 11beta-hydroxysteroid dehydrogenase type 2: a novel mechanism of apparent mineralocorticoid excess. *Journal of the American Society of Nephrology*, **18**, 1262–70.

69 Milford, D.V. (1999) Investigation of hypertension and the recognition of monogenic hypertension. *Archives of Disease in Childhood*, **81**, 452–5.

70 Kotelevtsev, Y., Brown, R.W., Fleming, S., Kenyon, C., Edwards, C.R., Seckl, J.R. and Mullins, J.J. (1999) Hypertension in mice lacking 11beta-hydroxysteroid dehydrogenase type 2. *Journal of Clinical Investigation*, **103**, 683–9.

71 Bailey, M.A., Paterson, J.M., Hadoke, P.W., Wrobel, N., Bellamy, C.O., Brownstein, D.G., Seckl, J.R. and Mullins, J.J. (2008) Switch from sodium-dependent to sodium-independent hypertension in the syndrome of apparent mineralocorticoid excess. *Journal of the American Society of Nephrology*, **19**, 47–58.

72 Palermo, M., Delitala, G., Sorba, G., Cossu, M., Satta, R., Tedde, R., Pala, A. and Shackleton, C.H. (2000) Does kidney transplantation normalise cortisol metabolism in apparent mineralocorticoid excess syndrome? *Journal of Endocrinological Investigation*, **23**, 457–62.

73 Robson, A.C., Leckie, C.M., Seckl, J.R. and Holmes, M.C. (1998) 11 Beta-hydroxysteroid dehydrogenase type 2 in the postnatal and adult rat brain. *Brain Research. Molecular Brain Research*, **61**, 1–10.

74 Roland, B.L., Li, K.X. and Funder, J.W. (1995) Hybridization histochemical localization of 11 beta-hydroxysteroid dehydrogenase type 2 in rat brain. *Endocrinology*, **136**, 4697–700.

75 Gomez-Sanchez, E.P. (1986) Intracerebroventricular infusion of aldosterone induces hypertension in rats. *Endocrinology*, **118**, 819–23.

76 Gomez-Sanchez, E.P. and Gomez-Sanchez, C.E. (1992) Central hypertensinogenic effects of glycyrrhizic acid and carbenoxolone. *American Journal of Physiology*, **263**, E1125–1130.

77 Hatakeyama, H., Inaba, S., Takeda, R. and Miyamori, I. (2000) 11Beta-hydroxysteroid dehydrogenase in human vascular cells. *Kidney International*, **57**, 1352–7.

78 Souness, G.W., Brem, A.S. and Morris, D.J. (2002) 11Beta-hydroxysteroid dehydrogenase antisense affects vascular contractile response and glucocorticoid metabolism. *Steroids*, **67**, 195–201.

79 Walker, B.R., Connacher, A.A., Webb, D.J. and Edwards, C.R. (1992) Glucocorticoids and blood pressure: a role for the cortisol/cortisone shuttle in the control of vascular tone in man. *Clinical Science (London)*, **83**, 171–8.

80 Hadoke, P.W., Christy, C., Kotelevtsev, Y.V., Williams, B.C., Kenyon, C.J., Seckl, J.R., Mullins, J.J. and Walker, B.R. (2001) Endothelial cell dysfunction in mice after transgenic knockout of type 2, but not type 1, 11beta-hydroxysteroid dehydrogenase. *Circulation*, **104**, 2832–7.

81 Seckl, J.R. and Holmes, M.C. (2007) Mechanisms of disease: glucocorticoids, their placental metabolism and fetal "programming" of adult pathophysiology. *Nature Clinical Practice. Endocrinology & Metabolism*, **3**, 479–88.

82 Bocchi, B., Kenouch, S., Lamarre-Cliche, M., Muffat-Joly, M., Capron, M.H., Fiet, J., Morineau, G., Azizi, M., Bonvalet, J.P.

and Farman, N. (2004) Impaired 11-beta hydroxysteroid dehydrogenase type 2 activity in sweat gland ducts in human essential hypertension. *Hypertension*, **43**, 803–8.

83 Melander, O., Frandsen, E., Groop, L. and Hulthen, U.L. (2003) No evidence of a relation between 11beta-hydroxysteroid dehydrogenase type 2 activity and salt sensitivity. *American Journal of Hypertension*, **16**, 729–33.

84 Poch, E., Gonzalez, D., Giner, V., Bragulat, E. and Coca A., and de La Sierra, A. (2001) Molecular basis of salt sensitivity in human hypertension. Evaluation of renin–angiotensin–aldosterone system gene polymorphisms. *Hypertension*, **38**, 1204–9.

85 Williams, T.A., Mulatero, P., Filigheddu, F., Troffa, C., Milan, A., Argiolas, G., Parpaglia, P.P., Veglio, F. and Glorioso, N. (2005) Role of HSD11B2 polymorphisms in essential hypertension and the diuretic response to thiazides. *Kidney International*, **67**, 631–7.

86 Booth, R.E., Johnson, J.P. and Stockand, J.D. (2002) Aldosterone. *Advances in Physiology Education*, **26**, 8–20.

87 Good, D.W. (2007) Nongenomic actions of aldosterone on the renal tubule. *Hypertension*, **49**, 728–39.

88 Campese, V.M. and Park, J. (2006) The kidney and hypertension: over 70 years of research. *Journal of Nephrology*, **19**, 691–8.

89 Young, M.J., Lam, E.Y. and Rickard, A.J. (2007) Mineralocorticoid receptor activation and cardiac fibrosis. *Clinical Science*, **112**, 467–75.

90 Gomez-Sanchez, E.P. (1997) Central hypertensive effects of aldosterone. *Frontiers in Neuroendocrinology*, **18**, 440–62.

91 Levy, D.G., Rocha, R. and Funder, J.W. (2004) Distinguishing the antihypertensive and electrolyte effects of eplerenone. *Journal of Clinical Endocrinology and Metabolism*, **89**, 2736–40.

92 Grossmann, C., Benesic, A., Krug, A.W., Freudinger, R., Mildenberger, S., Gassner, B. and Gekle, M. (2005) Human mineralocorticoid receptor expression renders cells responsive for nongenotropic aldosterone actions. *Molecular Endocrinology*, **19**, 1697–710.

93 Funder, J.W. (2006) Minireview: aldosterone and the cardiovascular system: genomic and nongenomic effects. *Endocrinology*, **147**, 5564–7.

94 Brilla, C.G. and Weber, K.T. (1992) Mineralocorticoid excess, dietary sodium, and myocardial fibrosis. *Journal of Laboratory and Clinical Medicine*, **120**, 893–901.

95 Sato, A. and Saruta, T. (2004) Aldosterone-induced organ damage: plasma aldosterone level and inappropriate salt status. *Hypertension Research*, **27**, 303–10.

96 Pitt, B., Remme, W., Zannad, F., Neaton, J., Martinez, F., Roniker, B., Bittman, R., Hurley, S., Kleiman, J. and Gatlin, M. (2003) Eplerenone, a selective aldosterone blocker, in patients with left ventricular dysfunction after myocardial infarction. *New England Journal of Medicine*, **348**, 1309–21.

97 Pitt, B., Zannad, F., Remme, W.J., Cody, R., Castaigne, A., Perez, A., Palensky, J. and Wittes, J. (1999) The effect of spironolactone on morbidity and mortality in patients with severe heart failure. Randomized Aldactone Evaluation Study Investigators. *New England Journal of Medicine*, **341**, 709–17.

98 Rocha, R. and Funder, J.W. (2002) The pathophysiology of aldosterone in the cardiovascular system. *Annals of the New York Academy of Sciences*, **970**, 89–100.

99 Swedberg, K., Eneroth, P., Kjekshus, J. and Wilhelmsen, L. (1990) Hormones regulating cardiovascular function in patients with severe congestive heart failure and their relation to mortality. CONSENSUS Trial Study Group. *Circulation*, **82**, 1730–6.

100 Nagata, K., Obata, K., Xu, J., Ichihara, S., Noda, A., Kimata, H., Kato, T., Izawa, H., Murohara, T. and Yokota, M. (2006) Mineralocorticoid receptor antagonism attenuates cardiac hypertrophy and failure in low-aldosterone hypertensive rats. *Hypertension*, **47**, 656–64.

101 Le Menuet, D., Isnard, R., Bichara, M., Viengchareun, S., Muffat-Joly, M., Walker, F., Zennaro, M.C. and Lombes, M. (2001) Alteration of cardiac and renal functions in transgenic mice overexpressing human mineralocorticoid receptor. *Journal of Biological Chemistry*, **276**, 38911–20.

102 Qin, W., Rudolph, A.E., Bond, B.R., Rocha, R., Blomme, E.A., Goellner, J.J., Funder, J.W. and McMahon, E.G. (2003) Transgenic model of aldosterone-driven cardiac hypertrophy and heart failure. *Circulation Research*, **93**, 69–76.

103 Beggah, A.T., Escoubet, B., Puttini, S., Cailmail, S., Delage, V., Ouvrard-Pascaud, A., Bocchi, B., Peuchmaur, M., Delcayre, C., Farman, N. *et al.* (2002) Reversible cardiac fibrosis and heart failure induced by conditional expression of an antisense mRNA of the mineralocorticoid receptor in cardiomyocytes. *Proceedings of the National Academy of Sciences of the United States of America*, **99**, 7160–5.

104 Funder, J.W. (2004) Is aldosterone bad for the heart? *Trends in Endocrinology and Metabolism*, **15**, 139–42.

105 Funder, J.W. (2005) RALES, EPHESUS and redox. *Journal of Steroid Biochemistry and Molecular Biology*, **93**, 121–5.

106 Mihailidou, A.S. and Funder, J.W. (2005) Nongenomic effects of mineralocorticoid receptor activation in the cardiovascular system. *Steroids*, **70**, 347–51.

107 Duprez, D.A. (2007) Aldosterone and the vasculature: mechanisms mediating resistant hypertension. *Journal of Clinical Hypertension (Greenwich)*, **9**, 13–8.

108 Cai, T.Q., Wong, B., Mundt, S.S., Thieringer, R., Wright, S.D. and Hermanowski-Vosatka, A. (2001) Induction of 11beta-hydroxysteroid dehydrogenase type 1 but not -2 in human aortic smooth muscle cells by inflammatory stimuli. *Journal of Steroid Biochemistry and Molecular Biology*, **77**, 117–22.

109 Hatakeyama, H., Inaba, S. and Miyamori, I. (1999) 11Beta-hydroxysteroid dehydrogenase in cultured human vascular cells. Possible role in the development of hypertension. *Hypertension*, **33**, 1179–84.

110 Brem, A.S., Bina, R.B., King, T.C. and Morris, D.J. (1998) Localization of 2 11beta-OH steroid dehydrogenase isoforms in aortic endothelial cells. *Hypertension*, **31**, 459–62.

111 Ullian, M.E. (1999) The role of corticosteriods in the regulation of vascular tone. *Cardiovascular Research*, **41**, 55–64.

112 Couture, R. and Regoli, D. (1980) Vascular reactivity to angiotensin and noradrenaline in rats maintained on a sodium free diet or made hypertensive with desoxycorticosterone acetate and salt (DOCA/salt). *Clinical and Experimental Hypertension*, **2**, 25–43.

113 White, R.E. and Carrier, G.O. (1986) Alpha 1- and alpha 2-adrenoceptor agonist-induced contraction in rat mesenteric artery upon removal of endothelium. *European Journal of Pharmacology*, **122**, 349–52.

114 Ullian, M.E. and Fine, J.J. (1994) Mechanisms of enhanced angiotensin II-stimulated signal transduction in vascular smooth muscle by aldosterone. *Journal of Cellular Physiology*, **161**, 201–8.

115 Schelling, J.R., DeLuca, D.J., Konieczkowski, M., Marzec, R., Sedor, J.R., Dubyak, G.R. and Linas, S.L. (1994) Glucocorticoid uncoupling of antiogensin II-dependent phospholipase C activation in rat vascular smooth muscle cells. *Kidney International*, **46**, 675–82.

116 Uno, S., Guo, D.F., Nakajima, M., Ohi, H., Imada, T., Hiramatsu, R., Nakakubo, H., Nakamura, N. and Inagami, T. (1994) Glucocorticoid induction of rat angiotensin II type 1A receptor gene promoter. *Biochemical and Biophysical Research Communications*, **204**, 210–5.

117 Chan, J.S., Ming, M., Nie, Z.R., Sikstrom, R., Lachance, S. and Carriere, S. (1992) Hormonal regulation of expression of the angiotensinogen gene in cultured opossum kidney proximal tubular cells. *Journal of the American Society of Nephrology*, **2**, 1516–22.

118 Sim, M.K. and Chan, C.S. (1992) Effect of experimentally-induced hypertension on angiotensin converting enzyme activity in

the aortic endothelium and smooth muscle cum adventitia of the Sprague Dawley rat. *Life Sciences*, **50**, 1821–5.

119 Meggs, L.G., Stitzel, R., Ben-Ari, J., Chander, P., Gammon, D., Goodman, A.I. and Head, R. (1988) Upregulation of the vascular alpha-1 receptor in malignant DOCA-salt hypertension. *Clinical and Experimental Hypertension A.*, **10**, 229–47.

120 Haigh, R.M. and Jones, C.T. (1990) Effect of glucocorticoids on alpha 1-adrenergic receptor binding in rat vascular smooth muscle. *Journal of Molecular Endocrinology*, **5**, 41–8.

121 Longhurst, P.A., Rice, P.J., Taylor, D.A. and Fleming, W.W. (1988) Sensitivity of caudal arteries and the mesenteric vascular bed to norepinephrine in DOCA-salt hypertension. *Hypertension*, **12**, 133–42.

122 Lockette, W., Otsuka, Y. and Carretero, O. (1986) The loss of endothelium-dependent vascular relaxation in hypertension. *Hypertension*, **8**, II61–66.

123 Handa, M., Kondo, K., Suzuki, H. and Saruta, T. (1984) Dexamethasone hypertension in rats: role of prostaglandins and pressor sensitivity to norepinephrine. *Hypertension*, **6**, 236–41.

124 Losel, R., Schultz, A., Boldyreff, B. and Wehling, M. (2004) Rapid effects of aldosterone on vascular cells: clinical implications. *Steroids*, **69**, 575–8.

125 Schmidt, B.M., Oehmer, S., Delles, C., Bratke, R., Schneider, M.P., Klingbeil, A., Fleischmann, E.H. and Schmieder, R.E. (2003) Rapid nongenomic effects of aldosterone on human forearm vasculature. *Hypertension*, **42**, 156–60.

126 Schmidt, B.M., Sammer, U., Fleischmann, I., Schlaich, M., Delles, C. and Schmieder, R.E. (2006) Rapid nongenomic effects of aldosterone on the renal vasculature in humans. *Hypertension*, **47**, 650–5.

127 Yee, K.M. and Struthers, A.D. (1998) Aldosterone blunts the baroreflex response in man. *Clinical Science (London, England)*, **95**, 687–92.

128 Schiffrin, E.L., Lariviere, R., Li, J.S., Sventek, P. and Touyz, R.M. (1995) Deoxycorticosterone acetate plus salt induces overexpression of vascular endothelin-1 and severe vascular hypertrophy in spontaneously hypertensive rats. *Hypertension*, **25**, 769–73.

129 Landmesser, U., Hornig, B. and Drexler, H. (2004) Endothelial function: a critical determinant in atherosclerosis? *Circulation*, **109**, II27–33.

130 Oberleithner, H. (2005) Aldosterone makes human endothelium stiff and vulnerable. *Kidney International*, **67**, 1680–2.

131 Nietlispach, F., Julius, B., Schindler, R., Bernheim, A., Binkert, C., Kiowski, W. and Brunner-La Rocca, H.P. (2007) Influence of acute and chronic mineralocorticoid excess on endothelial function in healthy men. *Hypertension*, **50**, 82–8.

132 Williams, G.H. (2005) Aldosterone biosynthesis, regulation, and classical mechanism of action. *Heart Failure Reviews*, **10**, 7–13.

133 Savoia, C. and Schiffrin, E.L. (2006) Inflammation in hypertension. *Current Opinion in Nephrology and Hypertension*, **15**, 152–8.

134 Kathiresan, S., Larson, M.G., Benjamin, E.J., Corey, D., Murabito, J.M., Fox, C.S., Wilson, P.W., Rifai, N., Meigs, J.B., Ricken, G. *et al.* (2005) Clinical and genetic correlates of serum aldosterone in the community: the Framingham Heart Study. *American Journal of Hypertension*, **18**, 657–65.

135 Berger, S., Bleich, M., Schmid, W., Cole, T.J., Peters, J., Watanabe, H., Kriz, W., Warth, R., Greger, R. and Schutz, G. (1998) Mineralocorticoid receptor knockout mice: pathophysiology of Na^+ metabolism. *Proceedings of the National Academy of Sciences of the United States of America*, **95**, 9424–9.

136 Berger, S., Wolfer, D.P., Selbach, O., Alter, H., Erdmann, G., Reichardt, H.M., Chepkova, A.N., Welzl, H., Haas, H.L., Lipp, H.P. *et al.* (2006) Loss of the limbic mineralocorticoid receptor impairs behavioral plasticity. *Proceedings of the National Academy of Sciences of the United States of America*, **103**, 195–200.

137 Ronzaud, C., Loffing, J., Bleich, M., Gretz, N., Grone, H.J., Schutz, G. and

Berger, S. (2007) Impairment of sodium balance in mice deficient in renal principal cell mineralocorticoid receptor. *Journal of the American Society of Nephrology*, **18**, 1679–87.

138 Girod, J.P. and Brotman, D.J. (2004) Does altered glucocorticoid homeostasis increase cardiovascular risk? *Cardiovascular Research*, **64**, 217–26.

139 Hammer, F. and Stewart, P.M. (2006) Cortisol metabolism in hypertension. *Best Practice and Research Clinical Endocrinology and Metabolism*, **20**, 337–53.

140 Wintermantel, T.M., Berger, S., Greiner, E.F. and Schutz, G. (2005) Evaluation of steroid receptor function by gene targeting in mice. *Journal of Steroid Biochemistry and Molecular Biology*, **93**, 107–12.

141 Cole, T.J., Blendy, J.A., Monaghan, A.P., Krieglstein, K., Schmid, W., Aguzzi, A., Fantuzzi, G., Hummler, E., Unsicker, K. and Schutz, G. (1995) Targeted disruption of the glucocorticoid receptor gene blocks adrenergic chromaffin cell development and severely retards lung maturation. *Genes and Development*, **9**, 1608–21.

142 Kellendonk, C., Tronche, F., Reichardt, H.M. and Schutz, G. (1999) Mutagenesis of the glucocorticoid receptor in mice. *Journal of Steroid Biochemistry and Molecular Biology*, **69**, 253–9.

143 Reichardt, H.M., Kaestner, K.H., Tuckermann, J., Kretz, O., Wessely, O., Bock, R., Gass, P., Schmid, W., Herrlich, P., Angel, P. *et al.* (1998) DNA binding of the glucocorticoid receptor is not essential for survival. *Cell*, **93**, 531–41.

144 Tronche, F., Opherk, C., Moriggl, R., Kellendonk, C., Reimann, A., Schwake, L., Reichardt, H.M., Stangl, K., Gau, D., Hoeflich, A. *et al.* (2004) Glucocorticoid receptor function in hepatocytes is essential to promote postnatal body growth. *Genes and Development*, **18**, 492–7.

145 Tronche, F., Kellendonk, C., Kretz, O., Gass, P., Anlag, K., Orban, P.C., Bock, R., Klein, R. and Schutz, G. (1999) Disruption of the glucocorticoid receptor gene in the nervous system results in reduced anxiety. *Nature Genetics*, **23**, 99–103.

146 Sainte-Marie, Y., Cat, A.N., Perrier, R., Mangin, L., Soukaseum, C., Peuchmaur, M., Tronche, F., Farman, N., Escoubet, B., Benitah, J.P. *et al.* (2007) Conditional glucocorticoid receptor expression in the heart induces atrio-ventricular block. *FASEB Journal*, **21**, 3133–41.

147 Newell-Price, J., Bertagna, X., Grossman, A.B. and Nieman, L.K. (2006) Cushing's syndrome. *Lancet*, **367**, 1605–17.

148 Whitworth, J.A., Williamson, P.M., Mangos, G. and Kelly, J.J. (2005) Cardiovascular consequences of cortisol excess. *Vascular Health and Risk Management*, **1**, 291–9.

149 Etxabe, J. and Vazquez, J.A. (1994) Morbidity and mortality in Cushing's disease: an epidemiological approach. *Clinical Endocrinology*, **40**, 479–84.

150 Arnaldi, G., Mancini, T., Polenta, B. and Boscaro, M. (2004) Cardiovascular risk in Cushing's syndrome. *Pituitary*, **7**, 253–6.

151 Magiakou, M.A., Smyrnaki, P. and Chrousos, G.P. (2006) Hypertension in Cushing's syndrome. *Best Practice and Research Clinical Endocrinology and Metabolism*, **20**, 467–82.

152 Birkenhager, A.M. and van den Meiracker, A.H. (2007) Causes and consequences of a non-dipping blood pressure profile. *Netherlands Journal of Medicine*, **65**, 127–31.

153 Aizawa, Y., Kamimura, N., Watanabe, H., Aizawa, Y., Makiyama, Y., Usuda, Y., Watanabe, T. and Kurashina, Y. (2006) Cardiovascular risk factors are really linked in the metabolic syndrome: this phenomenon suggests clustering rather than coincidence. *International Journal of Cardiology*, **109**, 213–8.

154 Chew, G.T., Gan, S.K. and Watts, G.F. (2006) Revisiting the metabolic syndrome. *Medical Journal of Australia*, **185**, 445–9.

155 Grundy, S.M. (2007) Metabolic syndrome: a multiplex cardiovascular risk factor. *Journal of Clinical Endocrinology and Metabolism*, **92**, 399–404.

156 Walker, B.R. (2006) Cortisol–cause and cure for metabolic syndrome? *Diabetic Medicine*, **23**, 1281–8.

157 Livingstone, D.E., Jones, G.C., Smith, K., Jamieson, P.M., Andrew, R., Kenyon, C.J. and Walker, B.R. (2000) Understanding the role of glucocorticoids in obesity: tissue-specific alterations of corticosterone metabolism in obese Zucker rats. *Endocrinology*, **141**, 560–3.

158 Livingstone, D.E., Kenyon, C.J. and Walker, B.R. (2000) Mechanisms of dysregulation of 11 beta-hydroxysteroid dehydrogenase type 1 in obese Zucker rats. *Journal of Endocrinology*, **167**, 533–9.

159 Rask, E., Olsson, T., Soderberg, S., Andrew, R., Livingstone, D.E., Johnson, O. and Walker, B.R. (2001) Tissue-specific dysregulation of cortisol metabolism in human obesity. *Journal of Clinical Endocrinology and Metabolism*, **86**, 1418–21.

160 Rask, E., Walker, B.R., Soderberg, S., Livingstone, D.E., Eliasson, M., Johnson, O., Andrew, R. and Olsson, T. (2002) Tissue-specific changes in peripheral cortisol metabolism in obese women: increased adipose 11beta-hydroxysteroid dehydrogenase type 1 activity. *Journal of Clinical Endocrinology and Metabolism*, **87**, 3330–6.

161 van Rossum, E.F. and Lamberts, S.W. (2006) Glucocorticoid resistance syndrome: a diagnostic and therapeutic approach. *Best Practice and Research Clinical Endocrinology and Metabolism*, **20**, 611–26.

162 Charmandari, E., Kino, T., Souvatzoglou, E., Vottero, A., Bhattacharyya, N. and Chrousos, G.P. (2004) Natural glucocorticoid receptor mutants causing generalized glucocorticoid resistance: molecular genotype, genetic transmission, and clinical phenotype. *Journal of Clinical Endocrinology and Metabolism*, **89**, 1939–49.

163 Chrousos, G.P., Detera-Wadleigh, S.D. and Karl, M. (1993) Syndromes of glucocorticoid resistance. *Annals of Internal Medicine*, **119**, 1113–24.

164 Huizenga, N.A., Koper, J.W., De Lange, P., Pols, H.A., Stolk, R.P., Burger, H., Grobbee, D.E., Brinkmann, A.O., De Jong, F.H. and Lamberts, S.W. (1998) A polymorphism in the glucocorticoid receptor gene may be associated with and increased sensitivity to glucocorticoids *in vivo*. *Journal of Clinical Endocrinology and Metabolism*, **83**, 144–51.

165 van Rossum, E.F. and Lamberts, S.W. (2004) Polymorphisms in the glucocorticoid receptor gene and their associations with metabolic parameters and body composition. *Recent Progress in Hormone Research*, **59**, 333–57.

166 Lin, R.C., Wang, X.L. and Morris, B.J. (2003) Association of coronary artery disease with glucocorticoid receptor N363S variant. *Hypertension*, **41**, 404–7.

167 Murray, J.C., Smith, R.F., Ardinger, H.A. and Weinberger, C. (1987) RFLP for the glucocorticoid receptor (GRL) located at 5q11–5q13. *Nucleic Acids Research*, **15**, 6765.

168 Koper, J.W., Stolk, R.P., de Lange, P., Huizenga, N.A., Molijn, G.J., Pols, H.A., Grobbee, D.E., Karl, M., de Jong, F.H., Brinkmann, A.O. *et al.* (1997) Lack of association between five polymorphisms in the human glucocorticoid receptor gene and glucocorticoid resistance. *Human Genetics*, **99**, 663–8.

169 van Rossum, E.F., Feelders, R.A., van den Beld, A.W., Uitterlinden, A.G., Janssen, J.A., Ester, W., Brinkmann, A.O., Grobbee, D.E., de Jong, F.H., Pols, H.A. *et al.* (2004) Association of the ER22/23EK polymorphism in the glucocorticoid receptor gene with survival and C-reactive protein levels in elderly men. *American Journal of Medicine*, **117**, 158–62.

170 van Rossum, E.F., Voorhoeve, P.G., Velde, S.J., Koper, J.W., Delemarre-van de Waal, H.A., Kemper, H.C. and Lamberts, S.W. (2004) The ER22/23EK polymorphism in the glucocorticoid receptor gene is associated with a beneficial body composition and muscle strength in young adults. *Journal of Clinical Endocrinology and Metabolism*, **89**, 4004–9.

171 Ross, R. (1999) Atherosclerosis is an inflammatory disease. *American Heart Journal*, **138**, S419–420.

172 Colao, A., Pivonello, R., Spiezia, S., Faggiano, A., Ferone, D., Filippella, M., Marzullo, P., Cerbone, G., Siciliani, M. and Lombardi, G. (1999) Persistence of increased cardiovascular risk in patients with Cushing's disease after five years of successful cure. *Journal of Clinical Endocrinology and Metabolism*, **84**, 2664–72.

173 Hermanowski-Vosatka, A., Balkovec, J.M., Cheng, K., Chen, H.Y., Hernandez, M., Koo, G.C., Le Grand, C.B., Li, Z., Metzger, J.M., Mundt, S.S. *et al.* (2005) 11betaHSD1 inhibition ameliorates metabolic syndrome and prevents progression of atherosclerosis in mice. *Journal of Experimental Medicine*, **202**, 517–27.

174 del Rincon, I., O'Leary, D.H., Haas, R.W. and Escalante, A. (2004) Effect of glucocorticoids on the arteries in rheumatoid arthritis. *Arthritis and Rheumatism*, **50**, 3813–22.

175 Morton, N.M., Holmes, M.C., Fiévet, C., Staels, B., Tailleux, A., Mullins, J.J., Seckl, J.R. (2001) Improved lipid and lipoprotein profile, hepatic insulin sensitivity, and glucose tolerance in 11beta-hydroxysteroid dehydrogenase type 1 null mice. *Journal of Biological Chemistry*, **276**, 41293–300.

176 Morton, N.M., Paterson, J.M., Masuzaki, H., Holmes, M.C., Staels, B., Fiévet, C., Walker, B.R., Flier, J.S., Mullins, J.J., Seckl, J.R. (2004) Novel adipose tissue-mediated resistance to diet-induced visceral obesity in 11 beta-hydroxysteroid dehydrogenase type 1-deficient mice. *Diabetes*, **53**, 931–8.

177 Endemann, D.H., Touyz, R.M., Iglarz, M., Savoia, C., Schiffrin, E.L. (2004) Eplerenone prevents salt-induced vascular remodeling and cardiac fibrosis in stroke-prone spontaneously hypertensive rats. *Hypertension*, **43**, 1252–7.