MiniReview

Protein Kinase D in the Cardiovascular System
Emerging Roles in Health and Disease

Metin Avkiran, Alexandra J. Rowland, Friederike Cuello, Robert S. Haworth

Abstract—The protein kinase D (PKD) family is a recent addition to the calcium/calmodulin-dependent protein kinase group of serine/threonine kinases, within the protein kinase complement of the mammalian genome. Relative to their alphabetically superior cousins in the AGC group of kinases, namely the various isoforms of protein kinase A, protein kinase B/Akt, and protein kinase C, PKD family members have to date received limited attention from cardiovascular investigators. Nevertheless, increasing evidence now points toward important roles for PKD-mediated signaling pathways in the cardiovascular system, particularly in the regulation of myocardial contraction, hypertrophy and remodeling. This review provides a primer on PKD signaling, using information gained from studies in multiple cell types, and discusses recent data that suggest novel functions for PKD-mediated pathways in the heart and the circulation. (Circ Res. 2008;102:157-163.)

Key Words: protein kinase D ■ signal transduction ■ contraction ■ hypertrophy ■ remodeling ■ ischemia

The protein kinase (PKD) family consists of 3 isoforms; these are the original PKD that was identified by Rozengurt and colleagues in 19941 and now is also referred to as PKD1 (gene name, PRKD1; UniProt accession nos. Q62101 [mouse], Q15139 [human]) and the more recently discovered isoforms PKD2 (PRKD2; Q8BZ03 [m], Q9BZL6 [h]), and PKD3 (PRKD3; Q8K1Y2 [m], Q94806 [h]). The kinase initially termed protein kinase C (PKC) was identified by Johannes et al2 in the same year as mouse PKD,1 and is its human ortholog. PKD is a 918-aa serine/threonine kinase that consists of an N-terminal regulatory domain (containing 2 cysteine-rich, zinc finger–like motifs and a pleckstrin homology domain) and a C-terminal catalytic domain,1,2 as illustrated in Figure 1. Also as illustrated in Figure 1, PKD2 and PKD3 share a similar structure and exhibit a high degree of homology to PKD1, exceeding 90% within the catalytic domain. Components of the regulatory domain inhibit the activity of the catalytic domain (such as deletion or mutation of the regulatory domain renders the catalytic domain constitutively active3) and also facilitate PKD association with intracellular and plasma membranes.4,5 The structural and enzymatic properties of PKD distinguish it from PKC isoforms (see review by Rozengurt et al6), and PKD does not phosphorylate several established PKC substrates.1,4 Indeed, the PKD family members have been classified into the calcium/calmodulin-dependent protein kinase (CAMK) branch of the human kinome, as distinct from the AGC branch that contains the PKC isoforms.7 This classification is based on catalytic domain structure and substrate specificity, and PKD is not regulated by any direct interaction with calcium/calmodulin; nevertheless, calcium may regulate PKD activation indirectly through altered diacylglycerol (DAG) production (see below).8

As with similar motifs in classic and novel PKC isoforms, the cysteine-rich, zinc finger–like motifs within the N-terminal regulatory domain of PKD (Cys1 and Cys2 in Figure 1) allow it to bind DAG and phorbol esters with high affinity,1,4 and such binding activates PKD in vitro.4 A second mechanism of PKD activation involves PKC-mediated PKD phosphorylation.9,10 The pertinent phosphorylation sites have been mapped to Ser744 and Ser748 within the activation loop of the PKD catalytic domain, and their PKC-mediated transphosphorylation is established as the principal mechanism of PKD activation by various stimuli in intact cell systems.11–13 Consistent with this, in fibroblasts, PKD activation by DAG analogs or phorbol esters, or by G protein–coupled receptor agonists (eg, bombesin, vasopressin, bradykinin and endothelin-1 [ET1]), is blocked by PKC inhibitors (eg, bisindolylmaleimide I and Ro 31-8220) that do not directly inhibit PKD.9,10 Alternative mechanisms of cellular PKD activation in response to specific stimuli, such as caspase 3–mediated proteolytic cleavage of the regulatory domain in response to genotoxic agents14 and Src/Abl-mediated phosphorylation of Tyr463 (Tyr469 in mouse) within the pleckstrin homology domain in response to oxidative stress,15 have been reported; nevertheless, their broader significance remains to be established. Recent evidence suggests that, in response to oxidative stress, Tyr95 (Tyr93 in mouse) may also become phosphorylated and create a docking site for PKCβ, which in turn phosphorylates the PKD...
of cell growth, proliferation, differentiation, migration, and death (see recent reviews). Specific cellular processes that appear to be under PKD control include vesicular transport from the Golgi network, integrin recruitment to focal adhesions, transcription factor activation, induction of antioxidant protein expression by reactive oxygen species (ROS), and transmembrane proton efflux in response to intracellular acidosis. Although the molecular mechanism through which PKD regulates the relevant process is unclear in many cases, several PKD substrates have been identified. PKD substrate proteins, their targeted residues, and the proposed functional consequences of their phosphorylation are summarized in the Table. The Table also illustrates that putative PKD substrates commonly conform to the optimal PKD phosphorylation motif, as identified through the use of combinatorial peptide libraries, which favors an aliphatic amino acid (Leu/Val/Ile) at the -5 position and a basic amino acid numbering refers to mouse PKDs). Pertinent upstream kinases (Src, Abl, nPKC) are also illustrated, with percentages indicating sequence homology of PKD2 and PKD3 domains with corresponding PKD1 domains. Cys1/Cys2 indicates cysteine-rich, zinc finger–like domains; PH, pleckstrin homology domain.

Figure 1. Functional domains and conserved phosphorylation sites of PKD isoforms (amino acid numbering refers to mouse PKDs). Pertinent upstream kinases (Src, Abl, nPKC) are also illustrated, with percentages indicating sequence homology of PKD2 and PKD3 domains with corresponding PKD1 domains. Cys1/Cys2 indicates cysteine-rich, zinc finger–like domains; PH, pleckstrin homology domain.

Table. Putative PKD Substrates, Their Targeted Residues, and Functional Consequences of Their Phosphorylation

<table>
<thead>
<tr>
<th>Substrate Protein</th>
<th>PKD Target Motif(s)</th>
<th>Functional Consequence</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidins220</td>
<td>Y117ROMSFVLTK136 (Ser919)</td>
<td>Polarized plasma membrane localization in neural cells</td>
<td>41</td>
</tr>
<tr>
<td>RIN1</td>
<td>L136RSMAAFCS156 (Ser351)</td>
<td>Binding to 14-3-3 protein and altered subcellular localization</td>
<td>80</td>
</tr>
<tr>
<td>PLKIII-β</td>
<td>K191RTSPNKVE209 (Ser294)</td>
<td>Increased lipid kinase activity and enhanced vesicular transport to plasma membrane</td>
<td>27</td>
</tr>
<tr>
<td>Hsp27</td>
<td>L175SQLSGVSE187 (Ser82)</td>
<td>Unknown</td>
<td>34</td>
</tr>
<tr>
<td>cTnl</td>
<td>P231PRRSSNYRA27 (Ser22)</td>
<td>Decreased myofibrillar calcium sensitivity</td>
<td>57</td>
</tr>
<tr>
<td>HDAC5</td>
<td>L245RKTASEPNLK154 (Ser259)</td>
<td>Binding to 14-3-3 protein and nuclear export</td>
<td>36,64</td>
</tr>
<tr>
<td>HDAC7</td>
<td>L152KTVSPNK160 (Ser155)</td>
<td>Binding to 14-3-3 protein and nuclear export</td>
<td>81</td>
</tr>
<tr>
<td>CREB</td>
<td>L123SRPRSYRK138 (Ser133)</td>
<td>Transcriptional activation of CREB-responsive genes via recruitment of co-activators CBP/p300</td>
<td>30</td>
</tr>
<tr>
<td>CERT</td>
<td>L123PRHSGMVL137 (Ser132)</td>
<td>Decreased affinity for PI3P and Golgi membrane dissociation</td>
<td>82</td>
</tr>
<tr>
<td>VR1</td>
<td>L151YDORSFEAV21 (Ser116)</td>
<td>Enhanced expression and activity of VR1 in response to capsaicin and low pH</td>
<td>42</td>
</tr>
<tr>
<td>HPK1</td>
<td>L156SPGYSGLP76 (Ser171)</td>
<td>Activation of JNK/SAPK and NF-κB pathways in the adaptive immune response</td>
<td>43</td>
</tr>
<tr>
<td>TLR5</td>
<td>L165MKHDGSRGF101 (Ser805)</td>
<td>p38 MAPK activation and increased production and release of inflammatory cytokines e.g. IL-8</td>
<td>83</td>
</tr>
<tr>
<td>c-Jun</td>
<td>L428RKHNSDDLTTS150 (Ser58)</td>
<td>Suppression of JNK signaling pathway, possibly by modulation of c-Jun phosphorylation by JNK</td>
<td>84,85</td>
</tr>
<tr>
<td>SPHK2</td>
<td>L419HVSVDLPL140 (Ser421)</td>
<td>Nuclear export</td>
<td>46</td>
</tr>
</tbody>
</table>

CBP indicates CREB-binding protein; CERT, ceramide transfer protein; CREB, CAMP-response element-binding protein; HPK1, hematopoietic progenitor kinase 1; IL-8, interleukin-8; JNK, c-Jun N-terminal kinase; Kidins220, kinase D–interacting substrate of 220 kDa; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor-κB; PI3P, phosphatidylinositol 3-phosphate; PKLII-β, phosphatidylinositol 4-kinase IIβ; RIN1, Ras and Rab interactor 1; SAPK, stress-activated protein kinase; SPHK2, sphingosine kinase 2; TLR5, toll-like receptor 5; underlined letters, preferred residues at -5 and -3 positions; bold letters, the phosphorylated residues.
acid (particularly Arg) at the −3 position, relative to the targeted Ser/Thr residue. Not surprisingly, synthetic peptide substrates for PKD, such as syntide-2 (PLARTLARTLSVA-GLPGKK; underlined letters indicate preferred residues at -5 and -3 positions, bold letters indicate the phosphorylated residue) and μ-peptide (AALVRQMSVAFFFK), conform perfectly to this motif.

Selective pharmacological inhibitors of PKD are currently unavailable. The indolocarbazole compound Gö6976, which is a PKC inhibitor that favors classic isoforms, inhibits PKD activity in vitro, and has been used by some investigators (eg, Vega et al and Wu et al) to infer cellular functions for PKD. Because Gö6976 inhibits PKD and classic PKCs with comparable potency, in some studies, other PKC inhibitors that cannot inhibit PKD directly (eg, bisindolylmaleimide I) have been used to control for the consequences of PKC inhibition per se. This approach is not entirely satisfactory, however, because PKC isoforms are critically involved in PKD activation by many stimuli (see above). Thus, PKC inhibition would be expected to inhibit PKD activation and produce an effect similar to that achieved by direct PKD inhibition, unless PKD is activated through a PKC-independent pathway in the relevant system. Resveratrol also inhibits PKD activity in vitro but exhibits poor potency and selectivity in intact cells. A recent study has revealed that derivatives of the Src tyrosine kinase inhibitor PP1 inhibit PKD, as well as a number of other kinases, and also showed that Gö6976 potently inhibits many kinases in addition to PKD and classic PKCs.

In the absence of selective inhibitors, several investigators have used dominant negative mutants, antisense oligonucleotides, and RNA interference in loss-of-function experiments to test the roles of PKD isoforms in intact cell systems. Single and double knockouts of PKD1 and PKD3 in an avian B-lymphocyte cell line have also been described. At the time of writing, there were no reports in the literature of genetically modified mouse models with targeted disruption of PKD isoforms.

**PKD in the Heart**

The key components of PKD signaling and putative PKD functions in cardiac myocytes are illustrated in Figure 2.

**Expression and Regulation**

The original descriptions of the pertinent genes reported PKD transcripts to be present in mouse and human myocardium. PKD protein expression in the heart was first described by Brooks et al, who reported that 2 bands migrating at ≈110 and ≈100 kDa were detected by immunoblot analysis of protein samples from adult rat left ventricular tissue, using an antibody raised against a synthetic peptide corresponding to the PKD C-terminus. This study also showed that the majority of PKD protein in ventricular tissue was associated with a fraction that was insoluble in 1% Triton. Subsequently, we reported that 2 such bands were detectable by different antibodies raised against the N terminus, the C terminus, or the central domain of PKD in ventricular tissue from rats of different ages and in ventricular myocytes isolated from neonatal rat hearts. We speculated that these PKD moieties may represent distinct isoforms, which had not yet been identified. It is now known that PKD1 and PKD2 migrate as distinct bands of ≈115 and ≈105 kDa, respectively, on SDS-PAGE and that PKD2 and PKD3 are of a similar mass. It is probable, therefore, that the larger protein that we detected in rat myocardium represented PKD1, whereas the faster migrating species comprised PKD2 and/or PKD3. Interestingly, adult rat ventricular myocytes expressed predominantly the ≈115-kDa protein. This suggests that PKD1 is the main PKD isoform expressed in adult rat ventricular myocytes, which is consistent with our recent observation that transcripts for PKD1, but not PKD2 or PKD3, are readily detectable in this cell type (R.S.H. and M.A., unpublished data, 2007). These early studies also established that PKD...
expression in myocardial tissue and isolated myocytes is subject to developmental regulation and declines significantly in adulthood. More recent work in our laboratory and by others has confirmed the expression of PKD protein in neonatal and adult rat ventricular myocytes and adult mouse, rat, rabbit, and human myocardium. In common with several other genes, cardiac PKD expression appears to revert to the fetal phenotype in disease, with evidence of increased expression in failing rat, rabbit, and human myocardium.

In the first detailed characterization of PKD regulation in myocardium, we have reported previously that a variety of stimuli, such as phorbol ester, norepinephrine, and FBS, increase PKD activity in neonatal rat ventricular myocytes. Norepinephrine-induced PKD activation was shown to be mediated via α1-adrenoceptors, and the responses to α1-adrenergic stimulation, phorbol ester, and FBS were blocked by the PKC inhibitor bisindolylmaleimide I, which did not directly inhibit PKD activity. Phorbol ester activated PKD also in adult rat ventricular myocytes. Subsequent work by us and others has confirmed the ability of a variety of stimuli (eg, norepinephrine, the α1-adrenergic agonist phenylephrine, ET1, angiotensin II, aldosterone, phorbol ester, and FBS) to induce PKD activation in neonatal and adult ventricular myocytes. In many cases, PKD activation was shown to be attenuated by PKC inhibition, highlighting the critical role of PKC isoforms in neurohormonal regulation of PKD activity in cardiac myocytes. Recently, we have reported evidence that PKCe plays a key role in ET1-induced PKD activation in adult rat ventricular myocytes, which is consistent with data on α1-adrenergic PKD activation in neonatal myocytes, and demonstrated that ET1-induced, PKCe-mediated PKD activation is counteracted by protein kinase A (PKA) activation. Notably, β-adrenoceptor stimulation (which activates PKA) does not affect PKD activity in cardiac myocytes, and the molecular mechanism behind this novel crosstalk process between PKCe and PKA pathways in PKD regulation is currently unclear.

Functions and Substrates

Regulation of Myofilament Phosphorylation
In an effort to identify myocardial substrates for PKD, we have performed a yeast 2-hybrid screen of a human cardiac library using as bait the PKD catalytic domain that had been mutated to render it catalytically inactive, to promote sustained interaction with substrate proteins. This work identified the inhibitory subunit of cardiac troponin I (cTnI) as a PKD-interacting protein and led to further studies that confirmed cTnI as a PKD substrate in vitro and identified Ser22 and Ser23 (“the PKA sites”) as the pertinent phosphoacceptor residues. Indeed, exposure of chemically permeabilized ventricular myocyte fragments to recombinant PKD catalytic domain induced cTnI dual phosphorylation at Ser22/23, reduced myofilament Ca2+ sensitivity, and accelerated isometric cross-bridge cycle kinetics. More recently, we have used adenoviral gene transfer to determine the role of full-length PKD, with functional regulatory domains that determine enzyme activity and localization in response to a variety of stimuli, in regulating cTnI phosphorylation and contractile function in intact adult ventricular myocytes exposed to ET1 or β-adrenoceptor stimulation. The pertinent data provided further evidence that PKD-mediated myofilament phosphorylation represents a novel signaling mechanism in the regulation of myocyte contractile function, particularly through altered Ca2+ sensitivity. Furthermore, through the adenoviral delivery of short hairpin RNA targeted at PKD1, we were able to confirm a significant contribution from PKD-mediated pathways to ET1-induced cTnI phosphorylation at Ser22/23.

The findings outlined above suggest that PKD-mediated myofilament phosphorylation may be of physiological significance in neurohormonal regulation of myocardial contractile function. In this context, it is interesting to note that the replacement of native cTnI in mouse myocardium by a Ser22/23Ala mutant potentiates the ability of ET1 to increase myofilament Ca2+ sensitivity. This suggests that, in response to ET1, the functional consequences of phosphorylation of PKC-targeted residues in cTnI (eg, Ser43/45 and/or Thr144) are partially opposed by the concurrent phosphorylation of Ser22/23, with the latter response likely to be mediated by PKD activation downstream of PKC.

Furthermore, Pieske et al have shown an attenuated inotropic response to ET1 in failing human myocardium, which might arise from increased PKD expression and activity in this setting. Indeed, in our recent studies, the positive inotropic effect of ET1 in adult rat ventricular myocytes was abolished by PKD overexpression.

The physiological role of PKD in regulating myocardial contractile responses to neurohormonal stimulation requires examination in more complex systems, particularly because loading conditions (eg, frequency and afterload) influence the functional impact of cTnI phosphorylation at Ser22/23. It is also important to note that, in cardiac myocytes, the molecular fingerprint of PKD-mediated phosphorylation appears to be distinct from that of PKA-mediated phosphorylation, with only the latter targeting sites such as Ser282 in cardiac myosin-binding protein C, Ser16 in phospholamban, and Ser68 in phospholamban. Thus, the functional consequences of PKD activation would be expected to differ from those of PKA activation. Furthermore, our recent data suggest that neurohormonal stimulation of PKD activity may be enhanced under conditions where PKA activity is downregulated, perhaps allowing PKD-mediated pathways to assume greater significance in the acute regulation of contractile function in settings such as heart failure.

Regulation of Myocyte Hypertrophy
Nuclear-localized class II histone deacetylases (HDACs) suppress the myocardial expression of prohypertrophic genes, in large part by disrupting their transcriptional induction by myocyte enhancer factor (MEF2). In response to pathological signals, class II HDACs are neutralized by phosphorylation, binding of 14-3-3 proteins to the newly created motifs (which masks nuclear localization sequences) and export from the nucleus, freeing MEF2 to induce downstream target genes that promote myocyte hypertrophy and pathological cardiac remodeling. On the basis that phosphory-
lation-dependent neutralization of class II HDACs (such as HDAC5) is a final common step required to unlock a pathological gene program in stressed myocardium, the expectation is that identification and inhibition of the pertinent HDAC kinase(s) may help maintain the integrity of MEF2-HDAC complexes in the nucleus and thereby suppress hypertrophy and remodeling.\textsuperscript{43} As part of their concerted effort to delineate pathologically relevant HDAC kinase pathways, the laboratories of Olson and McKinsey have identified PKD as a mediator of HDAC5 phosphorylation (through a direct interaction\textsuperscript{44}), which facilitates the binding of 14-3-3 proteins and induces the nuclear export and/or cytosolic retention of HDAC5 in neonatal rat ventricular myocytes exposed to prohypertrophic neurohormonal stimuli.\textsuperscript{36,44} The same investigators have also reported a significant increase in PKD expression and activity in the myocardium of spontaneously hypertensive heart failure rats, in which PKD activity was further exaggerated by thoracic aortic banding.\textsuperscript{44} Furthermore, they have shown that cardiосpecific expression of a constitutively active PKD mutant in transgenic mice leads to cardiac hypertrophy, followed by ventricular chamber dilation, wall thinning, and a marked deterioration of contractile function,\textsuperscript{44} suggesting that a chronic increase in PKD activity is sufficient to induce adverse myocardial remodeling. These exciting data, together with the preliminary evidence that PKD expression may be increased in human heart failure,\textsuperscript{56} necessitate further investigation of the role of PKD in the development of cardiac hypertrophy and failure in vivo in response to clinically relevant stresses such as pressure overload and myocardial infarction. In the absence of selective PKD inhibitors suitable for use in vivo, such work would be facilitated by the development of mouse models with genetic disruption of myocardial PKD expression or activity.

**Potential Role in Myocardial Ischemia?**

Some of the cellular actions of PKD that have been defined in noncardiovascular cells suggest that PKD activity may play a role in regulating myocardial responses to ischemia. A series of elegant studies by Storz and Toker have shown that PKD becomes activated in response to mitochondria-derived ROS and triggers the induction of nuclear genes that mediate cellular detoxification and survival, such as manganese-dependent superoxide dismutase (MnSOD), through an nuclear factor κB–mediated pathway.\textsuperscript{17,65,66} Interestingly, mitochondrial ROS, nuclear factor κB, and manganese-dependent superoxide dismutase have all been implicated in the myocardial protective mechanisms underlying delayed preconditioning,\textsuperscript{67} suggesting a possible role for PKD-mediated pathways in this phenomenon. Such a possibility may at first appear contradictory to evidence that, in adult rat myocardium, PKD is not activated by ischemic preconditioning.\textsuperscript{48} However, in the pertinent study,\textsuperscript{48} PKD activity was measured only in the Triton-soluble fraction, although the same study also showed that the majority of PKD protein in myocardium resided in the Triton-insoluble fraction; thus, the measured activity is unlikely to have been representative of the true myocardial PKD activity. Other potential mechanisms by which PKD may regulate myocardial responses to ischemia include the phosphorylation of vanilloid receptor type 1 (TRPV1) (also known as transient receptor potential vanilloid type 1 (TRPV1),\textsuperscript{42} and the 27-kDa heat shock protein (HSP27).\textsuperscript{34} Because both TRPV1\textsuperscript{68} and HSP27\textsuperscript{69} have been proposed to modulate the extent of myocardial ischemic injury. Further investigation is needed to determine whether PKD plays a role in regulating the outcome of myocardial ischemia and to delineate the underlying molecular mechanisms.

**PKD in the Circulation**

In recent years, evidence has emerged that endothelial cells\textsuperscript{70} vascular smooth muscle cells\textsuperscript{71} and platelets\textsuperscript{72} also express PKD. Although information on the expression of distinct PKD isoforms in these cell types is scarce, PKD activity in them has been shown to be increased by physiologically important stimuli, such as vascular endothelial growth factor (VEGF)\textsuperscript{70} and hydrogen peroxide\textsuperscript{73} in endothelial cells; angiotensin II,\textsuperscript{71,74} platelet-derived growth factor,\textsuperscript{71} and thrombin\textsuperscript{75} in vascular smooth muscle cells; and thrombin\textsuperscript{72} and a thromboxane mimetic\textsuperscript{72} in platelets.

Functionally, PKD activity appears to regulate proliferation,\textsuperscript{70,76} migration,\textsuperscript{76} and apoptosis\textsuperscript{73} in endothelial cells, hypertrophy in vascular smooth muscle cells,\textsuperscript{77} and activation in platelets.\textsuperscript{72} Furthermore, interfering with PKD signaling has been shown to inhibit VEGF-induced angiogenesis in an in vivo model.\textsuperscript{76} Although the pertinent PKD substrates have not been identified in endothelial cells or platelets, PKD-mediated HDAC5 phosphorylation may facilitate angiotensin II–induced hypertrophy in vascular smooth muscle cells.\textsuperscript{77} Notably, the transcription factor cAMP-response element-binding protein, which also contributes to angiotensin II–induced vascular smooth muscle cell hypertrophy,\textsuperscript{78} is itself a PKD substrate.\textsuperscript{50} Finally, PKD may impact on vascular function indirectly by mediating the physiological actions of aldosterone in renal epithelial cells.\textsuperscript{79}

**Concluding Remarks**

Investigation of the regulation and functions of PKD family members in the constituent cells of the heart and the circulation is in its infancy. Nevertheless, the emerging evidence already suggests that, in such cells, PKD is responsive to physiologically important stimuli and may regulate fundamental processes such as contraction, hypertrophy, proliferation, and death. Expanding interest among cardiovascular investigators in this intriguing signaling pathway should facilitate the determination of the roles of PKD in regulating cardiovascular function in health and disease and identification of the pertinent PKD substrates. Such progress may also reveal novel targets for therapeutic manipulation through pharmacological or genetic approaches.

**Sources of Funding**

The work on PKD by the authors was supported by the British Heart Foundation and the Medical Research Council.

**Disclosures**

None.
References


Protein Kinase D in the Cardiovascular System: Emerging Roles in Health and Disease
Metin Avkiran, Alexandra J. Rowland, Friederike Cuello and Robert S. Haworth

doi: 10.1161/CIRCRESAHA.107.168211
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2008 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/102/2/157

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/