Corticosteroids and vascular tone: mapping the messenger maze

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INTRODUCTION

The effects of corticosteroids on vascular tone have been recognized since the 1940s. However, despite repeated demonstration of pharmacological effects both in vitro and in vivo, their physiological significance as regulators of tone in health remains to be established. Evidence that abnormal vascular reactivity is important when adrenal steroid production is deranged is more convincing, and probably contributes to the changes in blood pressure seen in conditions of corticosteroid excess and deficiency. It remains speculative whether the same mechanisms contribute to the pathogenesis of other hypertensive syndromes and of circulatory shock.

Recently, the effects of corticosteroids have been studied in cultured vascular cells. These studies exploit our improving understanding of the actions of steroid hormones on their target cells and better appreciation of the diversity of biochemical signals in the vessel wall which mediate the response to vasoactive stimuli. They include measurements of endothelium-derived factors (which act on neighbouring vascular smooth muscle cells), contractile cell-surface receptors and intracellular second messengers (released within the contractile cell in response to the activation of surface receptors). Such studies have shown a number of interactions between corticosteroids and newly discovered biochemical messengers. In this review we assess whether our interpretation of the older evidence that steroids affect vascular tone is influenced by recent studies of their cellular effects, and whether we can now deduce the role of corticosteroids in vascular physiology and pathology.

MECHANISMS OF RESPONSE TO CORTICOSTEROIDS

Most effects of corticosteroids are mediated by binding to classical cytosolic steroid receptors (Fig. 1). These receptors vary in their intrinsic affinity for glucocorticoids (principally cortisol (= hydrocortisone) in man; also corticosterone in rat; and synthetic dexamethasone, prednisolone, budesonide, etc.) and mineralocorticoids (principally aldosterone; also deoxycorticosterone, fludrocortisone). Binding affinities approximate to physiological concentrations of free steroids (K_0 10^{-4}-10^{-9} mol/l). The steroid–receptor complex is translocated to the nucleus where it binds to steroid response elements on DNA. These act as promoter genes to influence mRNA transcription and thereby protein synthesis from a number of genes [1]. Changes in protein expression have multiple effects on the cell, some of which are direct (e.g. inhibition of phospholipase A_2 by glucocorticoid-induced synthesis of lipocortin [2]) and some of which depend on modulation of the response to other hormones (e.g. glucocorticoid-induced insulin resistance [3]).

Binding sites with the characteristics of classical glucocorticoid and mineralocorticoid receptors have been identified in vitro in homogenates of rabbit [4-7] and bovine [8] aorta and in cultured vascular smooth muscle cells from rat aorta [9] and human uterine artery [10]. Recently, the presence of mineralocorticoid receptors in vascular smooth muscle was confirmed immunohistochemically using an anti-idiotypic monoclonal antibody [11, 12]. Studies in vivo have shown binding of [3H]aldosterone in major vessels of dogs [13] and in rat mesenteric artery [14]. These binding sites seem to function as genomic promoters, since induction of glucocorticoid-responsive proteins has been demonstrated by two-dimensional gel electrophoresis in cultures of rat aortic vascular smooth muscle cells [15, 16] and bovine aortic endothelial cells [17] exposed to a variety of corticosteroids for 24 h. If the effects of corticosteroids on tone are mediated by activation of classical receptors, they should conform to certain characteristics. They typically have a time course from 20 min to several hours, involve the induction of expression of glucocorticoid-responsive genes and can be prevented by inhibitors of protein synthesis (e.g. actinomycin D or cycloheximide) or by competitive receptor antagonists (e.g. RU38486 for...
glucocorticoid receptors and spironolactone for mineralocorticoid receptors) [1].

In addition, some effects of corticosteroids may be independent of classical receptors and of steroid response elements ('non-genomic' effects; see Fig. 1). These include interactions between the lipid-soluble steroids and cellular lipid bilayer membranes, which induce effects over a faster time course than those resulting from genomic induction. Evidence suggests that this occurs in neural tissue [18], where recently a cell-surface receptor for corticosteroids was identified [19]. However, this mechanism is hard to confirm since specific antagonists are not available, and it has not yet been examined in vascular tissue.

Vascular responses to corticosteroids may not be uniform. Target-organ sensitivity to steroid hormones is not only dependent on the availability of receptors but also on the access of hormones to their receptors (Fig. 1). This access may be regulated by dissociation of the steroid from binding proteins [20], by local metabolism to inactive hormones and by the availability of final pathways for mediating the effect. Little information is available to suggest site-specificity in the distribution of receptors but we have recently shown that local metabolism of corticosteroids varies between different sites. The enzyme 11β-hydroxysteroid dehydrogenase converts cortisol to inactive cortisone and protects mineralocorticoid receptors in the kidney from excessive exposure to cortisol [21, 22]. Recently, 11β-hydroxysteroid dehydrogenase activity was described in rat vascular tissue [14] and we showed that it is particularly active in the resistance beds of mesentery and tail [23]. This may limit the access of cortisol to receptors and contribute to site-specific effects of corticosteroids in different vascular beds.

**CORTICOSTEROID EFFECTS ON VASCULAR TONE**

**Corticosteroid insufficiency**

The earliest studies highlighting the potential role of corticosteroids in the control of vascular tone examined vessel diameter in the exposed mesenteric vascular arcade of anaesthetized rats. In this model adrenalectomy had a profound vasodilating effect, which could be restored by topical administration of adrenocortical extract [24, 25]. Other investigators used the pressor response to catecholamine infusion as an indicator of vasoconstrictor sensitivity, and showed loss of pressor response to catecholamines after adrenalectomy in dogs [26, 27]. Interpretation of these studies is limited because they fail to account for the effect of glucocorticoids on cardiac output [28, 29] and because these animals also had excision of the adrenal medulla. Persuasive evidence that these changes were attributable to glucocorticoid deficiency came later with the demonstration that the pressor response to catecholamines was reduced in rats by administration of the specific glucocorticoid receptor antagonist RU38486 [30], and that the effect of adrenalectomy was reversed by replacement with the glucocorticoids dexamethasone [31] or corticosterone [32] but not by aldosterone [33].

Studies of the vascular response in humans with adrenal insufficiency are scarce because of the need for immediate therapy. While the postural hypotension associated with Addison's disease is in part attributable to depletion of intravascular volume, in adrenocorticotropic hormone (ACTH) deficiency there is often a significant postural fall in blood pressure which is independent of sodium balance and might be attributable to impaired vascular responsiveness. Mineralocorticoids may also be
involved in maintenance of tone in man, since administration of the mineralocorticoid receptor antagonist canrenoate led to hypotension associated with reduced peripheral vascular resistance [33].

Corticosteroid excess

Studies in vitro. A number of investigators have examined the effects of corticosteroids on isolated vessels in organ baths, sometimes with contradictory results. Steroids were added to baths for short periods of up to 20 min. They had no direct effects when added alone, except in one study when a dose of budesonide of $10^{-3}$ mol/l caused vasoconstriction [34]. In aortae from rabbit [35-38] and dog [39] deoxycorticosterone, corticosterone and hydrocortisone at doses of $10^{-3}$-10$^{-7}$ mol/l potentiated the constrictor response to catecholamines but not to other agents such as KCl, angiotensin II or vasopressin. These findings are broadly in keeping with observations in vivo described below. However, in doses of $10^{-4}$ mol/l, corticosteroids caused inhibition of vasoconstriction in response to all of these agents [34, 40, 41]. A dose-dependent biphasic effect was confirmed in vivo in studies of aorta and mesenteric artery in rats treated with high and low doses of hydrocortisone and methylprednisolone [40].

Interpretation of these studies in vitro is difficult. First, the dose of steroid was generally higher than would be achieved in vivo and inappropriate to the low $K_d$ of classical corticosteroid receptors or to the micromolar concentrations of steroids found in clinical glucocorticoid excess. Secondly, the time-course of the steroid effect observed was often rapid, within seconds or minutes. It seems likely that the results in vitro could comprise both classical receptor-mediated events and non-genomic effects of steroids on cell membranes. Which responses are relevant in vivo is hard to deduce. The major contribution of these studies has been to demonstrate that direct effects of corticosteroids on vessels do occur, and that effects in vivo are therefore not entirely dependent on secondary changes, such as altered renal sodium handling with suppression of angiotensin II production, or effects mediated by steroid actions in the central nervous system.

Studies in vivo. Clinical syndromes of glucocorticoid and mineralocorticoid excess provide a model from which many of the physiological functions of corticosteroids have been inferred. In Cushing’s syndrome due to adrenal adenoma, pressor responses to noradrenaline and angiotensin II [42] and digital vasoconstriction in response to noradrenaline [43] were increased. Systemic administration of cortisol or ACTH to healthy subjects mimicked these findings, except that the response to angiotensin II was not increased [44, 45]. These effects preceded the rise in blood pressure but took several days to develop [46]. Evidence is available to suggest that they reflect changes in vascular tone rather than in intravascular volume or cardiac output. First, the increase in the pressor response after oral cortisol was independent of sodium intake [47]. Secondly, brachial artery infusion of noradrenaline produced a greater fall in forearm blood flow after oral cortisol [48] and also after dexamethasone, deoxycorticosterone and fluorocortisone [49].

Local administration of glucocorticoids to specific vascular beds in vivo also affects tone. Infusion of hydrocortisone into the brachial artery had no direct effect on blood flow in the hand, but potentiated vasoconstriction in response to adrenaline [50]. Glucocorticoids cause vasoconstriction when applied to human conjunctiva [51, 52] or skin [53, 54]. Glucocorticoid-induced dermal vasoconstriction is now exploited in the skin vasoconstrictor assay, which is used to assess the relative potency of topical steroids [55]. This response was blocked by local [56] or systemic [57] glucocorticoid receptor antagonists and did not occur with mineralocorticoids.

Broadly similar observations have been reported in animal models. Systemic administration of dexamethasone to rats led to an increase in catecholamine vasoconstriction in isolated perfused mesenteric artery [58] and increased pressor responsiveness to noradrenaline [59, 60]. Vasoconstriction has also been observed after local application of glucocorticoids to vessels in rabbit ear [61] and rat hind limb [62].

In contrast with the lack of evidence that mineralocorticoid insufficiency affects vascular tone, mineralocorticoid excess has been associated with increased vascular reactivity. This was demonstrated in experimental mineralocorticoid excess in man by increased pressor responses [63-65] and increased catecholamine sensitivity of forearm vessels [49, 66]. Similarly, in animals sensitivity to catecholamines was increased in perfused caudal artery in deoxycorticosterone–salt hypertensive rats [67], and in aortae excised from deoxycorticosterone–salt hypertensive rabbits [68] or aldosterone hypertensive rats [69, 70]. In deoxycorticosterone–salt hypertension the change in vascular reactivity was shown to precede the elevation of blood pressure [71]. However, topical application or infusion of mineralocorticoids had no effect on vascular tone.

CORTICOSTEROID EFFECTS ON VASCULAR BIOCHEMISTRY

In summary, both glucocorticoids and mineralocorticoids have pharmacological effects which increase vascular tone. Furthermore, glucocorticoids, but probably not mineralocorticoids, appear to be required for the maintenance of normal tone. These effects are not direct, but rely on permissive potentiation of the response to catecholamines (particularly noradrenaline) and less consistently to other vasoactive agents studied (e.g. angiotensin II or vasopressin). By what biochemical mechanisms might these effects be mediated? In order to interact with other vasoactive hormones corticosteroids might influence (a) their local production and/or metabolism, (b) their membrane receptors and post-receptor second messengers or (c) the final effector mechanisms mediating contraction. Furthermore, these effects may occur in vascular smooth muscle cells themselves or in the vascular endothelium.
Some mechanisms of 'second messenger' transmission from cell membrane receptor to the functional response in vascular smooth muscle are illustrated in Fig. 2. Each of these elements is a potential target for one of the diverse proteins induced by corticosteroids.

**Effects on vascular smooth muscle cells**

**Hormone metabolism.** The earliest hypothesis to explain the vascular effects of corticosteroids was that they interfere with catecholamine metabolism, thus causing increased local catecholamine concentrations. At high dose *in vitro*, corticosteroids inhibited both extra-neuronal reabsorption of noradrenaline [72] and catechol-

**O-methyltransferase metabolism** [37, 38]. However, in a number of studies *in vivo* these effects could not be shown to be relevant [48, 62, 68, 73].

In the late 1970s and early 1980s the importance of the prostaglandin (PG) family in vascular biology emerged. Glucocorticoids inhibit phospholipase A₂ and thus reduce production of the vasorelaxant eicosanoids PGE₁ and prostacyclin [2, 74]. This action was invoked to explain the inhibition by glucocorticoids of ACTH-induced vasodilatation in rabbit fat pads [75] and may also indirectly explain changes in catecholamine sensitivity [74]. Glucocorticoid potentiation of catecholamine responses has been reproduced in a number of models by the phospholipase A₂/cyclo-oxygenase inhibitor indome-
thacain. These include human skin [76] and isolated perfused rat hind limb, where the effect of corticosterone was mimicked by indomethacin and abolished by excess arachidonic acid or prostacyclin [77]. The pressor response to noradrenaline in rats was potentiated by both indomethacin and by oral dexamethasone (0.1 mg/day), which reduced urinary PGE_2 excretion and attenuated the effect of indomethacin [59]. Urinary PGE_2 excretion was similarly reduced in patients with Cushing’s syndrome [42]. However, other investigators have not been able to confirm a pharmacological effect of glucocorticoids on vascular PG production in vivo [78]. Furthermore, when the dexamethasone given to rats was reduced to a sub-pharmacological dose which was not associated with weight loss but still increased blood pressure, i.e. 2 µg/day subcutaneously [79], an increased response to noradrenaline in the excised perfused mesenteric circulation was not affected by indomethacin and was not associated with a change in prostacyclin metabolites (6-keto-PGF_1α) in the effluent perfusate [58]. Also the activation of adenylyl cyclase by PGE_1, which usually produces relaxation, was potentiated by dexamethasone in vascular smooth muscle cells (see below and [80]). Thus involvement of PGs remains unexplained when physiological concentrations of glucocorticoids are administered in vivo.

Receptors and second messengers. In membranes from rat aorta the number of α₁-adrenoceptors was decreased by adrenalectomy and restored by dexamethasone replacement [81]. Similarly, in cultured rat aortic vascular smooth muscle cells the number of β-adrenoceptor-binding sites was increased by dexamethasone at physiological concentration over 20 h of incubation [82]. In addition to effects on receptor number, in the absence of glucocorticoid the coupling of adrenoceptors to guanine nucleotide-binding proteins (G-proteins) was disturbed [81] and the ratio of G_i (inhibitory) to G_s (stimulatory) subunits fell [83]. Functional significance of these observations is suggested by studies in vascular smooth muscle cells which confirmed that dexamethasone potentiated the production of inositol trisphosphate in response to noradrenaline (an α₁-adrenoceptor effect) [84], and that concomitant with the increase in β-adrenoceptor number there was a greater cyclic AMP response to β-adrenoceptor agonists [82]. This latter effect was blocked by cycloheximide and RU38486, suggesting that it is mediated through glucocorticoid receptors. Relevance to the intact animal is suggested by increased generation of inositol monophosphate by femoral artery tissue slices from deoxycorticosterone-salt hypertensive rats after incubation with noradrenaline [85]. Interestingly, one group described a direct effect of cortisol alone to increase inositol trisphosphate concentrations in cultured cells. This was blocked by RU38486, and was further increased by noradrenaline [86, 87]. However, in most laboratories direct effects of steroids on these second messengers have not been observed.

Changes in adrenoceptor number and function are not the only relevant effects of glucocorticoids on second messenger systems. Glucocorticoids affect cyclic AMP synthesis induced by other stimuli in other tissues [88]. The cyclic AMP responses to dopamine (DA) [89] and PGE_1[80] in cultured vascular smooth muscle cells from renal artery were increased by dexamethasone. These effects were maximal at 24 h, occurred at appropriate concentrations of dexamethasone but not aldosterone, and were inhibited by actinomycin D and cycloheximide [80, 89]. The response to forskolin (a direct activator of the catalytic unit of adenylyl cyclase) was increased, suggesting that the effect of glucocorticoid was not only post-receptor but post-G-protein [80, 89]. Phosphodiesterase inhibition was not implicated, since isobutylmethylxanthine did not interfere with the potentiation. Increased cyclic AMP levels would be expected to relax vessel tone, but in the same cells reduced cyclic GMP generation in response to atrial natriuretic peptide (ANP) after dexamethasone provides a possible contractile effect [90]. Similar insensitivity to ANP was seen in aortae from mineralocorticoid hypertensive rats [91]. These observations, together with the suppression of ANP production by glucocorticoids in vivo [92], suggest that recently recognized vasoactive agents may be highly significant in mediating corticosteroid effects.

Final common pathway of contraction. Early studies undertaken to determine the effects of corticosteroids (particularly mineralocorticoids because of the analogy with their effects on mucosal Na^+/K^+ exchange) focused on changes in vascular electrolyte permeability. Chronic administration of deoxycorticosterone to rabbits was associated with increased Na^+ influx into vascular smooth muscle cells [5], but administration of aldosterone to pig carotid arteries in vitro resulted in a fall in intracellular Na^+ concentration [93]. In rat tail artery, aldosterone had two actions: (1) a rapid increase in Na^+ efflux, which was not blocked by actinomycin D, and (2) a slow Na^+ efflux, which does appear to be receptor-mediated since it was blocked by actinomycin D and mineralocorticoid receptor antagonists [94]. These effects may in part result from potentiation of a response to vasopressin [95]. They are not exclusive to mineralocorticoid receptor agonists, but the change in Na^+ efflux exchange seen after glucocorticoids [96] appears to be mediated by a different ion transporter, since it was blocked by both amiloride and bumetanide, in contrast to the mineralocorticoid effect which was only blocked by amiloride [97].

Effects on endothelial cells

In recent years the endothelium has been the focus of research attention in vascular biology. A significant advance was the identification of 'endothelium-derived relaxant factor' as nitric oxide [98]. In addition to the Ca^{2+}-dependent calmodulin-dependent constitutive nitric oxide synthase of endothelium, there is another Ca^{2+}-independent endotoxin-inducible enzyme abundant in macrophages [99] and recently described in vascular endothelium [100] and smooth muscle [101, 102]. This inducible enzyme is inhibited by glucocorticoids [99, 101], an effect which is blocked by glucocorticoid receptor antagonists [100]. A recent study in vitro by Rees et al. [103] demonstrated the functional consequences of this
effect. In untreated rat aortic rings a time-dependent relaxation and loss of response to phenylephrine occurred between 2 and 8 h after starting the experiment, in rings both with and without endothelium. This was potentiated by nitric oxide synthase substrate (L-arginine), prevented by a nitric oxide synthase inhibitor (Nω-nitro-L-arginine) and associated with endotoxin induction of Ca^{2+}-independent nitric oxide synthase. Administration of dexamethasone at 10^{-7} mol/l over 8 h prevented this relaxation and loss of response to phenylephrine and inhibited the induction of nitric oxide synthase in vascular smooth muscle. This study was superior to any previous organ bath experiments because the time-course and dose of steroid application were more physiologically relevant (see above). However, although inhibition of nitric oxide synthase in human forearm caused vasoconstriction [104], implying that basal nitric oxide production is important, it remains to be seen whether the inductive enzyme makes a significant contribution in vivo.

The effects of glucocorticoids on PG synthesis described above apply similarly to endothelial cells. Glucocorticoids also induce angiotsintin-converting enzyme in endothelial cells [105]. The importance of the tissue renin-angiotensin system in controlling tone is unknown [106], but corticosteroids may provide a tool with which to examine it further.

SIGNIFICANCE OF THE NEW MESSENGER

The evidence that corticosteroids affect vascular tone is thus persuasive and is now supported by studies showing steroid-receptor-mediated effects on vascular biochemistry. However, a confusing variety of biochemical actions of corticosteroids has emerged and it remains difficult to develop a unifying model to explain their role in the control of vascular tone. While the predominant effect when tone has been measured is constriction, glucocorticoids have biochemical actions with the potential for both vasoconstriction (e.g. nitric oxide synthase inhibition, reduced prostacyclin production, increased phosphoinositide pathway products, increased α-adrenoceptor numbers, decreased cyclic GMP generation) and dilatation (e.g. increased cyclic AMP responses, increased β-adrenoceptor numbers). Corticosteroids may also act by non-genomic mechanisms which could potentially oppose their genomic effects (e.g. vasodilatation at high dose).

It is a characteristic of vascular physiology that responses may be site-specific. It seems most likely that a balance of opposing effects exists with the result depending on the local milieu. Examples include the well-established differential distribution of adrenoceptors in different vascular beds, and the more recently appreciated differences in basal nitric oxide production between arteries and veins [104, 107]. In addition to these we might now add the potential for site-specific sensitivity to corticosteroid hormones offered by the combination of variable distribution of steroid-metabolizing enzymes, receptors and target mechanisms. The most significant differences between corticosteroids and other vasoactive hormones are their lack of direct effect, their long time-course and their diverse effector mechanisms. These features suggest that corticosteroids can play a role not served by short-acting rapidly metabolized mediators with local actions, in providing a medium-term pattern of vascular responsiveness which is then adapted locally and in the short-term by other hormones. Another recently described vasoactive agent with a relatively long time-course is endothelin [108]. Interactions between corticosteroids and endothelin have not been described.

Clinical inferences from these observations remain limited. The mechanism for the hypertension observed with glucocorticoid excess has been discussed extensively elsewhere. The role of increased peripheral vascular resistance remains uncertain [42], although it is worth noting that in animal models increased vasoconstriction occurred before the rise in blood pressure [59, 71, 109]. Similarly, extensive debate has been conducted on the role of corticosteroids both in the pathogenesis and therapy of circulatory shock. Observations in vivo were evoked in this debate by proponents of endotoxin-induced nitric oxide production as a pathogenic mediator of circulatory failure in endotoxic shock [110] and in hepatic cirrhosis [111]. They have suggested that inhibition of nitric oxide production by dexamethasone should encourage the use of glucocorticoids in shock, although the data in vitro show prevention rather than reversal of endotoxin-induced nitric oxide synthesis, and therapeutic benefit of glucocorticoids in endotoxic shock remains unproven [40, 112–114].

CONCLUSIONS

The hypothesis that corticosteroids are important in the control of vascular tone has been with us for many years but remains difficult to confirm. In recent years we have been offered a wide variety of biochemical clues which increase our confidence that glucocorticoids have important effects to be explained, but also alert us to the diversity and complexity of the mechanisms involved. To account for these new findings glucocorticoids are best perceived in a permissive role setting a medium-term pattern which may dictate site-specific responses to short-acting hormones. With the development of tools to extrapolate these biochemical observations to experiments in vivo will follow a better understanding of the role of corticosteroids in sickness and in health.

ACKNOWLEDGMENTS

We thank Drs David Webb and Bill Haynes for their helpful comments. B.R.W. is a Medical Research Council Training Fellow, and our work is sponsored by the British Heart Foundation.

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