Vasoconstriction Caused by the ATP Synthase Subunit–Coupling Factor 6
A New Function for a Historical Enzyme

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Enhancement of arterial vasoconstriction and depression of endothelial cell–dependent relaxation have been reported by many investigators as characteristics of vascular dysfunction in hypertension. These dysfunctions support the increase in total peripheral resistance observed in experimental and human forms of hypertension. Because arterial smooth-muscle cell hyperreactivity and reduced vasoactive function of the endothelial cell are observed in response to multiple stimuli (eg, serotonin, norepinephrine, KCl in smooth muscle; acetylcholine, bradykinin, A23187 in endothelial cell) and are thus not necessarily agonist-specific, researchers have investigated the idea that more general mechanisms of signal transduction are altered and support global changes in vascular function. These include alterations in composition and function of elements as diverse as potassium channels, calcium channels, sodium-potassium ATPase, G-proteins, membrane lipid composition, etc. In this issue of Hypertension, the study by Osanai et al reveals a new function for a protein subunit of an enzyme that is ubiquitously expressed in all tissues, the ATP synthase.

Historic Function of ATP Synthase

Figure 1 depicts our classic understanding of the ATP synthase. This ancient enzyme is present in the inner membrane of mitochondria and is responsible for generation of ATP. Synthesis of ATP is generated by the movement of hydrogen ions from the intermembrane space into the matrix of the mitochondria through the transmembrane H+ carrier (F0) with subsequent movement of the F1 portion of the enzyme that coordinates the interaction of the subunits and ATP synthesis. This powerhouse of an enzyme can synthesize more than 100 molecules of ATP per second; this enzyme can also function in the reverse direction, hydrolyzing ATP. The H+ channel and the enzymatic portion of the ATP synthesis are connected by a protein stalk. This stalk is composed of 4 subunits: the oligomycin sensitivity-conferral protein, subunit b, subunit d, and coupling factor 6 (CF6).

Functions of CF6: Old and New

CF6 is synthesized in an immature form in the cell cytosol as a 108-amino acid peptide and is led to the mitochondria where a 32-amino acid signal peptide is cleaved, forming the mature 78-amino acid peptide (≈9 kDa in Western analyses) that is essential for energy transduction through the ATP synthase. Half a decade of studies have suggested that this protein has a function in the cardiovascular system previously unappreciated. Researchers have demonstrated that the ATP synthase, of which CF6 is a part, is a receptor for angiotatin and HDL (κ; Figure 2).

The models used in the preceding and present studies that focus on CF6 include human umbilical vein endothelial cells (HUVECs), rat vascular endothelial cells, and the Wistar Kyoto (WKY) and spontaneously hypertensive rat (SHR). The first line of evidence that CF6 was potentially important to the cardiovascular system was discovery of the localization of CF6 to the plasma membrane of endothelial cells using a rabbit-raised antibody; this and other important pieces of data suggested the presence of the CF6/ATP synthase in a site other than the mitochondria. CF6 is capable of being shedded or released. Several lines of evidence support this, including measurement of CF6 in the media of HUVEC and rat vascular endothelial cells and measurement of circulating CF6 in rats and humans. Circulating levels of CF6 in the normal rat is ≈0.1 nM. Figure 2 depicts a current understanding of potential physiological regulators of endothelial cell CF6.

The story of the cardiovascular function of CF6 begins with the observation that a peptide extracted from the hearts of SHR reduced prostacyclin synthesis. This protein was isolated and identified as CF6. Recombinant CF6 was made, and it reduced baseline and bradykinin-stimulated prostacyclin synthesis as well as arachidonate release when used in HUVECs (1 to 100 nM CF6). Further work has suggested that CF6 does not inhibit prostacyclin synthase directly but may inhibit activation of calcium-dependent phospholipase A2. The mechanism by which this occurs may be through a reduction in intracellular pH of endothelial cells; this activity is one focus of the study by Osanai et al.

Two lines of evidence suggest that CF6 has physiological function. First, administration of recombinant CF6 to WKY and SHR increased blood pressure of ketamine/xylazine-anesthetized rats. Administered in the left femoral vein,
recombinant CF6 (0.1 to 1 µg/kg) increased blood pressure a maximum of 5 mm Hg in WKY and 11 to 12 mm Hg in SHR. This blood pressure reduction was virtually abolished by indomethacin. Second, administration of an antibody to CF6 modestly reduced blood pressure of WKY (7 mm Hg), whereas it reduced blood pressure of SHR over 30 mm Hg. The CF6 antibody potentiated the depressor effects of bradykinin infusion in SHR but not in WKY. These studies suggest an increased sensitivity to CF6 in hypertension and potentially a greater function of CF6 in hypertension. This idea is supported by findings that circulating levels of CF6 in SHR were greater than in WKY. Importantly, CF6 plasma levels were greater in essential hypertensive humans compared with age-matched normotensive individuals. An interesting note is that vitamin C normalized levels of circulating CF6 in hypertensive individuals, building a potential connection between reactive oxygen species and regulation of CF6 levels.

How does CF6 elicit a biological function? Is there a receptor for CF6? Current evidence suggests that CF6 stimulates ATPase activity in HUVECs, whereas biological functions of CF6 can be blocked with efrapeptin, an ATP synthase inhibitor. Radioligand-binding studies presented in the present article suggest there is a saturable binding site in plasma membranes of HUVECs, though the pharmacological tools to perform these studies were limited and may be biased (eg, [125I-CF6] competed with cold CF6). The interaction of CF6 with the ATP synthase may be directly important for biological activity because administration of the β-subunit antibody dampened the function of CF6. Importantly, activity was not obliterated by the antibody, suggesting there may be other potential sites of action for CF6.

Unanswered Questions

This work leads to a number of interesting questions. The first set of questions pertains to the general function of CF6. Although CF6 is present in the plasma membrane of endothelial cells, the mechanism by which CF6 is transported to the membrane is unclear. Another question is whether endothelial cells are the primary source for circulating CF6, and the identification of physiological stimuli for the release of CF6, considered an endogenous inhibitor of arachidonate release (Figure 2). Given the ubiquitous expression of ATP synthase, it would be surprising that endothelial cells would be the only source. The receptor for CF6 also remains an issue: is ATP synthase the only receptor for CF6? If so, how does stimulation of ATP synthase by CF6 occur through the β subunit? There is something of a conundrum with the CF6-induced reduction in pH being association with vasoconstriction. As the authors point out, vasoconstriction is typically associated with an alkalinization of cells. It would be interesting to examine the effects of CF6 on the function of isolated blood vessels with respect to arachidonate-induced relaxation and general vessel contractility.

The second set of questions is in regard to the relevance of CF6 to hypertension. Reductions in prostacyclin synthesis have been observed in a number of different hypertension models, suggesting a common underlying dysfunction that lowers the levels of this important vasodilator. The authors have begun to build a case that CF6 may play a role in determination of prostacyclin levels in vivo. There are questions as to the application of the finding of higher plasma levels of CF6 to models other than SHR, as to how antioxidants affect the plasma levels of CF6, and as to whether CF6 could play an initiating or maintenance role in hypertension.

In summary, this interesting work by Osanai et al2 points to a new function—that of an endogenous prostacyclin inhibitor with potentially direct and/or indirect vascular effects—for CF6 and widens our view of the functions of the ancient enzyme ATP synthase.

References

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