The Janus-faced regulation of endothelial permeability by cyclic GMP

Wolfgang M. Kuebler1,2,3,4
1The Keenan Research Centre at the Li Ka Shing Knowledge Institute of St. Michael’s; 2Department of Surgery, University of Toronto, Ontario, Canada; 3Institute of Physiology, Charité-Universitätsmedizin Berlin and 4German Heart Institute Berlin, Germany
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FOLLOWING ITS IDENTIFICATION as the long-searched-for endothelial-derived relaxing factor and the subsequent recognition of its additional anti-inflammatory and antiaggregatory properties, nitric oxide (NO) was initially hailed as the new universal therapeutic weapon in pathologies associated with hypertension, inflammation, and/or coagulation. Hopes were particularly high for the treatment of acute lung injury (ALI), which characteristically displays all of these pathological features. Yet, although clinical trials showed initial improvement of oxygenation with inhalation of NO, these effects were not sustained and did not reduce mortality (8). These and other studies led to the realization that the role of NO in ALI and permeability-type lung edema is much more complex, in that NO, based on its site of action and interaction partners, may have both protective and detrimental effects. In the past, the beneficial effects of NO have at large been attributed to activation of its molecular target soluble guanylyl cyclase (sGC) and the formation of the second messenger 3',5'-cyclic guanosine monophosphate (cGMP) from guanosine triphosphate (GTP), whereas barrier-disruptive effects were ascribed to the formation of peroxynitrite (ONOO−) in the presence of superoxide (O2−) or conjecturally to S-nitrosylation of junctional proteins (15). Recent evidence, however, demonstrates that cGMP signaling by itself may play an ambivalent role in vascular barrier regulation, a recognition that not only incites new scientific perspectives and challenges but may be of considerable clinical relevance given the pending introduction of sGC activators as novel therapeutic strategy in cardiopulmonary disease (18).

Barrier-Protective Effects of cGMP

Compelling evidence for the barrier-protective role of cGMP comes from a series of in vitro and in situ studies that set to increase endothelial cGMP levels by administration of exogenous cyclic nucleotide analogs. In these experiments, membrane-permeant cGMP analogs were shown to protect cultured pulmonary artery endothelial cells (PAECs) from oxidant-induced endothelial barrier failure (10) and attenuated the increase in lung vascular filtration coefficient (Kf) in isolated perfused lungs exposed to ischemia and reperfusion (1) or hydrostatic stress (24). The barrier-enhancing properties of cGMP were further substantiated by use of a group of recently identified sGC stimulators and activators that either amplify NO-induced cGMP formation by native (reduced) sGC, or induce cGMP generation from heme-free or oxidized sGC independent of NO, respectively (18). In line with the proposed barrier protection by cGMP, the sGC stimulator BAY 41-2272 was shown to reduce lung vascular permeability in models of overventilation (17), elevated lung vascular pressures (24), or pulmonary ischemia-reperfusion injury (2).

The molecular mechanisms underlying these barrier-enhancing effects of cGMP are probably multifaceted (Fig. 1): cGMP signals predominantly through 1) activation of either of the two sGC-regulated protein kinases, cGKI with two isoforms, cGKII and cGKIβ, and cGKII (4); yet expression of cGKII in vascular endothelium has thus far not been detected; 2) regulation of cyclic nucleotide-gated membrane ion channels (23); or 3) allosteric regulation of cyclic nucleotide degrading phosphodiesterases (PDEs). In the latter respect, cGMP directly stimulates PDE2 and inhibits PDE3, which hydrolyzes both 3',5'-cyclic adenosine monophosphate (cAMP) and cGMP, whereas the stimulating effect of cGMP on its own hydrolysis by PDE5 is mediated via cGKI (5).

Experiments in PAECs show that the barrier-protective effect of exogenous cGMP analogs requires cGKI (10) and is associated with an attenuated intracellular Ca2+ response (12), suggesting that cGKI may regulate lung vascular permeability by interference with endothelial Ca2+ entry and/or removal mechanisms. Attenuation of endothelial Ca2+ signaling may consequently preclude endothelial barrier dysfunction by preventing the Ca2+/calmodulin-dependent activation of endothelial myosin light chain kinase. Polymodal cation channels of the transient receptor potential canonical (TRPC) and vanilloid (TRPV) subfamilies present likely target candidates in this context. TRPC6 and TRPV4 play important roles in endothelial barrier failure in permeability-type (6) and hydrostatic lung edema (24), respectively, and are negatively regulated by cGMP in a cGKI-dependent manner (21, 24). In addition, cytosolic Ca2+ levels in endothelial cells may be reduced by cGKI-mediated stimulation of endoplasmic reticulum Ca2+-ATPase facilitating Ca2+ store uptake (7). In smooth muscle cells, cGMP signaling via cGKI has furthermore been shown to inactivate RhO by phosphorylation at Ser188 (11) and to phosphorylate and thereby activate myosin light chain phosphatase (3), mechanisms that may stabilize the vascular barrier by a Ca2+-independent inhibition of endothelial actin-myosin contraction. cGMP also stabilizes intercellular junctional complexes, as it prevents the oxidant-induced loss of junctional VE-cadherin in PAECs (10). Notably, vasodilator-stimulated phosphoprotein, a well-known cGKI target and attractive downstream candidate mechanism for barrier protection owing to its role in actin polymerization and stress fiber bundling, does not seem to contribute significantly to the barrier-protective effects of cGMP (14).

Address for reprint requests and other correspondence: W. M. Kuebler, The Keenan Research Centre, Li Ka Shing Knowledge Institute of St. Michael’s Hospital 209 Victoria St., M5B 1W8 Toronto, Ontario, Canada (e-mail: kueblerw@smh.ca).

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Despite this well-based theoretical rationale and experimental evidence for a barrier-protective role of cGMP, an increasing number of reports suggest that cGMP may also be barrier-disruptive. This notion was first derived from measurements of hydraulic conductivity (Lp) by the Landis technique in frog mesenteric capillaries, demonstrating that agents which elevate intracellular cGMP levels such as atrial natriuretic peptide (ANP), exogenous NO donors, or zaprinast (an inhibitor of cGMP hydrolyzing PDEs) increase baseline capillary permeability (9). Schmidt and coworkers (17) established the relevance of cGMP-dependent barrier disruption in high tidal volume (HVT)-induced lung injury by showing that the C^-/-/Ca^2+ influx via transient receptor potential (TRP) channels, promoting Ca^2+ uptake into the endoplasmic reticulum (ER) via Ca^2+ ATPase, and inactivating RhoA. The latter events prohibit phosphorylation and, thus, inactivation of myosin light chain (MLC) phosphatase (composed of 3 subunits, MYPT1, M20, and PP1) while limiting the Ca^2+/calmodulin (CaM)-dependent activation of MLC kinase (MLCK), thus shifting the balance from phosphorylated (MLC-P) to unphosphorylated MLC, resulting in actin/myosin dissociation and barrier stabilization. Cyclic GMP furthermore stimulates its own hydrolysis to cAMP by cGKI-dependent activation of phosphodiesterase 5 (PDE5) and by direct allosteric activation of phosphodiesterase 2 (PDE2) while blocking phosphodiesterase 3 (PDE3). PDE2, however, will also hydrolyze 3',5'-cyclic adenosine monophosphate (cAMP), which is formed from adenosine triphosphate (ATP) by transmembrane adenylyl cyclase (AC). Subplasmalemml cAMP stabilizes the endothelial barrier via both protein kinase A (PKA) and Epac (exchange protein directly activated by cAMP)-dependent signaling pathways. Consequently, stimulation of cAMP hydrolysis by PDE2 will terminate this barrier-protective effect and promote barrier dysfunction in a cGMP-dependent manner.

**Barrier-Disruptive Effects of cGMP**

Despite this well-based theoretical rationale and experimental evidence for a barrier-protective role of cGMP, an increasing number of reports suggest that cGMP may also be barrier-disruptive. This notion was first derived from measurements of hydraulic conductivity (Lp) by the Landis technique in frog mesenteric capillaries, demonstrating that agents which elevate intracellular cGMP levels such as atrial natriuretic peptide (ANP), exogenous NO donors (activating sGC) or zaprinast (an inhibitor of cGMP hydrolyzing PDEs) increase baseline capillary permeability (9). Schmidt and coworkers (17) established the relevance of cGMP-dependent barrier disruption in high tidal volume (HVT)-induced lung injury by showing that sGC inhibition attenuates whereas sGC stimulation amplifies cGMP formation and lung microvascular leak in parallel. Their concomitant finding that 1) HVT increases the expression of the cAMP-hydrolyzing phosphodiesterase 2A (PDE2A) and that 2) PDE2A inhibition protects from HVT-induced endothelial dysfunction provided a first mechanistic explanation for the seemingly double-edged role of cGMP in the regulation of lung vascular permeability (Fig. 1): Subplasmalemmal cAMP as generated by transmembrane adenylyl cyclase enhances endothelial barrier properties by acting through protein kinase A (PKA) and Epac (exchange protein directly activated by cAMP) as recently highlighted in a comprehensive review (16). As PDE2A is stimulated by cGMP, elevated levels of both cGMP and PDE2A will promote the hydrolysis of cAMP, thereby terminating its barrier-stabilizing effect. Remarkably, this pathway appears to become prominent at high cGMP concentrations, whereas small increments in cGMP may conversely attenuate cAMP hydrolysis by inhibiting PDE3 (19). To complicate matters further, PDE2A activation will also hydrolyze cGMP, thereby potentially further aggravating endothelial barrier dysfunction by blocking the barrier-stabilizing effects of cGMP (20). The relevance of the cGMP-PDE2 pathway is not confined to overventilation-induced lung injury, since a similar protective role of pharmacological PDE2 inhibition was recently documented in a model of pneumococcal pneumonia (22). In a study published in this issue of the Journal (13), the group of...
David Pearse has now extended their previous findings on the role of PDE2A in ALI. First, in a model of LPS-induced ALI patients at risk for lung edema (18). Additional overventilation further amplified PDE2A expression in conjunction with expression of inducible NO synthase. The applied double hit thus increases the level of both the enzyme and its stimulatory pathway that will result in accelerated cAMP hydrolysis. Second, in an elegant approach using adenoviral-based in vivo transfection of a short hairpin RNA, Rentsendorj and colleagues (13) accomplished a partial knockdown of PDE2A in murine lungs. This strategy proved effective to reduce ALI subsequent to LPS and overventilation compared with mice transfected with a control vector. Notably, adenoviral transfection per se aggravated critical features of ALI and caused considerable mortality that was effectively prevented by PDE2A knockdown, further substantiating the proposed relevance of PDE2A in ALI. In line with its proposed mode of action, attenuation of ALI by PDE2A knockdown was associated with increased cAMP concentrations in the lung. PDE2A knockdown also reduced the expression of inducible nitric oxide synthase (iNOS) and attenuated the downstream increase in cGMP concentration in ALI lungs by a mechanism that remains to be determined. Of relevance, this finding identifies a potentially detrimental feedforward interaction between PDE2A and iNOS in ALI, in that PDE2A is required for iNOS upregulation, which in turn (via NO-dependent activation of sGC) will lead to cGMP generation that activates PDE2A.

The Janus Face of cGMP Signaling

The divergent effects of cGMP are reminiscent of the dual role of cAMP in the regulation of lung vascular permeability. Depending on its subcellular compartmentalization, cAMP not only confers barrier protection by its effects in the subplasma membrane compartment but may instead disrupt the endothelial barrier when it gains access to the cytosolic space (16). It may be speculated that the effects of cGMP are likewise governed by subcellular gradients that are generated by an intricate spatial arrangement of cGMP forming guanylyl cyclases, cGMP hydrolyzing phosphodiesterases, and molecular targets of cGMP such as cGK1 and PDE2A. The evolving role of cGMP and PDE2A as important regulators of pulmonary barrier function opens new perspectives for therapeutic targeting of lung vascular leakage. Notably, this mechanism may have contributed to the failure of inhaled NO strategies to improve primary outcome in clinical acute respiratory distress syndrome trials. More importantly, its recognition raises similar caution with respect to ongoing clinical trials addressing the safety and efficacy of pharmacological sGC stimulators in patients at risk for lung edema (18).

DISCLOSURES

No conflicts of interest are declared by the author(s).

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