Important Role of NAD(P)H Oxidase 2 in the Regulation of the Tubuloglomerular Feedback

Mattias Carlström, A. Erik G. Persson

The tubuloglomerular feedback (TGF) mechanism is a negative feedback loop that senses changes in luminal NaCl delivery at the macula densa (MD) in the juxtaglomerular apparatus and adjusts the vascular tone of the afferent arteriole accordingly. This mechanism contributes importantly to the kidney's ability to regulate renal microcirculation, fluid homeostasis, and, consequently, blood pressure. Adenosine appears to be the mediator of TGF, whereas angiotensin II, NO, and superoxide (O$_2^-$) have important roles in modulating the response. A decrease in NO in the juxtaglomerular apparatus is associated with increased TGF sensitivity and an increase in arterial blood pressure.

Oxidative stress results from a shift in balance between the production of reactive oxygen species and action of antioxidant systems, in favor of reactive oxygen species, and has been implicated in the pathogenesis of hypertension. O$_2^-$ is one of the main reactive oxygen species and is in the kidney predominantly formed by NADPH oxidases (NOXs) in the vasculature, cortex, and medulla. MD cells express the main units of NOX and produce O$_2^-$ in response to angiotensin II or increased NaCl load. O$_2^-$ has been shown to enhance the TGF response primarily by scavenging NO in the MD but also directly by constricting the afferent arteriole.

Five NOX isoforms (NOX1 to 5) with a distinct tissue distribution have been found, and the potential isoforms expressed in the kidney are NOX1, NOX2, and NOX4. Using isolated and perfused afferent arterioles from NOX2-deficient mice, it was demonstrated recently that NOX2 (also known as gp91phox) plays an important role in the renal microcirculatory responsiveness to angiotensin II and adenosine and may also contribute to angiotensin II–induced hypertension. Until recently, however, the expression and function of each NOX isoform in the MD have been unknown. For the biochemical analysis of cellular and molecular mechanisms, sufficient MD cell quantities are required; these studies have, therefore, been attended with great difficulties, because MD cells only represent a minor part of the renal cells and are located in very small clusters. This problem has been partially addressed by the development of an MD-like cell line (MMDD1).

However, evaluations of MD cells harvested directly from renal tissue have been warranted.

In the present issue of Hypertension, Zhang et al have performed an interesting study in which they identified the NOX isoforms expressed by the MD cells and their role in NaCl-induced O$_2^-$ production. For the first time, laser capture microdissection was applied to isolate MD cells from the frozen renal cortex of rats. The captured cells expressed neuronal NO synthase (NOS; nNOS or NOS1) but not endothelial NO synthase (eNOS or NOS3), thus confirming that the cells were MD cells and not contaminated by the surrounding tissues. Single-cell RT-PCR was used to identify the different NOX isoforms and demonstrated that NOX2 and NOX4, but not NOX1, were expressed in the MD cells. The expression profile in the captured MD cells was essentially identical to that obtained in the MMDD1, further verifying the similarity of these 2 cell types and their suitability for MD research. In a lucigenin-enhanced chemiluminescence assay, this cell line was used in attempt to determine which isoform is responsible for NaCl-induced O$_2^-$ production. The cells were exposed to either low- (70 mmol/L) or high- (140 mmol/L) NaCl conditions, and all of the O$_2^-$ measurements were performed in the presence of the NOS inhibitor N-nitro-l-arginine methyl ester (l-NAME) to eliminate quenching by NO. It has been demonstrated that uncoupling of NOS may generate O$_2^-$. Future experiments are needed to elucidate whether increased NaCl load can cause uncoupling of nNOS in the MD.

Small-interfering RNA gene silencers were used to knock down NOX2 (efficiency: 91%) or NOX4 (efficiency: 86%), and scrambled small-interfering RNA was used as a negative control. High-NaCl solutions increased the O$_2^-$ levels of O$_2^-$ but not significantly inhibited the NaCl-induced O$_2^-$ production. The cells were exposed to either low- (70 mmol/L) or high- (140 mmol/L) NaCl conditions, and all of the O$_2^-$ measurements were performed in the presence of the NOS inhibitor N-nitro-l-arginine methyl ester (l-NAME) to eliminate quenching by NO. It has been demonstrated that uncoupling of NOS may generate O$_2^-$. Future experiments are needed to elucidate whether increased NaCl load can cause uncoupling of nNOS in the MD.

Small-interfering RNA gene silencers were used to knock down NOX2 (efficiency: 91%) or NOX4 (efficiency: 86%), and scrambled small-interfering RNA was used as a negative control. High-NaCl solutions increased the O$_2^-$ concentration in control MMDD1 cells compared with that with low-NaCl conditions. Knocking down NOX2 did not affect the basal levels of O$_2^-$ but significantly inhibited the NaCl-induced increases in O$_2^-$. In contrast, blocking NOX4 with small-interfering RNA had no effect on high-NaCl-induced O$_2^-$ production. Thus, NOX4 might contribute to the basal production of O$_2^-$ in the MD. However, the mechanism by which NOX4 expression and function is regulated and its biological relevance remains to be investigated.

Zhang et al further investigated whether NOX is the main source of O$_2^-$ production, through NaCl changes in the MMDD1 cells, by examining the relative contributions of NOX, xanthine oxidase, and cyclooxygenase-2 with use of specific antagonists (ie, apocynin, oxypurinol, and NS-398, respectively). Inhibition of xanthine oxidase or cyclooxygenase-2 had no effect, whereas adding the NOX inhibitor apocynin reversed the NaCl-induced increase in O$_2^-$ concentration. On this basis, the authors conclude that NOX is the...
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NO-sensitive fluorophore, has demonstrated that an increased intracellular pH and depolarization of the MD mechanisms stimulating NOX2 are not yet fully understood, but and increase the intracellular pH (pH_i), leading to increases in the production of O_2\textsuperscript{•–} from NOX2. O_2\textsuperscript{•–} will activate eot-5\’-nucleotidase (5\’-ND) and thereby increase the adenosine concentration. Increased NaCl reabsorption may also increase leakage of ATP through basolateral channels and thereby increase the adenosine formation. Extracellular ATP degradation occurs through ecto-nucleoside triphosphate diphosphohydrolases (E-NTPD), ecto-nucleotide pyrophosphatases/phosphodiesterases (E-NPP), and 5\’-ND.\textsuperscript{13} Activation of adenosine A\textsubscript{1} receptors (A\textsubscript{1}) and purinergic receptors (P2) on the vascular smooth muscle cells (VSMC) increases the cytosolic calcium concentration in the afferent arterioles, with consequent constriction. In parallel, the increased NaCl load will stimulate nNOS-derived NO production (unknown mechanisms). Future experiments will also have to elucidate whether increased NaCl load may cause uncoupling of nNOS in the MD, with subsequent O_2\textsuperscript{•–} production. NO will interact with O_2\textsuperscript{•–} and reduce its concentration via formation of peroxynitrite (ONOO\textsuperscript{–}) but may also directly antagonize the arteriolar vasoconstriiction.

Figure. Schematic illustration of the suggested mechanisms by which an increased NaCl concentration in the thick ascending limb (TAL) of Henle’s loop increases NOX2-derived O_2\textsuperscript{•–} production and, hence, enhances the TGF response. Increased tubular flow in the TAL will be sensed by the MD cells. The higher NaCl concentration will stimulate the apical Na\textsuperscript{+}/K\textsuperscript{+}/Cl\textsuperscript{–} cotransporter and the Na\textsuperscript{+}/H\textsuperscript{+}-exchanger. This will depolarize (PD) the MD and increase the intracellular pH (pH_i), leading to increases in the production of O_2\textsuperscript{•–} from NOX2. O_2\textsuperscript{•–} will activate eot-5\’-nucleotidase (5\’-ND) and thereby increase the adenosine concentration. Increased NaCl reabsorption may also increase leakage of ATP through basolateral channels and thereby increase the adenosine formation. Extracellular ATP degradation occurs through ecto-nucleoside triphosphate diphosphohydrolases (E-NTPD), ecto-nucleotide pyrophosphatases/phosphodiesterases (E-NPP), and 5\’-ND.\textsuperscript{13} Activation of adenosine A\textsubscript{1} receptors (A\textsubscript{1}) and purinergic receptors (P2) on the vascular smooth muscle cells (VSMC) increases the cytosolic calcium concentration in the afferent arterioles, with consequent constriction. In parallel, the increased NaCl load will stimulate nNOS-derived NO production (unknown mechanisms). Future experiments will also have to elucidate whether increased NaCl load may cause uncoupling of nNOS in the MD, with subsequent O_2\textsuperscript{•–} production. NO will interact with O_2\textsuperscript{•–} and reduce its concentration via formation of peroxynitrite (ONOO\textsuperscript{–}) but may also directly antagonize the arteriolar vasoconstriiction.

main source of O_2\textsuperscript{•–} in these cells. However, this interpretation should be viewed with some caution, because it was suggested recently that apocynin is not an inhibitor of vascular NOX but a potent antioxidant.

Emerging evidence suggests that MD-derived O_2\textsuperscript{•–} is important in the regulation of TGF (Figure). The present study by Zhang et al\textsuperscript{7} clearly demonstrates that NOX2 is the major NOX isoform responsible for O_2\textsuperscript{•–} production, through increases in the NaCl load at the MD. However, the mechanisms stimulating NOX2 are not yet fully understood, but increased intracellular pH and depolarization of the MD enhance the production of O_2\textsuperscript{•–} from NOX.\textsuperscript{9,10} Confocal microscopy in isolated perfused thick ascending limbs, using NO-sensitive fluorophore, has demonstrated that an increased NaCl load increases the NO concentration in the MD.\textsuperscript{11} The reactive anion O_2\textsuperscript{•–} can be metabolized by O_2\textsuperscript{•–} dismutase to hydrogen peroxide or scavenged by NO to form peroxynitrite. The latter interaction between O_2\textsuperscript{•–} and NO can enhance afferent arteriolar tone and reactivity through potentiation of TGF.\textsuperscript{3} It has been demonstrated that oxidative stress via O_2\textsuperscript{•–} activates eot-5\’-nucleotidase, thereby increasing the production of renal adenosine.\textsuperscript{12} This redox regulatory mechanism of adenosine is important in the control of renal microcirculation, and it is plausible that a reactive oxygen species–induced increase in adenosine production may be an important mechanism mediating the enhanced TGF responsiveness in pathological conditions such as hypertension. On this basis, one could speculate that NOX2-derived O_2\textsuperscript{•–} is not only an important modulator of the TGF response but might also be involved in mediating TGF by regulating the adenosine production. At the same time, nNOS-derived NO in the MD is important in modulating the O_2\textsuperscript{•–} concentration. The mechanism by which an increased NaCl load at the MD stimulates nNOS-derived NO is not clear and needs further investigation.

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Disclosures

None.

References

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