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Systemic and local ACTH produced during inflammatory states promotes osteochondrogenic mesenchymal cell differentiation contributing to the pathologic progression of calcified atherosclerosis

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Abstract

There are many well-known roles for the proopiomelanocortin (POMC) derived peptides and their receptors, the melanocortin receptors (MC-R). The focus here is on the evolving role of the melanocortin system in inflammation. Chronic inflammatory states such as those occurring in diabetes and obesity are associated with both a hyperactive hypothalamic-pituitary-adrenal (HPA) axis as well as increased incidence of atherosclerosis. An inflammation-induced hyperactive HPA axis along with increased leukocyte infiltration can lead to significant exposure to melanocortin peptides, particularly ACTH, in an inflamed vasculature. Mesenchymal progenitor cells are present throughout the vasculature, express receptors for the melanocortin peptides, and respond to ACTH with increased osteochondrogenic differentiation. Coupled to the increased exposure to ACTH during HPA hyperactivity is increased glucocorticoid (GC) exposure. GCs also promote chondrogenic differentiation of mesenchymal progenitors and increase their expression of MC-R as well as their expression of POMC and its cleavage products. It is hypothesized that during inflammatory states systemically produced ACTH and glucocorticoid as well as ACTH produced locally by macrophage and other immune cells, can influence and potentiate mesenchymal progenitor cell differentiation along the osteochondrogenic lineages. In turn the increase in osteochondrogenic matrix contributes to the pathophysiological progression of the calcified atherosclerotic plaque. The roles of the melanocortin system in inflammation and its resolution have just begun to be explored. Investigations into the ACTH-induced matrix changes among mesenchymal cell populations are warranted. ACTH signaling through the MC-R represents a new therapeutic target for the prevention and treatment of calcified atherosclerosis.

Keywords

proopiomelanocortin; diabetes; obesity; mesenchymal progenitors

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Atherosclerosis in diabetes and obesity; a link to inflammation and HPA hyperactivity

There is a significant clinical correlation between increased stress and cardiovascular risk factors [15, 39]. Stress plays a significant role in susceptibility, progress and outcome of cardiovascular disease. Correlations between elevated stress and the development of atherosclerosis have been well documented [3, 30, 32]. The endocrine response to stress is manifest in the HPA axis and many clinical correlations between HPA axis dysregulation and atherosclerosis exist.

Diabetes and obesity are well known significant risk factors for the development of atherosclerosis. These diseases are characterized by a hyperactive HPA axis. In uncontrolled or poorly controlled diabetes upregulation of the HPA axis [6] is related to insulin deficiency as opposed to hyperglycemia; basal ACTH and corticosterone levels in streptozotocin (STZ) treated rats are normalized with insulin but not phloridzin, a specific blocker of glucose absorption in the small intestine [7]. There is also an increased stress response associated with obesity. In fact glucagon administration or the ingestion of a high carbohydrate meal will elicit an exaggerated HPA response, with both elevated ACTH and cortisol [14, 31, 40, 51, 56].

In addition to metabolic load, the HPA responds to inflammatory states like diabetes and obesity with an increase in activity. Increased production of inflammatory cytokines is associated with increased stimulation of the HPA axis [28, 44]. These findings provide a link between HPA dysregulation and the development of atherosclerotic cardiovascular disease in diabetes and obesity.

POMC peptides and MC-R; Expression in immune cells and roles in inflammation

POMC is the precursor peptide to ACTH and the melanocortin family of endocrine peptides. POMC is traditionally known as an endocrine prohormone secreted and processed by corticotropes of the anterior pituitary in response to hypothalamic corticotrophin releasing hormone (CRH). POMC is processed by prohormone convertases (PCs) into the melanocortin peptides. Cleavage via PC1/3 produces adrenocorticotropic hormone (ACTH) and β-lipotropic hormone (β-LPH), whereas PC2 is required for the generation of α-melanocyte-stimulating hormone (α-MSH), β-MSH, γ-MSH and the endorphins [1, 4, 9].

The receptors for the melanocortin peptides are a family of G-protein coupled receptors, collectively known as MC-R. To date, five MC-R have been identified and termed MC1-R to MC5-R. The MC-R can be distinguished through their agonist profiles. α-MSH is the primary agonist for MC1-R, MC4-R and MC5-R. The MC2-R or ACTH receptor is only activated by ACTH and γ2-MSH is most potent at the MC3-R. Ligand binding to the MC-R results in the activation of second messenger pathways such as accumulation of adenylyl cyclase and the mobilization of intracellular calcium ($[Ca^{2+}]_i$) [18, 20, 26, 58].

The MC-R are found both in the central nervous system (CNS) and throughout the body. Traditional roles for the MC-R have been well-established. In melanocytes MC1-R regulates the eumelanin-pheomelanin switch [33]. Activation of the MC1-R leads to increased pigmentation in the skin. MC-2-R regulates adrenocortical steroidogenesis when stimulated by ACTH [9]. MC3-R and MC4-R are highly expressed by hypothalamic neurons and are known to play a role in satiety and energy balance [2, 9, 49]. The MC5-R is the most ubiquitously expressed melanocortin receptor and primarily known for its functional role in exocrine tissues [9, 55].

Recently, there has been an increased focus on the role the melanocortin system plays in the resolution of inflammation. This area of investigation encompasses anti-inflammatory effects in the brain, gouty arthritis and during reperfusion injury [4, 5, 21, 22, 25, 34, 36, 37, 41]. The MC1-R, MC3-R and MC5-R have been implicated as the mediators of these actions in immune cells. In response to inflammatory mediators, leukocytes produce melanocortin peptides, i.e. ACTH and α-MSH, which then act in an autocrine fashion to reduce inflammatory cytokine production and leukocyte trafficking during inflammation [4, 22, 34, 41]. Therefore a damaged hypertensive vasculature in diabetes and obesity is not only exposed to systemic elevations in ACTH and glucocorticoid, but local elevations of ACTH via production by leukocytes.

ACTH and glucocorticoid promote mesenchymal progenitor differentiation along the osteochondrogenic pathway

MC-Rs are expressed in mesenchymal progenitor cell populations and when exposed to ACTH undergo osteochondrogenic differentiation. Mouse MSC express the MC2-R and these cells when exposed to ACTH display enhanced osteogenic differentiation [29]. We have detected the expression MC2-R, MC3-R and MC5-R in rat bone marrow derived mesenchymal progenitors [17]. When these cells are exposed to ACTH they differentiate along the chondrogenic pathway [17] (and data in submission). Some data also suggests that glucocorticoid plays a role in osteochondrogenic mesenchymal differentiation. Dexamethasone up regulates osteoblast-specific transcription factors such as *Cbfa1* and Osterix (*Osx*) in rat calvariae progenitor cells [35] while it can also up regulate chondrogenic transcription factors such as *Sox9* and promote chondrogenic differentiation in human mesenchymal stem cells [11].

Calcified atherosclerosis; a result of osteochondrogenic differentiation

Key bone metabolism proteins and bone related cells are found at sites of vascular calcification [12, 13]. Recent evidence also suggests that calcification of atherosclerotic plaques is mediated by chondrocyte-like cells undergoing terminal endochondral differentiation [8, 19, 42, 43, 53, 54]. Many of the characteristic ECM components and transcription factors expressed by chondrocytes are also expressed in calcified atherosclerotic lesions of both humans and rodents [8, 19, 42, 43, 53, 54].

Hypothesis

These data, outlined above, have led us to hypothesize that during inflammatory states like diabetes and obesity systemically produced ACTH and glucocorticoid as well as ACTH produced locally by macrophage and other immune cells can influence and potentiate vascular mesenchymal progenitor cell differentiation along the osteochondrogenic lineages. In turn the increase in osteochondrogenic matrix contributes to the pathological progression of the calcified atherosclerotic plaque.

da Silva Mierelles *et al.* have postulated that mesenchymal stem cells (MSC) are located just below the endothelial lining [10] and therefore represent a source of osteochondrogenic progenitors in atherosclerosis. In diabetes and obesity hypertension can lead to endothelial damage in the vasculature. After this damage, both vascular smooth muscle cells (SMC) and vascular mesenchymal progenitors are in direct contact with circulating hormones and infiltrating macrophage cells. This has been confirmed by immunohistochemical and electron microscope analysis of human atherosclerotic plaques [46].

The ability of ACTH to induce transient elevations in intracellular calcium ($[Ca^{2+}]_i$) and raise basal $[Ca^{2+}]_i$ in mesenchymal progenitors provides a mechanism for ACTH effects. Factors that increase the differentiation of both chondrocytes and osteoblasts from progenitors also increase $[Ca^{2+}]_i$, [45, 48, 57, 59]. In fact calcium ionophore induced elevations in $[Ca2+]_i$ can promote chondrogenesis though a calcineurin/nuclear factor of activated T-cells (NFAT) axis [52]. We have demonstrated that both mesenchymal progenitors from rat bone marrow (in submission) and mouse aorta-derived mesenchymal progenitors respond to ACTH with transient increases in $[Ca^{2+}]_i$ [16].

One might argue that the concentrations of ACTH used in the *in vitro* studies were high relative to those reported as circulating levels in the diabetic and obese. However, as described above local vascular exposure is likely increased due to autocrine and paracrine production by leukocytes. The ACTH precursors POMC and pro-ACTH are also present in the human circulation at concentrations 5-fold greater than that of ACTH [50] and these peptides have the potential to be processed peripherally [47]. The ingestion of a meal and bouts of hyperphagia also elevate circulating ACTH levels [23]. Contrasts in exposure time should also be considered when evaluating the *in vitro* results. *In vivo*, mammals experience a continual systemic exposure to ACTH with periodic spikes or pulses. In humans ACTH secretion has a 24 h mean pulse frequency of 18 in men and 10 in women, with a mean peak amplitude of 3.7 and 2.3 pmol/L, respectively [27]. With diabetes these values can increase 10-fold at the diurnal peak [38] in addition to increases that occur after ingestion of a meal. In the *in vitro* studies examining the influence of ACTH on osteochondrogenesis cells were exposed to one pulse of ACTH, every two to three days with the lowest effective dose tested being 10 nM. Therefore, although the dose of ACTH was relatively high, the frequency of exposure was much lower.

Conclusion

The development of atherosclerosis and subsequent increased risk of cardiovascular disease events is a significant health problem in today's Western world. Atherosclerotic lesions frequently become calcified and calcium deposition in atherosclerotic lesions can increase the likelihood of adverse cardiovascular advents. Further understanding of mechanisms responsible for vascular calcification could significantly improve current cardiovascular disease prevention and treatment strategies. ACTH signals through the MC-R; a family of G-protein coupled receptors (GPCR). GPCR's and components of G-protein effector pathways are excellent therapeutic targets. Fifty percent of existing pharmaceuticals target specific GPCRs [24]. Therefore if the current hypothesis is confirmed ACTH signaling through the MC-R represents a new therapeutic target in the prevention and treatment of calcified atherosclerosis.

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