Erythropoietin and the cardiorenal syndrome: cellular mechanisms on the cardiorenal connectors

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Coexistence of renal and cardiac disease is associated with high morbidity and mortality. This pathophysiological condition, in which combined cardiac and renal dysfunction amplifies a progression in the failure of the individual organ, has recently been denoted as severe cardiorenal syndrome (SCRS) (19, 20).

In patients with declined renal function measured by glomerular filtration rate (GFR), an independent increased risk for cardiovascular events has been found. The hazard ratio for cardiovascular events was 1.4 with a GFR <60 ml/min and increased with diminished renal function to 2.8 in patients with a GFR <30 ml/min (99). A 40% higher adjusted risk for adverse cardiovascular outcomes or death was observed in those with relatively minor degrees of renal dysfunction (120). The prevalence of chronic renal failure (CRF) is reportedly high in patients with congestive heart failure (CHF). Furthermore, the prevalence and severity of CRF correlate with the severity of CHF (44, 72). These data support that combined cardiac and renal failure is a profound problem, and a further understanding of the underlying pathophysiological and molecular mechanisms is needed to improve therapy.

Our group proposed an interactive network of cardiorenal connectors, i.e., the renin-angiotensin system (RAS), nitric oxide and reactive oxygen species (NO-ROS) balance, the sympathetic nervous system (SNS), and inflammation, as the cornerstones of the pathophysiology of SCRS (20). Because erythropoietin (Epo) production declines in renal failure and Epo sensitivity might decrease by the cardiorenal connectors in patients with the SCRS, it is not surprising that anemia is a commonly occurring state coinciding with CRF and CHF (52). About one-half of all patients with CHF have anemia, and the prevalence of anemia increases with the severity of CHF, approaching 80% in those with New York Heart Association (NYHA) class IV (153). Declining renal function is also associated with anemia, approaching 90% of patients with a GFR <30 ml/min (93). The coexistence of heart and renal failure and anemia has been denoted as the cardiorenal-anemia syndrome.

In this review, we focus on how Epo could dampen the cardiorenal connectors. We propose that Epo treatment in patients with SCRS not only acts via hematopoietic effects but also intervenes in the vicious circle of cardiorenal connectors with subsequent deteriorating effects on cardiac, renal, and vascular function. Indeed, it appears that regular Epo treatment in anemic patients with diminished renal function improves cardiac performance (36) and delays the progression of kidney disease (101). There is also evidence that Epo treatment can be of clinical benefit to patients suffering from CHF with relatively mild anemia (153). Despite growing evidence about Epo having positive effects on both renal and cardiac function, little is known about the underlying mechanisms of action. Although examples exist of how the cardiorenal connectors could diminish the production of Epo, Epo resistance is not central in this review.

We investigate mechanisms by which (exogenous supplementation of) Epo could dampen the cardiorenal connectors in combined cardiac and renal failure. First, we will briefly recapitulate the proposed model of the cardiorenal connectors in the SCRS and supply background on Epo. Subsequently, an analysis using existing literature about the effect of Epo on these cardiorenal connectors with its possible underlying mechanisms will be provided.
SCRS and the Cardiorenal Connectors

Recently, we proposed that in SCRS, the cardiorenal connectors, i.e., RAS, NO-ROS balance, SNS, and inflammation, display mutual interactions and induce positive-feedback loops at many points (20). Considering RAS, inappropriate activation of RAS in renal and cardiac failure causes (dys)regulation of extracellular fluid volume and vasoconstriction (174), results in formation of ROS via activation of NADPH oxidase (29, 64), leads to vascular inflammation via the NF-κB pathway (132, 138), and increases sympathetic activity (38).

On the other hand, the imbalance between NO and ROS, by increased ROS production (69), a low antioxidant status (66) and lower availability of NO (164), may increase activity of preganglionic sympathetic neurons (180) and stimulate RAS directly by damaging the renal tubular or interstitial cells or by afferent vasoconstriction with chronic inhibition of NO synthesis (92). ROS are also major initiators of the inflammatory response, resulting in a shift toward production of inflammatory cytokines (177).

The chronic inflammatory state that is present in both CRF and cardiac failure, in turn, can cause ROS production by activating leucocytes to release their oxidative contents (13). As part of the systemic stress response, renin secretion is stimulated by cytokines and proinflammatory cytokines can stimulate norepinephrine release from sympathetic neurons (122).

Finally, the increased SNS activity in both renal and heart failure may induce inflammation by norepinephrine-mediated cytokine production (105) and by releasing neuropeptide Y, which can alter cytokine release and immune cell function (144, 183). In this way, all four cardiorenal connectors can augment each other with their deleterious effects in SCRS as a consequence. (For a more detailed description of the interaction among the cardiorenal connectors in combined cardiac and renal failure, see Ref. 20.)

Epo in SCRS

Several clinical studies have demonstrated the protective effects of Epo on cardiac and renal function (165). In patients with heart failure, correction of anemia with Epo was associated with an improvement of NYHA class and left ventricular ejection fraction (LVEF), a reduction in the need for hospitalization and high-dose diuretics, and an amelioration of peak exercise oxygen utilization and quality of life. In addition, stabilization of serum creatinine has been shown with correction of anemia (152–154). Similar findings were observed when anemia is treated in diabetic and nondiabetic patients with severe CHF and mild to moderate CRF (151). In predialysis CRF patients with anemia, a retrospective study of Epo treatment not only showed lower rates of hospitalization and treatment costs at the start of dialysis but also a lower relative risk of cardiac disease and death, compared with patients who received no or infrequent Epo therapy (36). Regression of left ventricular hypertrophy (130) and delayed progression of kidney disease (101) in anemic CRF patients treated with Epo have also been observed. When CRF patients are treated with Epo, correction of hematocrit up to 36% is desirable, because ameliorated cardiac and renal function is seen at this level, whereas normalization to 42% might increase cardiovascular events (16).

Because Epo treatment effectuates erythropoiesis, supplemental iron is required. Especially when given intravenously, iron may incite free radical formation and oxidative stress, which may lead to injury of cells and enhanced atherosclerosis (145). However, opposite results contradict this association, in that higher serum iron concentrations were associated with decreased mortality from cardiovascular disease (39). Furthermore, hemoglobin is a crucial antioxidant; thus anemia means decreased antioxidant capacity. In the studies described above, improved cardiac and renal function were seen on Epo treatment, even with supplemental iron. Thus positive effects of Epo have been demonstrated in patients with combined cardiac and renal dysfunction. These effects could result from increased hemoglobin levels or from nonhematopoietic actions of Epo treatment.

Regulation of Epo Production

To understand how Epo could exert its effects on the cardiorenal connectors in SCRS, it is important to understand how Epo production is regulated, how intracellular signaling of Epo takes place, and where it can influence processes by the existence of Epo receptors (EpoR).

Epo is a member of the cytokine superfamily, with significant homology to mediators of growth and inflammation (48). Its expression is primarily limited to cells in the fetal liver and the adult kidney. Soon after birth, the kidneys become the main site of production of (circulating) Epo (84). Most evidence favors that peritubular interstitial cells are the primary renal site of regulated Epo production, but a tubular origin is also possible (45). Low levels of Epo expression have also been found in other organs, including the lung, spleen, brain, and testis of rats (49, 180). Epo is secreted and circulates in the plasma with concentrations ranging from 3 to 15 U/l (140).

The primary stimulus of Epo production is tissue hypoxia, which activates hypoxia-inducible factor-1 (HIF-1), which in turn induces transcription of the Epo gene. The increase in Epo mRNA reaches its maximum at 4–8 h after exposure to hypoxia, following the time course of HIF-1 activation. The mechanism responsible for the activation of HIF-1 proceeds via the oxygen-labile subunit HIF-1α. Hypoxia blocks degradation of HIF-1α by blocking its association with von Hippel Lindau protein that targets HIF-1α for proteolysis (33).

Other stimuli can modulate Epo expression via transcription factors GATA-2 and NF-κB (53). Cytokines such as IL-1 and TNF-α activate GATA-2 and NF-κB, suggesting that both transcription factors are involved in the inhibition of Epo gene expression in inflammatory diseases (85, 103). In vitro, proinflammatory cytokines such as IL-1α, IL-1β, and TNF-α dose dependently inhibit hypoxia-induced Epo gene mRNA, and protein expression (50, 88). In chronic diseases, anemia is seen together with enhanced IFN-γ, IL-1, and TNF-α (109). Conversely, other studies have demonstrated that IFN-1, IL-1, IL-6, and TNF-α can lead to enhanced Epo and EpoR expression (32, 33, 99). Moreover, NF-κB has been shown to play a key role in HIF-1-regulated Epo gene expression (53).

Several studies also describe contradictory effects of ROS on Epo gene expression and Epo production (77, 87) and, similarly, the antioxidative extracellular superoxide dismutase can either suppress or enhance hypoxia-induced Epo gene expression (162, 182), possibly via modulation of hydrogen.

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peroxide levels or a subsequent change in NF-κB expression (35). NO seems to play a role in the induction of Epo production by inhibiting HIF-1α activation and destabilization of HIF-1α (76).

Finally, activity of RAS and SNS were also shown to affect erythropoiesis. Administration of ANG II dose dependently increases Epo production (56, 57) and angiotensin-converting enzyme inhibitors (ACEi) decrease plasma Epo concentrations, likely by inhibiting ANG II formation (131). Several studies have suggested that the SNS can stimulate erythropoiesis, because reduced SNS activity is accompanied by anemia, which could be corrected by administration of Epo (18, 135). However, there is a discordance because increased SNS activity is present in both renal and cardiac failure (38, 97), whereas anemia is also present. This could be caused by the predominance of factors other than SNS activity, such as inflammation, that decrease erythropoiesis.

Taken together, Epo production is primarily induced by tissue hypoxia, but inflammation, ROS, NO, RAS, and SNS can also modulate Epo production (Fig. 1). However, the exact regulatory mechanisms underlying the effects of these cardio-renal connectors on Epo production need to be clarified.

**EpoR Expression**

The most well-known effect of Epo is activation of receptors expressed specifically on erythroid progenitor cells, thereby promoting the viability, proliferation, and terminal differentiation of erythroid precursors and accelerating the release of reticulocytes from the bone marrow, resulting in an increase in red blood cell mass (84). Recombinant Epo therapy has been shown to directly stimulate hematopoiesis, thereby increasing hemoglobin levels (42). The effects of Epo on the growth of erythroid precursors is augmented by other hormones, such as androgens, thyroid hormones, somatomedins, and catecholamines (86). However, the effects of Epo extend beyond hematopoiesis. In the embryo, EpoR are found in almost every embryonic tissue; Epo acts as a major regulator of vascular formation and organ growth (89). The expression of Epo and human Epo-binding sites in adults has been demonstrated in other tissues and organs, including the human kidney, heart, and vascular system (Table 1).

Surprisingly, mechanisms of regulation of EpoR expression have not been well studied (1). It has been demonstrated that expression of the EpoR may be enhanced in a variety of nonhematopoietic cell types by the presence of hypoxia. In the rat brain, upregulation of Epo and EpoR has been demonstrated after induction of ischemia (15). Increased hypoxia-associated Epo and EpoR expression has also been shown in different tumor types (51). Whereas HIF-1α is known to mediate the expression of Epo, HIF-1α has not been identified as a regulator of EpoR gene expression. Some studies in erythroid cell lines indicate that the transcription factors GATA-1 and Sp-1 could be involved in EpoR gene regulation (31, 115). Nevertheless, it seems that multiple pathways for EpoR regulation

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**Table 1. Cell physiological effects of the activated Epo receptor**

<table>
<thead>
<tr>
<th>Organ</th>
<th>EpoR (Ref.)</th>
<th>Effect of Activated EpoR (Ref.)</th>
</tr>
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<tbody>
<tr>
<td>Heart</td>
<td>Cardiomyocyte (24, 178)</td>
<td>Proliferation (172), antiapoptosis (24)</td>
</tr>
<tr>
<td></td>
<td>Cardiomyoblast (123, 129)</td>
<td>Proliferation (123), antiapoptosis (129)</td>
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<tr>
<td></td>
<td>Cardiacfibroblast (126)</td>
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<tr>
<td>Kidney</td>
<td>Tubular cell (46, 175)</td>
<td>Proliferation (175), antiapoptosis (147)</td>
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<td></td>
<td>Mesangial cell (175)</td>
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<td></td>
<td>Glomerular cell (46)</td>
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<tr>
<td>Vascular system</td>
<td>Endothelial cell (8, 14)</td>
<td>Migration, proliferation (8), antiapoptosis (26), angiogenesis (28, 134)</td>
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<tr>
<td></td>
<td>Endothelial progenitor cell</td>
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<tr>
<td></td>
<td>Vascular smooth muscle cell (7)</td>
<td>Mobilization from bone marrow (70), proliferation, differentiation (10)</td>
</tr>
</tbody>
</table>

EpoR, erythropoietin receptor.
exist and not all of them are GATA-1 dependent (115). In the mouse brain, EpoR transcripts decrease during development. A similar pattern is seen in erythropoiesis, in which there is also a rapid reduction in EpoR expression as cells progress toward terminal differentiation (176). The scant available information suggests tissue-specific EpoR regulation, in response to different stimuli such as hypoxia or developmental aspects. However, the exact regulation of EpoR expression in different cell types remains to be elucidated. It might even be that the cardiorenal connectors act on EpoR expression, thereby possibly explaining the occurrence of Epo resistance in patients with CRF.

**Intracellular Signaling of Epo**

The first step in Epo signaling is induction of homodimerization of EpoR by Epo. Subsequently, one of the receptor-associated Janus family of protein tyrosine kinases, JAK2, is activated, leading to tyrosine phosphorylation of the EpoR cytosolic domain (37). Phosphorylated tyrosines provide docking sites for proteins, such as the signal transducer and activator of transcription factor 5 (Stat5), phosphoinositide-3 kinase (PI-3K), MAPK, NF-κB, and SHP1 (37) (Fig. 2). SHP1 can lead to JAK2 dephosphorylation and inactivation, thereby negatively regulating EpoR signaling (94).

One well-studied nonhematopoietic action of Epo is antiapoptosis. Epo can prevent apoptosis by consecutive activation of PI-3K and Akt (110, 157). Akt also induces a variety of other effects, including the mediation of anti-inflammatory cellular responses. Moreover, EpoR activation leads to increased NF-κB, followed by decreased apoptosis in erythroid progenitor cells (139). Next to activation of the PI-3K/Akt pathway, Epo-EpoR interaction leads to MAPK activation (61, 117), which is involved in cell proliferation (61).

The third pathway activated by EpoR via JAK2 is Stat5, which is thought to be important for mitogenic activity (133) but also protects against apoptosis (133, 158). Stat5 induces a variety of cellular responses, including upregulation of antiapoptotic genes such as Bcl2, Bcl-xL, and heat shock protein 70 (149, 150, 179). Furthermore, STAT5b has shown to affect inflammation by inhibiting NF-κB-mediated gene transcription, probably by competing with coactivators necessary for NF-κB signaling (107). STAT5 induces suppressors of cytokine signaling-1 (SOCS1), SOCS2, SOCS3, and cytokine-inducible SH2-containing protein (CIS1) (83). These SOCS family members take part in the negative-feedback loop to attenuate Epo signaling by binding to JAK2 or the activated EpoR (SOCS1 and SOCS3, respectively) or by blocking STAT binding to the EpoR (CIS1) (104). In this way, they are intrinsic modulators of JAK/STAT signaling. SOCS3 appears to be the most relevant in erythropoiesis in vivo, as SOCS3−/− mice have severe erythrocytosis and mice overexpressing SOCS3 are anemic (111). SOCS protein modulation could well be important in the regulation of the cardiorenal connectors, as discussed below.

**Known Actions of Epo on Cardiac, Renal, and Vascular Cells**

Multiple responses of cardiac, renal, and vascular cells on binding of Epo to its receptor have been described (Fig. 1). Activation of the EpoR has shown to stimulate proliferation in cardiomyoblasts (123) and cardiomyocytes (172). In vitro, Epo prevents apoptosis in rat cardiomyocytes exposed to hypoxia, as well as to oxidative stress. Additionally, in vivo rodent models of coronary ischemia-reperfusion showed that administration of Epo reduces cardiomyocyte loss by ~50%, reduces infarct size, increases viable myocardium, and mitigates ventricular dysfunction after myocardial infarction (24, 74, 129). Van der Meer et al. (165) performed an ischemia-reperfusion experiment in isolated rat hearts. Administration of Epo reduced the cellular damage by 56% during reperfusion, diminished apoptosis by 15% and resulted in significantly improved recovery of left ventricular pressure and coronary flow. Prevention of cardiomyocyte apoptosis in rats after administration of a derivative of Epo, lacking erythropoietic activity, indicates hemoglobin-independent cardioprotective effects (54). This finding is supported by a rabbit study in which cardioprotective...
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effects of Epo after infarction were seen, without an increase in hematocrit (129). It is likely that Epo actions on left ventricular function and coronary flow are not only mediated via antiapoptotic effects of Epo but also involve other actions such as increased NO production (137).

Several studies implicated that Epo also acts on renal cells. In vitro, human proximal tubular cells exposed to hypoxia and coincubated with Epo showed reduced apoptosis and high levels of Epo even increased cell proliferation (170). The same group of investigators demonstrated that Epo administration enhanced tubular regeneration and renal function recovery in rats with uni- and bilateral ischemia-reperfusion injury. Moreover, in a pig kidney endothelial cell (EC) line and in mouse mesangial cells, darbepoetin, an Epo analog, reduced apoptosis after exposure to different toxic and hypoxic stimuli (55). Finally, enhanced renal tubular regeneration (168), renal blood flow, and GFR by Epo administration were observed in a rat model with acute renal failure induced by cisplatin (9).

The mechanism of the vascular-protective effects of Epo is largely unknown. Various studies have demonstrated that Epo may affect the vascular wall on binding to local receptors. Epo may promote vascular-reparative processes (41). It was demonstrated that Epo could enhance mobilization of bone marrow-derived endothelial progenitor cells (EPC) and augmented neovascularization in animal models (70), enhanced EPC proliferation and differentiation in patients with advanced renal failure (10), and promoted EPC proliferative and adhesive properties in patients with CHF (59). Patients with CRF have endothelial dysfunction and enhanced oxidative stress (60), whereas Epo seems to have antioxidative properties (30, 155) and proangiogenic effects on EC (134). Furthermore, Epo stimulates human and bovine EC proliferation and migration (8, 27) and capillary outgrowth in an angiogenesis assay using adult human myocardial tissue in vitro (82). Chronic treatment with darbepoetin prevented endothelial injury in an experimental model of progressive CRF (41). Taken together, these studies indicate that local binding of Epo to cardiac, renal, and vascular cells invokes signaling cascades, leading to a cytoprotective and proliferative response.

How Does Epo Influence the Cardiorenal Connectors?

Epo and NO/ROS balance: Epo and NO. In SCRS, the balance between NO and ROS is shifted toward the latter (66, 69, 164). Actions of Epo on NO synthesis and release, on vasodilator responses in (isolated) blood vessels, and responses of vascular cells are controversial. Several lines of evidence support that Epo can regulate endothelial NO synthase (eNOS) via the PI-3K/Akt pathway (21). Because the systemic environment reacts in a complex manner on Epo administration, including changes in blood pressure, the actions of Epo on cardiac, vascular, and renal cells in culture will be considered first.

Extended Epo exposure of EC obtained from human umbilical, coronary, dermal, and pulmonary vessels induces transcription of eNOS and increases NO activity. During hypoxia, the response of EC to Epo administration to produce NO by induction of eNOS is enhanced (11, 14). Additionally, cardiomyocytes exposed to anoxia-reoxygenation in vitro are protected by Epo and display increased eNOS protein expression and NO production (137). In rats, 14 days of Epo treatment induce eNOS protein mass in thoracic aorta. In contrast to these studies indicating enhanced eNOS expression and NO production, there was no change in eNOS expression in kidney tissue obtained from Epo-treated rats (90). Moreover, in vascular smooth muscle cells (VSMC) of rats (102), but also in EC of human coronary artery (173), Epo decreased NOS expression and NO production. Scalera et al. (140) observed an Epo-induced increase in the endogenous NO synthase inhibitor ADMA in EC, leading to decreased NO production. These differences in vitro are somewhat difficult to explain. Scalera et al. underscore that many of the in vitro studies have applied concentrations that are only compatible with peak concentrations reached after Epo administration in vivo. In this regard, the studies summarized in supplemental Table 2a (the online version of this article contains supplemental data; see also www.nephrogenomics.net/data/appendices) support the suggestion that in vitro lower Epo dosages may increase NO, whereas high doses may diminish NO. In addition, elevation of hematocrit and hemoglobin and possibly blood pressure is known to independently stimulate eNOS expression and NO production. Conversely, CRF could suppress eNOS expression (167).

Few studies evaluated the effect of Epo on endothelium-dependent vasodilatation. In rats treated with a low dose of Epo for 14 days, acetylcholine caused significantly augmented concentration-dependent vasodilatation in thoracic aorta segments precontracted with phenylephrine (90). On the other hand, in high-dose Epo-induced hypertension in CRF rats, thoracic aorta segments showed impaired vasodilation to NO donors, suggesting either NO scavenging by increasing ROS or downregulation of guanylate cyclase by NO (169). Similarly, in rabbits treated with high-dose Epo, the endothelium-dependent vasodilatory response was decreased. Additionally, a reduced effect of a selective NOS inhibitor (N\textsuperscript{G}-nitro-L-arginine methyl ester; L-NAME) on acetylcholine-induced vasodilatation was observed in the Epo group, indicating that NO activity had been inhibited or sensitivity of NOS to L-NAME was decreased in Epo-treated rabbits (180). Similar effects have been observed in healthy human cutaneous vessels, in which local infusion of high doses of Epo could revert endothelium-dependent vasodilatation induced by acetylcholine (22). Taken together, there are several indications that Epo can enhance and diminish NO release, which may depend on concentrations and perhaps the presence of high blood pressure or a uremic environment. These studies clearly indicate the urge to explore Epo’s actions on NO production further to separate these factors and potentially, different effects in various cell types.

Because Epo can induce hypertension and seems to modulate NO release, several mechanisms for Epo-induced hypertension by impairment of the NO pathway have been postulated. These and other possible mechanisms for Epo-induced hypertension are discussed below.

Epo and NO/ROS balance: Epo and ROS. Besides modulation of NO, Epo also showed the capability to modify ROS. Little information is available about the effect of Epo on ROS specifically produced by cardiac, renal, or EC and most in vivo studies have been performed among patients with end-stage renal disease (ESRD), whereas our interest is pointed toward CRF (see supplemental Table 2b; see also www.nephrogenomics.net/data/appendices). As described below, these studies
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indicate that Epo indeed modulates oxidative balance. It can be hypothesized that these results may also be applicable to CRF patients, because in vitro studies show the effects of Epo, apart from ESRD conditions, and the proposed mechanism of free radical capturing by Epo treatment will be applicable in CRF patients as well. In rats, administration of Epo significantly reduced the increase in lipid peroxidation in cardiomyocytes after head trauma (47). However, when treating EC with increasing amounts of Epo, Scala et al. (140) observed an increase in ROS production and allantoin, a marker of oxygen free radical generation. Parallel to what was observed for NO, the greatest increase in oxidative stress on Epo treatment has been observed at high dosages (100–200 U/ml), whereas in vitro studies demonstrating diminished oxidative stress were performed with lower Epo concentrations (30).

Several studies have been conducted on oxidative stress parameters in plasma of ESRD patients receiving Epo treatment. In this patient population, the antioxidative capacity of red cells increased (23), as supported by enhanced antioxidant defense enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (43, 79, 116). SOD converts superoxide anions to \( \text{H}_2\text{O}_2 \) and \( \text{H}_2\text{O}_3 \) is subsequently detoxified by catalase or glutathione peroxidase. A decline in GPx in patients with CRF could also be prevented by Epo (127). Besides amelioration of antioxidative capacity, Epo intervenes in oxidative stress by modulation of lipid peroxidation as well. It has been demonstrated that markers of lipid peroxidation, like malondialdehyde (MDA), in plasma and red cells of hemodialysis patients diminish following Epo treatment (79, 106). This observation, however, is not consistent with another study indicating an increase in MDA (116). In sum, most of the evidence indicates that Epo diminishes oxidative stress; results pointing toward an opposite effect might be explained by Epo concentration dependency. Moreover, it must be emphasized that hypertension associated with Epo treatment might explain elevated ROS levels, and concomitant iron supplementation could also take part in free radical formation.

How Epo diminishes ROS is not clear. One option is mentioned above, namely, that Epo strengthens the antioxidative defense systems, possibly by increasing the number of erythrocytes, which are highly effective free radical scavengers (67). However, Epo has also been shown to protect against oxidative stress independently of erythrocyte number. In vitro, Epo scavenges hydroxyl radicals generated by the reaction between the oxidant phenylhydrazine and erythrocytes (34). Furthermore, oxidative stress-induced cell death in cerebral ischemia models demonstrated that neuroprotection by iron chelators is, in part, exerted by activation of a signal transduction pathway, leading to increased Epo gene expression (181). This indicates that Epo could exert antioxidative properties in a hemoglobin-independent manner.

Epo and the SNS. Information about the effect of Epo on the sympathetic nervous system is lacking for CRF patients, and it has been sparsely examined in patients with ESRD. In some studies, Epo-induced hypertension in hemodialysis patients is accompanied by increased plasma norepinephrine (58, 98, 142) or enhanced vascular response to norepinephrine (3, 68), whereas other investigators could not confirm this association (124, 126, 130) or even showed a decrease in plasma norepinephrine (78, 119). Other autonomic functions, like orthostatic blood pressure and baroreflex, also did not alter in hemodialysis patients receiving Epo treatment (136, 160). Even in the absence of an effect on blood pressure after Epo administration, plasma norepinephrine have been reported to increase (81, 130). Taken together, there is little systematic study on the interaction between Epo and the SNS, and the available data are conflicting.

Epo and inflammation. Several studies demonstrated protection against inflammation by Epo administration. Animal studies showed that Epo administration decreased the infiltration of inflammatory cells after spinal cord compression (63, 91). Furthermore, diminished levels of IL-6, TNF-α, C-reactive protein (CRP), and MCP-1 are seen in Epo-treated rodents with collagen-induced arthritis, autoimmune encephalomyelitis, cyclosporin nephropathy, or cerebral ischemia (2, 13, 40, 171). Moreover, Epo inhibited the expression of the iNOS gene and diminished nitrite production in oligodendrocytes cultured by IFN-γ and LPS (99). However, little is known about how Epo exactly modulates inflammation.

As mentioned, SOCS are downstream regulators of Epo signaling in the JAK/STAT pathway (104, 111), and we propose that these proteins are relevant in anti-inflammatory effects of Epo. SOCS have been demonstrated to dampen inflammation by attenuating proinflammatory cytokines. In vitro studies and various knockout mouse studies indicate that SOCS1 negatively regulates the IFN-γ/STAT1 pathway (5, 80), and SOCS3 showed a dampening of IL-6 signal transduction (143, 159). Because Epo induces expression of SOCS1 as well as SOCS3 (83), it can be speculated that this forms the underlying mechanism of anti-inflammatory properties of Epo treatment (Fig. 3).

Despite the dampening effect of Epo on inflammation, Epo also induces NF-κB and activating protein-1. Activating protein-1 participates in enhanced transcription of the proinflammatory factor IL-2 (118, 146, 148), whereas NF-κB has well-known proinflammatory actions as well.

Besides the mentioned effects on erythropoiesis, inflammation also seems to affect Epo responsiveness in patients on hemodialysis. In this field of interest, investigations among CRF patients are not available. ESRD patients with a poor response to Epo treatment express high levels of IFN-γ and TNF-α (108), both cytokines known to inhibit erythropoiesis in the bone marrow (6). The proinflammatory cytokine IL-6 is also enhanced in patients who need higher doses of Epo to achieve target hematocrit (62), and CRP is a good predictor for Epo resistance in hemodialysis patients with low hemoglobin levels (12, 75). Finally, an improved Epo response coincided with reversal of enhanced IL-6 and CRP levels in ESRD by treatment with an ultrapure dialysate (156). As regulation of inflammatory pathways involves upregulation of SOCS, this potentially could interfere with intracellular signaling of Epo, thereby possibly explaining Epo resistance and hampered erythropoiesis.

Epo and RAS. Epo treatment has been shown to protect against organ damage, and it might be speculated that Epo diminishes RAS activation. RAS might be dampened in a direct way or in response to increased blood pressure seen after Epo therapy. However, one of the proposed mechanisms for Epo-induced hypertension is an increased activity of RAS.

Few in vitro studies have been performed to evaluate blood pressure-independent actions of Epo on RAS. In VSMC, Epo
exposure enhances mRNA for angiotensin type 1 (AT1) and AT2 receptors and increases ligand binding (13). Moreover, Epo has been shown to increase sensitivity to ANG II by enhancing ANG II-induced intracellular calcium mobilization in VSMC (3, 121). Furthermore, in rats treated with Epo, an increase in mRNA for renin and angiotensinogen in kidney and aorta was observed; however, no change in plasma renin was shown (13). Although most of the in vitro evidence points toward enhanced activation of RAS by Epo, this could not be confirmed by diverse in vivo studies. Despite blood pressure-lowering effects of ACEi in Epo-induced hypertension, changes in plasma renin activity, ANG II, or plasma aldosterone could not be demonstrated in CRF and ESRD patients treated with Epo (81, 124–126, 130, 142). In one study, plasma renin activity and aldosterone declined in ESRD patients receiving Epo treatment for up to 12 mo (96). In in vivo studies, it is difficult to discern whether changes in RAS components result from direct effects or if they are secondary to effects of Epo on blood pressure. It might be that RAS activity decreases in response to a rise in blood pressure due to other mechanisms induced by Epo, such as increased endothelin-1 production.

To understand how Epo could modulate RAS, it seems important to know which intracellular signaling pathways are induced on ANG II stimulation. Several studies have demonstrated activation of the JAK2/STAT pathway after interaction of ANG II with the AT1 receptor (95). Although several STATs can be activated in different culture conditions (65, 71, 112), phosphorylated STAT3 consistently was present in ANG II-stimulated cardiac myocytes. This is interesting, because STAT3 is known for its cardiac hypertrophic effects (73, 100). In addition to its role as a blood pressure-regulating hormone, ANG II also promotes inflammatory responses by facilitating the release of proinflammatory mediators such as IL-6, which is induced in a JAK/STAT-dependent manner (141). Studies with IL-6 and IL-6-related LIF (leukemia-inhibiting factor), also activators of STAT3, showed that cross talk within the JAK/STAT pathway on different stimuli is possible, because ANG II showed inhibition of IL-6- as well as LIF-induced STAT3 (17, 163). Although the potential interaction between the JAK/STAT-pathway components is intriguing, it is confusing that ANG II enhances IL-6 production but can also dampen the IL-6 signaling pathway. Epo could intervene at different levels following ANG II stimulation, that is, by upregulating SOCS with subsequent dampening of ANG II signaling, IL-6 production, or IL-6 signaling. The exact mechanism remains to be elucidated.

The role of ANG II and SOCS in renal diseases has also been investigated (71). In cultured mesangial and tubular epithelial cells, overexpression of SOCS proteins prevented ANG II-induced STAT activation. In rats infused with ANG II, SOCS1 and SOCS3 are enhanced via JAK2/STAT1 after activation of the AT1 receptor. Additionally, in SOCS3 knockout rats, JAK/STAT activation by ANG II is increased, resulting in renal damage. By enhancing negative-feedback regulators, Epo might also interfere in the JAK/STAT pathway induced by ANG II (Fig. 4), resulting in renal and potentially also cardiac and vascular tissue protection. Although most of the mentioned studies on Epo and RAS have been performed among ESRD patients, we postulate that the proposed mechanism with intracellular signaling via JAK/STAT will also be applicable to CRF patients.

**Epo’s Effects On Blood Pressure**

Besides protective effects, Epo treatment in renal patients induces hypertension in 20–30% of cases (114), with a rise in diastolic and systolic arterial pressure of ~5 mmHg (161). Postulated mechanisms for Epo-induced hypertension include increased blood viscosity, alterations in vascular smooth muscle intracellular calcium levels, direct vasopressor action, arterial remodeling through stimulation of vascular cell growth, and changes in production or sensitivity to endogenous vasopressors, such as endothelin-1, catecholamines, and RAS, and vasodilatory factors, such as prostaglandins and NO (157, 166). Several mechanisms for Epo-induced hypertension by impairment of the NO pathway have been postulated. Interaction between Epo and its receptor induces Ca2+ channel activity with calcium influx, resulting in a rise in intracellular calcium levels directly followed by vasoconstriction (113). Chronic treatment with Epo has been shown to raise cytosolic...
Ca\textsuperscript{2+} concentration, which potentially antagonizes the actions of NO and could be reversed by calcium channel blockade (173). In addition to disturbed NO sensitivity via increased intracellular calcium, upregulation of an inhibitor of NOS (ADMA) by Epo decreases NO production by EC in vitro (140).

Several studies have shown that increased hematocrit and erythrocyte mass do not mediate Epo-induced hypertension, and conflicting results about the effects of Epo on endothelin-1, RAS, catecholamines, and prostaglandins exist (166). Moreover, it is not clear to what extent each of the proposed mechanisms contributes to the development of hypertension. Because the mechanism on Epo-induced hypertension is not well understood, it is difficult to speculate why one-fourth of patients develop hypertension on Epo administration and others do not. Hypertension possibly develops because of rapid reversal of anemia-induced peripheral vasodilatation with a less than complete reversal of the anemia-induced rise in cardiac output. This may be explained by impaired myocardial compliance following cardiac hypertrophy, which is more or less present in individual patients. Other predisposing factors to Epo-induced hypertension have been designated, such as age, antecedent hypertension, and no antiplatelet therapy (25). Even so, increased blood pressure is obviously unwanted in patients suffering from SCRS.

**Conclusions and Perspectives**

In SCRS, the interaction between the cardiorenal connectors leads to progressive failure of the heart and kidney. We propose that diminished Epo production (as a result of this syndrome) and impaired responsiveness to Epo actions could further amplify this negative interaction. Administration of exogenous Epo by either resolving the absolute deficiency or the relative insensitivity could dampen the cardiorenal connectors, thereby interrupting the vicious circle and thus intervening in the pathophysiology of SCRS (Fig. 5). In this review, the role of Epo and its mechanism of action in the SCRS have been analyzed. Although little is known about cellular mechanisms, studies demonstrated a protective role for Epo on cardiac, renal, and vascular function.

It is not yet clear to what extent each of the cardiorenal connectors mediates the development of combined cardiac and renal dysfunction and whether Epo modulates inflammation, NO-ROS balance, SNS, or RAS in a greater or lesser degree. However, it appears that a disturbance of NO-ROS balance is preponderant in the pathophysiology of SCRS and therefore an important role is assigned to this component. The effect of Epo on NO seems dose dependent, with increased NO production at Epo plasma concentrations reached during clinical application of Epo treatment. Most evidence on inflammation indicates
dampening of inflammatory cytokine production by Epo. The effect of Epo on RAS remains difficult to interpret, because of the blood pressure effects that Epo can elicit. Finally, very little is known about Epo and its effects on SNS. It should be taken into consideration that Epo treatment comes along with iron supplementation. Iron may affect NO-ROS balance by increasing free radical production, whereas iron is required for erythropoiesis and thus helps to reverse the low antioxidative anemic state.

Thus far, there is also a lack of evidence about the underlying mechanism of Epo protection. As suggested in this review, intracellular signaling via the JAK/STAT pathway, with upregulation of SOCS, seems to be a feasible explanation for the dampening effects of Epo on inflammation, RAS, and possibly a shift in NO-ROS balance toward NO. However, to elucidate which cellular pathways Epo induces in patients, and to distinguish hematopoietic from nonhematopoietic effects of Epo, a clinical study is urgently needed.

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