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PERSPECTIVE

Deactivation of Vasodilator Responses by GRK2 Overexpression: *A* Mechanism or *the* Mechanism for Hypertension?

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In medicine, it is said that if a disease has many purported treatments, it generally means that there is no clear cure or ideal form of therapy. With regard to the disease primary hypertension—one of the leading causes of cardiovascular morbidity and mortality globally—an analogous statement can be made regarding its cellular pathophysiology: where many known pathophysiological defects are described, identification of the root cause(s) remains elusive. Among the cellular defects associated with the primary hypertension, the majority has been ascribed to alterations in the cell membrane. Alterations in function and expression of a range of membrane transporters, channels, and receptors have been reported and have been associated with the increase in vascular resistance, which is the hallmark of the disease. Perhaps the most studied of these membrane defects are those related to function of the G-protein coupled receptors (GPCRs)—especially those GPCRs expressed on endothelial and vascular smooth muscle cells regulating vasoconstriction and vasodilation. However, for many of these perturbations in GPCR function, it has been difficult to determine whether they are the cause or consequence of the disease. The article in this issue of *Molecular Pharmacology* by Eckhart et al. (2002) makes a useful contribution to our ability to determine the causal relationship between alterations in vascular GPCR function and the pathogenesis/maintenance of the hypertensive state.

Probably the most consistently described vascular GPCR-related defect in humans is impairment in response to activation of GPCRs linked to vasodilation via adenylyl cyclase activation (although enhanced activation of GPCRs linked to vasoconstriction has been reported in animal models of hypertension—e.g., α_1 adrenergic receptors, α_2 adrenergic receptors, and angiotensin receptors). The prototypes for these studies are those examining the impairment of β-adrenergic responses. However, especially in animal models of hypertension, impaired vasodilator responses and impaired agonist-mediated adenylyl cyclase activation have been described for a range of hormones that activate other G_s-linked GPCRs, including dopamine, adenosine, glucagon, prostanoids, vasopressin, and parathormone (reviewed by Feldman and Gros, 1998). Defects in G-protein function have been described in human hypertension. This has primarily been in the context of impaired G_s function (Feldman et al., 1995; Feldman and Chorazyczewski, 1997), although enhanced G_i function has been reported in animal models of hypertension (Marcil et al., 1997). However, the predominant defect explaining the impairment in GPCR-stimulated adenylyl cyclase activity in hypertension seems to be a functional uncoupling of these GPCRs from G_s.

The efficiency with which GPCRs interact with G-proteins is dependent, at least in part, on the phosphorylation state of the receptor. GPCR phosphorylation is mediated by at least two classes of serine-threonine kinases: the second messenger-dependent protein kinases (PKA, PKC) and members of the GPCR kinase family (GRKs). Increased GRK function has been described in both human and animal models of hypertension (Gros et al., 1997, 1999, 2000; Ishizaka et al. 1997). Furthermore, increased protein expression of a member of the GRK family (GRK2) has been implicated as the principal factor in the uncoupling of GPCR/G-protein in the hypertensive state—both in human hypertension (Gros et al. 1997, 1999) and in the spontaneously hypertensive rat model (Gros et al. 2000). In human hypertension, GRK2 expression is both inversely correlated with β-adrenergic-stimulated adenylyl cyclase activity as well as positively correlated with blood pressure (Gros et al. 1997, 1999).

However, as with the reports of other cellular defects described in hypertension, an *association*, not a causal relation-

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ABBREVIATIONS: GPCR, G protein-coupled receptor; GRK, G protein-coupled receptor kinase.

ship, has been described between increased GRK activity/expression and hypertension. Thus, the development of a model of vascular-targeted overexpression of GRK2 by Eckhart and colleagues and the demonstration of a hypertensive phenotype in these transgenic animals is an important step in building the case for such a causal relationship. Using a portion of the SM22α promoter ligated to the coding sequence of bovine GRK2, the authors developed animals with transgenic GRK2 overexpression in both vena cava and aorta (in the range of two to three times that observed in nontransgenic littermate control animals). Increased vascular GRK2 expression was associated with attenuation of β-adrenergic-mediated cAMP accumulation, ERK1/2 phosphorylation, and vasorelaxation (as well as blunted β-adrenergic–mediated decreases in diastolic blood pressure). Interestingly, although α-adrenergic–mediated vasoconstriction was intact in these animals, angiotensin II-mediated increases in blood pressure (in vivo studies) were attenuated. Paralleling these cellular defects, the authors report that these animals developed both vascular and cardiac hypertrophy and moderate levels of hypertension (with mean arterial pressure increases in the range of 20%).

These studies are seminal because they help to establish the pathophysiological consequence of impaired GPCR-mediated vasodilator function with regard to increased vascular resistance. Thus, although an impairment in β-adrenergic response in hypertension has been well established, it has been argued that "tonic" β-adrenergic tone (or tonic GPCR-mediated vasodilator responses in general) is not a major determinant of resting blood pressure (Abboud et al. 1964). Thus, impairment in this system was unlikely to be a substantive contributor to the maintenance of hypertension. The current studies strengthen the argument that in aggregate, an impairment of GPCR-mediated vasodilation (in the presence of an intact vasoconstrictor response) may be an important contributor to the pathogenesis/maintenance of primary hypertension. These studies are also notable in that they recapitulate the pattern of impaired β-adrenergic but intact α-adrenergic-mediated vascular responses in hypertension, suggesting that there is a differential impact of increased GRK2 expression on these pathways.

However, it also is important to appreciate that this model cannot be viewed as the "last word" in defining the role of GRK2 overexpression in the hypertensive state, because it diverges from the phenotype seen in human and genetic models of hypertension in several key respects. Firstly, the authors document that although α-adrenergic–mediated vasoconstriction in ring segments is intact in their model, angiotensin II-mediated blood pressure increases are almost entirely attenuated. If such results do represent an impairment in vascular angiotensin II-mediated effects, this would reflect a very notable divergence from the "natural" expression of the disease—wherein angiotensin II-mediated responses have been reported to be enhanced at both the cellular and integrative levels (reviewed by Touyz and Schiffrin, 2000). [It is notable that the angiotensin II and α-adrenergic responses were assessed in different systems (i.e., in vitro versus in vivo responses); ideally these will need to be studied in parallel experimental systems.] In addition, the only GPCR-mediated vasodilator response assessed was the β-adrenergic pathway. Whether this transgenic model demonstrates a comparable impairment of vasodilator responses for the range of GPCR agonists reported in other models of hypertension has yet to be established but would be critical in determining the fidelity of this model to the "natural disease state."

Second, the mechanism underlying increased GRK activity/protein expression, as well as the time course for development of this defect, differs from that described in genetic models of hypertension. In the widely used spontaneously hypertensive rat model, in the prehypertensive phase, GRK2 expression is reduced (versus normotensive WKY control rats) and the subsequent increase in expression (versus WKY rats) parallels the development of hypertension (Gros et al. 2000). In contrast, in the work by Eckhart et al. (2002), increased GRK2 expression would have been apparent throughout the life of the animals. Whether a more "faithful" pattern of the hypertensive phenotype will be seen in a conditional model of vascular-targeted GRK2 overexpression has yet to be determined. Additionally and more importantly, this model does not seem to recapitulate the mechanism of GRK2 overexpression in hypertension. The indication is that increased GRK2 protein expression in human hypertension reflects a post-translational mechanism, not an increase in expression of GRK2 mRNA (Gros et al. 1999). This suggests that the mechanism of GRK over-expression in hypertension has more to do with alterations in GRK2 stability than with transcription. Several mechanisms regulating GRK2 stability have been elucidated—including ubiquitination (Penela et al. 1998) and src-dependent tyrosine phosphorylation (Penela et al. 2001). Whether such pathways contribute to the phenotype of increased GRK2 expression in hypertension remains to be established.

Lastly, like every other important scientific contribution, this study raises as many questions as it answers. Although the impairment of β-adrenergic-mediated cAMP accumulation in vascular smooth muscle cells would have been "expected," the almost complete attenuation of β-adrenergic mediated ERK1/2 and JNK1/3 activity would not have been anticipated (at least by me). Activation of the MAPK signaling cascades by β-adrenergic receptor activation is at least partially non–PKA-dependent and has been linked to GPCR coupling with G_i and/or to arrestin signaling cascades—pathways expected to be enhanced with GRK2 over-expression (Chesley et al. 2000; Maudsley et al. 2000; Valladares et al. 2000). The apparently paradoxical results demonstrated in this model should be a stimulus to re-examine the dogma **"**nouveau**"** regarding alternative β-adrenergic signaling cascades (i.e., alternative to the traditional dogma of the "ternary complex" of GPCR-G_s-adenylyl cyclase). Furthermore, why GPCRs linked to vasodilatory mechanisms are preferentially affected by GRK2 overexpression remains unclear. The selectivity is unlikely to be dependent on preferential coupling to G_s—because GRK2 phosphorylates GPCRs preferentially coupled to a range of G-proteins (i.e., the molecular determinants of GPCR domains critical for G-protein and for GRK2 interactions seem to be discrete). It is conceivable that this pattern has more to do with the stoichiometry of coupling of G_s-linked vasodilatory systems (versus G_q-linked vasoconstrictor systems). This might be more related to downstream effectors in the contractile/relaxation process, well beyond the initial GPCR–G-protein interaction. The model described by Eckhart et al. may prove useful for addressing this question.

Despite these limitations, Eckhart et al. (2002) have demonstrated the "proof of principle" of the hypothesis that vascular overexpression of GRK2 impacts on GPCR-mediated vasodilation and affects the "bottom line" in the regulation of blood pressure. Although, the model is by no means a faithful reproduction of the disease state, it does provide a very important starting point from which to make the transition from association to causality with regard to alterations in GPCR regulation and hypertension.

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