Role of guanine-nucleotide exchange factor Epac in renal physiology and pathophysiology

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CAMP IS A UNIVERSAL SECOND messenger that plays a critical role in the intracellular signal transduction of various stimuli regulating a wide variety of cellular events, including secretion, cell proliferation, differentiation, migration, and apoptosis. The cAMP is produced from ATP by adenylate cyclase isoforms, the majority of which are localized within the cell membrane (89). These cyclases are activated by G protein-coupled receptors (4). Previously, serine- and threonine-directed protein kinase A (PKA) and cyclic nucleotide-gated channels were believed to be the only two direct effectors of cAMP until the identification of a novel category of cAMP mediators (22, 38). These proteins, known as exchange proteins directly activated by cAMP (Epac), function in a PKA-independent manner and therefore represent a novel mechanism for governing the signaling specificity within the cAMP cascade (9). The Epac proteins act as specific guanine nucleotide exchange factors (GEFs) for the Ras GTPase family members Rap1 and Rap2. Upon binding of cAMP, Epac activates Rap by exchanging bound GDP for GTP, whereas GTPase-activating proteins (GAP) in turn channel them to their inactive GDP-bound states. Rap1 was originally identified as a protein that is capable of reverting back the morphological phenotype of Ras-transformed cells (41). Initial studies showed that the Epac-Rap1 pathway is involved in the control of integrin-mediated cell adhesion (9, 66).

However, with the development of 8-pCPT-2-O-Me-cAMP, a cAMP analog that can selectively activate Epac, several additional functions of Epac have recently been elucidated. So far, Epac proteins are implicated in several diverse cellular responses, including secretion, integrin-mediated cell adhesion, formation of cell-cell junctions, cellular calcium influx/efflux, apoptosis, cardiac hypertrophy, cell proliferation, cell differentiation, as well as expression of various genes (8, 36, 39, 40). The multidomain structure of Epac indicates that it may have multiple binding partners. In this regard, compelling evidence has now accumulated indicating the formation of molecular complexes in distinct cellular compartments that influence Epac signaling and cellular functions. This review focuses on recent findings describing the role of Epac in the renal physiology and pathophysiology.

Epac Protein Structure and Mechanisms of Action

So far, two isoforms of Epac named Epac1 and Epac2 have been identified. They are encoded by two distinct genes, RAPGEF3 and RAPGEF4 (19, 22). RAPGEF3 encodes for Epac1, whereas RAPGEF4 generates a long and a short variant named Epac2A and Epac2B, respectively (56). Epac1 mRNA is expressed ubiquitously with a high expression in the kidneys, hearts, and thyroid glands, whereas Epac2 mRNA is detectable most notably in the brain, pituitary, and kidney (38, 56). Based on immunohistochemistry and quantitative RTPCR analysis, Epac1 has been shown to be more abundant than Epac2 in the renal tubular epithelial cells (TECs) (50). Both isoforms of Epac are multidomain proteins characterized by a regulatory region and a catalytic domain. The regulatory region
of Epac1 and Epac2B contains two identifiable domains, i.e., a disheveled-Egl-10-pleckstrin (DEP) domain and a high-affinity cAMP-binding domain. Epac2A also shares these domain structures but includes an additional cyclic nucleotide-binding domain that has low affinity for cAMP and thus uncertain biological functions (21, 67). The DEP domain of Epac proteins is responsible for membrane association and is required for the translocation of Epac1 to the plasma membrane (21, 62, 64). The catalytic region of Epac constitutes a Ras exchange motif and a CDC25 homology domain. The crystal structure of the inactive state of the Epac2 protein revealed that the cyclic mononucleotide-binding domain sterically hinders the access of Rap to the catalytic site in its CDC25 homology domain, the latter being responsible for mediating guanine nucleotide exchange activity (67, 68). Binding of cAMP to Epac induces marked conformational changes within the protein and releases the autoinhibitory effect of the NH2-terminal domain, leading to Rap activation (68).

The major catalytic function of both Epac1 and Epac2 is guanine-nucleotide exchange of Rap1. Originally, Rap1 was identified as a revertant of oncogenic Ras-induced cell transformation (10), but recently its role in integrin-mediated cell adhesion and E-cadherin-mediated cell-junction formation has drawn quite a bit of attention as well. Rap1 is activated by a large variety of extracellular stimuli, in part mediated by the common second messengers, i.e., Ca2+, diacylglycerol, and cAMP. Several GEFs for Rap1 have been identified including both Epac proteins. In addition, several Rap-specific GAPs have been identified that can inactivate Rap1 (30). Downstream from Rap1, a variety of effectors have been identified, most notably scaffold proteins, such as acute lymphoblastic leukemia gene-1 fused to chromosome 6 (7) and a protein known as regulator of adhesion and polarization enriched in lymphocytes (37, 47). These effectors have been implicated in the regulation of cell adhesion and cell-cell junction formation. However, Rap1 may not mediate all the downstream effects of cAMP-Epac, which are targeted by other GTPases. For instance, it has been reported that Epac1 also activates R-Ras, a Ras-like small GTPase that is implicated in the regulation of integrin-mediated cell adhesion-in HEK 293 kidney-derived cells (52).

Epac-Selective cAMP Analogs

Most of the available data regarding Epac-dependent functions in the renal system have been obtained by the use of both in vitro and in vivo Epac activators, which unfortunately do not discriminate between Epac1 and Epac2. These Epac-selective cAMP analogs incorporate a 2′-O-methyl substitution on the ribose ring of cAMP, a modification that impairs their ability to activate PKA (23, 32, 79). Among them, 8-pCPT-2′-O-Me-cAMP is the most widely used Epac agonist that exhibits high affinity for Epac [dissociation constant (Kd) 2.2 μM for Epac1] as well as reduced affinity for PKA (Kd 200–300 μM) (23). However, like all pharmacological agents, Epac-selective cAMP analogs may be metabolized, such as by phosphodiesterases into bioactive products, which may lead to exerting nonspecific effects (24, 63). Therefore, it would be appropriate here to mention that the involvement of Epac in a particular function can only be firmly established when pharmacological data are supplemented by other ancillary analytical measures.

Epac Functions in Renal Tubular Transport Physiology

In the kidney, many physiological processes of ion transport and cellular proliferation are mediated via cAMP, which classically activates PKA. Recently, however, two new cAMP targets, the exchange protein directly activated by cAMP (Epac1 and -2), were found expressed selectively in different regions of renal tubular segments, perhaps suggesting a conceivable role for Epac1/2 in transport physiology in corresponding segments of the tubules (50). Because cAMP influences many facets of the biology of renal tubular cells, it is thus pertinent to delineate how Epac influences various cellular processes, as described below (Fig. 2).

Epac and Na+/H+ Exchanger

Na+/H+ exchangers (NHE) in the brush border (luminal, apical) membrane of renal proximal tubules are responsible for transcellular reabsorption of NaHCO3 and NaCl. Several NHE genes, including NHE1, NHE2, NHE3, and NHE4, are expressed in the kidney. Among these, NHE3 is quite relevant to the tubular physiology, and its activity is downregulated with the increase in intracellular cAMP levels. Previous studies have shown that cAMP-regulated NHE3 activity is mediated via PKA-dependent pathways (82). In this regard, recent observations by Honenger et al. indicate a new role of Epac in the regulation of NHE3 (33). In their study, they showed that both PKA and Epac inhibited NHE3 activity without inducing changes in the expression of the transporter localized in the brush-border membrane. Moreover, they reported that the Epac-induced modulation was independent of PKA, as indicated by the lack of activation of the PKA and the insensitivity to the PKA inhibitor. Their results suggested that Epac activation may represent a previously unrecognized mechanism involved in the cAMP regulation of NHE3. Murtzanna et al. also found that, in renal proximal tubules, cAMP inhibited NHE3 activity via both EPAC-dependent and PKA-dependent mechanisms (55). Interestingly, Carraro et al. noted that the mitogen/extracellular signal-regulated kinase (MEK) 1/2 inhibitor U-0126 was capable of abolishing the effect of Epac activator-induced NHE3 inhibition, and thus they proposed that EPAC-MEK1/2 signaling may play a pivotal role in modulating the biology of NHE3 (11).

Last, the observations that the EPAC inhibits the activity of NHE3 may be of potential significance to maintain proximal tubular homeostasis in diabetic nephropathy. In diabetes, volume expansion and hypertension are thought to be related to increased Na+ reabsorption and retention that are due to the increased activity of NHE3 along with that of Na+-glucose cotransporter 1 (34). Thus, inhibition of NHE3 by Epac analogs in controlling the volume expansion or Na+ reabsorption in diabetes may have some therapeutic benefits.

Epac and Aquaporin-2

Aquaporins are a family of water channels that mediate movement of water (and other small solutes) across the lipid bilayer of the cells along the osmotic gradients. In the kidney, the biological effect of the antidiuretic hormone arginine vasopressin (AVP) renders the collecting duct highly permeable to water. This increase in water permeability is largely due to the translocation of water channel aquaporin-2 (AQP-2) from...
intracellular storage vesicles to the apical plasma membrane of principal cells of the collecting ducts (25). Determining how AQP-2 trafficking occurs at the molecular level is fundamental to the understanding of the physiology of water balance regulation and pathophysiology of water imbalance disorders. It has been demonstrated that Ca²⁺ release from ryanodine-sensitive stores plays an essential role in vasopressin-mediated AQP-2 trafficking via a calmodulin-dependent mechanism (13). Previous studies have shown that the antidiuretic effects of AVP in the renal collecting ducts are a result of a cascade of events that are initialized by the binding of AVP to type 2 vasopressin receptor; thereafter, elevated intracellular cAMP and activated PKA lead to phosphorylation of AQP-2 and insertion into the apical membrane via PKA-anchoring proteins (69). Yip (87) investigated the role of Epac as an effector of cAMP action in addition to PKA and found that PKA inhibitors did not prevent AVP-induced Ca²⁺ mobilization and oscillations while Epac-selective cAMP agonist (8-pCPT-2′-O-Me-cAMP) mimicked AVP in triggering Ca²⁺ mobilization and oscillations; these results indicated that activation of Epac by an exogenous cAMP analog triggers intracellular Ca²⁺ mobilization and apical exocytotic insertion of AQP-2 in the inner medullary collecting ducts (IMCD) (87). In addition, Kortenoeven et al. found that the cAMP-PKA pathway was involved only in the initial rise in AQP-2 levels following AVP stimulation, and this pathway is not involved in the long-term effect of AVP (45). Instead, long-term regulation of AQP-2 may involve the activation of Epac (74).

Relevant to cAMP-Epac signaling, Noda et al. described a PDZ domain-containing protein [signal-induced proliferation-associated gene-1 (SPA-1), which is a GAP for Rap1], and this SPA-1 could directly bind to AQP-2 and promote AQP-2 trafficking (57). These observations indicate that the cAMP-Epac signaling pathway could promote AQP-2 trafficking independent of Rap1. Collectively, it would suggest that vasopressin regulates AQP-2 trafficking via cAMP-Epac as well as cAMP-PKA pathways. Another relevant interesting observation described in adenylate cyclase 6-deficient mice includes that these animals have relatively low levels of cAMP in the inner medulla, and AQP-2 phosphorylation and trafficking that apparently leads to the causation of nephrogenic diabetes insipidus (NDI) (58). Conceivably, in such a scenario, use of EPAC analogs may be worth the consideration in the amelioration of the causal effects seen in NDI.

Epac and H⁺-K⁺-ATPase

The collecting tubule includes two main cell types: the principal cells and the intercalated cells. Both subtypes have H⁺-K⁺-ATPase exchanger in the basolateral membrane, and they can be stimulated by calcitonin to secrete H⁺ (54, 75). The H⁺-K⁺-ATPase is an ion pump that uses the energy of ATP hydrolysis to transport protons (H⁺) in exchange for potassium ions (K⁺). Renal H⁺-K⁺-ATPase plays an important role in H⁺ secretion and acid-base balance (83). The signals regulating H⁺-K⁺-ATPase are not fully understood but most likely
involve second messenger molecules such as cAMP. The protein kinases involved in these signaling pathways include PKA and protein kinase C (PKC) (43). Another group of investigators delineated the mechanism of the PKA-independent activation of H+-K+-ATPase by preincubating with or without either specific inhibitors or antibodies directed against intracellular signaling proteins (48). They observed that the stimulation of H+-K+-ATPase by calcitonin was mimicked by cAMP analogs, and the stimulatory effect was abolished by adenylyl cyclase inhibition. Calcitonin increased the phosphorylation of extracellular signal-regulated kinase (ERK) via the PKA pathway, and inhibition of ERK phosphorylation prevented the stimulation of H+-K+-ATPase by calcitonin antibodies directed against either the cAMP-activated guanine-nucleotide exchange factor Epac1, monomeric G protein Rap-1, or the kinase Raf-B. All three antibodies curtailed the stimulation of H+-K+-ATPase by calcitonin, whereas antibodies directed against the related monomeric G protein Ras or kinase Raf-1 had no effect. Basically this meant that calcitonin stimulates H+-K+-ATPase via the cAMP/Epac1/Rap-1/Raf-B/ERK cascade (48).

Epac and Urea Transporter

In mammalian kidney, urea transporters are essential for concentrating urinary/tubular fluid and thus maintaining body fluid homeostasis. The urea transporters of IMCD are essential for water conservation by preventing urea-induced osmotic diuresis. Movement of urea across the plasma membrane is modulated by specialized urea transporter proteins such as UT-A1. It has been demonstrated that AVP stimulation results in increased phosphorylation of UT-A1 and induces the accumulation of UT-A1 in the plasma membrane of cells lining the IMCD (5, 42). Additionally, the increased phosphorylation of UT-A1 is a result of increased PKA activation (6, 90). Thus, vasopressin acting via cAMP stimulates urea transport across the terminal IMCD by increasing the phosphorylation and accumulation of UT-A1 at the apical plasma membrane. In addition to acting through PKA, cAMP also activates Epac. Wang et al. described that Epac activator significantly increases urea permeability in isolated-perfused rat terminal IMCD (81). Similarly, by increasing the activity of Epac with the addition of forskolin, a stimulator of PKA increased the urea permeability remarkably (81). Incubation of rat IMCD suspensions with the Epac activator also significantly increased UT-A1 phosphorylation and its accumulation in the plasma membrane. Furthermore, forskolin-stimulated cAMP significantly increased ERK1/2 phosphorylation, which could not be dampened by inhibition of PKA. This implied that the activation of Epac increases urea transport by UT-A1 phosphorylation and its accumulation at the plasma membrane, and the phosphorylation seems to be also mediated via the Epac-MEK-ERK pathway.

Role of Epac/C3G in Renal Glomerular Cells

C3G in glomerular mesangial cells. Glomerular mesangial cells (GMCs) provide structural support for the renal glomerulus and regulate the blood flow of its capillaries by rhythmically contracting and modifying the spatial geometry of fenestrated endothelium and of the glomerular basement membrane (GBM) and in turn their surface area, and thereby affecting directly the glomerular filtration rate. The mesangial cells are embedded in the mesangial matrix, which is contiguous with the GBM that is lined by fenestrated endothelium. Thus, for the regulation of intraglomerular hemodynamics and filtration rate, there seems to be an intricate anatomical and functional relationship between the mesangial and endothelial cells, such that endothelin-1 (ET-1) synthesized by endothelium conceivably modulates the biology of mesangial cells. In this regard it is worth mentioning that C3G- regulated nonreceptor proline-rich tyrosine kinase 2 (Pyk2) is a mediator of ET-1 signaling in human GMC (71). Rufanova et al. described that the endogenous Pyk2 is important for GMC adhesion and spreading, and Rap1 is stimulated by ET-1 by a mechanism involving Pyk2 activation and recruitment of the p130Cas/BCAR3 complex in GMCs (71). Interestingly, another GEF, C3G, is one of the mediators of the ET-1 intracellular signaling cascade, which is also vital for kidney development and homeostasis. A recent study found that overexpression of C3G in rat mesangial cells results in enhanced activation of Rap1, leading to significant changes in GMC spreading and their migratory behavior in response to ET-1 stimulation and increased stress fiber formation (Fig. 2) (70). These findings suggest that C3G-Rap1 may contribute to the resolution stage of mesangioproliferative forms of glomerulonephritis by reducing GMC sensitivity to

Fig. 2. Role of Epac/C3G in glomerular cells. Epac activation increases glomerular endothelial (En) barrier function by increasing the expression and integrity of junctional molecules, such as integrins. This process can provide a certain degree of amelioration from proteinuria. In addition, activation of Epac/C3G can regulate motility/migration of glomerular mesangial cells (Me) and podocytes (Po) via different pathways. Depending on the positive or negative signal at the level of Epac/C3G mediating downstream events, one would anticipate different outcomes in different glomerular disease processes (see details in the text).
ET-1, while at the same time modulating cellular motility and actin-associated cytoskeletal cellular dynamics. Also, it has been reported in the literature that ET<sub>A</sub> and ET<sub>B</sub> receptor blockade reduces glomerular cellular proliferation in experimental immune complex glomerulonephritis (29). This may indicate C3G may have a potential use for the dampening of the progression of the proliferative form of glomerulonephritis, since it reduces the responsiveness of ET-1 and possibly its ET<sub>A</sub>/ET<sub>B</sub> receptor activity while thereby downstream modulating Rap1-GTP loading and shifting actin balance toward stress fiber formation and decreased basal cellular motility.

C3G in glomerular epithelial cells. To regulate proper glomerular filtration, the renal glomerular visceral epithelial cells, i.e., podocytes, have developed a microtubule-based cytoskeletal network with sophisticated cellular branching morphology and have thin actin-based projections that are traditionally known as podocyte foot processes. These processes spread and form cell-cell contacts in mature cells via slit diaphragms. Recent studies have found that injury to the glomerular podocytes plays a pivotal role in pathogenesis of proliferative forms of glomerulonephritis in experimental animal models and in humans (3, 30). However, the molecular mechanisms for the proliferation of glomerular epithelial cells are still unclear. Recently, a functional link was uncovered between C3G/Rap1 and the fibroblast growth factor 2/protein kinase B (Akt)/glycogen synthase kinase 3β/β-catenin pathway. Here, with the use of C3G-deficient mice, it was shown that C3G was involved in a self-limiting process for the proliferation signals in the cerebral cortex, and thus it could play an important role for regulating the signaling cascades in the proliferative form of glomerulonephritis in the kidney. (Fig. 2) (80). Rufanova et al. found that the expression of C3G protein was upregulated in glomerular epithelial cells in an experimental model of an accelerated form of crescentic glomerulonephritis in the kidney. (Fig. 2) (80). Rufanova et al. found that the expression of C3G protein was upregulated in glomerular epithelial cells in an experimental model of an accelerated form of crescentic glomerulonephritis. Also, overexpression of C3G led to a significant reduction in glomerular epithelial cell spreading and decreased E-cadherin expression, whereas it led to augmented cellular motility and a decrease in basal cellular motility.

Epac in glomerular endothelial cells. The glomerular endothelial cells are unique both in location and anatomy compared with most of the other endothelial cells in mammals. The absence of a diaphragm in the fenestrations of the endothelium lining the GBM enable these uniquely situated cells to have a key role in ultrafiltration performed by the kidney in concert with other glomerular cells, such as the podocytes and mesangial cells, as well as with circulating and infiltrating inflammatory cells, which contribute to the final impact on the biology of glomerular endothelial cells in the maintenance of body fluid homeostasis (26). It has been reported that Epac plays an important role in the regulation of vascular endothelial barrier function (Fig. 2) (18, 28). However, the role of Epac with its downstream target Rap1 in glomerular endothelial cell biology is not clear. Recently, Cope et al. discovered that in vitro exposure of glomerular endothelial cell monolayers to 1,000 U/ml of interferon (IFN)-β reduces their permeability, and IFN-β induced Rap1 activation at levels comparable to 8'-ME-cAMP, an activator of Epac (16). These authors also demonstrated that coinubation with a Rap1 inhibitor significantly reduced the restrictive tightening of the glomerular endothelial cell barrier induced by IFN-β exposure. This interesting finding suggested that Epac-Rap1 might also mediate tightening of the endothelial monolayer barrier and thus restrict the passage of various macromolecules. Conceivably, modulation of this Epac-Rap1 pathway in glomerular endothelial cells may have a therapeutic potential in a vast array of renal diseases that are associated with proteinuria.

Epac in renal disorders. Epac in diabetic kidney disease. It has been reported that a high concentration of filtered glucose with increased work load leads to consequential hyperactivity of Na<sup>+</sup>-glucose cotransporter or NHE, which may be responsible for the renal tubular cell hypertrophy, possibly via angiotensin II/ANG II-induced pathways (49, 61). Other conceivable mechanisms that have been implicated in the pathogenesis of tubular hypertrophy include perturbation in the intracellular Ca<sup>2+</sup> and activation of transforming growth factor (TGF)-β1 (57, 84). Besides ANG II, the activation of TGF-β1 has been shown to occur by the influence of PKC (14), reactive oxygen species (35), and thrombospondin (85). Here, it is worth mentioning that Epac1 interactions with TGF-β1 receptor are known, but they are independent of the cAMP-binding domain of Epac1, and thus it is likely that Epac1 uses a different pathway in the induction of tubular hypertrophy (15). In line with these above observations, a study was performed by us to assess if high glucose ambience could directly exert its effect via the cAMP-stimulated GEF, Epac1, and thereafter modulate the downstream events leading to tubular hypertrophy. We observed that high glucose induced a hypertrophic response in tubular epithelial HK-2 cells, along with the prominence of cytoskeletal organization and an increase in de novo protein synthesis (77). In addition, high glucose increased the proportion of cells in the G<sub>0</sub>/G<sub>1</sub> cell-cycle phase, and the expression of Akt and the cyclin-dependent kinase inhibitors p21 and p27 was increased while the activity of cyclin-dependent kinase 4 decreased. The hypertrophic response and other high-glucose-induced effects were substantially blunted with the transfection of Epac1-siRNA or Epac1 mutants lacking cAMP-binding and GEF domains (77). Similar blunting effects of Epac1-siRNA have been reported in β-adrenergic receptor-induced hypertrophy in cardiomyocytes (53). To directly assess the hypertrophic effect of Epac1, the HK-2 cells were either treated with an activator of Epac or transfected with Epac1 cDNA. Even under low-glucose ambience, this yielded a significant hypertrophic response. These data suggested that high glucose increases transcription and translation of Epac1, leading to cell cycle arrest and cellular hypertrophy via the Akt/p21 pathway. An interesting question that stems from these studies is what would be the outcome if EPAC-TGF-β1 receptor interactions are perturbed in terms tubulointerstitial fibrosis since TGF-β1, a fibrogenic cytokine, plays an important role in the pathogenesis of diabetic nephropathy.

Epac in acute ischemic kidney injury. Renal ischemia-reperfusion injury (I/R) injury frequently occurs in patients with acute renal failure (1). It is also an important contributing factor to chronic allograft dysfunction seen following renal transplantation (12). In either instance, ischemia impairs the capacity of
the tubular epithelium to maintain integrity of its cytoskeleton and adversely affects the adhesors-catenin complex, tight junction stability, and cell-matrix interactions (46, 73, 91). Loss of cell adhesion is one of the earlier responses of the tubular epithelium observed during I/R injury, preceding TEC death and influx of inflammatory cells (2). Loss of cell-cell or cell-matrix adhesion has been found to correlate with a loss of cell function with initiation of proapoptotic signaling and ultimately cell death (17). Activation of the Epac-Rap1 pathway preserves endothelial barrier integrity by promoting the maturation of both cadherin and integrin-modulated cell-cell adhesion complexes (44). As indicated earlier, the Epac is expressed in endothelial as well as epithelial cells in various organ systems, with the highest expression being reported in the kidney (22). Both isoforms of Epac (Epac1 and Epac2) are expressed in all three segments of renal proximal tubules where they are localized in the brush border (50). Like in endothelium, Stokman et al. demonstrated that pharmacological induction of Epac-Rap1 signaling, using 8-pCPT-2-O-Me-cAMP, preserves cell adhesions during hypoxia in vitro, and thus it maintains the barrier functions of the epithelial monolayer as assessed by measuring the transepithelial resistance (76). In addition, these investigators showed that intrarenal administration of 8-pCPT-2-O-Me-cAMP also reduces the extent of renal failure in a mouse model of I/R injury. This was accompanied with decreased expression of the tubular cell stress marker clusterin-α and lateral expression of β-catenin following ischemia, thus suggesting preservation of tubular barrier function (76). In line with these observations, Patschan et al. also described that activation of Epac-Rap1 signaling by 8-pCPT-2-O-Me-cAMP augments the anti-ischemic potential of endothelial progenitor cells in a renal artery clamp model in mice (60). These findings suggest that therapeutic strategies aimed at preservation of TEC-cell-cell or cell-matrix adhesion while specifically activating the Epac-Rap1 pathway during states of I/R injury would have important clinical implications. Such a value of potential beneficial effect by the activation of Epac-Rap1 signaling is reinforced by the fact that the overexpression of Rap1b leads to amelioration from mitochondrial DNA fragmentation, Bcl-2-Bax heterodimerization, and reduced cell survival induced by hyperglycemic assault in proximal tubular epithelial HK-2 cells (78).

**Epac in cisplatin-induced kidney injury.** Cisplatin has been widely used for the clinical treatment of cancer. However, severe adverse effects, in particular, acute renal failure, limit the dose that can be given to decelerate the neoplastic process effectively. The principal cause of cisplatin-induced nephrotoxicity is related to the direct damage to the proximal TECs (59), since it is well known that the tubular cells are highly polarized, and their function is dependent on the integrity of cell adhesions, intercellular junctions, and the actin cytoskeleton frame of network (88). The cisplatin-induced pathological alterations in tubular cells begin with disruption of cell adhesion and actin cytoskeleton reorganization, followed by depolarization and mislocalization of Na⁺-K⁺-ATPase. These changes eventually lead to cell detachment and/or cell death (51). Cell-cell and cell-matrix adhesions provide epithelial cells with the environmental signals that are necessary to maintain normal cellular processes, including cell survival; and the loss of cell adhesion has been reported to induce cell death via the process of apoptosis (51). Thus, conceivably enhanced adhesion signaling, whether inside-out or outside-in mediated by integrins, may represent a potential shielding strategy for reducing cisplatin toxicity in healthy tissues equipped with detoxifying enzymes, and such defensive mechanisms are effectively operational in the kidney. Epac is a GEF for the small GTPase Rap, the latter being a regulator of both integrin- and cadherin-mediated cell adhesion processes. The study by Qin et al. investigated the effects of Epac activation in a model of cisplatin-induced nephrotoxicity (65). The results showed that Epac activation by 8-pCPT-2-O-Me-cAMP stabilized cell-cell junctions and shielded proximal TECs from undergoing apoptosis following cisplatin treatment. Such a protection was not observed in Epac-deficient cancer cells from cisplatin-induced cell necrosis. This would imply that pharmacological activation of the Epac-Rap signaling pathway, mediated by integrins or cadherins, along with a boost by the administration of BGD peptide to stimulation of phosphatidylinositol 3-kinase (PI3K), may be a potential strategy to reduce nephrotoxicity of AKT-dependent tubular cell survival and consequent renal insufficiency induced by cisplatin in patients undergoing cancer treatment.

**Epac in renal interstitial fibrosis.** Most of the chronic glomerular diseases are accompanied with accumulation of extracellular matrix (ECM) proteins, including collagens, in mesangium and other glomerular regions as well as in the renal interstitial compartment. Various mechanisms responsible for the abnormal accumulation of ECM have been delineated; however, many others still remain to be defined. ANG II has been implicated in the development of chronic progressive glomerular diseases, and the underlying precise mechanism has been the subject of rigorous investigation for the last two decades. In this regard, suggestions have been made that ANG II induces glomerular injury via perturbations of intrarenal hemodynamics with stimulation of growth factor cytokines and thereby excessive synthesis of ECM by glomerular cells (20). Several factors, such as TGF-β and ET-1, have been identified as crucial elements responsible for such an outcome; however, details of signaling pathways leading to such an end result are still somewhat unclear. Recently, ANG II acting via the angiotensin type 1 receptor (AT1R) was shown to increase collagen synthesis through the transactivation of the PI3K signaling pathway (86). In addition, Yano et al. noted that inhibition of adenylyl cyclase by cell-permeable 2,5-dideoxynosine (2′,5′-DOA) ablated the ANG II-induced increase in PI3K activity, whereas H89, a PKA-specific inhibitor, exerted no significant effects. These results suggested that ANG II-induced PI3K activation is mediated via cAMP, and the requirement of PKA is nonessential. Further affirmation to this notion was derived from the experiments where ANG II/AT1R transactivation of epidermal growth factor receptor (EGFR) and PI3K was demonstrated to be modulated by the Epac-specific cAMP analog 8-pHPT-2-O-Me-cAMP. It is worth mentioning here that this was the first study to demonstrate such an Epac-dependent effect of cAMP in the increased synthesis and accumulation of ECM proteins via such complex cross talks and transactivation processes (86). Moreover, their study emphasizes the importance of AT1R-PI3K-Akt cross talk and activation of EGFR in mediating ANG II-induced collagen synthesis and thereby mesangial glomerulosclerosis; and inhibition of adenylyl cyclase by cell-permeable 2′,5′-DOA may serve as a useful pharmacological agent in dampening the dire irreversible process of renal fibrosis.
Conclusions and Future Directions

Besides the well-known cAMP substrate PKA, Epac is now recognized as an incontrollable factor leading to complex and diversified cAMP signaling pathways. The evidence reviewed here alludes to the fact that this cAMP-Epac complex is involved in the control of various renal functions such as transporting of ions and proliferation of glomerular cells. Although a lot of work has been carried out in regard to the functional characterization of Epacs (Epac1 and Epac2), the list of their cellular functions is still further expanding. Depending upon their relative abundance, distribution, binding partners, and localization, Epac and PKA may act independently or cooperate or oppose one another’s action in regulating a specific cellular function. The understanding of Epac and PKA as a cohesive functioning complex would be certainly a fertile area of research, but it is also crucial to study the individual contribution of Epac (as an isolated protein or integrated in other macromolecular complexes) within the domain of overall cAMP signaling. Further studies would presumably unravel new Epac regulators and effectors as well as specific functions for Epac isoforms within the various compartments of the cell. For instance, the contribution of Rap1 proteins in the functionality of Epac in renal tubular pathobiology is still not clearly defined. Similarly, the data are also missing concerning the ability of hormones or neurotransmitters to activate various Epac isoforms in differentially modulating the pathophysiology of various compartments of the kidney, i.e., glomerulus vs. tubular.

Importantly, Epac seems to interfere with the regulation of cellular mechanisms intimately involved in the manifestation of diseases, such as diabetic kidney disease, ischemic kidney injury, and renal interstitial fibrosis. Thus, Epac proteins are not only at the crossroads of different physiological processes but also may offer attractive common therapeutic targets for the treatment of various renovascular disorders. An in-depth analysis of the structure, their spatial-temporal regulation, and the mechanism of action of Epac in various mammalian tissues will allow the development of new pharmacological compounds that could be targeted toward sites where cAMP sensor is specifically operational. To date, the pharmacology of Epac proteins has not been comprehensively investigated, and the current studies on Epac functional effects clearly suffer from the lack of available Epac-selective or -specific pharmacological inhibitors. Certainly, development of Epac knockdown or transgenic mice or newer classes of pharmacological inhibitors may be some of the new avenues that can be pursued to enhance our understanding of the pathophysiology of Epac in various mammalian tissues. In all likelihood, development of newer classes of Epac inhibitors would be a great promise in the advancement of small G protein-pathobiology. In this regard, various analogs of “tetrahydroquinoline” are being developed by various drug companies. Among them, one of the analogs designated as “C634” is being currently investigated with respect to its efficacy in inhibiting Epac and its downstream signaling events/pathways that are responsible for the causation of various renal disorders.

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DISCLOSURES

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AUTHOR CONTRIBUTIONS


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