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A novel role for the mineralocorticoid receptor in glucocorticoid driven vascular calcification

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ABSTRACT

Vascular calcification, which is common in the elderly and in patients with atherosclerosis, diabetes and chronic renal disease, increases the risk of cardiovascular morbidity and mortality. It is a complex, active and highly regulated cellular process that resembles physiological bone formation. It has previously been established that pharmacological doses of glucocorticoids facilitate arterial calcification. However, the consequences for vascular calcification of endogenous glucocorticoid elevation have yet to be established. Glucocorticoids (cortisol, corticosterone) are released from the adrenal gland, but can also be generated within cells from 11-keto metabolites of glucocorticoids (cortisone, 11-dehydrocorticosterone [11-DHC]) by the enzyme, 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1). In the current study we hypothesized that endogenous glucocorticoids facilitate vascular smooth muscle cell (VSMC) calcification and investigated the receptor-mediated mechanism underpinning this process.

In vitro studies revealed increased phosphate-induced calcification in mouse VSMCs following treatment for 7 days with corticosterone (100 nM; 7.98 fold; $P < 0.01$), 11-DHC (100 nM; 7.14 fold; $P < 0.05$) and dexamethasone (10 nM; 7.16 fold; $P < 0.05$), a synthetic glucocorticoid used as a positive control. Inhibition of 11β-HSD isoenzymes by 10 μM carbenoxolone reduced the calcification induced by 11-DHC (0.37 fold compared to treatment with 11-DHC alone; $P < 0.05$). The glucocorticoid receptor (GR) antagonist mifepristone (10 μM) had no effect on VSMC calcification in response to corticosterone or 11-DHC. In contrast, the mineralocorticoid receptor (MR) antagonist eplerenone (10 μM) significantly decreased corticosterone- (0.81 fold compared to treatment with corticosterone alone; $P < 0.01$) and 11-DHC-driven (0.64 fold compared to treatment with 11-DHC alone; $P < 0.01$) VSMC calcification, suggesting this glucocorticoid effect is MR-driven and not GR-driven. Neither corticosterone nor 11-DHC altered the mRNA levels of the osteogenic markers *PiT-1*, *Osx* and *Bmp2*. However, DAPI staining of pyknotic nuclei and flow cytometry analysis of surface Annexin V expression showed that corticosterone induced apoptosis in VSMCs.

This study suggests that in mouse VSMCs, corticosterone acts through the MR to induce pro-calcification effects, and identifies 11β-HSD-inhibition as a novel potential treatment for vascular calcification.

1. Introduction

Vascular calcification is a marker of increased cardiovascular disease risk in aging, including in diabetes, atherosclerosis and chronic kidney disease (CKD) [23,50]. The etiology of mineral accumulation within the vasculature shares many similarities with that of bone formation. Indeed, several studies have reported that vascular smooth muscle cells (VSMCs), the predominant cell type involved in vascular calcification, can undergo phenotypic transition to osteoblastic, chondrocytic and osteocytic cells in a calcified environment [16,49]. Furthermore, phosphate accelerates this trans-differentiation process, with the loss of characteristic smooth muscle markers and the increased expression of osteoblastic markers (e.g. Osterix, PiT-1 and BMP2) [28,31,51]. Vascular calcification can also proceed through mechanisms involving the reciprocal loss of recognized calcification inhibitors including inorganic pyrophosphate (PPi), fetuin A and Matrix Gla Protein [16,25,30,36].

Physiological glucocorticoids — primarily cortisol in humans and corticosterone in rats and mice — are steroid hormones produced by the adrenal cortex. Local glucocorticoid action on target tissues is determined by intracellular metabolism by the two isozymes of 11β-hydroxysteroid dehydrogenase (11β-HSD) which catalyze interconversion of active cortisol and corticosterone with inert cortisone and 11-dehydrocorticosterone [11]. 11β-HSD type 1, a predominant reductase

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in most intact cells, catalyzes the regeneration of active glucocorticoids, thus amplifying cellular action. 11β-HSD2 is a high-affinity dehydrogenase that inactivates glucocorticoids [4]. Both isozymes of 11HSD are modestly expressed in the blood vessel wall, suggesting that they can influence vascular function by regulating local availability of active glucocorticoids [11].

In the absence of 11β-HSD2, endogenous glucocorticoids can bind to the mineralocorticoid receptor (MR) as well as the glucocorticoid receptor (GR) [37]. Both MR and GR belong to the same nuclear hormone receptor superfamily, and share high sequence identity. MR has higher affinity for glucocorticoids than GR, and both receptors are expressed in the cells of the vasculature [10]. Glucocorticoids can activate the MR in VSMCs (11β-HSD2 is not expressed here), inducing pathways that are central to cell proliferation and differentiation [27].

Glucocorticoids are frequently permissive, co-operative or synergistic [48]. Indeed a permissive role of glucocorticoids in triggering cell transdifferentiation has previously been established in the conversion of pancreatic cells into hepatocytes [38]. Furthermore, glucocorticoids exert complex actions on calcium mobilisation and bone metabolism, regulating bone resorption and formation [13], intestinal calcium absorption and renal calcium excretion [8]. Therefore it is essential to establish the consequences for vascular calcification of endogenous glucocorticoid elevation given the high circulating calcium levels commonly observed in patients with this pathology [2].

Dexamethasone, a potent synthetic glucocorticoid which is primarily active at the GR, induces an osteoblastic differentiation pathway in many different mesenchymal-derived cell types *in vitro* [1,5,47], including VSMCs [29,39]. Whilst the established pro-calcification actions of dexamethasone on VSMCs [18,29] are presumed to be mediated *via* GR, plausibly endogenous corticosteroids may modulate VSMC calcification *via* MR. This is therapeutically important to ascertain, as vascular calcification is independently correlated with adverse cardiac events [50], and MR antagonism is highly successful in reducing mortality in heart failure [45]; aldosterone antagonists such as spironolactone and eplerenone have been shown to improve cardiovascular outcomes and prevent ischaemic events in cardiovascular patients [32,33]. Corticosterone has been shown to induce rapid MR signaling in VSMCs that involves mitogen-activated protein kinase kinase (MEK)/extracellular signal-regulated kinase (ERK)-dependent pathways, suggesting that glucocorticoids may contribute to vascular disease *via* MR receptor signaling [27]. Recent studies have shown that aldosterone-induced activation of MR promotes osteoblastic differentiation and calcification of VSMCs [15] through a mechanism involving the stimulation of spironolactone-sensitive, PiT-1 dependent signaling [46].

An additional level of control over endogenous corticosteroid action is provided by the HSD isoenzymes, whose role in vascular calcification has yet to be elucidated. The induction of local glucocorticoid generation through increased 11β-HSD1 expression (>10 fold) and activity (>4 fold) by inflammatory cytokines and glucocorticoids is well documented in fibroblasts and osteoblasts [19,43], which both have the capacity to calcify [3,42].

It is therefore essential to establish the consequences for vascular calcification of endogenous glucocorticoid elevation and potential strategies for inhibition of calcification. The aims of this study were to undertake *in vitro* murine VSMC calcification studies to investigate both the

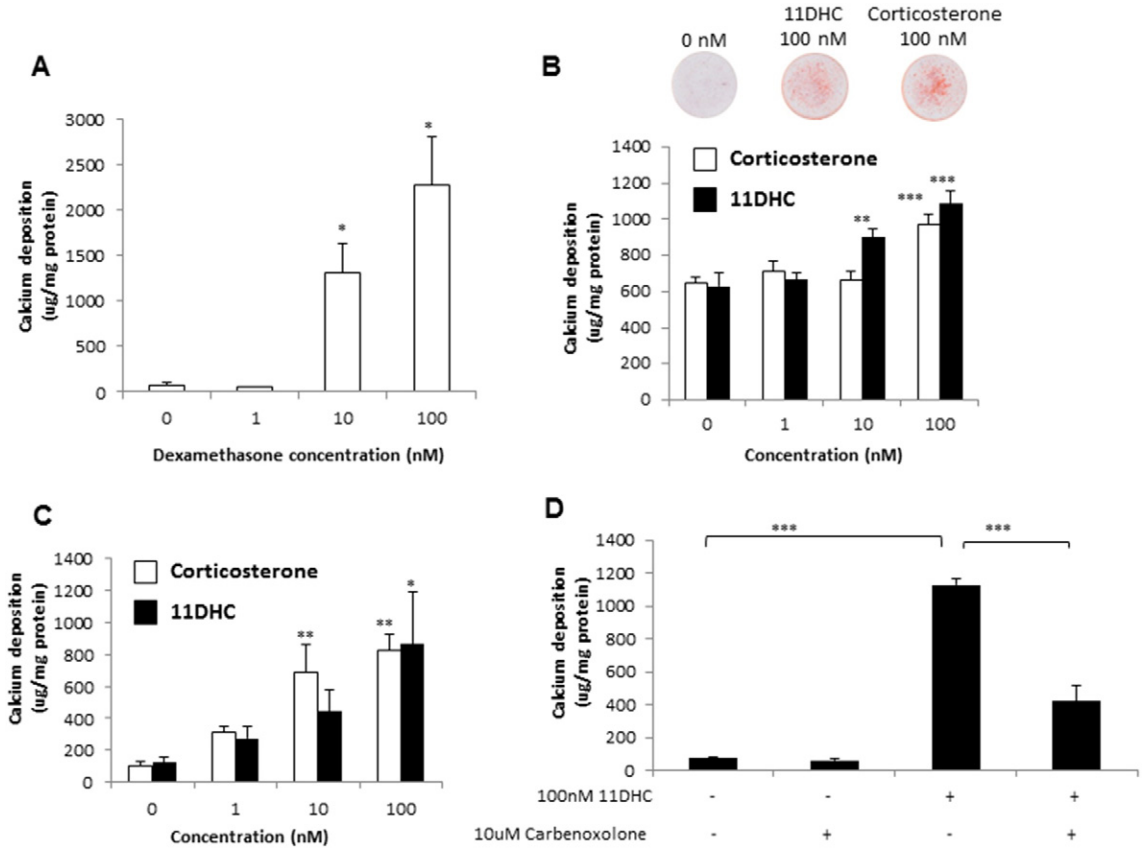

Fig. 1. Dexamethasone, corticosterone and 11-DHC all glucocorticoids induce VSMC calcification. Effect of (A) dexamethasone (1–100 nM), (B, C) corticosterone (white bar) and 11-dehydrocorticosterone (11DHC; filled bar) (1–100 nM) in the presence of standard and charcoal-stripped fetal bovine serum (FBS) respectively and (D) carbenoxolone (10 μM) in the presence or absence of 11DHC (100 nM) on calcium deposition in VSMCs cultured in high phosphate (Pi) (3 mM Pi) for 7 days, as determined by alizarin red staining and/or quantitative HCL leaching (μg/mg protein) ($n = 6$). Results are presented as mean ± S.E.M. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ compared with corresponding 0 nM treatment.