

The first *Highlighted Topics* article featured in this issue of the *Journal of Applied Physiology*, “Time course and heterogeneity of contractile responses in cultured human airway smooth muscle cells,” by Fabry and colleagues (p. 986–994), extends respiratory mechanics at the tissue and organ level to individual cells in culture. In their study, Fabry and colleagues used magnetic microbeads to apply forces to smooth muscle cells cultured on a rigid plastic substrate and measured the phase and amplitude of the resulting bead motion with video microscopy. Changes in cell stiffness and friction in response to relaxing and contracting agonists in single cells closely resembled those observed at the level of intact smooth muscle tissue. This outcome reinforces the observation that adherent smooth muscle cells can maintain contractile properties under culture conditions and demonstrates the feasibility of modeling bronchoconstriction in cell culture systems. The system developed by these investigators also allowed them to measure cell hysteresivity, which is closely related to cross-bridge cycling and unloaded shortening velocity. Contractile agonists caused the cells to rapidly increase their stiffness and to exhibit a transient increase in hysteresivity. The former likely reflects increased numbers of attached cross bridges and the latter the transient conversion of rapidly cycling cross bridges to slowly cycling (latch) bridges. Furthermore, cells decreased their stiffness when treated with relaxing agonists, indicating the existence of a considerable degree of baseline tone. This tone varied dramatically among cells and resulted in markedly heterogeneous responses to contractile and relaxing agonists. Thus the method developed by Fabry and colleagues provides a reliable and efficient way to evaluate mechanical responses to stimuli at the level of cells in culture.

The second *Highlighted Topics* article appearing in this issue, “Tryptase-induced PAR-2-mediated  $Ca^{2+}$  signaling in human airway smooth muscle cells,” by Berger and colleagues (p. 995–1003), examines signal transduction mechanisms implicated in the direct, receptor-mediated effect of tryptase, the major mast cell product in human airway smooth muscle. Tryptase plays an important role in airway inflammation and hyperresponsiveness, yet it appears to produce different, sometimes opposite, effects on airway responsiveness (e.g., bronchoprotection and/or airway contraction). Most of the biological effects of proteases are mediated via protease-activated receptors (PARs) that belong to the family of G-protein-coupled receptors. However, activation of PARs is

different from that of other seven-transmembrane domain G-protein-coupled receptors. Proteases cleave PARs within the extracellular  $NH_2$ -terminal domain, exposing a new  $NH_2$  terminus that acts as a tethered ligand by binding to extracellular domains of the receptor and thereby activating the cleaved receptor molecule. In this connection, most of the biological effects of tryptase are also receptor mediated via the PAR subtype, PAR-2. In the study by Berger and colleagues, PAR-2 receptors were detected at the site of the human airway smooth muscle cell by means of immunocytochemistry. These investigators discovered that PAR-2 activation mobilizes intracellular  $Ca^{2+}$  stores to a similar extent as that observed in response to “conventional” agonists (e.g., ACh or histamine). Tryptase increases intracellular  $Ca^{2+}$  concentration via phosphoinositide phospholipase C activation and via the inositol trisphosphate pathway. It was also shown that desensitization, a characteristic feature of PARs, involves protein kinase C. These findings may have both physiological and pathophysiological implications, given that mast cells, which may release significant amounts of tryptase, are localized close to airway smooth muscle cells.

The final *Highlighted Topics* article in this issue, “Effects of ischemia-reperfusion on vascular contractility and  $\alpha_1$ -adrenergic-receptor signaling in the rat tail artery,” by Seasholtz and colleagues (p. 1004–1010), demonstrates that ischemia-reperfusion increases the vascular contractile response to  $\alpha_1$ -adrenergic-receptor stimulation. The ischemia-reperfusion encountered under many clinical conditions such as myocardial infarction, acute renal failure, adult respiratory distress syndrome, and organ transplantation is hemodynamically characterized as increased vascular tone and reduced blood flow. Whereas almost all previous efforts have focused on the role of endothelial dysfunction in the development of ischemia-reperfusion injuries, the study of Seasholtz and colleagues addresses an important yet understudied aspect of vascular dysfunction following ischemia-reperfusion: altered vascular contractility. The increased vascular contractile response and endothelial dysfunction result in a shift in balance between vasoconstrictor and vasodilator tone that leads to a tremendous increase in vascular resistance and decreased organ perfusion. These investigators provide evidence that the coupling of  $\alpha_{1a}$ -adrenergic receptor to the associated  $G_{q/11}$  protein is selectively enhanced following ischemia-reperfusion. The tightened receptor-G protein coupling enables smooth muscle cells to generate high levels of inositol trisphosphate and intracellular  $Ca^{2+}$  concentration, even in

the absence of alterations in receptor expression. These marked increases in intracellular responses ultimately lead to an intensified contractile response of blood vessels to circulating catecholamines that are abundantly released under pathophysiological conditions such as ischemia-reperfusion. These results will help balance the

views concerning the relative contributions of vascular smooth muscle and endothelial cells to the development of vascular dysfunction following ischemia-reperfusion. This work identifies new targets that may guide the development of novel therapeutic strategies for the prevention and treatment of ischemia-reperfusion injuries.

