Focused Perspective

Cyclic AMP Response Element-Binding Protein in the Vessel Wall
Good or Bad?

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atherosclerosis and postangioplasty restenosis are leading causes of death in the Western world. Recent advances in our understanding of vascular biology would suggest that targeting specific signals in the post–balloon injury vessel or in persons prone to development of atherosclerosis could decrease lesion formation and in the long term decrease cardiovascular events. In light of this, numerous laboratories around the world are investigating the functional activities of the various cellular components of the atherosclerotic plaque, including vascular smooth muscle cells (SMCs), endothelial cells (ECs), and monocyte/macrophages.

One of the strategies for examining vascular wall response to injury in animal models has been the use of the balloon injury model. In this model, a balloon catheter is used to cause an injury and denuding of the endothelium. The animals are monitored serially over 7, 14, and 28 days for narrowing of the blood vessel lumen after injury. This model traditionally has been used to examine activation of SMCs and their proliferative response. In response to balloon injury, vascular SMCs (which are normally contractile and quiescent [nonproliferative]) are released into a proliferation phase by the injury and loss of the endothelium. Immunostaining studies have suggested that the majority of the cells that cause luminal narrowing are SMCs. However, recent studies have also demonstrated the presence of inflammatory cells in this model. Macrophages are observed both in the balloon injury model and in balloon injury/stent models. Thus, interfering with either SMC function or macrophage activation would be expected to have an impact on restenosis.

Agents that can be delivered either pharmacologically or directly at the time of angioplasty to decrease neointimal proliferation are considered to have therapeutic promise. The most useful are agents that decrease SMC proliferation. In the present issue of Circulation, Tokunou et al present a series of experiments that indicate that delivery of a DNA-binding dominant negative isofrom of the transcription factor CREB (cyclic AMP response element-binding protein) at the time of balloon injury leads to a decrease in neointimal thickening. At first glance, this study may appear to contradict our recent reports that CREB restrains mitogen-stimulated SMC proliferation and migration. In the present commentary, we will review the available data on CREB in macrophages and SMCs and offer our view of the studies by Tokunou et al.

CREB in Vascular Tissues

CREB is a 43-kDa nuclear transcription factor in the beta leucine zipper family of transcription factors, and it has been shown to have important functions in differentiation of numerous target tissues, including adipocytes, vascular SMCs, neurons, and cardiac myocytes. One additional function of CREB is the prevention of apoptosis. This has been carefully outlined in neuronal cells, fat cells, and most recently, beta cells. Additional unpublished studies suggest that CREB may also play a pivotal role in terminal macrophage differentiation (C.K. Glass, MD, Professor of Medicine, University of California San Diego, personal communication, 2003). No published studies have examined CREB in ECs. Given the potent impact of CREB on numerous vascular cell types, CREB would be expected to have pleiotropic effects on the vessel wall.

SMC proliferation is the primary end point for most investigators studying the balloon injury/restenosis model. The question is what the expected impact of CREB on vascular SMC proliferation and migration would be. Our laboratory has demonstrated both in vivo and in vitro that CREB content correlates negatively with proliferation. In addition, expression of active CREB decreases mitogen-stimulated proliferative capacity and migratory capacity. Expression of dominant negative CREB augments the ability of platelet-derived growth factor to stimulate proliferation and migration in vitro. CREB decreases expression of numerous cell cycle–regulatory proteins, as well as expression of the growth factor receptors endothelin, endothelin 1 receptor, and platelet-derived growth factor receptor α. In the study by Tokunou et al in the present issue of Circulation, the authors demonstrate in vitro that adenoviral delivery of dominant negative CREB into SMCs in culture leads to increased smooth muscle apoptosis and no appreciable change in entry through cell cycle.

So how do we resolve these divergent and paradoxical results? First, we should consider the noncontroversial aspects of this work. The fact that interference with CREB...
activity through a dominant negative isoform of CREB would augment apoptosis is an expected result, consistent with studies conducted in our laboratory (J.E.B. Reusch, MD, and P.A. Watson, PhD, unpublished data, 2003) and in numerous other cell types and cell culture systems. The second issue, the apparent discrepancy between acute CREB activation of prothrombotic genes (thrombin) and restraint of mitogen-stimulated proliferation, could be understood as follows. Loss of CREB function in unstimulated SMCs in culture does not affect proliferation in a robust way (observed by both authors interpret this decrease in proliferative index as evidence that we don’t yet know. The more speculative response (Figure 1) is that CREB is an immediate early gene, which responds in concert with AP1 factors and nuclear factor-κB to numerous toxic stimuli, not only in vascular SMCs, but also in other cell types such as neurons, beta cells, fat cells, and various lymphocytes. In the context of metabolic and cytokine stress, we have observed in vitro and in vivo that there is an acute and robust activation of CREB and numerous other inflammatory cytokines and immediate early genes. With time, chronic metabolic stress leads to loss of both acute signaling to CREB through the AKT signaling pathway and decreased CREB expression in numerous target tissues, including the heart, the vasculature, and the nervous system. An important study by Mabuchi et al15 demonstrated that acute neuronal injury to the brain activated cyclic AMP response element (CRE)-responsive genes very robustly. This was interpreted to be a cytoprotective response. It is also likely that the acute activation and phosphorylation of CREB with balloon injury is a cytoprotective response and that CREB working in concert with other immediate early genes is an appropriate response to injury.

Conclusions

The article in the present issue of Circulation by Tokunou et al3 demonstrates that adenoviral delivery of DNA-binding dominant negative CREB M1 at the time of balloon injury leads to decreased neointimal lesion formation and slows down restenosis. Our interpretation of these data is that dominant negative CREB acts by increasing apoptosis of ECs and vascular SMCs and interfering with recruitment and activation of macrophages. Because this CREB M1 vector decreases intimal lesions, it could be a reasonable pharmacological target. In contrast, the interpretation that adenoviral...
delivery of that dominant negative CREB interferes with SMC proliferation is unlikely, according to their in vitro studies and the literature. Transgenic or SMC-specific adenoviral delivery of CREB would be necessary to clear up any remaining controversy. In order to leave the reader with a more general understanding of how CREB may be contributing to the vascular lesion, the model shown in Figure 2 is proposed.

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References

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