Desensitization of vascular endothelin receptors by G protein-coupled receptor kinase 2

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This editorial refers to ‘Endothelin signalling in arterial smooth muscle is tightly regulated by G protein-coupled receptor kinase 2’ by G.E. Morris et al., pp. 424–433, this issue.

At the heart of receptor-mediated signal transduction are two intertwined processes: translation of signals carried by stimulating agents to cells through intracellular messenger molecules and termination/restoration of signalling by cells. The latter is a critical mechanism that protects cells from overreacting to an extended or appropriate stimulus and makes cells ready to react to forthcoming stimulation. G protein-coupled receptors (GPCRs) are a family of receptors composed of seven transmembrane domains that respond to various stimuli. GPCRs usually undergo rapid desensitization after activation, in which GPCR kinases (GRKs), a family consisting of seven members (GRK 1–7), play a critical role.1

Endothelins (ETs) are a family of peptides comprising ET1, ET2, and ET3.2 ETs exert their actions through two types of receptors, ETA and ETB, which belong to the GPCR family.3 Activation of ETA receptors results in vasoconstriction through the generation of 1,4,5-inositol triphosphate and diacylglycerol as intracellular messengers, which, in turn, trigger intracellular Ca2⁺ release and protein kinase C activation. Among the ET family members, ET1 is a potent vasoconstrictor that induces contraction at nanomolar concentrations in several vascular beds.3 Significant desensitization of ET1-induced cellular response was soon reported after ET was discovered in 1988,4 but the underlying mechanisms were not fully understood.

Morris et al.5 provide important, novel data regarding ETA receptor desensitization in vascular smooth muscle cells (SMCs) on several fronts. First, the study revealed a critical role of GRK2 in ETA receptor desensitization, with impressive evidence from multiple approaches, including ‘real-time’ confocal imaging, transfection with catalytically inactive GRK mutants and siRNA, and immunocytochemistry. The specificity of GRK2’s involvement was confirmed because transfection with catalytically inactive mutants of several other isoforms (GRK3, GRK5, and GRK6) was not effective. Second, the data provide a better representation of in vivo ET receptor regulation when compared with data from non-SMC lines with recombinantly expressed ET receptors. This is because receptor types, quantity, and their micro-environment better represent the naturally expressed when compared with data from non-SMC lines with recombinantly expressed ET receptors.5 Normal interaction between ETA and ETB receptors, as demonstrated in rat pulmonary arteries,7 may also be lost, if only one type of ET receptor is expressed. Because pharmacological properties such as agonist affinity, affinity, and antagonist sensitivity may be altered by functional interaction or dimerization of receptors when compared with those of the individual monomers, a response observed with recombinantly expressed receptors might not necessarily represent in vivo conditions. Interestingly, a recent study found that ETB receptors interacted with dopamine D3 receptors in renal tubule cells,8 suggesting that a broader spectrum of receptor interaction may exist in vivo. As rat mesenteric SMCs express ETA and ETB among many other receptors, native cells are certainly more suitable for receptor regulation studies than non-muscle cell lines. Third, the origin of the SMC makes the data more physiologically relevant, since resistance vessels are the main contributor to peripheral resistance, a key factor in blood pressure regulation. This may be especially important for ET1 because of its proven trophic and mitogenic effects via ETA receptors on SMCs in resistance arteries.9 In vivo, SMCs of resistance arteries may have more exposure to endothelium-derived ET than SMCs of non-resistance arteries, since less connective tissue exists between the endothelium and the smooth muscle layers in resistance vessels. Lastly, the finding by Morris et al.5 suggests that GRKs might be a new target for therapeutic intervention, in addition to a currently available ET receptor antagonist (bosantan), as overexpression of GRK2 attenuated ET-induced SMC proliferation.10

Although the finding by Morris et al.5 significantly extends current understanding of ETA receptor desensitization mechanisms in vascular SMCs, several questions remain. First, what is the role of GRK2-mediated GPCR desensitization in hypertension? Enhanced GRK2 expression has been found in hypertension,11 and GRK2 desensitizes receptors mediating contraction (such as angiotensin II12 and ET1) and vasodilation (such as β2 receptors13). The consequence of enhanced GRK2 on vessel tone will depend on which aspect prevails. Second, are ETB receptors desensitized in vascular smooth muscle and endothelial cells in a manner that is similar to the case with ETA receptors? The ETB receptor, also a member of the GPCR family, is present in SMCs (especially after organ culture),
where it mediates contraction, and predominantly in endothelial cells, where it mediates endothelium-dependent vasodilatation through NO and prostacyclin. It would be relevant to know whether ETB receptors are also desensitized by GRK2 in vascular tissues, as seen in transfected HEK 293 cells, which would potentiate vasoconstriction mediated by ETA receptors. In the present study, contractility of SMCs was not measured and endothelial cells were not employed. Third, is the extent of ETA receptor desensitization proportional to the concentration of ET and the duration of exposure at the initial challenge? This study used a high pharmacological concentration of ET (50 nanomolar) to maximize the desensitization, which does not seem to represent in vivo plasma ET (picomolar range) even under pathological conditions with elevated ET levels, although local ET concentrations in vascular smooth muscles remain unknown. It would be interesting to examine to what extent ET receptors are desensitized at the in vivo plasma range of ET concentrations. As the response to an initial challenge with ET1 was remarkable, ETA receptors in vascular SMCs are apparently not desensitized to any significant degree, which is probably due to a low basal concentration of ET.

Whether ETA receptors were desensitized by GRK2 through phosphorylation-dependent or -independent mechanisms, or both, remains to be determined, since ET receptor phosphorylation was not directly measured in this study. Moreover, GRK2 may have a broader role than previously thought, since it also suppresses GPCR expression or mediates cross-talk between two different categories of receptors. Hence, further investigation is required to gain full understanding of GRK-mediated ET receptor desensitization in vascular tissues.

References

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