Enzymes are critical components of every cell, and their activity is tightly regulated by substrate availability, competitive or allosteric inhibitors, and the presence of cofactors. Enzymatic activity may also be modulated by posttranslational modifications, such as cleavage of the polypeptide chain or phosphorylation/dephosphorylation of amino acid residues. Under pathological conditions, other modifications, such as oxidation or nitration, may occur, thereby disturbing enzyme activity. Beside these well-known principles of enzyme biology, temperature and pH are crucial determinants of enzyme activity, but corresponding changes in the cellular environment have attracted surprisingly little attention as putative disease mechanisms. Changes in intracellular pH can affect the ionization state of acidic or basic amino acid residues, which may disrupt ionic bonds that help to determine the 3-dimensional shape of the enzyme. These conformational alterations can lead to inactivity of the enzyme, because they may prevent binding of the substrate or cofactors. In addition, the modification of intracellular pH may change the charge properties of the substrate, so that either the substrate cannot bind to the active site or it cannot undergo catalysis.

In general, our knowledge about how the intracellular pH may affect disease development and vice versa is still rudimental. The acidification of cellular endosomes and lysosomes is crucial for the correct trafficking of these organelles. For example, the inhibition of proton pumps responsible for acidification induces apoptosis in tumor cells. This has lead to the development of specific inhibitors of proton ATPases as anticancer therapeutics. Because aberrant endosomal trafficking also affects degradation pathways and results in accumulation of degraded proteins, disturbances in intracellular pH (pHi) have been implicated in the development of neurodegenerative diseases. In the vasculature, the effects of pHi changes are even more complex, because they have been shown to modulate the activity of different enzyme systems including endothelial NO synthase (eNOS), endothelin-converting enzyme, ion channels, and actin/myosin filaments.

Vascular integrity and function is critically determined by the balance between NO production and its deterioration by reactive oxygen species. Therefore, the regulation and activity of the eNOS has attracted particular interest during the past 2 decades. To date, numerous mechanisms have been identified that regulate eNOS activity. At the level of gene expression, eNOS levels may be influenced by direct binding of transcription factors to the eNOS promoter or by affecting mRNA stability. The subcellular location may also modulate eNOS activity. Within the cell, eNOS targets the Golgi complex and plasmalemmal microdomains called caveolae, whereas the interaction of eNOS with the caveolae coat protein Caveolin-1 can reversibly inhibit eNOS activity. The eNOS substrate l-arginine is abundant at levels far above the K_m of the enzyme, but deficiency of l-arginine transporters or the presence of competitive endogenous eNOS inhibitors, in particular asymmetrical dimethyl arginine, may nevertheless prevent substrate utilization with prognostic implications. In addition, proper eNOS catalytic function requires several cofactors, including NADPH, flavin mononucleotide, flavin-adenine dinucleotide, and tetrahydrobiopterin. There has been particular interest in tetrahydrobiopterin biology, because it can be oxidized to dihydrobiopterin by strong oxidants such as peroxynitrite, which will result in eNOS uncoupling, a condition that turns this enzyme from an NO-producing enzyme into a superoxide source. Oxidative stress and subsequent peroxynitrite formation may in addition disrupt the zinc thiolate cluster and thereby cause eNOS uncoupling.

In this issue of Circulation, Boedtkjer et al demonstrate that a lower steady-state intracellular pH, via disruption of the Na^+HCO_3^-cotransporter NBCn1, leads to diminished eNOS activity. The finding of eNOS regulation by intracellular pH is not entirely new, and has been previously described in cultured endothelial cells, but its significance for vascular function in vivo is addressed for the first time in the current study. A drop in pHi led to diminished acetylcholine-triggered NO production, compatible with endothelial dysfunction in NBCn1 knockout mice. Importantly, this impaired NO bioavailability was solely attributable to a diminished NO production, because antioxidant supplementation did not restore endothelial function. Endothelial dysfunction is associated with increased clinical events in patients with vascular disease, highlighting the importance of the current findings. Because pHi may be an underestimated modulator of endothelial function, investigators should take this into account whenever unexplained changes in endothelial function are encountered.

Another important finding of the current study is the identification of Rho as another functionally important pH-sensitive component in the contractile machinery of the vasculature. Boedtkjer et al show for the first time that pH-related changes in Rho kinase activity have important functional implications in vivo, because NBCn1 knockout mice showed no blood pressure increase in response to angiotensin II. This observation may account for changes in blood pressure related to nutrition or other environmental factors. Under physiological conditions, exercise may constitute another important reason for transient changes in systemic pH or pHi, and it is tempting to speculate that acidification during exercise may limit the blood pressure increase induced by...
the autonomous nervous system. Clinicians may have encountered a similar phenomenon in patients with severe systemic acidosis (eg, after successful resuscitation), when catecholamine therapy is less effective with respect to the increase in blood pressure.

Despite these intriguing observations, several unresolved issues remain. First, we have only a very limited knowledge about how physiological events or diseases may affect the pH. Some associations of decreased pH seem obvious, as in the case of ischemia/hypoxia, when anaerobic metabolism leads to the accumulation of acidic end products. This may be further complicated by the time course of these pH changes, eg, transient in the case of hypoxia during exercise or sustained as in the case of severe arterial stenosis and subsequent permanently impaired tissue perfusion.

Within the cell, pH may vary considerably because of cellular compartmentalization. For example, membrane-enclosed organelles, such as lysosomes, generate an acidic pH required for the dissociation and recycling of endocytosed materials, whereas mitochondria use the flux of electrons to generate a proton gradient for ATP production. Because most cellular enzymes are exquisitely pH sensitive, the pH will determine the biochemical reactions occurring in each cellular compartment. Trafficking of enzymes within the cell may therefore already cause changes in enzymatic activity because of the different environments. These compartment-dependent changes in pH can be visualized in living cells by using pH-sensitive fluorescent probes, nuclear magnetic resonance, or pH microelectrodes, but their assessment and modulation in vivo remains a challenge.

A general problem is that pH changes will always affect a broad variety of intracellular enzymes, and these may involve opposing systems that are activated or inhibited at the same time. This is also evident in the current study, where a lower pH had opposing systems that are activated or inhibited at the same time. Trafficking of enzymes within the cell may therefore already cause changes in enzymatic activity because of the different environments. These compartment-dependent changes in pH can be visualized in living cells by using pH-sensitive fluorescent probes, nuclear magnetic resonance, or pH microelectrodes, but their assessment and modulation in vivo remains a challenge.

Figure. Mechanisms by which pH can affect vascular function. A low pH in endothelial cells—as in the case of a disruption of the Na\(^+\)HCO\(_3\)\(^-\) cotransporter NBCn1—will impair NO bioavailability by decreasing eNOS catalytic activity. In vascular smooth muscle cells, decreases in pH will attenuate contractility by several independent mechanisms. The SERCA, which facilitates calcium cycling between the sarcoplasmic reticulum and the cytosol, is inhibited by a low pH. In addition, cellular acidification attenuates the activity of Rho kinase and reduces the opening probability of L-type calcium channels and Ca\(^{2+}\)-dependent potassium channels (BK\(_{Ca}\)). pH indicates intracellular pH; NO, nitric oxide; eNOS, endothelial NO synthase; SERCA, sarcoplasmic reticulum calcium ATPase; and ER, endoplasmic reticulum.

With respect to endothelial cells, the relative importance of NO and endothelium-derived hyperpolarizing factor for vasodilation may vary among different regions of the vascular tree. This may explain why the modulation of vascular tone by pH depends on the type of blood vessel and on the pattern of regulatory signals; and why the opposite effects (eg, in large arteries versus resistance arteries) are possible. Without a distinct control of pH in different tissues or subcellular compartments, the use of such a fundamental regulator of cellular function as a therapeutic target remains elusive.
Intracellular pH: A Fundamental Modulator of Vascular Function
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