Rac regulates cardiovascular superoxide through diverse molecular interactions: more than a binary GTP switch

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Gregg, David, Frederick M. Rauscher, and Pascal J. Goldschmidt-Clermont. Rac regulates cardiovascular superoxide through diverse molecular interactions: more than a binary GTP switch. Am J Physiol Cell Physiol 285: C723–C734, 2003; 10.1152/ajpcell.00230.2003.—The small G protein Rac has been implicated in multiple cardiovascular processes. Rac has two major functions: 1) it regulates the organization of the actin cytoskeleton, and 2) it controls the activity of the key enzyme complex NADPH oxidase to control superoxide production in both phagocytes and nonphagocytic cells. In phagocytes, superoxide derived from NADPH has a bactericidal function, whereas Rac-derived superoxide in the cardiovascular system has a diverse array of functions that have recently been a subject of intense interest. Rac is differentially activated by cellular receptors coupled to distinct Rac-activating adapter molecules, with each leading to pathway-specific arrays of downstream effects. Thus it may be important to investigate not just whether Rac is activated but also where, how, and for what effector. An understanding of the biochemical functions of Rac and its effectors lays the groundwork for a dissection of the exact array of effects produced by Rac in common cardiovascular processes, including cardiac and vascular hypertrophy, hypertension, leukocyte migration, platelet biology, and atherosclerosis. In addition, investigation of the spatiotemporal regulation of both Rac activation and consequent superoxide generation may produce new insights into the development of targeted antioxidant therapies for cardiovascular disease and enhance our understanding of important cardiovascular drugs, including angiotensin II antagonists and statins, that may depend on Rac modulation for their effect.

small G proteins; antioxidants; atherosclerosis; NADPH oxidase

The small GTP-binding protein Rac is an important molecular switch integrating diverse stimuli in the cardiovascular system and transducing key signaling functions such as superoxide production (2, 25), cytoskeletal organization (73, 74), and gene expression essential for cellular proliferation and hypertrophy (21, 59). Many investigations, including recent animal models, have placed Rac as a central mediator in cardiovascular physiology, including vascular reactivity and blood pressure regulation (62), as well as in pathological processes such as cardiac hypertrophy (87), vascular hypertrophy (55, 81), leukocyte migration (42), and platelet activation (35, 85) (Fig. 1). This review highlights the importance of Rac-mediated signaling in the cardiovascular system and particularly the vascular wall, with emphasis on the role of Rac as a regulator of the NADPH complex and the ability of Rac to be differentially activated by diverse mechanisms.

The role of Rac as a regulator of the NADPH oxidase complex was first described in phagocytes, where the isoforms Rac1 and Rac2 control respiratory burst oxidation. Recently, an NADPH oxidase complex, regulated by Rac1, has been characterized in nonphagocytic cells, such as vascular smooth muscle, cardiac myocytes, and endothelial cells (for review, see Ref. 32). Rac-regulated production of superoxide by the nonphagocytic NADPH oxidase has proved to be a core element in the transduction of cardiovascular signals including angiotensin II (ANG II) (77, 81), PDGF (74), thrombin (85), endothelin (18), and leukotriene B4 (LTB4) (108) (Table 1). In contrast to phagocytes, nonphagocytic cells exhibit low-intensity basal production of superoxide, tightly controlled by Rac1, as well as small bursts of NADPH oxidase activity upon Rac1 stimulation. The control of redox-dependent cellular events represents a relatively new paradigm in cell signaling that has been recently reviewed (30, 49). We will highlight the importance of examining superoxide regulation by Rac, because the cross talk and spatial confinement that it confers may help explain the com-
plex and sometimes contradictory finding with oxidant modulation.

**BIOCHEMICAL STRUCTURE AND FUNCTION**

Rac is a member of the Rho (Ras homology) family of small (20–40 kDa) monomeric GTP-binding proteins (small G proteins), all of which undergo regulatory control by binding GTP for activation and hydrolysis to GDP for inactivation. In all known cases, the interaction of Rac with effector molecules requires that Rac be bound to GTP; thus Rac “activity” is traditionally viewed as being synonymous with GTP binding. Although Rac may mediate multiple stimuli originating both in the cytosol and at the plasma membrane, GTP-binding appears to be a common pathway of Rac activation. Binding of GTP is constitutively inhibited by guanine dissociation inhibitors (GDIs) and is enhanced by guanine nucleotide exchange factors (GEFs), whereas hydrolysis to GDP is promoted by GTPase-activating proteins (GAPs), which activate Rac’s intrinsic GTPase activity (Fig. 2). GEFs for Rac, which often share binding with other small G proteins, comprise a growing group of proteins containing tandem DH (Dbl homology) and PH (pleckstrin homology) domains. When a PH domain undergoes phosphorylation or interaction with membrane-associated phosphatidyl inositides [such as phosphatidylinositol 3,4,5-trisphosphate (PIP3)], GEF activity, catalyzed by the proximate DH domain, is enhanced (33). Of the eight GEF proteins that activate Rac in vitro (Sos1, Vav1–3, Trio, Ost, Bcr, Abr, Ect2, and Tiam1), all but Tiam1 have so far been shown to activate other small G proteins, particularly Ras, which helps explain frequently observed intracellular small G protein “cross talk” (80, 98).

Individual GEFs, by possessing distinct lipid- and protein-binding motifs in addition to sites to bind Rac, seem to dictate which subset of a multitude of down-

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**Table 1. Cardiovascular signal involving Rac**

<table>
<thead>
<tr>
<th>Signal</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angiotensin II</td>
<td>77, 81, 92</td>
</tr>
<tr>
<td>Platelet-derived growth factor</td>
<td>26, 47</td>
</tr>
<tr>
<td>Epidermal growth factor</td>
<td>86</td>
</tr>
<tr>
<td>Insulin-like growth factor</td>
<td>61</td>
</tr>
<tr>
<td>Vascular endothelial growth factor</td>
<td>84</td>
</tr>
<tr>
<td>TNF-α</td>
<td>86, 107</td>
</tr>
<tr>
<td>Leukotriene B4</td>
<td>108</td>
</tr>
<tr>
<td>Thrombin</td>
<td>85, 101</td>
</tr>
<tr>
<td>Integrins</td>
<td>16, 24</td>
</tr>
<tr>
<td>Endothelin-1</td>
<td>18, 36</td>
</tr>
<tr>
<td>Lysoosphatidic acid</td>
<td>38</td>
</tr>
</tbody>
</table>

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**Fig. 2. Rac regulation by GTP cycling.** Inactive Rac is bound to GDP and constitutively inhibited by guanine dissociation inhibitors (GDIs) to prevent unsignal GTP exchange. Once an agonist signals Rac activation, guanine nucleotide exchange factors (GEFs) catalyze the exchange of GDP for GTP and membrane localization. GTPase-activating proteins (GAPs) then regulate the hydrolysis of GTP back to inactive GDP-bound Rac. P, phosphate.
stream effectors are targeted upon Rac activation (13, 23). For example, whereas GEF-independent activation of Rac stimulates both p21-activated kinase (PAK) and c-Jun NH2-terminal kinase (JNK) pathways, the Rac-specific GEF Tiam1 preferentially activates PAK, with minimal JNK activation (115). This bears physiological significance because PAK and JNK mediate distinct cellular events (cytoskeletal reorganization and gene expression, respectively). Thus, although Rac is always activated through a common pathway of GTP binding, the GEFs potentially allow diverse upstream signals to culminate in downstream cellular events that are equally diverse and specific.

Once bound to GTP, Rac is self-inactivated through slow intrinsic GTPase activity, which is greatly enhanced by interaction with GAPs. The enhancement is through a conserved GAP domain, found for example in N- and β-chimerins, that inserts into the active site of Rac to stabilize the transition state and thus favors GTP hydrolysis (10). In addition to regulating the speed of GTP-GDP cycling, individual GAPs also contain unique domains that mediate downstream effects. In fibroblasts, microinjection of N- and β-chimerins, including mutants lacking GAP activity, induces Rac-dependent lamellipodia formation (46). In this sense, GAPs may be viewed as a special set of Rac effector proteins, not only capable of binding to activated Rac to transduce downstream signals but with the additional ability to turn off their own upstream activation. Coordinated cell function may also be facilitated by the ability of GAPs to inactivate multiple members of the small G protein family, which occurs with p190 RhoGAP. Moreover, phosphorylated p190 can bind to the SH2 domain of the Ras-specific GAP p150, producing cross talk between effector proteins (82). In addition, more complex molecules such as Bcr can exhibit both GEF activity for one small G protein and GAP activity for another, allowing it to activate one pathway while silencing another (14).

In addition to domains for binding and hydrolyzing GTP, Rac activity also requires a COOH-terminal geranylgeranyl moiety. This modification, essential for the localization of Rac to the plasma membrane upon activation (73, 90), arises from posttranslational addition by the enzyme geranylgeranyl transferase (GGT) (12). Interestingly, geranylgeranyl groups, like cholesterol, are derived from the mevalonate pathway, and their production is blocked by HMG-CoA reductase inhibitors (statins) (73, 90). Thus modification of small G proteins by statins may contribute to their beneficial effect in cardiovascular disease (91). The geranylgeranyl moiety of inactive Rac can be sequestered within the hydrophobic pockets of GDIs. These cytosolic proteins stabilize both GDP- and GTP-bound Rac to prevent unnecessary GTP cycling and inhibit inactive Rac from interacting with effector proteins, such as PAK, through concealment of key structural regions (24). Live cell imaging has revealed that proper cellular localization of Rac is crucial because certain effector molecules, such as PAK, bind to Rac only after successful membrane targeting (47). However, GDI-mediated presentation of Rac to other effectors, such as the p67phox component of the NADPH oxidase complex, appears to occur in the cytosol (17, 22).

**RAC AND NADPH OXIDASE REGULATION**

Once activated, Rac is able to complex with its effectors to initiate downstream cellular events including NADPH oxidase, the predominant source of superoxide in both phagocytic and nonphagocytic cells and a central player in redox-dependent signaling (32, 40). Whereas Rac regulation of the NADPH has been observed in the homologous nonphagocytic oxidase of the cardiovascular system (86), as well as in phagocytes where it was first described, most molecular details of oxidase function are derived from leukocytes with extrapolation to the nonphagocytic oxidase. The NADPH oxidase is composed of four functional components whose assembly requires the presence of Rac at the plasma membrane. Two components are constitutively located in the membrane and comprise the cytochrome: Gp91phox (or its homologue in vascular smooth muscle cells, Nox1) and p22phox, a second smaller membrane subunit. The other components, p47phox and p67phox, are cytosolic adapter proteins, which complex with Rac to regulate oxidase activity. Although this model provides a general guideline to understand oxidase activation in phagocytes, important differences in molecular structure and function exist and need to be further explored in different cell types. For example, in vascular endothelial cells, gp91phox and p22phox have been recently observed as preassembled complexes in the cytoplasm rather than membrane localized (55), and smooth muscle cells do not express the adapter protein p67phox.

Interestingly, the affinity of Rac for the oxidase differs depending on which GEF is bound to Rac. In COS cells engineered to express the oxidase components Gp91phox, p22phox, p47phox, and p67phox, a constitutively active Vav1 GEF mutant exhibits greater superoxide production than active Vav2 or Tiam1 mutants, despite greater PAK binding by the Vav2 and Tiam1 mutant (68) (Fig. 3). The importance of Rac GEFs in differentially regulating the activation of the oxidase in human disease is illustrated in oncogenic transformation, where the same NH2-terminal deletion of Vav1 used to create constitutively active mutants acts as a protooncogene (43). Inherited defects in Rho family GEFs have been identified recently in facio genital dysplasia and X-linked mental retardation (48, 63). Although a cardiovascular disease related to polymorphism in Rac or Rac GEFs has yet to be identified, subtle genetic variations resulting from Rac and Rac GEF polymorphisms may potentially shift the Rac balance between different effectors to impact predisposition to cardiovascular disease.

**RAC ACTIVATION BY SURFACE RECEPTORS**

The specificity of activation conferred by GEF mutants (68, 115) lays the conceptual groundwork for an understanding of differential activation by surface re-
ceptor-coupled agonists. Each receptor may initiate a different signaling pathway to Rac activation, with different molecular signals and kinetics presumably resulting in unique physiological effects. Although the full details and complexity of the activation of Rac by different agonists are still missing, hints of how it differs depending on the stimulus with respect to time course, mechanism of activity, and time to inactivation are emerging. For example, assays of Rac binding to its effector protein PAK show brisk and transient activation by Gi stimuli such as LTB4 that peaks within 2 min and no longer binds PAK at 5 min (77). In contrast, the more complex activation through the Gi- and Gq-coupled receptor ANG II also rapidly activates Rac but is sustained through 30 min (81). Studies with inhibitors of various signaling cascades have also revealed that different signaling pathway requirements are agonist dependent. For example, LTB4 activation of Rac in neutrophils is via Gi-coupled receptors that can be inhibited by pertussis toxin and requires phosphatidylinositol 3-kinase (PI3K) but not PKC (4), whereas thrombin-induced Rac activation appears independent of PI3K (85). Rac's supporting cast of GAPs, GEFs, and GDIs are likely to contribute to these unique modes of activation and regulate a balance among Rac's affinities for its multiple effectors.

**RAC ACTIVATION BY RECEPTOR TYROSINE KINASES**

The richness of signaling that may be afforded by the supporting cast of GEFs and GAPs is best illustrated by the well-characterized pathway of Rac activation by receptor tyrosine kinases (RTK) (Fig. 4). When an agonist, such as PDGF, EGF, or insulin, activates a RTK, the receptor oligomerizes, leading to autophosphorylation of a key tyrosine residue. The SH2 domain of an adapter protein, such as Grb2, recognizes this receptor change, binds the receptor, and is itself phosphorylated. This allows for a signaling complex assembly comprising E3b1, Eps8, and son of sevenless (Sos). This in turn confers GEF function to Sos, which catalyzes guanine nucleotide exchange for both Rac and Ras (11, 27, 83). The key event in GEF activation appears to be the adapter protein localizing the GEF to the membrane (6, 70). Initial evidence showed that once this complex was formed and localized to the membrane, it activated Ras, which in turn activated Rac (6, 78). The specifics of this activation of Rac through Ras have remained unclear, but it requires PI3K, whose product PIP3 is capable of activating the Rac-specific GEF Vav (33). New evidence, however, has now emerged showing both in vivo and in vitro that the Sos-Eps8-E3b1 complex can interact directly with Rac through the interaction of Eps8 with Rac. Eps8 may also contribute to proper Rac subcellular localization through the interaction of Eps8 with F-actin (39, 79). It thus appears that RTK activation of Rac can be activated directly through Sos or through a more complex Ras-dependent pathway; what still remains unclear is how these multiple pathways of activation may enhance the complexity of Rac signaling with each likely resulting in a slightly different affinity of Rac for downstream effector proteins.

**RAC ACTIVATION BY ANG II**

The molecular details of ANG II activation of Rac have also been of intense interest, and the requirement
of Rac to mediate many well-characterized physiological consequences of ANG II signaling has been defined. ANG II-induced gene expression and hypertrophy can be blocked with a dominant negative Rac (92). Furthermore, ANG II-induced superoxide is inhibited by a dominant negative Rac or Clostridium difficile toxin A, a Rac inhibitor (81). Similarly, inhibiting Rac-regulated superoxide production with antioxidants or antisense to the p22phox component of the oxidase blocks ANG II signal transduction (31, 71, 97). ANG II is also capable of inducing PAK binding by Rac (77).

ANG II signaling proceeds through a heterotrimeric G protein-coupled receptor through both a Gq/11 pathway and a G1 pathway (94) (Fig. 5). With two parallel pathways resulting in Rac activation through the same receptor, investigations attempting to define the signaling requirements of each pathway have been complicated by the possibility of redundant pathways. Subtle differences in agonist dosing and kinetics also appear to produce different effects and may explain the seemingly contradictory investigations exploring the signal transduction pathways seen by Schmitz et al. (77) in contrast to Seshiah et al. (81). The most comprehensive model of ANG II activation of Rac and superoxide derives from work by Ushio-Fukai et al. (96) and Seshiah et al. (81). They propose that AT1

![Diagram](image1.png)

Fig. 4. Rac activation by receptor tyrosine kinase (RTK). Once an agonist, such as PDGF or EGF, binds an RTK, the receptor oligomerizes and is auto-phosphorylated. The adapter molecule Grb2 recognizes the receptor change and signals the assembly of the E3b1-Eps8-Sos GEF complex. Sos then catalyzes Rac's exchange of GDP for GTP to become active. A complementary activation pathway proceeds through Sos activation of Ras, which then activates Rac through a pathway requiring phosphatidylinositol 3-kinase (PI3K) and, likely, the Rac GEF Vav. GAPs then regulate the inactivation of Rac through the hydrolysis of GTP.

![Diagram](image2.png)

Fig. 5. Angiotensin II (ANG II) activation of Rac. ANG II activates Rac through transactivation of the EGF receptor (EGFR). After ANG II binds the Gq-coupled receptor, PKC is activated, which leads to early superoxide release necessary for Src activation of the EGFR. The signaling linking PKC to superoxide release is unclear and may involve transient activation of Rac; however, NADPH oxidase is required for transactivation, because it can be blocked by antioxidants. Rac is then activated through a process requiring PI3K to result in more sustained superoxide production. SO, superoxide.
receptor binding leads to initial PKC activation, which directly results in rapid early reactive oxygen species (ROS) production. Sustained superoxide then proceeds through transactivation of the epidermal growth factor receptor (EGFR) by a Src kinase activated by the initial ROS burst. EGFR subsequently activates PI3K with resultant activation of the small GTP-binding protein Rac, a key regulator of the NADPH oxidase complex, to result in long-term superoxide production. This model is well supported by their data, showing that inhibition of PKC with GF-109203x significantly blocks hydrogen peroxide production. Additionally, Src kinase appears to be activated by superoxide to transactivate the EGFR, because antioxidant treatment with diphenylene iodonium, an inhibitor of flavin oxidases, tiron, a superoxide scavenger, N-acetylcysteine, or ebselen, a glutathione peroxidase mimetic, will block both ANG II-induced EGFR and Src activation, whereas hydrogen peroxide treatment or treatment with the superoxide-releasing compound LY-83583 will mimic ANG II-induced EGFR phosphorylation. Similarly, inhibition of Src with protein phosphatase 1 (PP1) blocks EGFR activation and limits Rac-PAK binding and superoxide production. Further supporting this model, inhibition of EGFR kinase with AG-1478 or PI3K inhibition with wortmannin attenuated Rac-PAK binding. In contrast, Schmitz et al. (77) found that ANG II induction of Rac activity as measured by PAK-PBD binding is inhibited by the global RTK inhibitor genistein but not the Src kinase inhibitor PP1, suggesting that activation requires a non-Src tyrosine kinase. Furthermore, although they found that activation also requires PKC, because downregulation of PKCs with the inhibitor phorbol 12,13-dibutyrate limits Rac-PAK binding, the low-dose PKC inhibitor GF-109203x had only minimal effect (77). The precise explanation for these apparently contradictory findings is unclear. Furthermore, Seshiah et al. (81) found that early PKC-derived superoxide precedes Rac activation by EGFR transactivation; however, whether this initial NADPH oxidase activation through PKC involves Rac as well remains unclear. In their different model system, Schmitz et al. (77) show Rac-PAK binding 15 s after stimulation, whereas EGFR transactivation has not been shown earlier than 1 min, suggesting perhaps that Rac is directly activated to effect the early superoxide burst and later activated through EGFR to perpetuate a more sustained activation.

**RAC IN CARDIOVASCULAR DISEASE**

As a key signaling molecule in the transduction of multiple important cardiovascular agonists resulting in the production of superoxide, cellular motility necessary for chemotaxis and vessel wall repair, and gene expression controlling proliferation and hypertrophy, Rac plays a prominent role in the cardiovascular system in both health and disease. Several important themes have emerged demonstrating that Rac and ROS production are core signaling elements in a diverse spectrum of cardiovascular disease and biology as well as a likely player in the action of important cardiovascular drugs including angiotensin-converting enzymes and statins.

**Cardiac hypertrophy.** Myocardial hypertrophy can be an adaptive, or maladaptive, response to increased wall stress resulting from chronic hypertension or altered cardiac geometry after myocardial infarction; furthermore, excessive hypertrophy leads to impaired myocardial function and eventual heart failure. In addition to potentially promoting cytoskeletal and myofibrillar derangement, Rac also contributes to myocardial hypertrophy by activation of cellular growth pathways (19). Hyperactivation of growth signals underlies the oncogenic effects of Rac in mitotically competent cells (41), whereas similar activation in postmitotic cells, such as cardiomyocytes, appears to result in a hypertrophic phenotype. In investigations of this analogy, early evidence of the role of Rac in oncogenic transformation and the regulation of important pathways controlling genes involved in growth and proliferation, such as p38 mitogen-activated kinase (MAPK), JNK, Akt, and ERK, sparked interest in a possible role of Rac in cardiac hypertrophy. Similarly, Rac GEFs, such as Vav and Sos, had been observed to be involved in oncogenesis, further suggesting that Rac and its associated GEFs might be involved in cardiac hypertrophy. Subsequent work positioned Rac and superoxide as a necessary downstream signal of the small G protein Ras, a key regulator of mitogenesis and oncogenic transformation (41, 69), and in neonatal cardiomyocytes, adenoviral infection with a dominant negative form of Ras blunts the activation of MAPK, suggesting a possible relationship of Rac in cardiac hypertrophy (66). These findings were subsequently extended to directly implicate Rac when it was shown in neonatal rat cardiomyocytes that constitutively active Rac V12 induced sarcomeric reorganization and hypertrophy indistinguishable from phenylephrine-induced changes. Rac also resulted in increased expression of the embryonic gene atrial natriuretic peptide. Furthermore, dominant negative Rac N17 blocks phenylephrine-induced hypertrophic changes (67). In vitro work has continued to implicate Rac as a messenger of other important hypertrophic pathways including signal transduction downstream of ANG II, TNF-α, and EGF. Finally, a mouse model in which a constitutively active Rac V12 gene was expressed selectively in the myocardium confirms that Rac activation leads to cardiac hypertrophy in vivo. These transgenic mice show two phenotypes: a lethal dilated cardiomyopathy associated with neonatal expression, and a viable hypertrophy in young mice that resolved with age. Interestingly, excessive Rac activation did not lead to any myofibrillar derangement, and Rac V12 hearts were in fact hypercontractile (87).

Although PAK binding has been used to document increased Rac activation in cardiac hypertrophy, the downstream effector most likely mediating Rac-induced hypertrophy is superoxide produced by NADPH oxidase. By infection of fibroblasts with PAK mutants unable to bind Rac, Westwick et al. (106) showed that...
Rac-induced mitogenesis, transformation, and lamellipodia formation as well as activation of growth-related genes JNK, p38, and serum response factor (SRF) are PAK independent. Hinting that Rac-activated NADPH oxidase is the critical mediator of hypertrophy, EGF-induced calcium release in fibroblasts correlates with hydrogen peroxide production and can be blocked by dominant negative Rac or the antioxidant enzyme catalase (52). In addition, catalase and SOD mimetics also blunt mechanical stress-induced hypertrophy and activation of JNK and ERK in cardiomyocytes (65). These findings provide a mechanism by which Rac activation by ANG II can induce cardiac hypertrophy independent of blood pressure elevation (77), through increased superoxide generation (9). Indeed, ANG II-induced hypertrophy is blunted by the Rac inhibitor C3 exotoxin as well as dominant negative Rac and SOD (92). Furthermore, statins, in addition to their cholesterol-lowering effects, may also regulate cardiac hypertrophy by inhibiting Rac prenylation and oxidant signaling (51, 92). Treatment of rat cardiomyocytes with simvastatin in vitro blocks ANG II-induced hypertrophy by inhibiting Rac prenylation and oxidant signaling (51, 92). Treatment of rat cardiomyocytes with simvastatin in vitro blocks ANG II-induced hypertrophy and fetal gene expression, which correlates with decreased Rac activity and superoxide production. Convincingly, simvastatin also inhibits cardiac hypertrophy by inhibiting Rac prenylation and oxidant signaling (51, 92).

Vascular remodeling. Vascular remodeling is a compensatory response to vascular insults such as hypertension and increased shear stress and is implicated in the development of atherosclerosis. Evidence supporting the role of Rac in vascular hypertrophy stems from its role in regulating gene expression, controlling superoxide production, and sensing and transducing shear stress. Although the upstream signals are yet unidentified, Rac is activated by vascular shear stress, resulting in transduction of growth and hypertrophy signals. In vascular smooth muscle cells (VSMC), dominant negative Rac N17 blocks cyclic strain-induced activation of p38 MAPK and ERK (53, 54). Not surprisingly, this regulation appears to be superoxide dependent, with shear-induced MAPK activation equally blocked by antioxidants or RacDN (111). Heat shock protein expression and activation in response to both cyclic stress and heat stress also require Rac (110). As in cardiac hypertrophy, Rac is essential for ANG II-induced vascular remodeling, with ANG II Rac-dependently activating ERK and JNK pathways in VSMC (77). In addition, vascular NADPH oxidase components are upregulated in vivo upon ANG II infusion (15, 29). Furthermore, ANG II-induced vascular hypertrophy is blocked by catalase, SOD mimetics, and antisense p22phox cDNA in vitro (97, 113) or by knockout of gp91phox in mice (102).

Hypertension. There has been intense interest in the role of superoxide and oxidative stress in hypertension (114), because as both a key regulator of NADPH-derived superoxide and as a mediator of the potent vasoconstrictor ANG II, Rac is likely an important regulatory target in hypertension. Illustrating the importance of Rac-derived superoxide, angiotensin treatment results in increased superoxide production with significantly increased membrane-bound NADH and NADPH oxidase. Impaired relaxation to calcium ionophore, acetylcholine, and nitroglycerin are also seen after ANG II treatment, and these changes can be reversed with an SOD mimic (71). Direct inhibition of the oxidase in a mouse model with a chimeric protein gp91ds-tat, which inhibits the interaction of p47phox and gp91phox, or use of p47phox knockout mice blunts the superoxide production and hypertension induced by ANG II treatment and clearly demonstrates that in vivo NADPH oxidase-produced superoxide is the essential mediator of angiotensin's hypertensive response (50, 72). Interestingly, the spontaneous hypertensive rat (SHR) shows enhanced superoxide production with increased expression of the oxidase component p22 and impaired vasodilatation to acetylcholine (29). Moreover, treating normocholesterolemia SHR with statins reduces blood pressure with a concomitant decrease in superoxide production as well as angiotensin receptor NADPH oxidase component expression (104). Mechanically, Rac-derived superoxide production may act as an antagonist to vasodilatory nitric oxide (NO) through the rapid reaction of the two reactive species to form peroxynitrite, thus decreasing NO bioactivity (114).

Rac has now been directly implicated in hypertension in vivo through the development of a transgenic mouse model expressing constitutively active RacV12 under an α-actin promoter for smooth muscle-selective expression. These mice show hypertension compared with wild-type littersmates, caused by enhanced superoxide production. Treatment of these mice with the antioxidant N-acetylcysteine results in reversal of the hypertension (Goldschmidt-Clermont, unpublished data). Similarly, Rac mediates the arteriolar constriction response to increased transmural wall pressure. In studies using isolated mouse tail arterioles, increased wall pressure has been shown to result in superoxide production and arteriolar contraction that could be attenuated with RacDN or superoxide inhibition (62).

Atherosclerosis. There has long been interest in the role of oxidative stress and antioxidants in the development of atherosclerosis. Recently, it was shown that cellular oxidative stress predicts mortality in patients with coronary artery disease (37). Smooth muscle cells from p47phox−/− knockout mice lacking a key component of the oxidase exhibit decreased proliferation and superoxide production. Furthermore, knockout of p47phox attenuates the development of atherosclerosis in ApoE−/− mice (8). As the core regulator of superoxide production through the NAD(P)H oxidase, Rac is positioned as a critical atherogenic signal. In mice, Rac appears to be directly linked to the consequences of hypercholesterolemia, which include increased NADPH-derived superoxide production, increased expression of the AT1 receptor, and impaired endothelium-dependent vasorelaxation. In addition, hypercholesterolemic mice have increased macrophage infiltration of the vascula-
tured and enhanced plaque rupture that are attenuated with AT1 receptor antagonism (103). Similarly, in vascular injury resulting from balloon-induced carotid injury, there is a significant upregulation of the oxidase component Nox1 with associated increased superoxide production, pointing to a central role of NADPH oxidase-generated superoxide in restenosis (89). Coordinated Rac activity may also be important in smooth muscle migration from the media to intima required for the development of atherosclerosis, because both Ra N17 and, to a lesser extent, RacV12 inhibit smooth muscle migration (26). Similarly, endothelial migration and cytoskeletal changes controlling the integrity of the vascular wall are regulated by Rac and superoxide (60). Moreover, because Rac activation requires geranylgeranyl synthesis, which is inhibited by statins, Rac deactivation may account for the cholesterol-independent antiatherosclerotic effects of statins demonstrated in clinical trials (57, 91). In addition to lowering cholesterol, statins limit smooth muscle cell hypertrophy and vascular inflammation, improve vasoactivity, stabilize plaques, and regulate platelet aggregation all through pathways that appear to be regulated by Rac and Rho family GTPases (91). Moreover, in VSMC, atorvastatin blocks ANG II-induced Rac activation and downstream superoxide production, an effect that is mimicked by GGT inhibitors and reversed by mevalonate (105). Interestingly, in contrast, treatment of endothelial cells with cerivastatin increases Rac activation as measured by Rac-PAK binding in total cell lysates, although statin treatment decreases the fraction of Rac that was membrane localized (100).

Atherosclerotic vessels also demonstrate reduced levels of NO, which may be mediated by NO inactivation by superoxide to produce peroxynitrite. In vivo investigations in hypercholesterolemic rabbits demonstrate statin inhibition of Rac and superoxide with resultant restoration of NO levels and plaque stabilization (93). Moreover, Rac activity enhances the expression of plaque-destabilizing cytokines such as IL-6 and monocyte chemotactrant protein (MCP-1) through superoxide (28, 56). In fact, Rac-dependent MCP-1 expression is blocked by NO, suggesting an inactivation of Rac-derived superoxide by NO (109).

Leukocyte migration, chemotaxis, and platelet aggregation. Communication of the vasculature with both leukocytes and platelets is crucial in the vascular flux of inflammatory cells leading to both chronic atherosclerosis and acute thrombus formation. Rac signaling is central to leukocyte attraction, migration, and vascular attachment via production of MCP-1, regulation of cellular motility, and expression of adhesion molecules. In addition to shear stress-induced endothelial injury that sums leukocytes, Rac also contributes to the cytoskeletal rearrangement necessary for the movement of inflammatory cells through the endothelium (42, 76). Both dominantly negative and constitutively active Rac inhibit macrophage migration (5, 42) and PDGF-induced chemotaxis of fibroblasts and VSMC. The supporting cast of both the dominant negative and constitutively active Rac to inhibit these processes indicates that a balanced, coordinated regulation of Rac is necessary to regulate cell movement. The potent chemotactrant LTβ4 also activates Rac and ROS production (108), and blockade of the leukocyte LTβ4 receptor attenuates atherosclerosis in mice by diminishing monocytic infiltration of the vasculature (3).

In platelets, thrombin signaling and interaction with adhesion molecules requires Rac and contributes to subsequent thrombus formation (85). Cytoskeletal reorganization is necessary for platelet activation and morphological changes (34, 35). Rac and PAK are activated in the process of platelet spreading on collagen through a pathway that requires Src kinase and, to a lesser extent, PI3K. This appears to be independent of integrin α2β1, because surfaces coated with anti-α2β1 also induce Rac binding of PAK with a requirement of Src and PI3K (88). Through the use of PAK binding assays, thrombin, which signals through a heterotrimeric G protein coupled receptor, and collagen, which signals through an RTK, have now both been demonstrated to rapidly activate Rac with the requirement of phospholipase C and calcium mobilization. Activation by collagen also requires PI3K, whereas thrombin is PI3K independent (85). Interestingly, integrins appear to predominantly regulate Rac activation not through effects on GTP loading but, rather, through the plasma membrane localization of Rac that is already GTP bound. Transfection of cells with V12 Rac does not result in global PAK binding but, rather, is limited to specific plasma membrane locales where integrin activation is occurring. Thus integrins provide spatiotemporal regulation of Rac’s binding to effectors, which appears to be mediated by GDI release (24).

FUTURE

Knowledge and implication of Rac and its regulation of superoxide in the cardiovascular system has burgeoned over the last decade since the discovery that Rac regulates NADPH oxidase. Rac has been clearly defined to be a regulator in a diverse array of cardiovascular conditions initiating largely redox-dependent signaling cascades to mediate adaptive and maladaptive responses to stress such as hypertrophy, vasoconstriction, platelet aggregation, and leukocyte migration into the vasculature. Rac inhibition is also likely to be a major mechanism leading to some of the clinical benefits of both statins and anti-angiotensin drugs. Rac regulation, however, is much more complex than a simple binary GTP switch. Most research has defined whether Rac is involved in disease processes by demonstrating whether the switch was on or off, but often without questioning “on or off where and for what effectors?” The supporting cast of Rac GEFs, GAPs, and GDIs, through their unique effector domains, modulates the affinity of Rac for effectors and facilitates intracellular cross talk. Whereas oxidant production under Rac regulation is central to the development of multiple pathological states, the clinical efficacy of antioxidant drugs for cardiovascular diseases has been disappointing (1, 112). An understanding of Rac-acti-
vated oxidant production helps explain the frequently observed ineffectiveness of global antioxidant therapy. In contrast to respiratory burst oxidation in inflammatory cells, vascular superoxide production is mostly involved in the alteration of local redox conditions that influence the propagation of molecular signaling pathways (30, 49). Because of the inherent short half-life of free radical second messengers, cells have evolved necessary mechanisms for localizing oxidant production. Similar to the localization of NO signaling afforded by the spatial distribution of NO synthase isoforms, Rac likely mediates cardiovascular physiology through a spatiotemporal regulation of superoxide production (7). Thus, for an antioxidant therapy to work, it must be targeted to the desired physiology and cellular locale. Rac GEFs and GAPs as well as other pathway-specific signaling molecules resulting in Rac activation are more likely to be successful drug targets, because they would control a subset of redox-dependent signals rather than alter the global cell redox state. To achieve this goal, future investigations should aim to expand our understanding of how Rac-associated molecules modulate downstream binding affinities to result in varied temporal and spatial regulation and differentially regulated physiological processes. As we head into the future of molecular medicine, the next frontier of the GISSI-Prevenzione trial. Gruppo Italiano per lo Studio della Sopravvivenza nell’Infarto miocardico. Lancet 354: 447–455, 1999.


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