MINIREVIEW

Renal Interactions of Renin-Angiotensin System, Nitric Oxide and Superoxide Anion: Implications in the Pathophysiology of Salt-Sensitivity and Hypertension

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Summary
Renin-angiotensin system (RAS) plays a key role in the regulation of renal function, volume of extracellular fluid and blood pressure. The activation of RAS also induces oxidative stress, particularly superoxide anion (O$_2^-$) formation. Although the involvement of O$_2^-$ production in the pathology of many diseases is known for long, recent studies also strongly suggest its physiological regulatory function of many organs including the kidney. However, a marked accumulation of O$_2^-$ in the kidney alters normal regulation of renal function and thus may contribute to the development of salt-sensitivity and hypertension. In the kidney, O$_2^-$ acts as vasoconstrictor and enhances tubular sodium reabsorption. Nitric oxide (NO), another important radical that exhibits opposite effects than O$_2^-$, is also involved in the regulation of kidney function. O$_2^-$ rapidly interacts with NO and thus, when O$_2^-$ production increases, it diminishes the bioavailability of NO leading to the impairment of organ function. As the activation of RAS, particularly the enhanced production of angiotensin II, can induce both O$_2^-$ and NO generation, it has been suggested that physiological interactions of RAS, NO and O$_2^-$ provide a coordinated regulation of kidney function. The imbalance of these interactions is critically linked to the pathophysiology of salt-sensitivity and hypertension.

Key words
Renin-angiotensin system • Superoxide anion • Nitric oxide • Renal function • Salt-sensitivity • Hypertension

Introduction

In recent years, the pathophysiological aspects of excessive salt intake as an important contributor to the high prevalence of cardiovascular diseases including hypertension have been investigated in many studies (Franco and Oparil 2006, Mancia et al. 2007). Responsible mechanisms have been examined in clinical as well as experimental research by evaluating the responses during manipulation of dietary sodium intake (Hall et al. 1980, Jackson and Navar 1986, Sagnella et al. 1989, Midgley et al. 1996, Poch et al. 2001). The blood pressure response to changes in salt intake displays an individual variability leading to salt resistance or salt sensitivity (Weinberger et al. 2001, Haddy 2006). A various salt intake induces changes in regulation of sodium and extracellular volume homeostasis particularly by the kidney (Guyton et al. 1972). The renal hemodynamic and tubular function maintaining physiological sodium and extracellular fluid volume are regulated by several systems such as renin-angiotensin system (RAS), system of nitric oxide (NO) and reactive oxygen species (ROS), primarily superoxide anion (O$_2^-$) (Romero and Reckelhoff 1999).

It is generally recognized that RAS plays an important role in the regulation of blood pressure and renal function (Hall et al. 1980, Jackson and Navar 1986, Poch et al. 2001). In the kidney, the key role of RAS in the regulation of sodium and extracellular fluid homeostasis has been studied extensively and an inverse relationship between salt intake and RAS activity at
physiological state is now well established (Barri and Wilcox 1998, Husková et al. 2006). The relationship between fluid volume, sodium and endogenous RAS activity is critical for maintenance of normal blood pressure level. An enhanced activity of RAS can alter this relationship leading to the development of hypertension (Navar 2004, Kopkan et al. 2005, Husková et al. 2006). Angiotensin II (ANG II), as a major vasoactive agent, is responsible for physiological as well as pathophysiological effects of RAS (Navar 2004). Moreover, ANG II is a known stimulus for the formation of O$_2^-$ via activation of NAD(P)H oxidase (Nox) enzyme (Rajagopalan et al. 1996, Romero and Reckelhoff 1999). 

Three different Nox isoforms have been isolated in the kidney: Nox1, gp91phox called also as Nox2 and Nox4; however, Nox4 seems to be most abundant (Gill and Wilcox 2006). Nox enzymes are expressed along the renal vessels and nephrons and Nox activity is upregulated not only by ANG II but also by high salt intake (Hannken et al. 1998, Kitiyakara et al. 2003). Although O$_2^-$ is produced by many other oxidizing enzymes and mitochondria, their induction by ANG II or high salt is not clearly defined. Furthermore, prolonged effects of both ANG II and high salt diet can reduce the expression of defense system against O$_2^-$ such as superoxide dismutase (Chabrashvili et al. 2003, Kitiyakara et al. 2003, Welch et al. 2005). Thus increased production of ROS and their reduced degradation can lead to the oxidative stress with wide physiological and pathophysiological consequences. In the kidney, it has been clearly shown that increased O$_2^-$ activity induces vasoconstriction and enhances tubular sodium reabsorptive function (Majid and Nishiyama 2002, Makino et al. 2002). These direct renal effects may be significantly involved in the pathophysiology of salt-sensitive hypertension.

Another system that plays an important role in the regulation of blood pressure and renal function is a group of enzymes producing NO (called NO synthases; NOS). There are three isoforms of NOS (neuronal, nNOS; inducible, iNOS and endothelial, eNOS). Although the kidney contains all three isoforms, they are expressed along the blood vessels and nephron segments differently (Bachmann et al. 1995, Wu et al. 1999, Tojo et al. 2000, Herrera et al. 2006). NO is a very short-lived active free radical that exerts a wide range of physiological action (Romero and Reckelhoff 1999, Wink et al. 2001, Wilcox 2005). NO is characterized as a major vasodilator agent regulating basal vascular tone and it also inhibits renal tubular transport of sodium and thus plays an important role in overall excretory function of the kidney (Majid and Navar 2001, Wilcox 2005). Interestingly, eNOS that is considered to be the main source of NO production can be also induced by ANG II (Chin et al. 1999, Moreno et al. 2002). On the other hand, an excessive salt intake may also activate iNOS and nNOS in the kidney (Mattson and Higgins 1996, Tan et al. 2000, Schneider et al. 2008). Thus NO provides a protective role against several factors such as salt loading, oxidation or vasoconstrictors in the control of renal function (Zou et al. 1998, Chin et al. 1998, Lopez et al. 2003, Kopkan and Majid 2005, Červenka et al. 2008). The functional NO system is required for the maintenance of sodium and extracellular fluid homeostasis and any alterations in NO production lead to the impairment of organ function including the kidney.

Thus, in this minireview, we would like to focus on the current understanding of the involvement of renal interactions between RAS, NO and O$_2^-$ in the coordinated regulation of kidney function. As a central role of the kidney in the sodium and extracellular fluid homeostasis and thus long-term blood pressure regulation is still supported by current evidence, a further aim of our minireview is to consider the consequences of imbalance in these interactions that are critically linked to the pathophysiology of salt-sensitivity and hypertension.

**ANG II, NO and O$_2^-$ interactions and renal function**

Several mechanisms of the interaction between ANG II, NO and O$_2^-$ that are involved in the regulation of kidney function have been demonstrated in many studies (Wilcox 2005, Patzak and Persson 2007). Although direct acute effect of ANG II on renal hemodynamic and excretory function is well known, new findings have been described when NO or O$_2^-$ is pharmacologically interfered acutely. In rats (Lopez et al. 2003), it has been shown that acute ANG II infusion into the kidney caused a dose-dependent reduction in glomerular filtration rate (GFR) and NO level in the renal cortex. Moreover, the dose of ANG II administered into the dog kidney, that unaffected GRF under intact conditions, reduced GFR after NOS inhibition (Majid et al. 2005). This response was greatly attenuated by concomitant administration of a O$_2^-$ scavenger, tempol, or Nox inhibitor, apocynin (Lopez et al. 2003, Majid et al. 2005). These data clearly indicate an interactive role of ANG II, NO and O$_2^-$ in the
regulation of GFR, where NO displays protective properties against vasoconstrictors to maintain GFR. Furthermore, the sodium retaining effect of acute ANG II infusion was also influenced by concomitant O$_2^-$ generation (Lopez et al. 2003) and such effects were greatly accelerated under the conditions of NO blockade in dogs (Majid et al. 2005). Acute systemic infusion of ANG II in mice lacking catalytic subunit of Nox, gp91phox (gp91phox KO), which exhibit lower production of O$_2^-$, caused a lesser degree of decreases in renal blood flow (RBF) and increases in renal vascular resistance (RVR) compared to wild-type mice (Haque and Majid 2004). These observations demonstrate that the acute renal effects of ANG II are enhanced by an increased Nox-derived O$_2^-$ production, particularly under the conditions of NO deficiency, and they can be partially attenuated by the use of O$_2^-$ scavengers or Nox inhibitor and also in gp91phox KO mice.

These in vivo observations are well supported by in vitro studies testing the resistance and responsiveness of afferent and efferent arterioles, tubuloglomerular feedback (TGF) mechanisms and tubular transport in the isolated tubules. Glomerular arterioles account for the major part of renal vascular resistance and thus play important role in the control of RBF and GFR. It has been clearly demonstrated that constrictor responses of glomerular arterioles to ANG II were increased after NOS blockade (Ikenaga et al. 1996a). These responses to ANG II are also attenuated by addition of superoxide dismutase or tempol to this preparation (Schoonmaker et al. 2000, Ozawa et al. 2004). Thus O$_2^-$ appears to be partially responsible for ANG II-induced constriction of that vascular segment. Moreover, NO is diminished by increasing O$_2^-$ and thus vasoconstriction can be further enhanced (Wilcox and Welch 2000). These interactions directly modulate the TGF mechanism and tubular function in nephrons. Although ANG II is essential for TGR responsiveness (Ikenaga et al. 1996b, Schnermann et al. 1997), its increased production leads to the exaggerated TGF responses in hypertensive animals (Huang et al. 1988, Brannstrom et al. 1999, Welch et al. 2000). Furthermore, O$_2^-$ enhances the basal TGF response and limits NO signaling from the macula densa. On the other hand, NO particularly derived from the macula densa inhibits TGF to antagonize enhanced ANG II and O$_2^-$ effects (Braam and Koomans 1995, Ichihara and Navar 1999, Wilcox and Welch 2000, Wang et al. 2002). The role of ANG II and NO interaction in the regulation of tubular sodium transport within nephron segments has been studied conclusively (Wang and Giebisch 1996, Dickhout et al. 2002, Mori and Cowley 2003, Zhang and Edwards 2007, Silva and Garvin 2008) as these substances counteract each other. Significant finding has been made by Garvin’s group when a direct stimulatory effect of O$_2^-$ on sodium transport in thick ascending limb of loop of Henle has been found. And this stimulatory O$_2^-$ effect on tubules is also counteracted by NO (Ortiz and Garvin 2002a, 2002b, Juncos et al. 2006). Thus it is obvious that these mechanisms play an important role in the regulation of sodium excretion and may contribute in the development of salt-sensitivity and hypertension. This notion is further supported by observations that high salt intake induces production of NO as well as O$_2^-$ in the glomerular arteries, macula densa and tubules where they modulate physiological function of the kidney (Ortiz et al. 2003, Varela et al. 2004, Abe et al. 2006).

Collectively, these in vivo and in vitro studies propose new insights into the involvement of ANG II, NO and O$_2^-$ interactions in the regulation of renal hemodynamics and tubular function and suggest possible mechanisms that may contribute to the development of salt-sensitivity and hypertension (Fig. 1).

[Fig. 1. Pathophysiological impact of the imbalance between renin-angiotensin system, superoxide anion and nitric oxide in the kidney.]

Pathophysiological impact of RAS, NO and O$_2^-$ interactions in ANG II-dependent hypertension

Many studies have confirmed the prominent importance of RAS in the pathophysiology of
hypertension (Reckelhoff and Romero 2003, Navar 2004). Hypertension induced by chronic infusion of subpressor ANG II dose is a simple model which does not have elevated circulating or tissue renin activity. Although the enhanced plasma and mainly kidney ANG II levels has been confirmed in this model, renal hemodynamic and tubular function are maintained at relatively normal range or can be just slightly reduced (Von Thun et al. 1994, Wang et al. 1997, Kopkan et al. 2006). This observation strongly suggests that counteracting renoprotective mechanisms are activated. As seen in ANG II-induced hypertensive rats, NO partially counteracts the vasoconstrictor influence of elevated ANG II levels to maintain renal hemodynamics (Chin et al. 1998, Nishiyama et al. 2001). In addition, the exogenous L-arginine can significantly blunt angiotensin II-dependent hypertension and associated renal damage by increasing NO bioavailability in hypertensive animals (Rajapakse et al. 2008). The relation between high salt intake and blood pressure responses to chronic ANG II administration were also evaluated in rats (Sasser et al. 2002, Pech et al. 2006). As high salt intake exaggerated ANG II-induced increase in blood pressure, this model of hypertension exhibits an obvious salt-sensitivity. Moreover, the development of salt-sensitive hypertension has been demonstrated after transient exposure to ANG II in rats with progressive organ damage (Lombardi et al. 1999, Rodriguez-Iturbe et al. 2001).

There is a clear link between oxidative stress and the development of ANG II-induced hypertension (Wang et al. 2001, Reckelhoff and Romero 2003), but the exact mechanisms are not yet fully understood. It has been shown that production of O$_2^-$ is enhanced via NAD(P)H oxidase. Moreover, defense antioxidative enzymes (superoxide dismutase, catalase and glutathione peroxidase) in the kidney were reduced during ANG II exposure (Cifuentes et al. 2000, Chabrashvili et al. 2003, Welch et al. 2005, Pech et al. 2006). The development of hypertension induced by chronic low-dose infusion of ANG II was shown to be attenuated by chronic treatment with tempol in mice and rats (Kawada et al. 2002, Welch et al. 2005). An enhanced ROS production during chronic ANG II administration might not be only involved in the progression of organ damage. As it has been demonstrated that an enhanced O$_2^-$ activity mediates acute renal responses to ANG II (Lopez et al. 2003, Haque and Majid 2004, Majid et al. 2005), the possible role of enhanced O$_2^-$ generation in the regulation of renal function has examined in ANG II-induced hypertensive rats (Kopkan et al. 2006). This study showed that acute tempol infusion into the renal artery in anesthetized rats increased RBF, GFR and U$_{Na}V$ in hypertensive rats, but not in normotensive rats, implying that enhanced generation of O$_2^-$ modulates renal hemodynamic and excretory function leading to sodium retention in this model.

In the transgenic rat model of ANG II-dependent hypertension with constitutive mouse renin gene expression (TGR) which exhibits increased circulating as well as tissue ANG II levels and also oxidative stress since prehypertensive phase (Kopkan et al. 2005, Vaněčková et al. 2005), it has been observed that tempol administered directly into the renal artery acutely increased renal plasma flow (RPF) and GFR in these prehypertensive animals. Moreover, NOS inhibitor, nitro-L-arginine methylester (L-NAME) caused greater decreases in RBF and sodium excretion in prehypertensive TGR compared to age-matched control rats and these responses to L-NAME were abolished by co-administration of tempol (Kopkan et al. 2007). These data suggest that renal function is modulated by enhanced O$_2^-$ generation induced by ANG II in prehypertensive TGR and further support the notion that NO serves a protective role maintaining normal renal function in this model. To examine the role of this enhanced O$_2^-$ generation in the pathophysiology of hypertension in TGR, chronic antioxidant treatment was applied during the development of hypertension in these animals (Kopkan et al. 2009). However, despite of reduction in oxidative stress by tempol and apocynin administration in TGR, hypertension was not attenuated in this model. These data suggest that the markedly enhanced ANG II activity is almost entirely responsible for the development of hypertension with a negligible contribution of oxidative stress to the pathophysiology of hypertension in this model of ANG II-dependent hypertension (Kopkan et al. 2009).

In TGR, high-salt diet induced significant increases in blood pressure at all ages when compared with age-matched TGR fed normal-salt diet (Husková et al. 2006). Interestingly, salt-sensitive responses in early stages of hypertension were more pronounced in female than in male TGR. On the other hand, salt restriction led to lower progression of hypertension in both female and male TGR. This observation cannot be explained just by unresponsiveness of RAS activity to various levels of salt intake in these animals and thus other unknown mechanism needs to be considered (Husková et al. 2007). These studies indicate a strong salt-sensitive component
of hypertension with unanticipated sexual dimorphism. However, the exact role of the interaction between ANG II, NO and O$_2^-$ in the salt-sensitivity remains to be clarified in this model.

Another important model of ANG II-dependent hypertension is the two-kidney, one-clip (2K1C) Goldblatt model in which hypertension is induced by unilateral stenosis of the renal artery (Welch et al. 2003, Navar 2004, Červenka et al. 2008). The reduced renal perfusion pressure stimulates renin release from the clipped kidney triggering a whole RAS cascade with a slow progression of hypertension. Similarly to the previous models of ANG II-dependent hypertension, the augmented RAS activity leads to the induction of oxidative stress that may be involved in the pathophysiology of hypertension in this model (Lerman et al. 2001). This was further supported by acute administration of tempol that reduced RVR and increased RPF, GFR and sodium excretion in both clipped and nonclipped kidney (Guron et al. 2006). Moreover, chronic treatment with tempol reduced blood pressure and also improved renal function in the clipped kidney (Welch et al. 2003). A protective role of NO in the kidney has been also suggested in this model where enhanced NO release buffers ANG II-induced contraction of isolated afferent arteriole from the nonclipped kidney of 2K1C hypertensive rats (Helle et al. 2009). It seems that NO protective mechanism might be impaired in 2K1C hypertensive rats (Sánchez-Mendoza et al. 1998, Červenka et al. 2008). The administration of L-arginine, as a substrate for NO generation, has been shown to attenuate hypertension in 2K1C rats suggesting that NO production is insufficient in this model (Abreu et al. 1999). Moreover, treatment with losartan prevented increased vascular responses to ANG II, and this effect was associated with a restoration of NOS activity and NO release (Martínez et al. 2002). These data suggest that ANG II reduces NOS activity directly or via enhanced ROS production blunting vascular and renal effects of NO during the development of renovascular hypertension. Given together, these investigations collectively help in understanding these interactions and their possible involvement in the pathophysiology of hypertension.

Pathophysiological impact of RAS, NO and O$_2^-$ interactions in salt-induced models of hypertension

Dahl salt-sensitive rats exhibit progressive increases in arterial pressure in response to high salt intake leading to the endothelial dysfunction, inflammation and renal damage. An impaired NO system and oxidative stress have been suggested to significantly contribute to the development of hypertension in this model (Tan et al. 2000, Zicha et al. 2001, Mori et al. 2007, Zhou et al. 2009). However, exaggerated local RAS activity is also involved in the pathophysiological processes. Although Dahl salt-sensitive rats have low renin level and high salt intake suppresses plasma ANG II, it has been observed that kidney ANG II level is not decreased by high salt (Kobori et al. 2003). Moreover, high salt intake increases renal angiotensinogen level in Dahl salt-sensitive rats. This paradoxical enhancement may be an important contributor to the salt-sensitivity in this model (Kobori et al. 2003). It has been also indicated that sympathetic hyperactivity in Dahl salt-sensitive rats plays a major role in the maintenance of salt-sensitive hypertension (Zicha et al. 2001). These sympathetic responses to high salt intake are also closely associated with enhanced RAS and O$_2^-$ activity and NO deficiency and these interactions may influence renal function in hypertensive animals. There is a good agreement between these results and studies indicating that RAS inhibition attenuates hypertension, endothelial dysfunction and renal damage in Dahl rats (Tian et al. 2006, Liang and Leenen 2008, Zhou et al. 2009). Similar protective effects have been shown during antioxidant treatments, when renal function was improved in these hypertensive rats (Tomohiro et al. 1997, Tian et al. 2008).

In other salt-induced model of hypertension – deoxycorticosterone acetate (DOCA) rats – oxidative stress, insufficient NO production and local ANG II seem to play a role in the development of hypertension and impaired organ function (K-Laflamme et al. 1998, Shelat et al. 1999, Rhaleb et al. 2001, Beswick et al. 2001a, Sullivan et al. 2002, Obst et al. 2004, Erdely et al. 2007). Similarly to Dahl rats, antioxidant treatment or RAS inhibition attenuated hypertension and organ damage in DOCA-salt rats and improves endothelial dysfunction by increased NO generation in these hypertensive animals (Gross et al. 1999, Beswick et al. 2001a, 2001b, Xu et al. 2002, Ghosh et al. 2004). It could be argued that blood pressure lowering effect of O$_2^-$ scavengers such as tempol might be due to inhibition of increased sympathetic activity in DOCA rats. However, it was also demonstrated that SOD inhibition enhancing O$_2^-$ level caused stimulation of renal sympathetic activity and tempol ameliorated this effect (Shokoji et al. 2004), indicating that tempol-induced inhibition of sympathetic
activity could be related to its ability to scavenge $O_2^-$. In DOCA rats, it has been observed that NO plays a protective role against hypertensive DOCA effect, as orally administered L-NAME induced a further increase in blood pressure (Alvarez et al. 2000). These results suggest that NOS inhibition may also affect sodium retention induced by DOCA and, therefore, increases blood pressure in this model. Overall, this provides further evidence that ANG II, NO and $O_2^-$ interactions and their impact on regulation of renal function contribute to the development of salt-sensitive hypertension.

**Pathophysiologic impact of RAS, NO and $O_2^-$ interactions in NO-deficient form of hypertension**

The most conclusive evidence for the essential role of NO in the regulation of blood pressure and organ function was obtained from a model using pharmacological inhibition of NO production that primarily targets the constitutive eNOS as a major source of NO under normal condition. Although this is a simple experimental model, it reveals in fact very complex mechanisms that contribute to the pathophysiology of NO-deficient hypertension (Zatz and Baylis 1998). Acute cardiovascular and renal effects of NOS inhibition have been studied intensively in many *in vivo* and *in vitro* preparations, as mentioned above. In conscious animals, chronic administration of nonselective NOS inhibitors produced sustained hypertension, increased vascular resistance and an impairment of organ function including the kidney (Baylis et al. 1992, Lahera et al. 1992, Tolins and Shultz 1994, Kopkan and Majid 2006). An activation of RAS and sympathetic tone may also account, at least in part, for the vasoconstrictor activity after NOS inhibition (Fortepiani et al. 1999, Pechňová et al. 2004, Zicha et al. 2006). Moreover, an enhanced $O_2^-$ activity has been indicated as an important contributor for the pathophysiology of NO-deficient form of hypertension (Usui et al. 1999, Rauchová et al. 2005). Although an exact explanation for the exaggerated activities of vasoconstrictor systems during NO inhibition is still indefinite, this model could afford more pathophysiological mechanisms of the imbalance between those vasoactive substances than other hypertensive models.

Previous observations have shown that NO not only counteracts the vascular effects of vasoconstrictors, but it exhibits crucial properties in the maintenance of sodium and extracellular fluid homeostasis. This notion has been further supported by observation that NO-deficient hypertensive animals evidently exhibit salt-sensitivity (Tolins and Shultz 1994, Kopkan and Majid 2005) leading to the progression of renal injury in this model (Yamada et al. 1996, Graciano et al. 2004). Interestingly, after 3 week exposure to L-NAME and the 1 week washout period, a salt-sensitive state has been still observed in rats (Quiroz et al. 2001). This founding was explained by irreversible renal damage and inflammation after prolong NO blockade. Furthermore, it was observed in NO-deficient animals, that this exaggerated hypertensive response to high salt intake was abolished by concomitant administration of tempol with L-NAME (Kopkan and Majid 2005). These data indicate that $O_2^-$ contributes to the development of salt-sensitivity, which is involved in the pathophysiology of NO-deficient hypertension. The pathophysiological role of enhanced $O_2^-$ activity in the kidney in salt-sensitive responses was more than evident in this model. Indeed, acute treatment with an $O_2^-$ scavenger, tempol, increased RBF, GFR, and urinary sodium excretion in L-NAME-induced hypertensive rats (Kopkan and Majid 2006). Taken together, these results demonstrate that enhanced $O_2^-$ activity under the conditions of NO deficiency modulates renal hemodynamics and excretory function, which contribute to the development of salt-sensitivity and hypertension induced by chronic NOS inhibition.

Although specific contributions of particular NOS isoforms in the regulation of renal function and blood pressure have been studied in many models of hypertension, acute and chronic administration of the potentially selective inhibitors for nNOS or iNOS have yielded inconclusive results that were mostly limited by missing selective eNOS inhibition (Ollerstam et al. 1997, Mattson et al. 1998). However, studies in genetically modified mice strongly support the notion that eNOS isoform is a predominant and essential source of NO maintaining several physiological processes in the body (Ortiz and Garvin 2003). It has been observed that knockout mice lacking the gene for the eNOS exhibit higher blood pressure and impaired vasodilator responses (Huang et al. 1995, Faraci et al. 1998), whereas deletion of the genes encoding the nNOS or iNOS isoforms did not significantly alter cardiovascular system (Ortiz and Garvin 2003). In the mouse kidney, NO derived from eNOS regulates renal hemodynamics (Mattson and Meister 2005, Patzak et al. 2008), but it remains to be
determined if an increased salt and fluid absorption by the kidney may be involved in the hypertension exhibited by eNOS-deficient mice. Although nNOS and iNOS may partially compensate deficient production of NO in these mice (Mattson and Meister 2006, Patzak et al. 2008), the study by Ortiz et al. (2003) suggested that eNOS mediates NO production and thus regulates sodium chloride transport in thick ascending limb. This transport mechanism is impaired in eNOS deficient mice, in which it can be reversed by eNOS gene transfer to the thick ascending limb (Ortiz et al. 2003). These data indicate that genetic deletion of eNOS is not compensated for the thick ascending limb by other NOS isoforms. Thus an increased blood pressure response to a high-salt diet that has been observed in eNOS-deficient mice (Leonard et al. 2006) is likely promoted by reduced production of NO in the tubules. It remains to be resolved whether other mechanisms such as increased \( \text{O}_2^- \) may contribute in the salt-sensitivity and hypertension in this mouse model.

**Conclusions and Perspectives**

This minireview demonstrates the important interactive role between RAS, \( \text{O}_2^- \) and NO in the regulation of renal hemodynamics and tubular sodium excretion and its impact on blood pressure supporting the hypothesis that imbalance between these substances may contribute to the development of salt-sensitivity and hypertension (Fig. 1). These observations suggest that the activation of RAS, which is associated with an enhanced \( \text{O}_2^- \) activity, causes reduction of NO availability leading to the disparity between oxidative and antioxidative mechanisms in the tissues, which is involved in many pathophysiological processes in the body. It should be emphasized that further experiments are needed to characterize and delineate these interactions at the enzymatic level of the production of vasoactive substances such as ANG II, oxidative and nitrosative radicals in normal conditions as well as during the development of salt-sensitive hypertension. These new findings would increase our understanding of physiological as well as pathophysiological processes of many cardiovascular and renal diseases.

**Conflict of Interest**

There is no conflict of interest.

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